myPresto 4.2

USER MANUAL

Version 1.0

About copyright

The program myPresto includes software distributed to with "program use written consent".

Copyright indication as follows.

myPresto version 4 :

Software License Agreement

See the separate document.

Overview of myPresto version 4

tplgene: topology generator for protein. Available force fields are AMBER/CHARMm.

tplgeneL: topology generator for protein. Available force field the general AMBER force field (GAFF).

cosgene :Molecular dynamics simulation program. NVE/NVT/NPT ensemble, SHAKE, rigid model, multicanonical MD,various umbrella sampling, GBSA, etc.

sievgene : protein-compound docking program

Matrix : in silico screening (Multiple Target Screening method, Docking Score Index method)

LigandBox : compound 3D database generation tools

Hgene : add/remove H atoms of molecule, Gasteiger charge calculation, etc.

VCOL : combinatorial compound generation tool

confgene/ confgeneC :conformer generator for compound

MVO: modeling of protein-compound complex structure by the maximum volume overlap method

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Cited references

Please refer to the following works when using this software.

myPresto and the filling potential method

1) "The filling potential method: A method for estimating the free energy surface for protein-ligand docking", Yoshifumi Fukunishi, Yoshiaki Mikami, and Haruki Nakamura, J. Phys. Chem. B. (2003) 107, 13201-13210.

cosgene multicanonical MD

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3) "Multicanonical molecular dynamics algorithm employing adaptive force-biased iteration scheme", Jae Gil Kim, Yoshifumi Fukunishi, Haruki Nakamura, Phys. Rev. E 70, 057103 (2004).

Particle Mesh Ewald(PME)

4) U.Essmann, L.Perera, M.L.Berkowitz, T.Darden, H.Lee and L.G.Pedersen. A smooth particle meth Ewald method. *J. Chem. Phys.* 103, 8577-8593(1995)

Accessible surface area (ASA)について

5) Kinjo, A. R., Kidera, A., Nakamura, H. & Nishikawa, K. Physicochemical evaluation of protein folds predicted by threading. Eur Biophys J 30, 1-10. (2001).

Fast Multipole Method (FMM)

6) J. A. Board, Z. S. Hakura, W. D. Elliott, and W. T. Rankin. "Scalable variants of multipole-accelerated algorithms for molecular dynamics applications" In *Proceedings of the Seventh SIAM Conference on Parallel Processing for Scientific Computing*, February 1995.

7) W. T. Rankin, "*Efficient Parallel Implementations of Multipole Based N-Body Algorithms*." PhD thesis, Duke University, Department of Electrical and Computer Engineering, P.O.Box 90291, Durham, NC 27708-0291, April 1999.

8) W. T. Rankin, DPMTA?Distributed Parallel Multipole Tree Algorithm, Duke University, Durham, NC (2002).

sievgene

9) "Similarity among receptor pockets and among compounds: Analysis and application to in silico ligand screening", Y. Fukunishi, Y. Mikami, and H. Nakamura, The Journal of Molecular Graphics and Modelling 24 (2005) 34-45.

Multiple Target Screening (MTS) method

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Modelling,25, 61-70 (2005).

11) "Noise reduction method for molecular interaction energy: application to in silico drug screening and in silico target protein screening", Y. Fukunishi, S. Kubota, H. Nakamura, Journal of Chemical Information and Modeling, 46, 2071-2084 (2006).

12) "Improvement of protein-compound docking scores by using amino-acid sequence similarities of proteins", Y. Fukunishi, H. Nakamura, Journal of chemical information and modeling, 48, 148-156 (2008)

Docking Score Index (DSI) method

13) "Classification of chemical compounds by protein-compound docking for use in designing a focused library", Y. Fukunishi, Y. Mikami, K. Takedomi, M. Yamanouchi, H. Shima, H. Nakamura, Journal of Medicinal Chemistry, 49, 523-533 (2006).

14) "An efficient in silico screening method based on the protein-compound affinity matrix and its application to the design of a focused library for cytochrome P450 (CYP) ligands", Y. Fukunishi, S. Hojo, H.Nakamura, Journal of chemical information and modeling, 46, 2610-22 (2006).

Maximum Volume Overlap (MVO) method

15) "Prediction of protein-ligand complex by docking software guided by other complex structures", Y. Fukunishi, H. Nakamura, Journal of Molecular Graphics and Modelling, 26 (2008) 1030-1033.

Other references are listed at the end of this document.

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This software was developed as part of a research project advanced by the late Dr. Yoshimasa Kyogoku.

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Table of Contents

1 Overview

1.1 Molecule dynamics simulation system: myPresto

myPresto is a molecule dynamics simulation system for biomolecules which is equipped with a conformation search engine based on a highly efficient conformation search algorithm. myPresto was developed with the goal of creating an efficient general purpose system for the simulation of three-dimensional dynamic biomolecule structures and free energy calculation. The main areas of application include protein modeling, protein - pharmaceutical low molecule modeling, pharmaceutical docking, and calculation of film proteins.

myPresto version 4.0 is composed of the subsystems indicated below. The procedure for using the system is divided into the following stages: initial molecule coordinates and topology file preparation (tplgene/tplgeneL), energy minimization and MD calculation (cosgene) , and analysis of results using the analysis tools.

① Topology generator: tplgene
② Low molecule topology generator: tplgeneL
③ Conformation search engine：cosgene
④ Assembly of tools
⑤ Compound database：LigandBOX

Initial molecule coordinates and topology file preparation (tplgene/tplgeneL)
Energy minimization and MD calculation (cosgene)
Analysis of results using analysis tools

myPresto configuration

1.2 Topology generator : tplgene

When performing energy minimization and MD calculation using myPresto, a topology file must first be created for the molecule system. This file can be easily created using the tplgene subsystem.

Using tplgene, even when incomplete Cartesian coordinates that are missing some information (such as for a hydrogen atom) are used in standard input, complete Cartesian coordinates can be obtained as an initial structure for performing the conformation energy calculation. Supported force fields are AMBER and CHARMm.

1.3 Low molecule topology generator: tplgeneL

The low molecule topology generator tplgeneL can be used to create topology files for ligands and other low molecules that are not supported by tplgene.
Supported force fields are AMBER parm99 and AMBER General Amber Force Field(GAFF).

Calculation of the MD of a high molecule - low molecule compound can be performed by combining the topology files created with the tplgene and tplgeneL subsystems into a single file.

1.4 Conformation search engine: cosgene

cosgene performs energy minimization and MD calculation using the initial molecular coordinates and topology file that were prepared with tplgene as input. The main functions of cosgene are described below.
(Current version does not support the fast multipole method.)

Main functions of cosgene

Function type	Description
Energy minimization	Steepest descent method, Steepest descent method with SHAKE, Conjugate gradient method
MD calculation	Micro-Canonical, Canonical, Force-biased Multi-Canonical
	Tsallis Dynamics (under development)
Integrator	Leap-frog (Verlet), Velocity Verlet, RESPA
Thermostat	Hoover-Evans Gaussian constraint, Nose-Hoover
Barostat	Andersen, Parrinello-Rahman
Long distance interaction	Direct summation, Direct summation & Cutoff, Ewald, Particle Mesh Ewald, Fast Multipole Method
Restraint method	SHAKE, RATTLE, Rigid-body, Position restraint, Distance restraint
Boundary conditions	Sphere, ellipsoid, periodic boundary conditions

※Shaded items in the above table are not supported in this release.

1.5 Installation

（1）System requirements

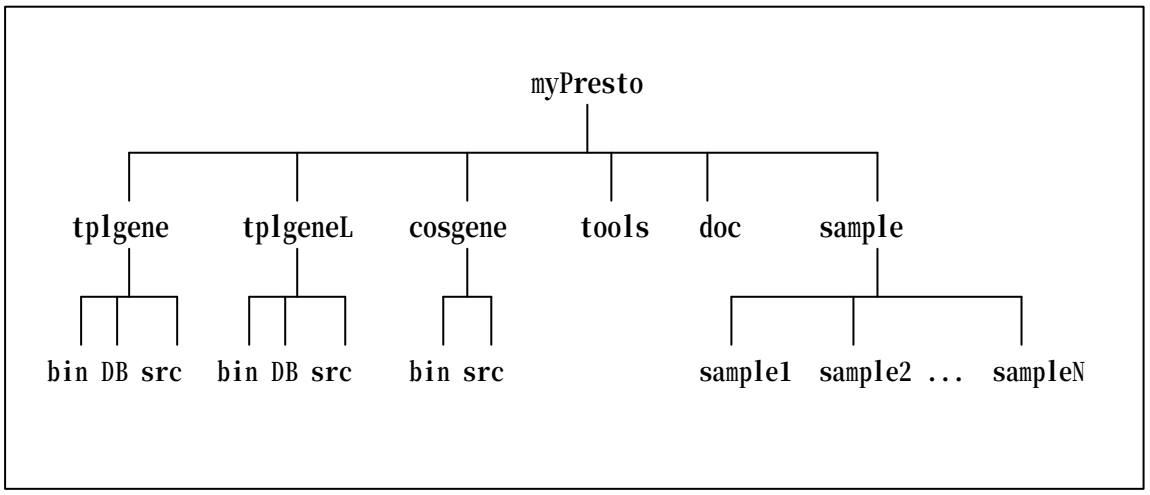
・UNIX (Linux) environment : Environment in which myPresto is run.
・C compiler : Used to build tplgene and tplgeneL.
・Fortran90 compiler : Used to build cosgene.

（2）Installation method

Copy the myPresto directory and its subdirectories to the desired installation directory.

The myPresto directory consists of the following subdirectories:

・tplgene : tplgene main module
・tplgeneL : tplgeneL main module
・cosgene : cosgene main module
・tools : Tool set
・doc : Documentation
・sample : Sample data (goes with chapter 5, "Calculation Examples", in this manual)



Use the "make" command in the "src" directory of tplgene, tplgeneL, and cosgene. Compile the tools in "tools" as needed.

【Note】It may be necessary to modify Makefile to suit your compiler environment.

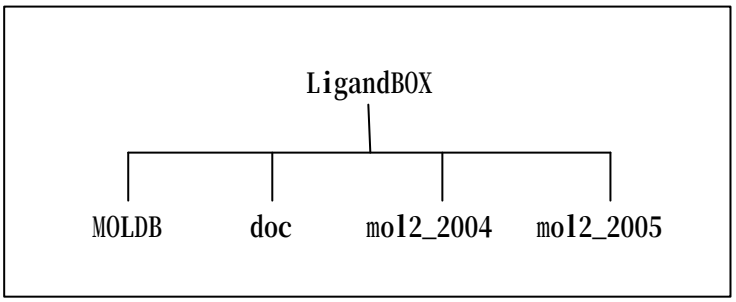
1.6 Compound database：LigandBOX

（1）Compound database LigandBOX

Mol2 file format dataset made by adding hydrogen atoms and estimating the total molecular charge from the 2D electron catalog distributed by Namiki Shoji Co., Ltd. in order to convert 2D molecular data into 3D data.

The directory configuration is as follows:

- MOLDB ：Compound database preparation tool
- doc ：Document
- mol2_2004 ：3D compound data prepared based on 2D electron catalog in 2004.
- mol2_2005 ：3D compound data prepared based on 2D electron catalog in 2005.

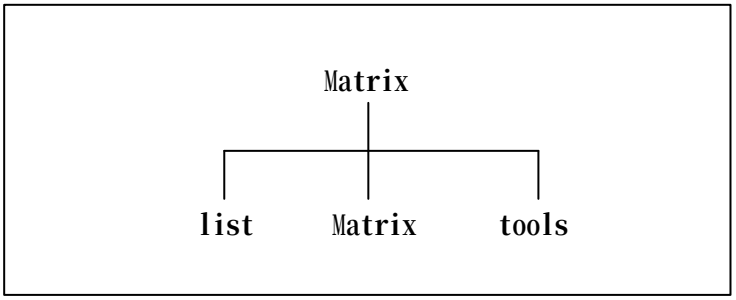


（2）Protein－compound interaction matrix

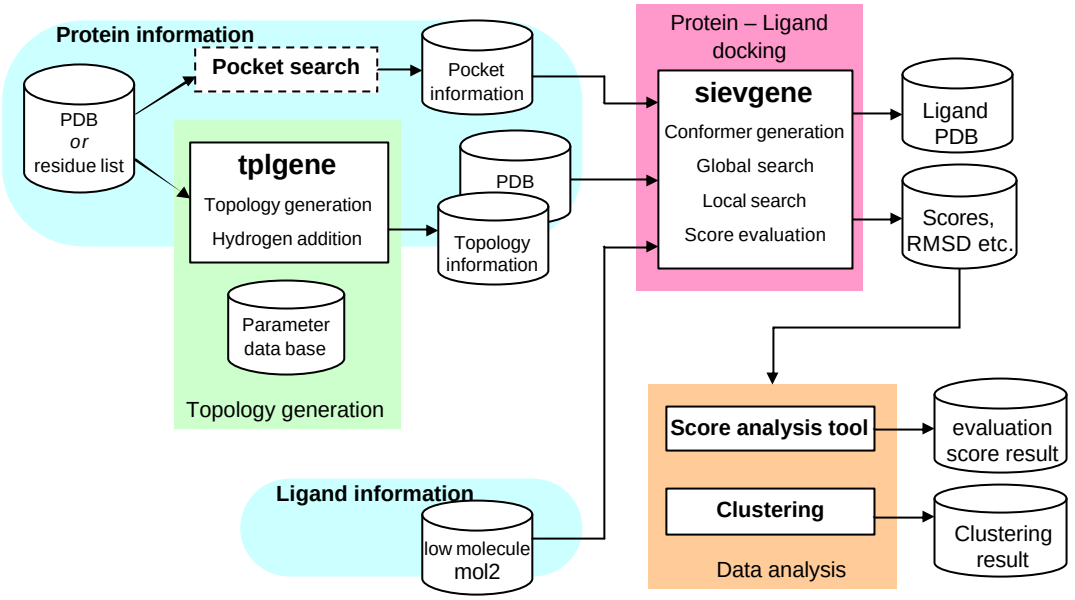
Protein－compound interaction matrix prepared from 3D compound data based on LigandBOX 2D electron catalog in 2004.

The directory configuration is as follows:

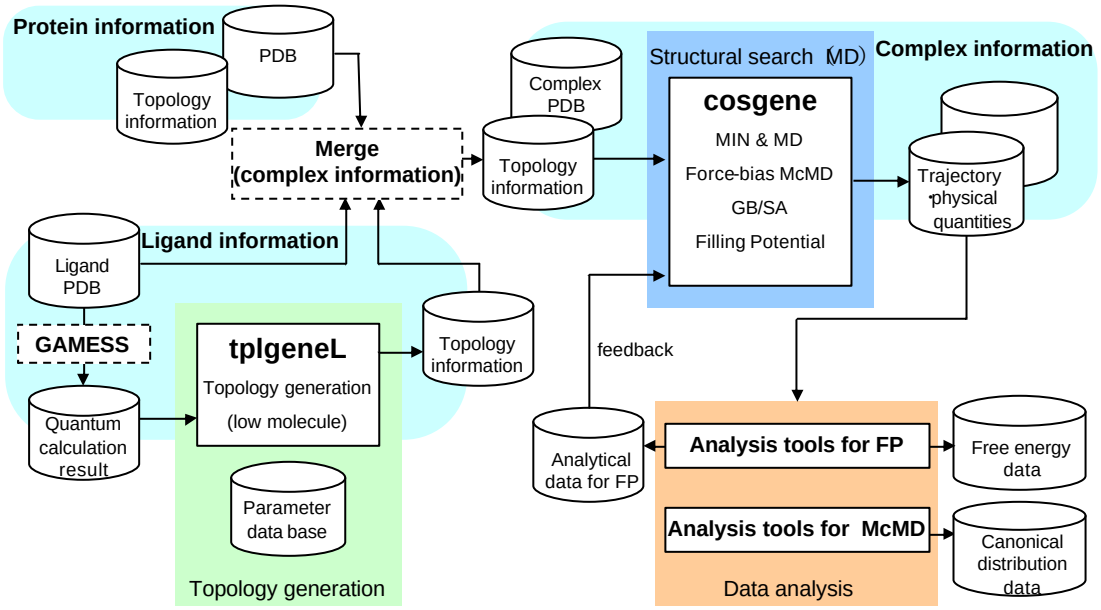
- list ：List of proteins and compounds
- Matrix ：Protein－compound interaction matrix
- tools ：Protein－compound interaction matrix analysis tool



◆Execution examples



rough *in silico* screening ～ tplgene & sievgene ～



Structural optimization and free energy calculation ～ tplgeneL & cosgene ～

2 tplgene

2.1 Execution

tplgene creates initial coordinate and topology files for a molecule using data related to the structure of the target molecule (PDB files and DIHED files) as input.

Directories referenced during calculation (directories for input files, output files, and force field DB files) can be set in environment variables. If environment variables will not be used, copy the input files and force field DB files to the execution directory ahead of time, as the current directory will be used.

When tplgene is executed, the following items are specified. These items can be entered interactively from the screen, or using command line options.

■Input items

- Title of topology file
- Molecule name
- Molecule type (1： Peptide chain, 2： DNA or RNA chain)
- Input file format (1：PDB, 2：DIHED)
- Force field DB file name
- Input file name
- Output PDB filename
- Output TPL filename

```
% tplgene
  or
% tplgene (option)
```

Items specified using command line options are skipped during interactive input. Only items that were not specified using command line options are entered interactively.

-title <title_name>
Specify the title in <title_name>
-molname <molculer_name>
Specify the molecule name in <molculer_name>
-i <input_coord>
Specify the input coordinate file name in <input_coord>
-db <db_file>
Specify the force field DB file name in <db_file>
-chain [pep | nuc]
Specify the type of molecule calculated
Peptide ⇒ pep
Nucleotide ⇒ nuc
-filetype [pdb | dihed]
Specify the type of input file
PDB file format ⇒ pdb
DIHED file format ⇒ dihed
-outcrd <output_coord>
Specify the output coordinate file name in <output_coord>
-outtpl <output_tpl>
Specify the output topology file name in <output_tpl>

■Example of option specifications (underlined parts are entered)

```
% tplgene -i vas.dih -chain pep -filetype dihed -db C96_aa.tpl
```

Instructions for using tplgene can be viewed by specifying the option "-h" or "-help".

```
% tplgene -h
  or
% tplgene -help
```

Items entered interactively (control specification) can be saved in a file (control_file) to eliminate the trouble of entering the items each time tplgene is executed.

```
% tplgene < control_file
```

■Control file example

```
ALA-ALA                 : Title line. Anything can be entered in 10 lines or less.
END                     : Enter "END" at the end of the title lines.
ALA-ALA                 : Molecule name. Any name can be entered
GLY-GLY                 : To calculate multiple molecules, write each molecule name
                          on its own line.
END                     : Enter "END" as the final line of the molecule names.
1                       : Enter "1" for a peptide chain, or "2" for DNA or RNA.
1                       : Enter "1" for PDB input, or "2" for DIHED input.
C96_aa.tpl              : Enter the force field DB file name.
ALA-ALA-input.pdb       : Enter the input file name.
ALA-ALA_out.pdb         : Enter the output PDB file name.
ALA-ALA-out.tpl         : Enter the output TPL file name.
```

The results output by tplgene are a topology that takes into account all atoms of the molecule system, and coordinate information. These two sets of information can be used to perform many conformation energy calculations.

If you wish to use separate directories for the data related to the structure of the target molecule, the force field used, and the output files, each path can be specified in an environment variable. If no environment variables are configured, the current directory at the time of execution is used (refer to "2.2.4 Environment Variables").

2.2 Creating input data

2.2.1 PDB files

Files in standard PDB format are used. The required information is indicated below.

(1) Amino acid residue name and residue sequence information

(2) Names of atoms of amino acid residues and cartesian coordinate information

(3) Disulfide bond information

(4) Circular molecule information

(1) and (2) above are required. (3) and (4) can be specified as necessary.

Disulfide bonds are defined according to normal PDB format. When calculating a circular molecule, specify the keyword "CIRCLE" on the line prior to the ATOM lines (see the example below).

When information on several molecules (several chains) is included in the PDB file, calculations of all included molecules are performed.

If your system includes metal ions and water molecules, these atoms must be specified by "HETATM" instead of "ATOM" in the PDB file.

■Example of PDB file

```
SSBOND   1 CYS A    6    CYS A   11
CIRCLE
ATOM     20  N   GLU A   4      33.037  -5.952  10.469
ATOM     21  CA  GLU A   4      33.629  -7.247  10.859
ATOM     22  C   GLU A   4      32.721  -7.845  11.909
ATOM     23  O   GLU A   4      32.470  -9.061  11.856
ATOM     24  CB  GLU A   4      35.029  -7.100  11.439
ATOM     25  CG  GLU A   4      36.081  -6.452  10.545
ATOM     26  CD  GLU A   4      35.906  -5.028  10.096
ATOM     27  OE1 GLU A   4      35.591  -4.102  10.842
ATOM     28  OE2 GLU A   4      36.158  -4.867   8.851
.
.
.
TER
HETATM   29  Zn  ZN    1      29.157   3.021  20.624  1.00 41.80          Zn
HETATM   30  Zn  ZN    2      20.538  16.287   4.630  1.00 43.88          Zn
HETATM   32  O   HOH   1      29.669  21.569  37.480  1.00 49.12           O
HETATM   33  O   HOH   2      20.132   6.585  18.359  1.00 60.57           O
HETATM   34  O   HOH   3      23.610  26.063  37.625  1.00 62.85           O
```

2.2.2 DIHED files

If you wish to generate Cartesian coordinates for one molecule, an effective method is to use a file in DIHED format. This format makes it possible to use the system by providing only amino acid residue and disulfide bond information, together with circular molecule information. The required information is indicated below.

(1) Amino acid residue name and residue sequence information
(2) Circular molecule information
(3) Disulfide bond information
(4) Dihedral angle information

(1) above is required. (2) through (4) can be specified as necessary.

If dihedral angle information is not specified, an elongated chain structure will be generated. If dihedral angle information is specified, a chain structure will be generated according to the provided values.

■Example of a DIHED file

The information entered in a DIHED file for a DODECA-PEPTIDE is as shown below. This peptide chain consists of 12 residues, and there is a disulfide bond between the 6th and 9th residues (CYS-CYS).

```
PRE>SEQUENCE
ASP                  :1
LYS                  :2
CYS                  :3 -----+
CYS                  :4      |
HIS                  :5      |
HIS                  :6    S-S BRIDGE
LEU                  :7      |
TRP                  :8      |
CYS                  :9 -----+
GLN                  :10
GLU                  :11
GLU                  :12

PRE>SSBONDS
    3    9
```

For amino acid residues in the PDB, enter the keywords below. Entries are from several groups beginning with "PRE>".

(1) Amino acid sequence (PRE>SEQUENCE)

Describe the amino acid residue sequence. Starting from the next line after "PRE>SEQUENCE", enter amino acid names in succession from the N terminal side. Enter one amino acid name per line.

Amino acids that can be used are as follows, for both the C96 and C99 data bases.
ACE (N terminal acetyl group) / ALA / ARG / ASN / ASP / ASH (ASP neutral) / CYS / CYSS / GLN / GLU / GLH (GLU neutral) /GLY / HIS / HISE / HIS / ILE / LEU / LYS / MET / PHE / PRO / SER / THR / TRP / TYR / VAL / NMEC (C terminal methyl group) / NHEC (C terminal amino group)
/ ABA (2-aminobutanoic acid) / NLE (2-aminohexanoic acid) / SEP (SER phosphate) / TYP (TYR phosphate) / THP (THR phosphate) / LYN (LYS neutral) / CYM (S^- non-protonated CYS)

(2) Specification of a circular molecule (PRE>CIRCULAR)

This indicates that the molecule is circular.

(3) Specification of S-S bond (PRE>SSBOND)

If the molecule has a disulphide bond, specify the bond as shown below.

PRE>SSBOND	: 1st line. Indicates that the molecule has a disulfide bond.
3 9	: #3 and #9 residues are joined by the SS bond.

(4) Specification of dihedral angles (PRE>DIHEDRAL-ANGLES)

Enter dihedral angle information. Enter ϕ, ψ, ω, and χ from the N-terminal to the C-terminal. Up to ten angles can be entered on each line. The angle definition follows ECEPP.

The following processing is performed within the program;
'+' ; Added to the end of the group name when LYS, ARG, or HIS is protonated.
'-' ; Added when ASP or GLU is non-protonated.
'E' ; Added if HIS has an AN HE hydrogen instead of an ANHD hydrogen.
'S' ; Added if CYS forms a disulphide bond.

The following processing is performed for N and C terminals.
'N+' ; Protonated N terminal
'N ' ; Neutral N terminal
'C-' ; Non-protonated C terminal
'C ' ; Neutral C terminal

The following processing is performed automatically in the current version.

'N+' is automatically added for an N terminal.
'C-' is automatically added for a C terminal.
'+' is automatically added for LYS, ARG.
'-' is automatically added for ASP, GLU.
'S' is automatically added to disulfide bonded CYS.

2.3 Force field database file

At present, the system has four types of force field databases for amino acids, two types of force field databases for nucleotides, and one type of force field dababase for water and metal ions.

Amino acid force field databases

C96_aa.tpl	Contains topology information for all amino acid monomers for the AMBER96 force field.
C99_aa.tpl	Contains topology information for all amino acid monomers for the AMBER96 force field.
charmm19_aa_all.tpl	Contains topology information for all amino acid monomers for the CHARMm19 force field.
charmm22_aa_all.tpl	Contains topology information for all amino acid monomers for the CHARMm22 force field.

Nucleotide force field databases

C96_na.tpl	Contains topology information for all nucleotides for the AMBER96 force field.
C99_na.tpl	Contains topology information for all nucleotides for the AMBER99 force field.

Force field database of water molecules and metal ions

metals.tpl	Contains topology information for water molecules and ions.

2.4 Environment variables

If you wish to calculate using separate directories for data relating to the structure of the applicable molecule, the force field to be used, and the output file, you can designate the path of each directory using environment variables.

The following 3 types of environment variables can be set. If you do not set environment variables, the current directory will be accessed during execution.

■Environment variables

Environment variable name	Explanation
TPL_INPUT_PATH	: Directory for tplgene input file (Must include path)
TPL_OUTPUT_PATH	: Directory for tplgene output file (Must include path)
TPL_DB_PATH	: Directory for tplgene force field DB (Must include path)

(Setting example)

Suppose you wish to set the directory for the tplgene force field DB to "/home/user01/myPresto/tplgene/DB". To set the environment variables, follow the setting method of the shell you are using.

(The underlined part indicates the part to be input.)

（1）In case of bash

```
% export TPL_DB_PATH=/home/user01/myPresto/tplgene/DB
```

（2）In case of csh

```
% setenv TPL_DB_PATH /home/user01/myPresto/tplgene/DB
```

※If the path to the directory (set in the environment variable) will not change, it is convenient to write it into a login script (.bashrc, .cshrc) or a dedicated script.

The shell which is currently being used can be checked using the following command.

```
% ps
```

3 tplgeneL

3.1 Execution

Using data (tplgeneL original format files or mol2 files) on the structure of the target molecule as input, tplgeneL creates initial coordinate and topology files for the molecule.

Directories (for input files, output files, and force field DB files) accessed during calculation can be set in environment variables. If environment variables are not configured, the current directory is used, and thus the input files, atom type definition files, and force field parameter DB files must be copied to the execution directory prior to execution.

When executing tplgeneL, specify the following items. These items can be entered interactively from the screen, or specified using command line options.

■Items entered

- Input file format (1：tplgeneL original format, 2：Sybyl mol2 format)
- Input file name
- Processing method when parameters are missing.
  (1： Use default parameters, 2： Automatically calculate parameters,
  3：Use default parameters. Dynamically calculate parameters for items with no parameters.)
- Parameter DB file name
- Use fragment DB? (yes: use, no: do not use)

```
% tplgeneL
  or
% tplgeneL  (option)
```

When an item is specified using a command line option, input of that item by interactive entry is skipped. Only items that are not specified using command line options are entered interactively.

-ft [1 | 2]

Specify the input file format.

tplgeneL original input file : 1

Sybyl mol2 input file : 2

-i <file>

Specify the input file name in <file>

-d <db_file>

Specify the parameter DB file name in <db_file>.

-r <resname>

Specify the residue name to be indicated in the output topology file in <resname> (4 characters or less).

-f [yes | no]

Specify whether or not the fragment DB is used.

Use ⇒ yes or y

Do not use ⇒ no or n

-p [1 | 2]

Select the method for compensating for missing parameters

Default parameters : 1

Dynamic compensation : 2

Default parameters + dynamic compensation : 3

■Example of specifying options (underlined parts are entered)

```
% tplgeneL -i methanol -d prm_gaff.db -f no
```

The option "-h" or "-help" can be specified to view the instructions for using tplgene.

```
% tplgeneL -h
  or
% tplgeneL -help
```

Interactive input (control) items can be stored in a file (control file) to eliminate the trouble of having to enter the items interactively each time tplgeneL is executed.

```
% tplgeneL  <  control_file
```

■Control file example

```
1               : Input file format (1:original、2:mol2)。
methanol        : File name (omit the extension).
1               : Compensation method for missing parameters
                : (1: default, 2: dynamic compensation).
prm_gaff.db     : Parameter DB file name
no              : Indicate whether or not fragment DB is used (yes/no)。
```

The high molecule topology file obtained with tplgene and the topology file obtained with tplgeneL can be combined to perform MD simulation of the high molecule - low molecule complex using cosgene.

If you wish to use separate directories for the target molecule structure data, the force field used, and the output files, the paths of the directories can be specified in environment variables. When environment variables are not configured, the current directory at the time of execution is used (refer to "3.2.6 Environment variables").

3.2 Creating input data

The input files that contain information on the molecule used in tplgeneL can be created in either tplgeneL original format (bond file, charge file, and zmat file) or Sybyl mol2 format (mol2 file).

3.2.1 tplgeneL original format files

When using input files in tplgeneL original format, the following three files are used:

(1) Charge information file (XXX.charge file (where "XXX" is the file name excluding the extension)).

This contains the atom name (item 1), the element symbol (item 2), Mulliken charge information (item 3), and Resp charge information (item 4).

(2) Bond information file (XXX.bond file)

This shows the combinations of the numbers of the bonded atoms (items 1 and 2), the bond length (item 3), and the bond order (item 4).

(3) Coordinate information file (XXX.zmat file)

Contains the Z-matrix information of the input molecule.

Files (1) and (2) above are required. When file (3) exists, the information in the file is reflected in the topology file.

■Example of charge information file

```
C1          C        -0.1320         -0.1320
O2          O        -0.7323         -0.7323
H3          H         0.1340          0.1340
H4          H         0.1653          0.1653
H5          H         0.1644          0.1644
H6          H         0.4006          0.4006
```

■Example of bond information file

```
  1    2      1.4130      0.7600
  1    3      1.1160      0.9530
  1    4      1.1200      0.9480
  1    5      1.1200      0.9480
  2    6      0.9630      0.7970
```

■Example of coordinate information file

```
C
 O      1   1.4132350
 H      1   1.1159340  2  112.6746
 H      1   1.1195330  2  107.3658  3  121.0117  0
 H      1   1.1196880  2  107.4483  3 -121.0171  0
 H      2    .9627370  1  107.7002  5 -120.0241  0
```

3.2.2 Sybyl mol2 files

In addition to files in tplgeneL original format, files in Sybyl mol2 format can also be used in tplgeneL.

Sybyl mol2 files contain information such as molecule information (@<TRIPOS>MOLECULE information), atom information (@<TRIPOS>ATOM information), and bond information (@<TRIPOS>BOND information). Among these, tplgeneL uses atom information and bond information. The following information is included in atom information and bond information.

(1) Atom information (@<TRIPOS>ATOM information)

- Atom ID : Consecutive number beginning from 1.
- Atom name : Atom name. The first and second characters are the element symbol.
- Coordinates : Coordinates in Cartesian coordinates.
- Atom type : Sybyl atom type.
- SuID : ID of substructure that includes the atom.
  <Not used in tplgeneL.>
- Substructure name : Name of substructure that includes the atom.
  <Not used in tplgeneL.>
- Charge : Information on charge of each atom.
- status bit : Status information unique to Sybyl.
  <Not used in tplgeneL.>

(2) Bond information (@<TRIPOS>BOND information)

- Bond ID : Consecutive number beginning from 1.
- Atom ID1 : Number of bound atom 1 (matches the above atom ID in the atom information).
- Atom ID2 : Number of bound atom 2 (matches the above atom ID in the atom information).
- Bond type : Bond type (1, 2, 3, am, ar, du, un, nc).
- status bit : Status information unique to Sybyl.
  <Not used in tplgeneL.>

【Reference】 Refer to the following for information on the Sybyl mol2 file format:
Tripos Online Mol2 File Format
URL http://www.tripos.com/custResources/mol2Files/index.html

■Example of Sybyl mol2 file

```
@<TRIPOS>MOLECULE
methanol.mol2
 6 5 0 0 0
SMALL
NO_CHARGES

@<TRIPOS>ATOM
      1 C          0.7253    0.0134    0.0001 C.3     1  <1>         -0.1320
      2 O         -0.6859   -0.0645   -0.0000 O.3     1  <1>         -0.7323
      3 H          1.0981    1.0651    0.0186 H       1  <1>          0.1340
      4 H          1.0901   -0.5342    0.9059 H       1  <1>          0.1653
      5 H          1.0900   -0.5012   -0.9251 H       1  <1>          0.1644
      6 H         -1.0287    0.8351    0.0005 H       1  <1>          0.4006
@<TRIPOS>BOND
     1    1    2    1
     2    1    3    1
     3    1    4    1
     4    1    5    1
     5    2    6    1
```

【Supplemental information】Reading mol2 files into tplgeneL

- **Mol2 file information referenced by tplgeneL**

tplgeneL does not reference @<TRIPOS>MOLECULE information.

Only atom and bond information

```
@<TRIPOS>ATOM
      :
@<TRIPOS>BOND
      :
```

in the specified mol2 file is obtained and processed. For this reason,

- If there is a format error in the ATOM or BOND sections, tplgeneL will show and error message and end.
- If there is a format error in @<TRIPOS> of other than ATOM or BOND, processing will continue.

- **Handling the status bit in the ATOM and BOND sections**

The status bits in MOL2 files are specified internally by SYBYL. The effective status bits are shown below. However, these items are not set by the user, and thus tplgeneL does not perform an error check on these items.

(Reference) Effective status bits for ATOM
DSPMOD, TYPECOL, CAP, BACKBONE, DICT, ESSENTIAL, WATER, DIRECT

(Reference) Effective status bits for BOND
TYPECOL, GROUP, CAP, BACKBONE, DICT, INTERRES

- **Handling bond types**

The following bond types are defined in MOL2 files.

```
1  = single
2  = double
3  = triple
am = amide
ar = aromatic
du = dummy
un = unknown
nc = not connected
```

When the bond type "am" is specified, the bond is processed internally as a single bond in tplgeneL. When the type "ar" is specified, it is processed as an aromatic bond.

When the type "du", "un", or "nc" is specified, processing is not possible in tplgeneL. An error message is output and the program ends.

【Error messages and causes】

No.	Error message	Cause
1.	ERROR> ltgReadMOParmMol2 Contents Error : *filename.mol2* Start of next line must not begin with "@" or "#", if line is continued with a back slash "\". Please check following information. *(data in vicinity of error)*	The first character of a line following the continue symbol is "@" or "#".
2.	ERROR> ltgReadMOParmMol2 Contents Error : *filename.mol2* It is necessary to describe sign "@" and "#" in column 1 of the line. Please check following information. *(data in vicinity of error)*	"@" or "#" is in a position other than the beginning of the line.
3.	ERROR> ltgReadMOParmMol2 Contents Error : *filename.mol* Atom format is wrong. Please check following information. *(data in vicinity of error)* or ERROR> ltgReadMOParmMol2 Contents Error : *filename.mol* Bond format is wrong. Please check following information. *(data in vicinity of error)*	With respect to the ATOM section, data continues after the continue symbol. 1st item includes characters other than numbers (atom ID). First character of 2nd item (atom name) is a number. 3rd item (x coordinates) includes characters other than numbers, "-", or "." 4th item (y coordinates) includes characters other than numbers, "-", or "." 5th item (z coordinates) includes characters other than numbers, "-", or "." First character of 6th item (atom type) is a number. 7th item (substructure ID) includes characters other than numbers. 9th item (atom type) includes characters other than numbers. Less than 6 items or more than 10 items are entered. With respect to the BOND section, data continues after the continue symbol. 1st item (bond ID) includes a character string. 2nd item (atom ID) includes a character string. 3rd item (atom ID) includes a character string. Less than 4items or more than 5 items are entered.
4.	ERROR> ltgReadMOParmMol2 Contents Error! The Bondtype ("*bond type*") that Mol2 Format does not support is found. Please check following information. *(data in vicinity of error)*	The 4th item (bond type) of the BOND section specifies a character string not defined in the MOL2 file format.

No.	Error message	Cause
5.	ERROR> ltgReadMOParmMol2 Contents Error! The Bondtype ("bond type") that tplgeneL does not support is found. Please check and modify following information. (data in vicinity of error)	The 4th item (bond type) of the BOND section is defined in the MOL2 file format, however, a bond type not supported by tplgene (du, un, or nc) is specified.
6.	ERROR> ltgReadMOParmMol2 File Format Error : *.mol2 File Format is not correct.	No ATOM line or no BOND line.
7.	ERROR> ltgDefineBond Contents Error! Isolated Atom ("*atom name*") that it has not any Bond is detected in Input File. Please check Input Files. : *.bond or *.mol2.	ATOM items and BOND items do not correspond. Too many ATOM items.
8.	ERROR> ltgReadMOParmMol2 Contents Error! Bond information does not match to Atom information. Please check mol2 file "*filename.mol2* ".	ATOM items and BOND items do not correspond. Too many BOND items.
9.	ERROR> ltgDefineBond Contents Error! The Bond Information is overlapped. : *(Duplicate atom combinations)* Please confirm Input Files. : *.bond or *.mol2.	The same atom combination is entered more than once in the BOND section.

3.3 Atom type definition file

tplgeneL first assigns the atom type of each atom in the molecule to be calculated, and then assigns force field parameters corresponding to each combination of atom types.

The atom type definition file contains atom type information corresponding to the environment (element symbol, number of bonds of atom, bond order, whether or not atom is in a ring, aromatic or not) of each atom.

Atom type definition files for the following two types of force fields are available in tplgeneL.

Atom type definition file

atomtype_gaff.db	DB file of atom type assignment rules for AMBER GAFF force fields
atomtype_amber99.db	DB file of atom type assignment rules for AMBER parm99 force fields

3.4 Force field parameter database file

tplgeneL assigns force field parameters based on the atom type assigned in "3.2.3 Atom type definition file".

The force field parameter database consists of the "prm_XXXX.db" file, which contains bond parameters for each atom type, bond angle parameters, dyhedral angle parameters, and improper dihedral parameter information, and the "nonbond_XXXX.db" file, which contains function parameters and nonbond parameters.

Force field parameter database files are currently available for AMBER parm99 and AMBER GAFF.

Force field parameter database files

prm_gaff.db	DB file for AMBER GAFF force field parameters
prm_amber99.db	DB file for AMBER parm99 force field parameters
nonbond_gaff.db	DB file for AMBER GAFF force field nonbond parameters
nonbond_amber99.db	DB file for AMBER parm99 force field nonbond parameters

【Supplemental information】AMBER GAFF parameters

Calculation can be performed in tplgeneL using AMBER ver. 7 GAFF (GAFF7) and AMBER ver. 8 GAFF (GAFF8) parameters. Using GAFF7, calculation is possible for almost all low molecules. Fewer molecules can be calculated using GAFF8, however, an accurate structure can often be calculated.

GAFF7 and GAFF8 cannot be used at the same time by a present specification. Copy the necessary files in the force field parameter DB directory before use.

GAFF7 calculation is selected by default.

Files for GAFF8

atomtype_gaff8.db, prm_gaff8.db, nonbond_gaff8.db

Files for GAFF7

atomtype_gaff7.db, prm_gaff7.db, nonbond_gaff7.db

Example) Copying files

Use the following commands to copy the necessary files.

Using GAFF8 parameters

```
cp prm_gaff8.db prm_gaff.db
cp atom_type_gaff8.db atom_type_gaff.db
cp nonbond_gaff8.db nonbond_gaff.db
```

Using GAFF7 parameters

```
cp prm_gaff7.db prm_gaff.db
cp atom_type_gaff7.db atom_type_gaff.db
cp nonbond_gaff7.db nonbond_gaff.db
```

3.5 Fragment database file

In addition to assigning parameters from AMBER as explained in "3.2.4 Force field database file", a part of a molecule can be regarded as a fragment in tplgeneL, and the user can assign unique parameters to that fragment.

The following information is entered in the fragment database file for each registered fragment (fragment block).

(1) Atom parameter information of fragment
(2) Bond parameter information of fragment
(3) Bond angle parameter information of fragment
(4) Dihedral angle parameter information of fragment
(5) Improper dihedral angle parameter information of fragment

Items 1 and 2 above are required to use a fragment database file. The parameters of 3 to 5 are used if stored.

The following two types of force field fragment database files are available in tplgeneL.

Fragment database files

frg_gaff.db	Fragment database file for AMBER GAFF force fields
frg_amber99.db	Fragment database file for AMBER parm99 force fields

3.6 Environment variables

When you wish to store data related to the structure of the target molecule, the force field used, and the output files in separate directories, environment variables can be used to specify the path of each directory.

The following three types of environment variables can be configured. If no environment variables are configured, the current directory at the time of execution is used.

■Environment variables

Environment variable name	Description
TPLL_INPUT_PATH	: Directory for tplgeneL input files (indicate with path included)
TPLL_OUTPUT_PATH	: Directory for tplgeneL input files (same)
TPLL_DB_PATH	: Directory for tplgeneL force field parameters DB (same)

Setting examples

Setting the directory for the tplgeneL force field parameter DB to "/home/user01/myPresto/tplgeneL/DB":

Environment variables are configured using the same method as for configuring the shell that is used.

(Enter the underlined parts.)

(1) For bash

```
% export TPLL_DB_PATH=/home/user01/myPresto/tplgeneL/DB
```

(2) For csh

```
% setenv TPLL_DB_PATH /home/user01/myPresto/tplgeneL/DB
```

※If the path to the directory set in the environment variable is fixed, it is convenient to write it in a login script (.bashrc, .cshrc) or dedicated script.

To check the currently used shell, use the following command.

```
% ps
```

(Blank)

4 cosgene

4.1 Execution

cosgene performs system energy minimization and MD calculation using information on the target molecule such as the initial coordinates and topology file prepared with tplgene and tplgeneL. The results of the calculations can be analyzed using the analysis tools.

Molecular information such as the initial coordinates, topology file, and calculation conditions are specified in the control file. cosgene loads the file by standard input.

```
% cosgene < control_file > output
```

4.2 Input data creation

4.2.1 Control file

The control file consists of the following groups. Each group is ended with "QUIT".

- **EXE> INPUT group** : Specifies the main input file names.
- **EXE> MINI group** : Specifies options for energy minimization.
- **EXE> MD group** : Specifies options for MD.
- **EXE> OUTPUT group** : Specifies output of the final results.
- **EXE> END** : Indicates the end of the control file.

```
EXE> INPUT
     TOPOLOGY= FORM   NAMETO= thrp.tpl   ; Topology file
     COORDINA= PDB    NAMECO= thrp.pdb   ; Initial coordinates
     QUIT
EXE> MINI
     METHOD=  CONJ                    ; Energy minimization using the conjugate gradient method
     LOOPLI=  40      UPDATE=  20     ; Calculate 40 times, update interaction table every 20 times.
     CUTMET=  RESA    CUTLEN= 8.0D0   ; Set CUTOFF length for interaction to 8A
     DIEFUN=  DIST    DIEVAL= 2.0D0   ; Use distance-dependant dielectric constant
     QUIT
EXE> OUTPUT
     COORDINATE= PDB  NAMECO=   thrp_mini.pdb   ; Final structure in PDB format
     QUIT
EXE> END
```

Example of control file for energy minimization

```
EXE> INPUT
     TOPOLOGY=   FORM     NAMETO=  serp.tpl   ; Topology file
     COORDINA=   PDB      NAMECO=  serp.pdb   ; Initial coordinates
     QUIT
EXE> MD
     LOOPLI=     20000          ; Number of MD steps
     UPDATE=     20             ; Frequency of interaction table updating
     TIMEST=     0.5D0          ; Time step of time integral
     METHOD=     CANONICAL      ; NVT canonical MD
     SETTEM=     300.0D0        ; Temperature setting
     INITIA=     SET      STARTT=     300.0D0   ;  Initial temperature setting
     RANDOM=     654321
     CUTMET=     RESA     CUTLEN=  10.0D0      ; Specification of energy CUTOFF
     DIEFUN=     CONS     DIEVAL=  1.0D0       ; Dielectric constant
     QUIT
EXE> OUTPUT
     COORDINATE=  PDB     NAMECO=   serp_md_1p.pdb   ; Final structure in PDB format
     QUIT
EXE> END
```

Example of control file for MD

Explanation of each control file command

Mandatory	◎
Can be omitted	△
Mandatory when the user designates certain functions	○

4.2.1.1 EXE> INPUT group

The INPUT group specifies external files that specify the topology, initial coordinates, and various atoms to be restrained or monitored (for the format of the external files, refer to "A File Formats" at the end of the manual). The same input group input is used for both "EXE> MIN" and "EXE> MD".

Items specified in the INPUT group :

(1) Topology of the system
(2) Coordinates of the system
(3) SHAKE atoms and restraining distance
(4) Fixed atoms and free atoms
(5) CAP potential
(6) Assignment of extended CAP potential
(7) Specifications for calculation of RMSD (when using MIN or MD)
(8) Position restraint
(9) Restraint of distance between atoms
(10) Dihedral angle restraint
(11) Monitored items
(12) System GB/SA and ASA parameters
(13) Umbrella restraint
(14) Alignment of center of mass of system
(15) QUIT

(1) Specification of topology of system

TOPOLOgy : Format of topology file (◎)
=NOREad ; No topology file input (default)
=FORMAtted ; Formatted ASCII file
=BINAry ; Binary file
UNITTOpology : IO units of topology file (△)
=10 ; (default)
NAMETOpology=(Topology file name, 80 characters or less. When TOPOLOgy=[FORM|BINA])

(2) Specification of system coordinates

COORDInate: Format of 3-dimensional coordinate file in PDB format (◎)
=NOREad ; No coordinate input (default)
=PDB ; PDB file format

=BINAry ; Binary file
UNITCOordiante : IO units of coordinate file (△)
=11 ; (default)
NAMECOordinate = (Coordinate file name, 80 characters or less. When COORD=[PDB|BINA])

(3) Specification of SHAKE/RATTLE atoms and restraint distance

If SHAKE/RATTLE is used, the atomic number of the target atom and the restraint distance may be designated by a file or the information may be automatically prepared. In this file, in addition to regular restraint of distance between two atoms, special distance restraint can also be specified in a 3-atom triangle (CH_2, H_2O) or 4-atom tetrahedron (CH_3, NH_3) topology. SHAKE/RATTLE is automatically prepared by the following method:

(a) Other than water molecule (molecule name is not "WAT")

If one to three hydrogen atoms bind covalently to an atom that is not hydrogen, their atomic distances are calculated and respectively set as SHAKE/RATTLE information of two to four atoms.

(b) Water molecule (molecule name is "WAT")

Set as SHAKE/RATTLE information of three atoms based on the bond distances of water held in the program.

(3－1) SHAKE/RATTLE information input designation

SETSHAke : Read file specifying atoms to which SHAKE/RATTLE will be applied. (○)
=NOREad ; Do not use SHAKE/RATTLE (default)
=READ ; Use SHAKE/RATTLE
UNITSHake: IO Units of SHAKE specification file (△)
=12 ; (default)
NAMESHake=(SHAKE file name, 80 characters or less (○))

【Note】 When using SHAKE/RATTLE, "SHAKEMethod= [HBON | ALLB]" must also be specified in the EXE> MD or EXE> MIN group.

【Note】 There are limitations on the range of application of SHAKE/RATTLE.

Range of application of SHAKE/RATTLE

		SHAKE	RATTLE
Energy minimization (EXE> MIN)	Steepest gradient method (METHOD=STEEP)	○	×
	Conjugate gradient method (METHOD=CONJ)	×	×
MD calculation	Leap Frog Verlet (INTEGR=LEAP)	○	×

(EXE> MD)	Velocity-Verlet (INTEGR=VELO)	×	○
	Multi Time Step (INTEGR=MTS)	×	×

(3－2) SHAKE/RATTLE automatic preparation information output designation

If SHAKE/RATTLE information is automatically prepared, the prepared information can be output as a file. The format of the output file is the same as the input file.

DBGSHA : SHAKE/RATTLE automatic preparation information output designation (△)
=NOWRite : Do not output file (default) .
=ASCIi : Output file.
UNITDS : IO units of SHAKE/RATTLE automatic prepration information file (△)
=84 : (default)
NAMEDS = (SHAKE/RATTLE automatic preparation information file name, 133 characters or less)

(4) Specification of fixed atoms and free atoms

Atoms specified as fixed atoms are not subject to MIN/MD calculation, and are treated as points where a force field is applied. Free atoms are subject to the normal MIN/MD calculation. Atoms to be fixed can be specified by atom number, or by specifying a particular center and radii R1 and R2 such that atoms at a distance R from the center where R1 < R < R2 are specified. For this purpose, a control file is necessary. Free atoms are specified in the same way. If these specifications are not made, all atoms in the system are treated as free atoms.

SETVARiables=:Format of fixed/free atom designation file (△)
=NOREad ; No fixed atom designation (Default)
=READ ; Designate fixed atoms
UNITVAribles : IO unit of fixed atom designation file (△)
=13 ; (Default)
NAMEVAriables =(Name of file designating fixed atoms, 80 chars. max.)

(5) Designation of CAP potential

This designates the atoms to which CAP potential is applied, coordinates of the CAP center, and constants for radius and force. You can designate atoms in the CAP designation file, and information like center coordinates can be designated either in the CAP designation file, or in the control file. However, control file input will take priority.

```
SETBOUndary : Designates atoms for applying CAP potential, and CAP radius and force
              constants (○)
    =NOREad          ; Do not use CAP
    =READ            ; Use CAP
UNITBOundary : IO unit of CAP designation file (△)
    =14              ; (Default)
NAMEBOundary=(Name of CAP designation file, 80 chars. max. (○) )
```

【Note】 In EXE>MD, you must add "CALCAP=CALC", and also add the designation of CAP parameters. It is best to designate "STOPCE=[TRANIBOTH]" and fix the 1st chain of the system (start molecule) in space, so that CAP potential does not shift from the 1st chain.

(6) Designation of ExtendCAP potential

Specify atoms to which an ExtendCAP potential, restraint range, and force constant are applied. A spherical orellipsoidal body can be designated for the restraint range.

```
SETEXtendCap : Specify atoms to which an ExtendCAP potential, restraint range,
               and force coefficient are applied. (○)
    =NOREad          ; ExtendCAP is not used.  (default)
    =READ            ; ExtendCAP is used.
UNITExtendCap : IO Units of ExtendCAP designation file (△)
    =23              ; (default)
NAMEExtendCap=(ExtendCAP designation file name, 133 characters or less (○) )
```

【Note】 "EXTCAP=CALC" should be added to EXE> MD. It is desirable to prevent the CAP potential from deviating from the first chain by specifying "STOPCE= [TRAN|BOTH]" and spacially fixing the first chain (leading molecule) of the system.

(7) Designation for RMSD calculation (when using MIN or MD)

```
REFCOOrdinate : Reference file. The coordinate file in PDB format which serves as the basis.
    =NOREad      ; Do not use (Default)
    =PDB         ; Use
UNITREfcoordi : IO unit of reference file (△)
    =15          ; (Default)
NAMEREFcoordi=(Reference file name, 80 chars. max.)
```

【Note】 Add "BESTFIt=YES" to EXE>MD or EXE>MIN for RMSD calculation.

(8) Designation of position restraint

You must prepare the following two files in order to use position restraint.

- A restraint designation file which designates the atoms to be restrained and information about the force constant
- Reference file in PDB format listing coordinates to be restrained

REFCOOrdinate : Reference file, same as for RMSD (○)
=NOREad ; Do not use (Default)
=PDB ; Use
UNITREfcoordi : IO Unit of reference file (△)
=15 ; Default
NAMEREFcoordi=(Reference file name, 80 chars. max. (○))
POSITIonrestrain : Designation of applicable atoms and force constant etc. (○)
=NOREad ; Do not use (Default)
=READ ; Use
UNITPOsition : IO unit of file designating atoms to be constrained
=16 ; (Default) (△)
NAMEPOsition=(Name of file designating atoms to be constrained, 80 chars. max. (○))

【Note】 You must also designate "CALPSR=CALC" and position restraint parameters in EXE>MIN or EXE>MD.

(9) Designation of restraint distance between atoms

Prepare a file designating the distance restraint between atoms.

DISTANcerestrain : Use restraint distance between atoms
=NOREad ; Do not apply (Default)
=READ ; Apply
UNITDIstance : IO unit of distance designation file
=17 ; (Default) (△)
NAMEDIstance=(Name of file for designating distance between atoms, 80 chars. max.)

【Note】 You must designate "CALDSR=CALC" and restraint potential weight parameters in EXE>MIN or EXE>MD.

(10) Specification of dihedral angle restraints

Prepare a dihedral angle restraint file.

```
DIHEDRalrestrain : Use dihedral angle restraints
    =NOREad        ; Do not apply (default)
    =READ          ; Apply
UNITDH : IO units of dihedral angle restraint file.
    =18            ; (default) (△)
NAMEDH= (Name of dihedral angle restraint file, 80 characters or less)
```

【Note】 "CALDHR=CALC" and restraint potential weight parameters must be specified in EXE>MIN or EXE>MD.

(11) Specification of monitored items

During MD, real-time monitoring is possible of coordinates of specific atoms, the distance between atoms, angles, dihedral angles, and other items, with the results output to a file. Prepare a file designating the atoms and pairs of atoms to be monitored.

```
OUTMONitoritems : Monitor information file
    =NOREad        ; Apply (default)
    =READ          ; Do not apply
UNITMO : IO units of monitor file
    =19            ; (default) (△)
NAMEMO= (Name of monitor file, 80 characters or less (○))
```

【Note】 Set the following items in EXE> MD.

```
OUTTRJ= n   : Output every n steps.
NAMETR= (Monitor information output file)
MNTRTR= [ASCI | BINAry]   : Output format
```

(12) System GB/SA and ASA parameters

```
ASAREA : File specifying GB/SA and ASA parameters (○)
    =NOREad        ; No file input (default)
    =READ          ; File input
```

UNITSA： I/O units of GB/SA and ASA parameter file (△)
=77 ; (default) (△)
NAMESA= (Name of GB/SA and ASA parameter file, 80 characters or less (○))

【Note】 The GB/SA and ASA parameter file can be created using a special tool. The radius of each atom, atomic solvation parameter, and other information are specified in the file (for the specification method, see "A File Formats" at the end of this manual).

(13) Specification of umbrella restraint

UMBREL：Umbrella restraint file (○)
=NOREad ; Do not apply (default)
=READ ; Apply
UNITUI：I/O units of umbrella restraint file (△)
=22 ; (default) (△)
NAMEUI= (Name of umbrella restraint file, 80 characters or less (○))

【Note】 The umbrella restraint file is used when the Filling Potential method is applied (for the specification method, see "A File Formats" at the end of this manual).

(14) Specification of alignment of center of mass of system

SETORIgin：Place center of mass of system at coordinate origin.
=NO ; Do not apply (default)
=YES ; Apply

(15) QUIT

Ends input of the EXE> group.

4.2.1.2 EXE> MINimize group

Items required for energy minimization such as the method, convergence conditions, calculation result output, energy terms used in calculation, and boundary/restraint conditions are specified in this group.
Almost all specifications related to energy calculation are the same as those for the EXE>MD group.

MIN/MD input items

		MIN	MD
1	Energy minimization control parameters (same as for STEEP/CONJ)	○	
1－1	Control parameters for steepest descent method (STEEP)	○	
1－2	Control parameters for conjugate gradient method (CONJ)	○	
1－3	Output of calculation results (same as for STEEP/CONJ)	○	
1	MD control parameters		○
1－1	Calculation upper limit settings		○
1－2	Time step and number of loop iterations for MD		○
1－3	MD calculation type		○
1－4	Expanded ensemble		○
1－5	Temperature setting		○
1－6	MD calculation conditions		○
1－7	Job restart setting		○
1－8	Calculation result output		○
2	Data output for analysis (energy variation)	○	
2	Data output for analysis (trajectory, parameters)		○
3	Control parameters related to energy calculation	○	○
3－1	Interaction CUTOFF method	○	○
3－2	Interaction calculation switch	○	○
3－3	Filling Potential method		○
4	Restraint conditions	○	○
4－1	SHAKE/RATTLE specifications	○	○
4－2	Rigid body model		○
5	PME, Ewald, FMM specifications	○	○
6	Solvent effect	○	○
7	Boundary conditions	○	○
8	LIST	○	○
9	QUIT	○	○

(1) Energy minimization control parameters (same for STEEP/CONJ)

METHODofmini : Energy minimization method (◎)
=STEEpest ; Steepest descent method (Default)
=CONJugate ; Conjugate gradient method
CPUTIMelimit : CPU time upper limit (secs.) (○)
=60.0 ; (Default)
LOOPLImit : Number of energy minimization cycles.
If this is 0, the program only calculates energy for initial coordinates. (○)
=0 ; (Default)
UPDATEinterval : Update cycle of coordinate information. (△)
If CUTOFF is used for 1-5 interaction energy, this designates the update cycle for the interaction table. In case of periodic boundary conditions, this designates the update cycle for calculation to correct the coordinates of an item (which has jumped out of the unit cell) to back within the cell.
=20 ; (Default)
CONVGRadient : Convergence determination condition (△)
If the root mean square summation of force (R.M.S.F.) is less than the designated value, the calculation is determined to have converged, and the calculation is terminated. Units (kcal/mol/A)
=0.1 ; (Default)
ISTEPLength : Movement distance of atoms in the first step (R.M.S.D.(A) with initial coordinates)
=0.01 ; (Default) (△)

(1－1) Control parameters for steepest descent method (STEEP)

This sets step length parameters for the steepest descent method.

UPRATE : If a low energy structure can be obtained in the previous step, this extends the movement distance by multiplying UPRATE with the step length.
=1.2 ; (Default)
DOWNRAte : If energy has increased in the previous step, this reduces the movement length by multiplying DOWNRATE with the step length.
=0.6 : ; (Default)

(1－2) Control parameters for conjugate gradient method (CONJ)

This sets search parameters in the conjugate gradient method.

LINESEarchlimit : Number of loop iterations of line search. Do not make this too small.
=10 ; (Default)
CONVLInesearch : Threshold value for determining convergence of line search.
Convergence is determined when (DIRGRD/DIRGRS) ≦ CONVL.
DIRGRD : Current Directional Derivative.
DIRGRS : Initial Directional Derivative.
=0.1 ; (Default)

(1－3) Calculation result output designation (same for STEEP/CONJ)

MONITOrinterval : Output cycle for standard output
Designates cycle for calculating energy, RMSD etc. (△)
=10 ; (Default)
LOGFORmat : Format of standard input (△)
=SHORt ; Simple output within 80 chars. in 1 line (Default)
=DETAil ; Detailed output within 80 chars. in l line. Add each energy.
BESTFItmini : In energy minimization, this provides standard output of RMSD for the 1st chain of the system relative to the reference structure. In the EXE>INPUT phase, "REFCOORD" or "NAMERE" must be designated as the reference structure. (△)
=NO ; Do not calculate (Default)
=YES ; Calculate.

(2) Data output for analysis (Energy variation)

MIN energy trajectory

・This outputs energy for each step during energy minimization to a file.
No input designation file.

NAMEAN= (Name of MIN energy trajectory file)
UNITAN : IO Unit of file for MIN energy trajectory
=30 ; (Default)

【Note】 the MIN energy trajectory is output for each step.

(3) Control parameters relating to energy calculation (same for STEEP/CONJ)

(3－1) Interaction CUTOFF method

CUTMEThod : Interaction CUTOFF method
=RESC ; Residue base cutoff (Default)
Calculates the interactions between all atoms included in residues if the distance between the residue centers of mass is at or below the CUTOFF distance.
=ATOM ; Atom base cutoff
Calculates the interactions between atoms if the distance between the atom centers of mass is less than the CUTOFF distance.
=RESA ; Residue base cutoff
Calculates interaction between all atoms included in residues if the minimum distance between two atoms of a residue is less than the CUTOFF distance.

【Note】 Ordinarily RESA is recommended (if boundary conditions are not periodic). RESC is recommended for periodic boundary conditions.

CUTLENgth : Cutoff length (Å)
=8.0 ; (Default)
DIEFUNction : Format of relative dielectric function in space
=CONS ; Dielectric function is constant (Default)
=DIST ; Dielectric constant is proportional to the distance. ε=DIEVAL *Distance (Å)
DIEVALue : Dielectric constant of space
=1.0 ; (Default)

【Note】 Ordinarily, "DIEVAL=1.0" is used if "DIEFUN=CONS". If "DIEFUN=DIST" is designated in a vacuum, DIEVAL is set to a value of about 1.0~4.0.

USESPL ; Application of spline interpolation
=NO ; Do not apply (default)
=YES ; Apply
CUT-ON ; Spline interpolation start distance
=6.0 ; (default)

【Note】 If input topology is CHARM potential, apply spline interpolation by CHARMM.

(3－2) Interaction calculation switch

Use the following switch to calculate (or not calculate) a specific interaction.

【Note】 Very Important
- With the 1-5 interaction switch, you must switch energy calculation of van der Waals/electrostatic interaction/hydrogen bonding between the case when an interaction table (CUTOFF) is used, and the case when it is not used.
- If you use restraint (CAP, position restraint etc.), you must turn on the switch for the corresponding energy calculation.

(3－2－1) 1-2, 1-3 and 1-4 interaction switches

All default values are used in ordinary MIN/MD calculation. Although it is extremely rare, this is used only when you do not wish to calculate a certain interaction.

```
CALBONd : 1-2 interaction calculation
    =CALC       ; Calculate (Default)
    =NOCAlc     ; Do not calculate
CALANGle : 1-3 interaction calculation
    =CALC       ; Calculate (Default)
    =NOCAlc     ; Do not calculate
CALTORsion : Torsion interaction calculation
    =CALC       ; Calculate (Default)
    =NOCAlc     ; Do not calculate
CALIMProper : Improper torsion calculation
    =CALC       ; Calculate (Default)
    =NOCAlc     ; Do not calculate
CALV14 : 1-4 van der Waals calculation
    =CALC       ; Calculate (Default)
    =NOCAlc     ; Do not calculate
CALE14 : 1-4 electrostatic interaction calculation
    =CALC       ; Calculate (Default)
    =NOCAlc     ; Do not calculate
```

(3－2－2) 1-5 interaction switch

This changes the switch designation when calculating using CUTOFF (calculation using an interaction table), and when calculating all 1-5 interactions for all atoms without using CUTOFF (direct calculation). The default setting is to use CUTOFF. Normally (default), all of the following are calculated: van der Waals, 1-5 electrostatic interaction, and hydrogen bonding. Although it is extremely rare, please use this only when you do not wish to calculate a certain interaction. If you use a force field which does not include hydrogen bonds (12-10 Potential), hydrogen

bonds are not calculated, regardless of the value of the switch CALHYD. When using the PME method or Ewald method, please use an interaction table.

When using an interaction table (using CUTOFF)

The following CALV15, CLAE15 and CALHYD are set to =CALC, and CALV5N, CALE5N and CALH5N are set to =NOCALC. (Default)

```
CALV15 : 1-5 van der Waals
    =CALC       ; Calculate(Default)
    =NOCAlc     ; Do not calculate
CALE15 : 1-5 electrostatic interaction calculation
    =CALC       ; Calculate(Default)  ※Mandatory with *PME/FMM
    =NOCAlc     ; Do not calculate
CALHYD : Hydrogen bonds
    =CALC       ; Calculate(Default)
    =NOCAlc     ; Do not calculate
```

When not using an interaction table

The above CALV15, CLAE15 and CALHYD are set to =NOCALC, and CALV5N, CALE5N and CALH5N are set to =CALC.

【Note】 With this setting, calculation cannot be done using the PME method, Ewald method and soft core.

```
CALV5N : 1-5 van der Waals
    =NOCAlc     ; Do not directly calculate 1-5 van der Waals (Default)
    =CALC       ; Calculate
CALE5N : 1-5 electrostatic interaction
    =NOCAlc     ; Do not directly calculate 1-5 electrostatic interaction (Default)
    =CALC       ; Calculate
CALH5N : Hydrogen bonds
    =NOCAlc     ; Do not directly calculate hydrogen bonds (Default)
    =CALC       ; Calculate
```

(3－2－3) Restraint potential

Restraint potential settings are all set to NOCALC (no calculation) by default.

Please set the corresponding energy calculation term when using soft core (soft repulsion) for CAP restraint, position restraint, distance/angle/torsion restraint or van der Waals repulsion etc. Also, ordinarily you should designate the applicable

atoms for these potentials (see the section on EXE>INPUT), and input parameters where they are required for force constants etc.

All of these restraint potentials are added to the potential energy term of the entire system.

```
CALPSR : Position restraint
    =NOCAlc   ; Do not calculate(Default)
    =CALC     ; Calculate
        Designate the following in the EXE>INPUT phase.
            POSITION=READ
            NAMEPO= (Position restraint designation file)
            REFCOORD=PDB
            NAMERE= (Reference coordinate file)
CALDSR : distance-restraint
    =NOCAlc   ; Do not calculate(Default)
    =CALC     ; Calculate
        Designate the following in the EXE>INPUT phase.
            DISTANcerestrain =READ
            NAMEDIstance= (Distance restraint designation file)
CALDHR : dihedral-restraint
    =NOCAlc   ; Do not calculate(Default)
    =CALC     ; Calculate
        Designate the following in the EXE>INPUT phase.
            DIHEDRalrestrain =READ
            NAMEDH= (Dihedral restraint designation file)
CALREP : simple repulsion
    =NOCAlc   ; Do not calculate(Default)
    =CALC     ; Calculate
CALCAP : CAP restraint
    =NOCAlc   ; Do not calculate(Default)
    =CALC     ; Calculate
        Designate the following in the EXE>INPUT phase.
            SETBOUndary =READ
            NAMEBOundary = (CAP boundary designation file)

EXTCAP : ExtendCAP restraint
```

=NOCAlc ; Do not calculate (default)
=CALC ; Calculate
The following is specified in the EXE>INPUT phase:
SETEXC =READ
NAMEEC = (ExtendCAP designated file)

Parameters necessary for restraint potential

Weight factors: With position, distance, repulsion or dihedral restraint, the hardness of the restraint potential is determined by the temperature andweight etc. If these parameters restrain the system and the hardness is good and appropriate, then that is generally sufficient. Ordinarily there is no need to be particularly precise about these values.

TEMPERature: Temperature used for restraint (K) (Position, Distance, Repulsion, Dihedral).
=300.0 ; (default) (△)
WETDSR : distance restraint weight
=1.0 ; (default)
WETPSR : position restraint weight
=5.0 ; (default)
WETDHR : dihedral restraint weight
=10.0 ; (default)

Simple repulsion parameters

WETREP : simple repulsion weight
=1.0 ; (default)
REPSCAle : van der Waals radius scale factor
=1.0 ; (default)
REPDELta : Permissible tolerance
=1.0 ; (default)

CAP restraint parameters

To use CAP restraint, a file specifying the atoms subject to CAP restraint (see the EXE>INPUT section) and "CALCAP=CALC" must be specified, as well as parameters for the CAP center, CAP radius, and the type and force coefficient of the repulsion potential which forms the CAP wall. Default values exist, however, the user normally specifies parameters other than the force constant (FORCAP).

RADCAP : Radius of CAP restraint (A).

(The restraint force is 0 inside this radius and is determined by the potential outside this radius.)

=20.0 : (default)

FORCAP : Force constant of repulsion potential forming the CAP wall

=150.0 : (default)

FUNCAP : Shape of repulsion potential forming the CAP wall

=HARMonic : Quadratic parabola potential (default)

F = 0.5 * FORCAP * (R - RADCAP) **2

where R = (center of mass of chain) - (CAP center).

=BIQUadratic : Biquadratic potential

F = 0.25 * FORCAP * (R**2 - RADCAP**2) **2

where R = (center of mass of chain) - (CAP center).

SETCEN : Sets center of CAP at center of mass of 1st chain of system.

=NO : Do not set. Specify the center with CENTRX/CENTRY/CENTRZ (default)

=YES : Set. Set center of CAP with Cartesian coordinates (A).

CENTRX= 0.0 : (default)

CENTRY= 0.0 : (default)

CENTRZ= 0.0 : (default)

【Note】 If these parameters are specified in both the CAP file and the control file, the specifications in the control file will take precedence.

【Note】 The CAP potential is applied to the center of mass of the molecule or residue. If the molecule or residue is large, some atoms in the molecule/residue may protrude outside the CAP radius even if the center of mass is inside the radius.

ExtendCAP restraint parameter

In ExtendCAP restraint, the target atom, target range and force coefficient are all designated by the ExtendCAP designation file. The control file does not designate anything other than the Extend designation file (see the EXE>INPUT chapter) , "EXTCAP=CALC".

(4) Specification of restraint conditions

(4－1) SHAKE/RATTLE

To use SHAKE/RATTLE, a SHAKE file that specifies the atom numbers of the target atoms and the restraint distance must be specified in the EXE>INPUT group. The calculation method and convergence conditions are specified in the EXE> MIN group.

SHAKEMethod : Specification of the SHAKE method

=NOSHake ; Do not perform SHAKE (default)

=HBON ; Do not perform together with other restraints; calculate as an independent restraint.
=ALLB ; Calculate all interrelated restraints using the iterative method.

COVSHK : Threshold value for determination of SHAKE convergence.
When (CBL - IBL) / IBL is less than this value, SHAKE is determined to have converged.
CBL: Calculated and corrected bond length, IBL : Input bond length
= 1.0D-6 ; (default) (△)

LIMSHK : Upper limit on number of iterations of SHAKE iterative method
=1000 ; (default) (△)

【Note】 When restraint conditions that overlap with and interrelate with other restraints are specified in the SHAKE file, "SHAKEMethod= ALLB" must be used.
【Note】 Special restraints in 3-atom triangle and 4-atom tetrahedron topologies are calculated using the iterative method, regardless of the "SHAKEMethod= [HBON | ALLB]" specification.
【Note】 In large systems with extreme deformity, calculation may stop without SHAKE converging.
【Note】 There are restrictions on the range of SHAKE/RATTLE application.

Range of SHAKE/RATTLE application

		SHAKE	RATTLE
Energy minimization (EXE> MIN)	Steepest descent method (METHOD=STEEP)	○	×
	Conjugate gradient method (METHOD=CONJ)	×	×
MD calculation (EXE> MD)	Leap Frog Verlet (INTEGR=LEAP)	○	×
	Velocity-Verlet (INTEGR=VELO)	×	○
	Multi Time Step (INTEGR=MTS)	×	×

(5) PME, Ewald, FMM specification

(5－1) Specification of Particle Mesh Ewald method and Ewald method

When a periodic boundary condition is specified, the PME (Particle Mesh Ewald) method or the Ewald method can be used for the calculation of 1-5 electrostatic interaction. Only one of the methods can be used.

CALPME : Apply the PME method.
=NOCALC : Do not apply (default)
=CALC : Apply

PMESPD : Adjust the calculation interval in the PME method.
=NORM : Calculate every time (default)

=HIGH : Calculate at each "UPDATE" cycle of the coordinate information.

PMEUPD : PMESPD= HIGH calculation method.

=CUT : Use cut off calculation (default)
Update calculation at each step for short-distance interaction. Synchronize long-distance interaction calculation with update cycle “UPDATE” of coordinate information.

=RECI : Use reversal space term calculation.
Only calculation of interaction for real space is updated at every step. Calculation of interaction from wavenumber space is synchronized with update cycle “UPDATE” of coordinate information.

【Note】 The recommended value of PMEUPD option is PMEUPD= CUT. This method is opportunistic, however, it has better qualities for retaining conserved quantity.

【Note】 Calculation by PMEUPD= RECI has lower qualities for retaining conserved quantity than PMEUPD= CUT.

CALEWA : Apply the Ewald method.

=NOCALC : Do not apply (default)

=CALC : Apply

【Note】 The PME and Ewald methods require the following specifications:

CALE15= CALC ; For nearby atoms, calculate by the CUTOFF method using the interaction table.

BOUNDA= PERI ; PME or Ewald can only be used when a periodic boundary condition is specified.

DIEFUN= CONS ; With PME and Ewald, the spatial dielectric constant of the coulomb force must be a constant.

PME, Ewald control parameters

EWAPRM : Ewald parameter for the PME, Ewald method

This is a convergence parameter for real space and inverse space. When increased, real space converges more quickly. When decreased, inverse space converges more quickly. Specify a value from 0.0 to 1.0.

= 0.35 ; (default)

REATOL : Permissible tolerance (ε) for cutoff in real space of the Ewald method
(erfc(β Rcut) / Rcut < ε)
= 1.0d-19 ; (default)

Specification of mesh count in PME

MESHLX= 16 : X-axis direction (default)
MESHLY= 16 : Y-axis direction (default)
MESHLZ= 16 : Z-axis direction (default)
PMEORD : Order of spline function fit when representing charge distribution with mesh points
=5 : Recommended value when EWAPRM = 0.35 (default)

【Note】 It is best to set the mesh count so that there is a distance of about 1 Å between mesh points.

(5－2) Specification of Fast Multiple Method

USEFMM : Application of Fast Multipole Method
Specify whether or not the Fast Multipole Method (FMM) is used as a method for not cutting off the coulomb force. This can be used when there is no periodic boundary condition.
=NO ; Do not apply FMM (default)
=YES ; Apply FMM.

FMMSPD : Adjustment of calculation interval for 1-5 electrostatic interaction in the FMM method.
=NORM : Calculate each time (default)
=HIGH : Calculate at "UPDATE" cycle of coordinate information.

FMTREE : Tree depth of Fast Multipole Method
Tree depth of the Fast Multipole Method. The number of minimum cells is 8**FMTREE, and the minimum cells are set so that they include several atoms to several tens of atoms. If stopping due to overflow occurs, increase the FMTREE value.
= 3 ; (default)

FMPOLE : Order of multipoles in Fast Multipole Method
Order of multipoles in the Fast Multipole Method. The larger the FMPOLE value, the better the accuracy but the slower the speed. However, the effect on speed is not that great.

= 8 ; (default)

FMNUMA : Maximum atom number included in minimum cell of Fast Multipole method

= 1000 ; (default)

【Note】 If the atom number included in the the minimum cell of the Fast Multipole method exceeds the value specified by the FMNUMA option, an error will occur And execution of cosgene will stop. In this case, increase the FMTREE option value or the FMNUMA option value. “DIEFUN=CONS” must be indicated.

(6) Solution effect

(6－1) Specification of Accessible Surface Area method

CALASA : Apply the SA method in the ASA method and the GB/SA method

Apply the accessible surface area method to calculation of the solvation in the implicit water model.

= NOCALC ; Do not apply (default)

= CALC ; Apply

ASAPRO : Probe radius (Å)

Set the radius for using solvent water for the probe in the ADA method. Set to approximately 1.4 to 1.6 Å, the radius of a normal water molecule.

= 1.4 ; (default)

ASAWEI : ASA weight

Scale factor of contribution to the energy item of the ASA item in the ASA method.

= 1.0 ; (default)

ASACUT : ASA cut-off length (Å)

The distance between atoms used to determine ASA overlapping in the ASA method.

Specify a length longer than (atom radius + probe radius)×2, however, if the length is too long, the calculation speed will decrease.

= 4.5 ; (default)

【Note】 Do not use solvents such as solvent water or counter ion.

(6－2) Specification of Generalized Born and Surface Area method

The Generalized Born method and the Accessible Surface Area method can be simultaneously specified (specify both GB calculation "CAL-GB= CALC" and ASA

calculation "CALASA= CALC") to perform calculation by the GB/SA method.

CAL-GB : Use the Generalized Born method
Use the Generalized Born method for calculation of the electrostatic field in the implicit water model.
= NOCALC ; Do not apply (default)
= CALC ; Apply

CALASA : Use the SA method in the GB/SA method
Use the Surface Area method for calculation of the solvation in the implicit water model.
= NOCALC ; Do not apply (default)
= CALC ; Apply

GBWELE : Dielectric constant of water
Set the dielectric constant of the solvent water region in the GB method. This varies depending on the temperature. A dielectric constant near 298 K is used as the default.
= 78.3 ; (default)

GBMELE : Dielectric constant of protein
Set the dielectric constant of the protein region in the GB method. This varies depending on the temperature and type of protein. Usually a value from about 1 to 4 is set.
= 1.0 ; (default)

GBDELT : Correction value for the Born radius (Å)
Correction value for the Born radius in the GB method (corresponds to "δ" introduced in Onufriev's work).
Born radius used for calculation = Born radius - GBDELT.
= 0.0 ; (default)

GBOFFS : van der Waals radius correction value (Å)
van der Waals radius correction value in the GB method.
van der Waals radius used for calculation = van der Waals radius - GBOFFS.
= 0.09 ; (default)

【Note】 The Born radius correction value "GBDELT" corresponds to "δ" introduced in Onufriev's work. If taken from the work of Still and Hawkins, "GBDELT=0.00". If taken from the work of Onufriev, "GBDELT=0.15". (For these works, see "References" at the end of this manual.)

【Note】 The default value of the van der Waals radius correction value "GBOFFS" originates in the work of Still WC (for this work, see "References" at the end of this manual).

【Note】 The Atomic Solvation parameter used in calculation by the ASA method and the

GB/SA method changes automatically.

【Note】Do not use solvents such as solvent water or counter ion.

(7) Boundary conditions

The boundary conditions which can be used in myPresto are a sphere/ellipsoid, or periodic boundary conditions (a cell with the 6 faces of a rectangular parallelepiped). A rigid wall which provides elastic collision is used in a sphere or ellipsoid. Common names are used for some variables (like designation of the center). When using periodic boundary conditions, be sure not to forget to designate the cycle (UPDATE) for returning coordinates to the unit cell. In contrast with CAP restraint, there is no need for a file designating applicable atoms.

```
BOUNDAry : Boundary condition type
    =NO          ; No boundary (Default)
    =PERI        ; Periodic boundary conditions
    =ELLIPSoid   ; Ellipsoid boundary
    =SPHERE      ; Sphere boundary
```

【Note】 Do not designate NO CUTOFF (CAL15N=CALC) for periodic boundary conditions. Also, RESC is the recommended value. The PME and Ewald methods can be used with a periodic system, but CUTOFF must be designated.

【Note】 Calculation may stop in the following cases with periodic boundary conditions.

- Switches for the necessary interaction calculation are OFF.
- An unnaturally large force has appeared.
  (Potential causes: Strain of initial coordinates, long UPDATE interval, large time step etc.)
- The sum of residue size and CUTOFF distance is larger than half the cell size.

(7－1) Boundary condition center setting

With a periodic system, the boundary condition center is set to the center of mass of the rectangular unit cell, and with a sphere or ellipsoid, the center is set to the center of mass of the sphere or ellipsoid.

```
SETCEN : Sets center of mass of the 1st chain of the system to the center of boundary conditions.
    =NO    : Do not apply (Default). Designate with CENTRX/CENTRY/CENTRZ.
    =YES   : Apply
```

If SETCEN=NO, the boundary condition center is designated with Cartesian coordinates

```
CENTRX=          0.0    ; (Default)
CENTRY=          0.0    ; (Default)
CENTRZ=          0.0    ; (Default)
```

(7－2) Boundary condition size setting

For periodic boundary conditions:
This sets the length along the X, Y and Z axes of the unit cell

```
LXCELL=          40.0      ; (Default)
LYCELL=          40.0      ; (Default)
LZCELL=          40.0      ; (Default)
```

For an ellipsoid:
This designates the radius in the X, Y and Z directions, assuming the major and minor axes of the ellipsoid are aligned with the XYZ coordination directions.

```
ELLIPA=          30.0      ; (Default)
ELLIPB=          30.0      ; (Default)
ELLIPC=          30.0      ; (Default)
```

For a sphere:
This designates the radius of the sphere.

```
RADIUS=          30.0      ; (Default)
```

【Note】 An error will occur if any of the atoms at the initial coordinates are such that their atomic nucleus is outside the boundary. Be careful because this is not the center of mass of the molecule or residue. After starting calculation, coordinates are corrected and processing is done to return the system inside the boundary only when the atom goes outside the boundary.

(7－3) Designation of method to pull back coordinates to unit cell

Designate coordinate pull back to a unit cell in a periodical system. Specify atom, residue, or chain for the unit cell.

```
REPLAC : coordinate pull back method
    =ATOM  : atom unit (default)
    =RESI  : residue unit
```

=CHAI ：chain unit

【Note】REPLAC option is enabled only when BOUNDA= PERI or HEXA.

【Note】If residue base cut off (CUTMET= RESA or RESC) is used with REPLAC= ATOM specified, coordinates are pulled back by residue unit.

(8) LIST

If you add the command "LIST", current parameter settings will be displayed. No parameters.

(9) QUIT

This indicates the end of EXE> group input. No parameters.

4.2.1.3 EXE> MD group

MIN/MD input items

		MIN	MD
1	Energy minimization control parameters (same for STEEP/CONJ)	○	
1－1	Control parameters for steepest descent method (STEEP)	○	
1－2	Control parameters for conjugate gradient method (CONJ)	○	
1－3	Specification of output of calculation results (same for STEEP/CONJ)	○	
1	MD control parameters		○
1－1	Calculation upper limit settings		○
1－2	Designation of time step and number of loop iterations for MD		○
1－3	MD calculation type		○
1－4	Expanded ensemble		○
1－5	Temperature setting		○
1－6	MD calculation conditions		○
1－7	Job restart setting		○
1－8	Specification of output of calculation results		○
2	Analysis data output (energy variation)	○	
2	Analysis data output (trajectory, parameters)		○
3	Control parameters related to energy calculation	○	○
3－1	Interaction CUTOFF method	○	○
3－2	Interaction calculation switch	○	○
3－3	Filling Potential method		○
4	Restraint specification	○	○
4－1	Specification of SHAKE/RATTLE	○	○
4－2	Specification of rigid body model		○
5	Specification of PME, Ewald, FMM	○	○
6	Solvent effect	○	○
7	Boundary conditions	○	○
8	LIST	○	○
9	QUIT	○	○

(1) MD control parameters

(1－1) Calculation upper limit setting

SETTIMelimit : Simulation time setting (ps).
If the time value (ps) given by "Loop iterations (LOOPLI) x Time step (TIMEST)" (described below) is large, the calculation is stopped at the time set here. (◎)
=5.0 ; (default)
CPUTIMelimit : Upper limit in CPU time (seconds) (◎)
=60.0 ; (default)

(1－2) Specification of MD time step and loop iterations

LOOPLImit : Number of loop iterations for MD simulation (◎)
=0 ; (default)
TIMESTep : Time step (fs).
Normally this is 0.5 to 1.0 fs. Set to 1.0 to 2.0 fs when using SHAKE or rigid model for all H in the system. (◎)
=1.0 ; (default)

(1－3) MD calculation type

(1－3－1) Calculation method: Integrator

Method of time integration. When using multi time step (RESPA method), the method of varying the time step must also be specified.

INTEGR : Method of time integration
=LEAPfrog ; Leap-frog Verlet method (default)
=VELOcity ; Velocity-Verlet method
=MTS ; Multi Time Step (RESPA)
=PRCO ; Predictor-Corrector
=RK4O ; Quartic Runge-Kutta method
=GEAR ; GEAR method
=EXVP ; EXtended phase space Volume Preserving integrator

【Note】 In this version, only execution files are provided for Predictor-Corrector, quartic Runge-Kutta method, GEAR method, and EXtended phase space Volume Preserving integrator. Source programs are not provided. Therefore, these functions cannot be used in an execution file obtained by compiling source

programs.

When using multi time step, the force f is separated into three types: fa having a long movement period, fb having an intermediate period, and fc having a short movement period. The time steps ⊿ta, ⊿tb, and ⊿tc corresponding to each force are controlled. These time steps are specified by the following relationships using the calculation frequencies K and L:

⊿tb = K⊿tc,
⊿ta = L⊿tb = LK⊿tc (K and L are natural numbers)

FREQMEdium: Specify the calculation frequency (interval - medium) (corresponds to K in the above equation: ⊿tb = K⊿tc)
=1 ; (default)
FREQLOng : Specify the calculation frequency (interval - long) (corresponds to L in the above equation : ⊿ta = LK⊿tc)
=1 ; (default)

In addition, specify which time step governs each interaction calculation. Specify in the same way as the next calculation.

CALBON : Calculation of 1-2 interaction
=NOCAlc ; Do not calculate
=CALC ; Calculate. Governed by time step ⊿tc (default)
=MEDIum ; Calculate. Governed by time step ⊿tb
=LONG ; Calculate. Governed by time step ⊿ta
CALANG : Calculation for 1-3 interaction
CALTOR : Calculation for torsion interaction
CALIMP : Improper torsion calculation
CALV14 : 1-4 van der Waals calculation
CALE14 : Calculation for 1-4 electrostatic interaction
CALV15 (or CALV5N) : 1-5 van der Waals
CALE15 (or CALE5N) : for 1-5 electrostatic interaction
CALHYD (or CALH5N) : hydrogen bond
CALPSR : position restraint
CALDSR : distance-restraint
CALDHR : dihedral-restraint
CALREP : simple repulsion
CALCAP : CAP restraint
CALUMB : UMBRELLA potential calculation

CALFLW : FLOW potential calculation
CAL-GB : GB calculation
CALASA : ASA calculation

【Note】 If "MEDI" or "LONG" is specified for the interaction calculation switch when other than Multi time step is specified ("INTEGR=MTS"), and error will result and the program will stop.
【Note】 It is best to specify the time step ⊿ta ("TIMEST"×"FREQME"×"FREQLO") of long-period movement fa so that is does not exceed 4.0 fs.
【Note】 When using RATTLE with NVT ensemble, "THERMO= NOSE" must be specified.
【Note】 There are limits on the range of application of SHAKE/RATTLE.

Range of application of SHAKE/RATTLE

		SHAKE	RATTLE
Energy minimization (EXE> MIN)	Steepest descent method (METHOD=STEEP)	○	×
	Conjugate gradient method (METHOD=CONJ)	×	×
MD calculation (EXE> MD)	Leap Frog Verlet (INTEGR=LEAP)	○	×
	Velocity-Verlet (INTEGR=VELO)	×	○
	Multi Time Step (INTEGR=MTS)	×	×

(1－3－2) Calculation method: Ensemble generation method

METHOD : Specify the ensemble type.
=MICRocanonical ; Micro-canonical(NVE) (default)
=CANOnical ; Canonical(NVT)
=NPT ; NPT
=EXPAnded ; Expanded ensemble
=TSAL ;Tsallis Dynamics

【Note】 The NPT ensemble "METHOD=NPT" can only be specified when a periodic boundary condition is specified.
【Note】 Tsalli Dynamics "METHOD=TSAL" is enabled only when the quartic Runge-Kutta method is used.

(1－3－3) Calculation method: Temperature/Pressure control method

THERMOstat：Temperature control method.
This is applied when "METHOD = CANO". Note carefully the range of application.
=CONStant ; Hoover-Evans Gaussian constraint method (default)
=NOSE ; Nose-Hoover method
COUPLIngtime：Coupling time in the Nose-Hoover method (τ：fs) .
This is applied when "THERMO = NOSE".
=100.0 ; (default)

BAROSTat：Pressure control method
This is applied when "METHOD = NPT". Note carefully the range of application.
=ANDErsen ;Andersen method (default)
=PARA ;Parrinello Rahmann method
SETPRE： Target pressure in NPT (atm) .
= 1.0 ; (default)
COUPHB： Coupling time of pressure control in NPT (fs).
= 1000.0 ; (default)
COUPPI： Coupling time of pressure control in NPT (fs).
= 1000.0 ; (default)
MODIFIcation： Cell shape in NPT. Only effective when Parrinello-Rahman method is specified.
= FLEX ; 6 degrees of freedom, rhombic cell (default)
= MONOclinic ; 4 degrees of freedom. Expands and contracts in 3 directions, and the angle of the base cell γ (the angle formed by the a axis and the b axis) changes.
= ORTHorhombic ; 3 degrees of freedom. Expands and contracts in 3 directions.
= ISOTropic ; 1 degree of freedom. Shape varies isotropically.
= SINGle_direction ; 1 degree of freedom. Shape varies only in the z direction.

(1－4) Expanded ensemble method

EXPAND：Specify the expanded ensemble method.
= FORC : Force-Biased McMD
= SIMU : Simulated Tempering
= GST : Generalized Simulated Tempering
= EFFE : Effective Temperature

(1－4－1) Force-biased Multicanonical MD method

RESETC : Step cycle of histogram creation

F.B.McMD is a histogram-based iterative method that recreates the energy histogram at the step cycle set in "RESETC". If too short, the system does not search the space sufficiently and the histogram becomes biased. If too long, a long time is required for calculation. It is best to make "RESETC" longer for a larger system. For several residue peptides in a vacuum, use a step number of about 200000 to 400000.

= 300000 ; (default)

DUMMYL : Number of dummy loop steps discarded before F.B.McMD is begun.

If starting F.B.McMD from the equilibrium state, set to "1".

= 1 ; (default)

TEMMAX : Upper limit (K) of temperature range searched by F.B.McMD.

= 700 ; (default)

TEMMIN : Lower limit (K) of temperature range searched by F.B.McMD.

= 250 ; (default)

ENEMAX : Upper limit (kcal/mol) of histogram created by F.B.McMD.

The energy distribution at "TEMMAX" must be covered, and thus this should be set sufficiently higher than the average energy at "TEMMAX". Perform a canonical calculation using the temperature "TEMMAX" to obtain the average energy, and set "ENEMAX" to a value higher than this.

= 10000 ; (default)

ENEMIN : Lower limit (kcal/mol) of histogram created by F.B.McMD.

The energy distribution at "TEMMIN" must be covered, and thus this should be set sufficiently lower than the average energy at "TEMMIN". Perform a canonical calculation using the temperature "TEMMIN" to obtain the average energy, and set "ENEMIN" to a value lower than this.

= -10000 ; (default)

BINSIZ : The bin size (kcal/mol) when a histogram is created with F.B.McMD.

If too fine, the histogram will not be smooth and differentiation will not be possible. If too rough, it will not be possible to express the shape of the histogram. It is best to set this to "ENEMAX" - "ENEMIN" divided by about 100 to 200.

= 5.0 ; (default)

LIMITS : Threshold determining the range of use of the histogram.

This is the lower limit of the histogram of the energy range sampled by F.B.McMD. It is best to set LIMITS = LIMITC. Normally a value in the range 0.0005 to 0.001 is set.

= 0.001 ; (default)

LIMITC : Threshold for updating the histogram.

In the iterative method, the histogram is only updated within the range that the histogram value is larger than "LIMITC". When the histogram is lower than "LIMITC", it is considered that there is too much noise and γ cannot be accurately calculated. "LIMITC" is normally set within the range 0.0005 to 0.001.

= 0.001 ; (default)

FBRSTO : Distribution data restart file output format

Specify ASCII or binary format for the output file of distribution data (scale factor, histogram) of F.B.McMD.

= NOWR ; Do not output (default)

= ASCI ; ASCII format

= DOUB ; Double precision binary format

NAMEFO= (Distribution data output file name, 133 characters or less)

FBRSTI : Distribution data restart file input format

Specify ASCII or binary format for the restart-input distribution data (scale factor, histogram) of F.B.McMD.

NAMEFI= (Distribution data input file name; 133 characters or less)

UNITFR : IO units of distribution data restart file

= 85 ; (default)

(File example in ASCII format)

```
# PREVIUS POTENTIAL, LOCAL LOOP, TOTAL LOOP, CURRENT STEP
   19.6646786598415                1           1           1
 # LOCAL HISTGRAM          351
   2.00000000000000
  0.000000000000000E+000
    :
   2.00000000000000
  0.000000000000000E+000
# TOTAL HISTGRAM          351
   2.00000000000000
   1.00000000000000
   5.00000000000000
    :
   4.00000000000000
# PREVIOUS SCALING FACTOR
  0.750000000000000
  0.750000000000000
  1.16322986359255
  0.991723974379762
    :
  0.750000000000000
 # BIN-LOWER, BIN-UPPER, PREVIOUS-LOWER, PREVIOUS-UPPER
        117         155         112         158
```

【Note】 Set "SETTEM" as the reference temperature for F.B.McMD. The kinetic energy is fixed at this temperature. Generally this is set higher than "TEMMIN" and lower than "TEMMAX".

(1－4－2) Simulated Tempering Multicanonical MD method

RESETC : Step cycle for histogram creation

S.T.McMD recreates the coefficient used to determine temperature transition at the step cycle set in "RESETC". If too short, the system does not search temperature space sufficiently and the histogram becomes biased. If too long, a long time is required for calculation. It is best to make "RESETC" longer

for a larger system. For several residue peptides in a vacuum, use a step number of about 200000 to 400000.
= 300000 ; (default)

DUMMYL : Number of dummy loop steps discarded before S.T.McMD is begun.
If starting S.T.McMD from the equilibrium state, set to "1".
= 1 ; (default)

TEMMAX : Upper limit (K) of temperature range searched by S.T.McMD.
= 700 ; (default)

TEMMIN : Lower limit (K) of temperature range searched by S.T.McMD.
= 250 ; (default)

ENEMAX : Upper limit of energy histogram created by S.T.McMD (kcal/mol)
The energy distribution at "TEMMAX" must be covered, and thus this should be set sufficiently higher than the average energy at "TEMMAX". Perform a canonical calculation using the temperature "TEMMAX" to obtain the average energy, and set "ENEMAX" to a value higher than this.
= 10000 ; (default)

ENEMIN : Lower limit of energy histogram created by S.T.McMD (kcal/mol)
The energy distribution at "TEMMIN" must be covered, and thus this should be set sufficiently lower than the average energy at "TEMMIN". Perform a canonical calculation using the temperature "TEMMIN" to obtain the average energy, and set "ENEMIN" to a value lower than this.
= -10000 ; (default)

BINSIZ : The energy bin size (kcal/mol) when an energy histogram is created with S.T.McMD.
= 5.0 ; (default)

LIMITS : Threshold determining the range of use of the temperature histogram
This is the lower limit for determination of the temperature range sampled by S.T.McMD. It is best to set LIMITS = LIMITC. Normally a value in the range 0.0005 to 0.001 is set.
= 0.001 ; (default)

LIMITC : Threshold for updating the temperature histogram.
The histogram item γ is used only in the range that the histogram value is larger than "LIMITC". When the histogram is lower than "LIMITC", it is considered that there is too much noise and γ cannot be accurately calculated. "LIMITC" is normally set within the range 0.0005 to 0.001.
= 0.001 ; (default)

STTNUM : Temperature division number
The temperature range from TEMMIN to TEMMAX is divided into STTNUM divisions and S.T.McMD is executed. A more natural temperature transition is expressed when a higher value is set for STTNUM, however, the sampling time required for convergence also increases.
= 100 ; (default)

STEBAS : Base energy

Set the minimum value of the energy that can be taken in by the system. Estimate this ahead of time by means of a canonical calculation at the temperature "TEMMIN".
= 0.0 ; (default)

【Note】 Set "SETTEM" as the reference temperature for S.T.McMD. The kinetic energy is fixed at this temperature. Generally this is set higher than "TEMMIN" and lower than "TEMMAX".

(1－4－3) Generalized Simulated Tempering method

Common energy distribution P (E) can be expressed by a Canonical distribution overlapped at various temperatures $\beta_i(=1/k_B T_i)$ by weight F_i. In the Simulated Tempering method, the ensemble Ξ in which the subensemble Q determined by β is overlapped by weight $F(\beta)$ has been considered. In Generalized Simulated Tempering, the parameter λ is introduced and it is expressed by the overlapping of a common subensemble determined by λ.

GSTMIN : Lower limit of parameter λ
= 0.001d0 ; (default)
GSTMAX : Upper limit of parameter λ
= 0.006d0 ; (default)
GSTNUM : Number of parameter λ
= 20 ; (default)
GSTUPD : Update interval of parameter λ
= 100 ; (default)
GSTCON : Parameter λ convergence MD number
= 10000000 ; (default)
GSTSAM : Sampling number before scaling parameter λ
= 50000 ; (default)
GSTBAS : Base energy
Set minimum energy value that can be taken by the system.
= 25.5 ; (default)
GSTETA :
= 0.5 ; (default)

(1－4－4) Effective Temperature method

RESETC : Step cycle to preparing histogram

If too short, the histogram deviates because the system temperature space is not investigated enough. If too long, the histogram takes a long time to calculate. As the system gets larger, it is desirable to set "RESETC" longer. In a vacuum, use approximately 200000 to 400000 steps for several peptide residues.

= 300000 ; (default)

ENEMAX : Upper limit of energy histogram prepared by Effective Temperature method (kcal/mol) .

= 10000 ; (default)

ENEMIN: Lower limit of energy histogram prepared by Effective Temperature method (kcal/mol) .

= -10000 ; (default)

BINSIZ : Energy bin size when preparing energy histogram byEffective Temperature method (kcal/mol) .

= 5.0 ; (default)

LIMITS : Threshold to determine the range used by the temperature histogram.

This is the lower limit for determining the temperature range sampled by the Effective Temperature method. This should be LIMITS= LIMITC. Generally, the range is 0.0005 to 0.001.

= 0.001 ; (default)

LIMITC : Threshold for updating temperature histogram

Term γ of the histogram is used only inthe range in which the histogram value is larger than "LIMITC". If the histogram is lower than "LIMITC", it is considered that there is too much noise and γ cannot be calculated correctly. Generally, the range is 0.0005 to 0.001.

= 0.001 ; (default)

(1－4－5) Extend ensemble data file output

Designate the file name to which theprobability , scale factor, and energy data is output when Extend ensemble (Effective Temp, Force Bias, Simulated Tempering, Generalized Simulated Tempering) is used.

NAMEEP : Probability data filename (133 characters or less)

= expand.prob ; (default)

UNITEP : IO units of probability data file

= 78 ; (default)

NAMEES : Scale factor data file name (133 characters or less)
= expand.scale ; (default)
UNITES : IO units of scale factor data file
= 77 ; (default)
NAMEEE : Energy data filename (133 characters or less)
= expand.energy ; (default)
UNITEE : IO units of energy data file
= 79 ; (default)

(1－5) Tsallis Dynamics

【Note】 In this version, only execution files are provided for Tsallis Dynamics . Source programs are not provided. Therefore, Tsallis Dynamics cannot be used in execution files obtained by compiling source programs.

(1－5－1) Setting distribution density function parameters

It is possible to change the distribution density function of Tsallis Dynamics using the potential energy range.

ELOWER : Low energy side threshold
= 0.0 ; (default)
EUPPER : High energy side threshold
= 0.0 ; (default)
ROF1DR : Condition of parameter d of ρ 1
= 0.5 ; (default)
ROF2XI : parameter ξ value of ρ 2
= 10 ; (default)
ROF2VX : Condition of parameter δ of ρ 2
= 1000 ; (default)
ROF2VY : Condition of parameter γ of row 2
= 0.5 ; (default)

(1－5－2) File output option

MNTRZT : Output format of zeta value monitor file
=NO ; Do not output (default)
=ASCI ; ASCII file

```
    =SING       ; Single precision binary file
    =DOUB       ; Double precision file
UNITZT : IO units of zeta value monitor file
    = 80        ; (default)
NAMEZT= (Zeta value monitor file name, 133 characters or less.)
OUTZET : Zeta value monitor file output interval
    = 1         ; (default)

MNTRCK : Output format of Tsallis integral check value monitor file
    =NO         ; Do not output (default)
    =ASCI       ; ASCII file
    =SING       ; Single precision binary file
    =DOUB       ; Double precision binary file
UNITCK : IO units of Tsallis integralcheck value monitor file
    = 75        ; (default)
NAMECK= (Tsallis integral check value monitor filename, 133 characters or less.)
OUTCHK : Tsallis integralcheck value monitor file output interval
    = 1         ; (default)

MNTRPK : Output format of energy monitor file
    =NO         ; Do not output (default)
    =ASCI       ; ASCII file
    =SING       ; Single precision binary file
    =DOUB       ; Double precision binary file
UNITPK : IO units of energy monitor file
    = 81        ; (default)
NAMEPK= (Energy monitor filename, 133 characters or less.)
OUTZPK : Energy monitor file output interval
    = 0         ; (default)
FLGPKT= (Energy monitor file output item. Potential, kinetic and total energy
         are expressed in three characters. "+" is output target, "-" is not
         output target.)

MNTRQU : Output format of physical quantity monitor file
    =NO         ; Do not output (default)
    =ASCI       ; ASCII file
```

=SING ; Single precision binary file
=DOUB ; Double precision binary file
UNITQU : IO units of physical quantity monitor file
= 82 ; (default)
NAMEQU= (Physical quantity monitor filename, 133 characters or less.)
OUTQUA : Physical quantity monitor file output interval
= 1 ; (default)

MNTRDO : Output format of Tsallis distribution density function monitor file
=NO ; Do not output (default)
=ASCI ; ASCII file
=SING ; Single precision binary file
=DOUB ; Double precision binary file
UNITDO : IO units of Tsallis distribution density function monitor file
= 82 ; (default)
NAMEDO= (Tsallis distribution density function monitor filename, 133 characters or less.)
OUTDOF : Tsallis distribution density function monitor file output interval
= 1 ; (default)

(1－6) Temperature settings

(1－6－1) Target temperature setting

SETTEMperature : Target temperature of system (K).
Target temperature in the fixed temperature ensemble Canonical (NCT) and NPT.
Reference temperature in F.B.McMD. Not used in Micro-canonical(NVE).
=300.0 ; (default)
TEMPCOntrol2 : Method of system temperature control.
There are two control methods: controlling the temperature (kinetic energy) of the entire system (TEMPCO=NO), and controlling the temperature of each molecule (TEMPCO=YES). When TEMPCO=NO, protein and water may have different temperatures when protein is in water. When TEMCO=YES, it is easier for different molecules (such as protein and water, and ligand molecules) to have the same temperature. However, there are cases where this is unnatural, such as when the temperature of single atom ions is kept fixed.

=NO ; (default)
=YES

(1－6－2) Initial temperature setting

INITIAlvelocity : Method of setting the initial velocity.
=ZERO ; Initial velocity = 0, Initial temperature = 0 (default)
=SET ; Using random numbers defined by RANDOM below, the initial velocity of each atom is set so that the velocities form a Gaussian distribution whose mean value is "STARTT".
=RESEt ; Change initial conditions of restart job (refer to (1－7))
STARTTempearture : Mean value of initial temperature (K)
=300.0 ; (default)
RANDOMseed : Random seed number for obtaining velocity distribution
(integer value < 2**31 - 1)
=584287 ; (default)
HEATLOop : Number of iterations of MD loop for raising the temperature using the Hoover-Evans Gaussian constraint method.
=0 ; (default)

(1－7) MD calculation conditions

UPDATEinterval : Update cycle of coordinate information. (△)
When CUTOFF is used for the 1-5 interaction energy, this designates the update cycle for the interaction table. When a periodic boundary condition is set, this designates the update cycle for calculation to correct the coordinates of an atom that has jumped out of a unit cell so that the coordinates are within the cell.
=20 ; (default)
STOPCEnterofmass : Method of fixing center of mass during MD calculation
When applying CAP restraint ora periodic boundary condition, thisperforms fixing of the center of mass so that translational or rotational motion is zero.
=NO ; Do not fix center of mass (default)
=TRAN ; Set translational momentum of 1st chain of system to zero.
=ROTA ; Set rotational momentum of 1st chain of system to zero.
=BOTH ; Set translational and rotational momentum of 1st chain of system to zero.
BESTFIt : Provide standard output of changes in RMSD in the 1st chain of the system relative to the reference structure in MD. For the reference structure, "REFCOORD" and "NAMERE" must be specified in the EXE>INPUT phase. (△)

=NO ; Do not calculate (default)
=YES ; Calculate

(1－8) Job restart settings

To stop an MD calculation at any step and then restart and continue the calculation, use the restart file that is output at the end of the job. To restart a job, specifications for loading the restart file and for resuming calculation are needed. The initial velocity and initial coordinates of a restart job can be changed.

(1－8－1) Specification of name of restart file: MD calculation before restart

NAMERO : File name of restart file. Binary format.
UNITRO : IO units of restart file
=41 ; (default)

【Note】 A restart file is always output at the end of MD calculation. If a file name is not specified, the system default file name will be used.

(1－8－2) Specify loading of restart file: Perform at restart

RESTARt : Restart
=NO ; Do not restart. The initial velocity setting (*see above) is applied (default)
=YES ; Restart.
NAMERI= Name of previously prepared restart file.
UNITRI : IO units of restart file.
=40 ; (default)

(1－8－3) Checkpoint setting : Specify the output interval for the automatic restart file

OUTReSTartfile : CPU time interval for output of automatic restart file.
=0 ; Do not output automatic restart file (default)
OUTReStartfileLoop : Loop interval for output of automatic restart file.
=0 ; Do not output automatic restart file (default)

【Note】 When OUTRST is specified, output of the automatic restart file takes place at the CPU time interval for output of automatic restart files. When OUTRSL is specified, output of the automatic restart file takes

place at the loop interval for output of automatic restart files.

【Note】 The file name is in the form “name.num” ("name" is the restart file name specified in the NAMERI option and "num" is a sequence number), and thus the file is not overwritten.

(1－8－4) Initial condition change: Change the initial velocity and initial coordinates for restart

Set the initial velocity and initial coordinates for the restart job.

INITIAlvelocity : Method of setting the initial velocity
=ZERO ; Initial velocity = 0, initial temperature = 0 (default)
=SET ; Using random numbers defined by RANDOM below, the initial velocity of each atom is set so that the velocities form a Gaussian distribution whose mean value is "STARTT"
=RESEt ; Change initial conditions of restart job.
RANDOMseed : Random seed number for obtaining velocity distribution (integer valueRandom seed number for obtaining velocity distribution (integer value < 2**31 - 1)
=584287 ; (default)
NAMETrajectryIn = Input trajectory file name
NUMberTRJ : Position of coordinate trajectory
=0 ; (default)

(1－9) Specification of result output

Among the intermediate results of MD, this provides standard output of data such as energy terms, RMSD, temperature, pressure, and CPU time. Monitor output and trajectories are output to separate files using a different command.

OUTLOG : Number of MD steps for output of intermediate results.
=1 ; Output results at each step (default)
LOGFORmat : Output format
=SHORt ; Simple output within 80 characters on 1 line (default)
=DETAil ; Detailed output within 80 characters on 1 line. Each energy is added.

(2) Analysis data input/output (trajectory, parameters)

The following trajectories are all output to files:

・MD energy trajectory: Each energy is output to a file at a fixed step cycle of the MD calculation. There is no input specification file.
・Specified monitor trajectory: Coordinates of specific atoms, distances between atoms, angles between atoms, and dihedral angles between atoms are output to a file at a fixed step cycle of the MD calculation. Perform input specification by file input and specify "OUTMON" and "NAMEMO" in "EXE>INPUT". Specify atom names in the control file.
・Coordinate trajectory: The coordinates (only) of all atoms included in the system (other than fixed atoms) are output to a file at a fixed step cycle of the MD calculation. There is no input specification file.
・Velocity trajectory: The velocities (only) of all atoms included in the system (other than fixed atoms) are output to a file at a fixed step cycle of the MD calculation. There is no input specification file.
・Total energy data: The total potential energy of the system is output to a file at each step of the MD calculation. There is no input specification file.

MD energy trajectory

```
MNTRENergy : Format of MD energy trajectory file
    =NO       ; Do not output file (default)
    =ASCIi    ; Output in ASCII format
    =SINGle   ; Output in single-precision binary
    =DOUBle   ; Output in double-precision binary
OUTENEergy : Output timing
    =0        ; Every 0 steps (Default)
UNITENergy : IO unit of MD energy trajectory file
    =44       ; (Default)
NAMEENergy = MD energy trajectory file name
```

Monitor designation trajectory

```
MNTRTRajectory : Format of monitor designation trajectory file
    =NO       ; Do not output file (Default)
    =ASCIi    ; Output in ASCII format
    =SINGle   ; Output in single-precision binary
    =DOUBle   ; Output in double-precision binary
OUTTRJ : Output timing
    =0        ; Every 0 steps (Default)
```

```
UNITTR : IO unit of monitor designation trajectory file
    =50       ; (Default)
NAMETR = Monitor designation trajectory file name
```

Coordinate trajectory

```
MNTRCOordinate : Format of coordinate trajectory file
    =NO       ; Do not output file (Default)
    =ASCIi    ; Output in ASCII format
    =SINGle   ; Output in single-precision binary
    =DOUBle   ; Output in double-precision binary
OUTCOO : Output timing
    =0        ; Every 0 steps (Default)
UNITCO : IO unit of coordinate trajectory file
    =42       ; (Default)
NAMECO = Coordinate trajectory file name
```

Velocity trajectory

```
MNTRVElocity : Format of velocity trajectory file
    =NO : Do not output file (Default)
    =ASCIi    ; Output in ASCII format
    =SINGle   ; Output in single-precision binary
    =DOUBle   ; Output in double-precision binary
OUTVEL : Output timing
    =0        ; Every 0 steps (Default)
UNITVE : IO unit of velocity trajectory file
    =43       ; (Default)
NAMEVE = Velocity trajectory file name
```

Total energy data

```
MNTRTOtalenergy : Format of total energy data file
    =NO       ; Do not output file (Default)
    =ASCIi    ; Output in ASCII format
    =SINGle   ; Output in single-precision binary
    =DOUBle   ; Output in double-precision binary
UNITTOtalenergy : IO unit of total energy data file
```

=59 ; (Default)
NAMETOtalenergy = Total energy data file name

【Note】 The total energy data file is output for each step of the MD calculation.

(3) Control parameters relating to energy calculation (Same for MIN/MD)

(3－1) Interaction CUTOFF method (Same for MIN/MD)

See the main section on the "EXE>MINimize group".

(3－2) Interaction calculation switch

See the main section on the "EXE>MINimize group".

(3－3) Filling Potential method

CALUMB : Apply or do not apply the Filling Potential method

Specify whether or not the Filling Potential method, a type of umbrella potential method, is applied. To use the Filling Potential method, a control file specifying the umbrella potential and the result analysis tool are necessary.

= NOCALC ; Do not apply (default)
= CALC ; Apply

(4) Specification of restraint conditions

(4－1) Specification of SHAKE/RATTLE

To use SHAKE/RATTLE, a SHAKE file that specifies the atom numbers and restraint distances of the target atoms must be specified in the EXE>INPUT group. In addition, the calculation method, convergence conditions, and other information must be specified in the EXE> MD group.

See this item in "EXE> MINimize Group".

(4－2) Specification of rigid body model

The rigid body model allows any atoms of a molecule to be treated as rigid bodies with internal degrees of freedom fixed. This function is mandatory when using the TIP4P model. To use the rigid body model, you must specify the molecule and the part of the molecule to be treated as a rigid body in a rigid-body-model file, or you just select the automatic mode.

If the automatic mode is selected, the rigid body model is specified as follows.

(a)For a non-water molecule, whose name is not "WAT".

When a non-hydrogen atom is covalently bound to 1 to 3 hydrogens, they are treated as a rigid body.

(b)For a water molecule, whose name is "WAT".

All atoms of the water molecule are treated as a rigid body. The molecule is treated as TIP3P if the number of atoms is 3 and treated as TIP4P If the number is 4.

```
RIGIDModel : Specify use of the rigid body model (△)
    =NO     ; Do not apply rigid body model (default)
    =YES    ; Apply rigid body model
    =AUTO   ; Apply automatic rigid body model
UNITRM : IO units of specification file of rigid body model (△)
    =58     ; (default)
NAMERM =(Rigid body model file name, 80 characters or less.)
```

If rigid-body is automatically prepared (RIGIDM= AUTO), the prepared information can be output to a file. The format of the output file is the same as the input file.

```
DBGRIG : Rigid-body automatic preparation information output designation (△)
    =NOWR   ; Do not output file (default)
    =ASCI   ; Output file
UNITDR : IO units of rigid-body automatic preparation information file (△)
    =84       : (default)
NAMEDR =(Rigid-body automatic preparation information filename, 133 characters
        or less.)
```

【Note】 The rigid body model file can be created using a special tool. The group of

atoms to be treated as a rigid body and the 3-dimensional coordinates to be fixed are specified in the file. (For the specification procedures, see "A. File Formats" at the end of this manual.)

(5) Specification of PME, Ewald, FMM

(5－1) Specification of Particle Mesh Ewald method, Ewald method

When a periodic boundary condition is in effect, the PME (Particle Mesh Ewald) method or the Ewald method can be used to calculate 1-5 electrostatic interaction. Only one of the methods can be used.

Refer to this item in "EXE> MINimize Group".

【Note】The following specifications are necessary for the PME method and Ewald method.

CALE15= CALC ; Calculate nearby atoms with the CUTOFF method using an interaction table.

BOUNDA= PERI ; PME and Ewald cannot be used unless a periodic boundary condition is in effect.

DIEFUN= CONS ; When using PME or Ewald, the spatial dielectric constant of the coulomb force must be constant.

(5－2) Specification of Fast Multiple Method

See this item in "EXE> MINimize Group".

(6) Solvent effect (same for MIN and MD)

(6－1) Specification of Accessible Surface Area method (same for MIN/MD)

See this item in "EXE> MINimize Group".

【Note】Solvents such as solvent water molecules and counter ions must not be used.

(6－2) Specification of Generalized Born / Surface Area method (same for MIN/MD)

See this item in "EXE> MINimize Group".

【Note】Solvents such as solvent water molecules and counter ions must not be used.

(7) Boundary conditions (same for MIN/MD)

(7－1) Boundary condition center setting (same for MIN/MD)

See this item in "EXE> MINimize Group".

(7－2) Boundary condition size setting (same for MIN/MD)

See this item in "EXE> MINimize Group".

(8) LIST

If list "LIST" is added, the current parameter settings will be displayed. No arguments.

(9) QUIT

Indicates the end of EXE>Group input.

4.2.1.4 EXE> OUTPUT group

The OUTPUT group designates external files for outputting topology files and final coordinates. Input of these OUTPUT groups is used in common in both "EXE>MIN" and "EXE>/MD".

【Note】 For the format of each external file, see "A File formats" at the end of this Manual.

Items designated in the OUTPUT group：

（1）Designation of system topology

（2）Designation of system coordinates

（1）Designation of system topology

TOPOLOgy：Format of topology file (○)

=NOWRite ; No topology file output (Default)

=FORMAtted ; Formatted ASCII file

=BINAry ; Binary file

UNITTOpology：IO unit of topology file (△)

=90 ; (Default)

NAMETOpology= (Topology file name. When TOPOLOgy=[FORMIBINA])

（2）Designation of system coordinates

The coordinate file output here outputs coordinates for the N+1st step when the MD step is N.

COORDInate：Format of 3-dimensional coordinate file in PDB format (○)

=NOWRite ; No coordinate input (Default)

=PDB ; PDB file format

=BINAry ; Binary file

UNITCOordiante：IO unit of coordinate file (△)

=91 ; (Default)

NAMECOordinate= (Coordinate file name. When COORD=[PDBIBINA])

（3）QUIT

This indicates the end of EXE> group input.

4.2.1.5 EXE> END group

END indicates the end of the cosgene control file.
The only item input is the single line "EXE>END".

5 Sample calculations

5.1 Sample-1 : Peptide in a vacuum - Calculation of Vassopressin -

(1) Preparing initial coordinate and topology files

Initial coordinates and a topology file in PDB format are required as mandatory input for MD/energy minimization. These can be prepared using tplgene. Roughly speaking, there are two ways of preparing these files.

Method 1 : Generating the initial coordinates and topology file (in PDB format) by indicating the amino acid/DNA sequence and its structure to tplgene using a dihedral angle system

Method 2 : Generating the initial coordinates and topology file (in PDB format) while automatically supplementing insufficient atoms with tplgene, using coordinates in PDB format downloaded from the PDB etc.

Sample-1 provides an explanation of Method 1, and Sample-2 provides an explanation of Method 2.

(2) Generating initial coordinates and topology file with a dihedral angle system

Create the dihedral angle system input file as "vas.dih".

① Amino acid sequence (PRE>SEQUENCE)

In the lines below "PRE>SEQUENCE", list the amino acid sequence using 3-letter abbreviations. For N terminal acetylation or C terminal methylation, write ACE/NME, and if the C terminal is NH2, write "NHE".

② Designation of S-S bonds (PRE>SSBOND)

If the nth residue and the mth residue are bonded with an S-S bond, write the location of the S-S bond below "PRE>SSBOND" in the form "n m". If there are multiple S-S bonds, continue to input them on the next line.

③ Designation of dihedral angles (PRE>DIHEDRAL-ANGLES)

Dihedral angles are listed under "PRE>DIHEDRAL-ANGLES". In accordance with the ECEPP definition, these are listed in the sequence ϕ-ψ-ω-$\chi 1$-$\chi 2$. Please be aware that in ordinary amino acids, the ω angle is 180°. If you do not understand the dihedral angles well, in many cases you can obtain a plausible structure via energy minimization later if you substitute a suitable angle, as indicated in the diagram below.

■Example of input file for dihedral system (vas.dih)

```
PRE>SEQUENCE
CYS
TYR
PHE
GLN
ASN
CYS
PRO
ARG
GLY
NMEC-

PRE>SSBOND
    1     6

PRE>DIHEDRAL-ANGLES
     180     180     180       0       0       0       0       0       0       0
     180     180     180       0       0       0       0       0       0       0
     180     180     180       0       0       0       0       0       0       0
     180     180     180       0       0       0       0       0       0       0
     180     180     180       0       0       0       0       0       0       0
     180     180     180       0       0       0       0       0       0       0
     180     180     180       0       0       0       0       0       0       0
     180     180     180       0       0       0       0       0       0       0
     180     180     180       0       0       0       0       0       0       0
     180     180     180       0       0       0       0       0       0       0
```

In the following example, "vas.dih" generates initial coordinate and topology files (with the names "vas-dih.pdb" and "vas-dih.tpl") for the molecule "VAS". This section explains the method of placing the input file and database file in the run-time directory.
(An example where an arbitrary directory is designated using an environment variable is given in "5.2 Sample-2".)

■Execution method (Method of placing input file and database file in the run-time directory)

① Copy the input file (assumed here to be "vas.dih"), and the database file to be used, into the execution directory.

② Execute tplgene in the execution directory. This has no parameters and processing begins interactively when you type "tplgene".

③ In sequence, input: the title (arbitrary), molecule name (arbitrary), protein or nucleic acid, input format, database used, input file names, and output file names.

■Sample execution

```
%% Input Title for this molecules %%
    If end, type end and (cr).
 %% Input Title less than 79 char. %%
Vassopressin test
end
%% Input Molecular Name less than 39 char. %%
    If end, type end and (cr).
VAS
end
%% Select Chain Species by the next number. %%
   1 : peptide    chain
   2 : nucleotide chain
1
%% Select Input File by the next number. %%
   1 : pdb   file
   2 : dihed file
2
%% Which Force Field Files do you use ? %%
    Please choose from the following files .
    and write the File Name .
./
C96_aa.tpl      charmm19_aa_all.tpl   vas.dih
C96_na.tpl      charmm22_aa_all.tpl
C96_aa.tpl
%% Input File Name of Atom Coord. or Dihed. %%
./
vas.dih
%% Input File Name of Output Coord. %%
./
vas-dih.pdb
%% Input File Name of Output Topology %%
./
vas-dih.tpl
   :

   :
%% Program is done. %%
%% This program ended normally. %%
```

5.2 Sample-2: Protein in a vacuum - Calculation of Lysozyme -

(1) Preparation of initial coordinate and topology files: When using PDB coordinate files

Prepare the initial coordinates of the protein in PDB format. Ifthere is a shortage of hydrogen (H) it is added automatically. Here it is assumed that "1LZA.pdb" is downloaded from the PDB. "tplgene" creates the PDB file used in MD, and the topology file where force field parameters are assigned.

【Note】 Check that molecules are delimited with "TER". If there is no "TER" between protein chains/molecules, write "TER" into the PDB file yourself.

【Note】 If the molecule is comprised of multiple chains, enter the chain name in PDB format in front of the residue no. A topology file is created even if there is no chain name, but multiple molecules with no chemical bonds will be handled as a single molecule.

【Note】 Only atoms in the ATOM line are subject to processing. The METATM line is not processed. If a non-standard atom name is designated, the program may stop processing. In particular, if an irregular name is used for a hydrogen (H) atom, the system may ignore the given coordinates and recreate a new H at coordinates built into the program.

【Note】 The types of amino acids which can be processed with tplgene are limited to those stored in the database. Amino acids other than these are not processed unless you expand the database yourself.

In the following example, the file in which the HETATM line is removed from "1LZA.pdb" is treated as "lys_0.pdb". As a result, initial coordinate and topology files (with the names "lys_1.pdb" and "lys_1.tpl") are created for the molecule called "LYZ". This section explains the method of handling database files by placing them in an arbitrary directory.
(An example of the method of placing files in the run-time directory is given in "5.1 Sample-1".)

■Execution method (Method of placing input file and database file in an arbitrary directory)

① Prepare the input file and database file in an arbitrary directory. Next, set the path of the directory for the input file, and the directory for the database file, in environment variables (for the setting method, see "2.2.5 Environment variables"). If environment variables are not set at the time of tplgene execution, the directory (input, output or force field DB directory) for the unset variables will be the current directory.

② Execute tplgene. This has no parameters and processing begins interactively when you type "tplgene".

③ In sequence, input: the title (arbitrary), molecule name (arbitrary), protein or nucleic acid, input format, database used, input file names, and output file names.

■Sample execution

※In this example, calculation is performed assuming that environmental variables have been set as follows.

Directory for input file : /home/user01/myPresto/sample/sample2

Directory for database file : /home/user01/myPresto/tplgene/DB

```
%% Input Title for this molecules %%
    If end, type end and (cr).
 %% Input Title less than 79 char. %%
LYSOZYME test
end
%% Input Molecular Name less than 39 char. %%
    If end, type end and (cr).
LYZ
end
%% Select Chain Species by the next number. %%
   1 : peptide    chain
   2 : nucleotide chain
1
%% Select Input File by the next number. %%
   1 : pdb   file
   2 : dihed file
1
%% Which Force Field Files do you use ? %%
    Please choose from the following files .
    and write the File Name .
/home/user01/myPresto/tplgene/DB/
C96_aa.tpl      C96_na.tpl      charmm19_aa_all.tpl   charmm22_aa_all.tpl
C96_aa.tpl
%% Input File Name of Atom Coord. or Dihed. %%
/home/user01/myPresto/sample/sample2/
1LZA.pdb        lys_0.pdb
lys_0.pdb
%% Input File Name of Output Coord. %%
./
lys_1.pdb
%% Input File Name of Output Topology %%
./
lys_1.tpl
```

(Continued on next page)

(Continued from previous page)

```
INFORMATION> tgReadAminoSequence
             Molecule Number           :1
             Total number of residues  :129

INFORMATION> tgReadAminoSequence
             Amino acid Sequence of the protein

Molecule number :1
LYS+N+ VAL    PHE    GLY    ARG+   CYSS   GLU-   LEU    ALA    ALA
ALA    MET    LYS+   ARG+   HIS    GLY    LEU    ASP-   ASN    TYR
ARG+   GLY    TYR    SER    LEU    GLY    ASN    TRP    VAL    CYSS
ALA    ALA    LYS+   PHE    GLU-   SER    ASN    PHE    ASN    THR
GLN    ALA    THR    ASN    ARG+   ASN    THR    ASP-   GLY    SER
THR    ASP-   TYR    GLY    ILE    LEU    GLN    ILE    ASN    SER
ARG+   TRP    TRP    CYSS   ASN    ASP-   GLY    ARG+   THR    PRO
GLY    SER    ARG+   ASN    LEU    CYSS   ASN    ILE    PRO    CYSS
SER    ALA    LEU    LEU    SER    SER    ASP-   ILE    THR    ALA
SER    VAL    ASN    CYSS   ALA    LYS+   LYS+   ILE    VAL    SER
ASP-   GLY    ASN    GLY    MET    ASN    ALA    TRP    VAL    ALA
TRP    ARG+   ASN    ARG+   CYSS   LYS+   GLY    THR    ASP-   VAL
GLN    ALA    TRP    ILE    ARG+   GLY    CYSS   ARG+   LEUC-

INFORMATION> tgReadInputTopology
             Amber Type Topology Database File is read

INFORMATION> tgOutputTopology
             Write formatted Topology File

INFORMATION> tgSetCoordinate
             All the atom positions are now set.

INFORMATION> tgOutputCoordinate
             Output pdb-formatted coordinates

CALC. TIME = 0.490000 sec.

%% Program is done. %%
%% This program ended normally. %%
```

This completes preparation of the protein PDB and topology file.

【Note】 For proteins not containing an S-S bond, the PDB ATOM line is sufficient. However, for molecules containing an S-S bond, tplgene determines the presence of S-S bonds by reading the line designating S-S bonds in the PDB.

In this example, there is an S-S bond designation line, so "CYS" is replaced with "CYSS". If the S-S designation line is deleted, "CYS" remains as is, S-S bonds are not formed, and H is added (as in "S-H"), as indicated in the example below. Please check whether the conversion has been done correctly.

■Results when S-S bonds have not been designated (Partial)

```
Molecule number :1
LYS+N+ VAL   PHE   GLY   ARG+  CYS   GLU-  LEU   ALA   ALA
ALA    MET   LYS+  ARG+  HIS   GLY   LEU   ASP-  ASN   TYR
ARG+   GLY   TYR   SER   LEU   GLY   ASN   TRP   VAL   CYS
ALA    ALA   LYS+  PHE   GLU-  SER   ASN   PHE   ASN   THR
GLN    ALA   THR   ASN   ARG+  ASN   THR   ASP-  GLY   SER
THR    ASP-  TYR   GLY   ILE   LEU   GLN   ILE   ASN   SER
ARG+   TRP   TRP   CYS   ASN   ASP-  GLY   ARG+  THR   PRO
GLY    SER   ARG+  ASN   LEU   CYS   ASN   ILE   PRO   CYS
SER    ALA   LEU   LEU   SER   SER   ASP-  ILE   THR   ALA
SER    VAL   ASN   CYS   ALA   LYS+  LYS+  ILE   VAL   SER
ASP-   GLY   ASN   GLY   MET   ASN   ALA   TRP   VAL   ALA
TRP    ARG+  ASN   ARG+  CYS   LYS+  GLY   THR   ASP-  VAL
GLN    ALA   TRP   ILE   ARG+  GLY   CYS   ARG+  LEUC-
```

(2) Energy minimization and MD calculation

There are two methods of energy minimization: the steepest descent method and the conjugate gradient method. However, please be aware that SHAKE cannot be used with the conjugate gradient method.

Energy minimization is performed in a vacuum using the previous created topology file and initial coordinates. For interaction, this example uses an 8 Å cutoff and a distance dependent dielectric function, and updating of the interaction table is done every 20 steps. In order to observe the displacement of coordinates from initial coordinates as energy minimization progresses, the following are designated: "REFCOORD=PDB NAMERE=lys_1.pdb" and "BESTFI=YES". SHAKE cannot be designated with the conjugate gradient method, so a semi-colon (;) is used to comment out the "SETSHAKE" line.

If convergence is poor with the conjugate gradient method (i.e. in a large system, or a system with large strain etc.), it is often better to apply the conjugate gradient method after first applying the steepest descent method (METHOD=STEEP) for a few tens of steps.

■Control file (min_vac.inp)

```
    EXE> INPUT
         TOPOLOGY=   FORM     NAMETO=    lys_1.tpl
         COORDINA=   PDB      NAMECO=    lys_1.pdb
         REFCOORD=   PDB      NAMERE=    lys_1.pdb
;        SETSHAKE=   READ     NAMESH=    lys.shk
         QUIT
    EXE> MINI
         METHOD=  CONJ        CPUTIM=     360000.0
         LOOPLI=  4000        UPDATE=     20
         MONITO=    5         CONVGR=     0.1D0
         CUTMET=  RESA        CUTLEN=     8.0D0
         DIEFUN=  DIST        DIEVAL=     2.0D0
         BESTFI=  YES
         QUIT
    EXE> OUTPUT
         COORDINATE=  PDB     NAMECO=   lys_1_min.pdb
         QUIT
    EXE> END
```

(3) MD calculation

MD is performed, based on the energy minimized coordinates, to achieve the equilibrium state of the system. The topology file is the same as that used with initial coordinates. In order to measure the discrepancy from the initial structure, the following are designated in the control file: "REFCOORD=PDB NAMERE=lys_1.pdb" and "BESTFI=YES".

Now, let's try measuring the distance between amino acid residues in a protein. Here we create a monitor designation file, and monitor the distance between atom A and atom B. This is designated as follows.

- Chain no. including A Residue no. in molecule containing A Atom name of A
- Chain no. including B Residue no. in molecule containing B Atom name of B

■Monitor designation file (lys.mntinp)

```
MONITOR> DISTANCE
1  46  ND2  1 109 CB
1  73  CZ   1 101 CG
END
```

The above designates the distance between ND2 in the 46th residue and CB in the 109th residue, and the distance between CZ in the 73rd residue and CG in the 101st residue.

To output this to "lys_vac.mnt" in ASCII format every 100 steps, designate "OUTTRJ=100", "NAMETR=lys_vac.mnt" and "MNTRTR=ASCI".

In addition to the above calculationconditions, the following also indicates input for NVT calculation at 300K, interaction cutoff at 10Å, fixing translational and rotational movement of the protein around the center of mass, and random generation of initial velocities.

The time step setting is normally 0.5fs, and 1.5fs if SHAKE is applied to all hydrogen, but it is okay to perform NVE calculation beforehand with the same system, and set to a time step where all energy is saved.

■Control file (md_vac.inp)

```
EXE> INPUT
     TOPOLOGY=   FORM     NAMETO=  lys_1.tpl
     COORDINA=   PDB      NAMECO=  lys_1_min.pdb
     OUTMONIT=   READ     NAMEMO=  lys.mntinp
     REFCOORD=   PDB      NAMERE=  lys_1.pdb
     QUIT
EXE> MD
     LOOPLI=     2000
     SETTIM=     500.0D0   CPUTIM= 3600000.0D0
     UPDATE=     20
     TIMEST=     0.5D0
     OUTTRJ=     100
     OUTLOG=     100
     LOGFOR=     DETA      STOPCE= BOTH

     METHOD=     CANONICAL
     SETTEM=     300.0D0
     INITIA=     SET
     STARTT=     300.0D0
     RANDOM=     654321

     NAMETR= lys_vac.mnt  MNTRTR=  ASCI
     BESTFI=     YES
     CUTMET=     RESA     CUTLEN=  10.0D0
     DIEFUN=     DIST     DIEVAL=  2.0D0
     CALV15=     CALC
     CALE15=     CALC
     CALHYD=     NOCALC
     CALV5N=     NOCALC
     CALE5N=     NOCALC
     CALH5N=     NOCALC
     QUIT
EXE> OUTPUT
     COORDINATE=  PDB      NAMECO=   lys_1_md.pdb
     QUIT
EXE> END
```

5.3 Sample-3: Protein in water - Calculation of Lysozyme -

(1) Preparation of initial coordinates and topology file

Prepare protein data using the same procedure as for the vacuum case.
In this example, these files are assumed to be "lys_1.pdb" and "lys_1.tpl".

(2) Boundary condition setting

Determine boundary conditions before preparing solvent water. The following 3 boundary conditions can be used.

- Sphere or ellipsoid having a rigid repulsive wall
- CAP water
- Periodic boundary conditions (Cubic cell)

With a sphere or ellipsoid with a rigid repulsive wall, designate the radius (with the center) for a sphere, or the major/minor axes for an ellipsoid, as indicated below. Designation is done in the control file. (The following example uses a sphere.) An ellipsoid is good for calculating the structure in water of a flat, long protein which deviates from the form of a sphere.

```
BOUNDARY=  SPHERE
RADIUS=    30.0
CENTRX=     3.500
CENTRY=    27.800
CENTRZ=    34.000
```

For CAP water, designate the CAP restraint parameters (like CAP center and CAP restraint radius) in the control file, as indicated below. In the CAP designation file, designate the protein and water molecules as the object of CAP calculation. In some cases the CAP center is indicated clearly with coordinates, and in some cases the center of mass of the molecule is designated. There are two types of repulsion potential , a quadratic function and a biquadratic function , and the quadratic function is used in this example. This method is suitable for protein-ligand docking calculation.

```
CALCAP=     CALC
CENTRX=     3.500
CENTRY=    27.800
CENTRZ=    34.000
RADCAP=      10.0
FORCAP=      20.0
FUNCAP=      HARMonic
SETCEN=      NO
```

■CAP designation file (lys_w.capbc)

```
BOUND> INCLUDE
LYZ        1       1     YES
WAT        1    4592     YES
```

Designate **periodic boundary conditions** in the control file as follows. In addition to designating the cell center, please designate the length of the cell sides.

```
BOUNDARY= PERI
CENTRX=     3.500
CENTRY=    27.800
CENTRZ=    34.000
LXCELL=    20.00
LYCELL=    20.00
LZCELL=    20.00
```

【Note】 For the method of interaction cutoff, please designate "CUTMET=RESC". A molecule dipole split will occur with ATOM base cut off, so myPresto is designed so that this feature cannot be used. If the molecule size is close to the cell size with "CUTMET=RESA", it may be impossible to definitely calculate interactions. In this case, the program will issue a warning.

(3) Generation of water

① Generate water molecules using the program tool "setwater".
(For information on using this tool, see "setwater" in "B Utilities".)

In this example, setwater is used to generate spherical TIP3P water molecules "wat.pdb" in the region from the center of mass of the protein "lys_1.pdb" to a radius of 34 Å.

【Note】 When adding water, there is a tendency for the density of water molecules to be somewhat smaller than the actual density due to the form of occupation of the protein space. If there appear to be gaps in the hydration system after NVT execution, adjust the number of water molecules. When adding CAP water, arrange molecules (depending on the system) so that they are somewhat larger than the CAP radius (0.5 to 2 Å). In other cases, it may be necessary to decrease the damping factor of the vdW radius, or adjust the density to a slightly higher value.

② Add PDB data for the created water molecules to the protein PDB data, and create an in-water PDB file. (Add the PDB to the end of the PDB of the target system.)

```
% cp  lys_1.pdb  lys_w.pdb
% cat wat.pdb >> lys_w.pdb
```

③ Using the program tool "mergetpl", merge the information in the water molecule topology file with information in the protein topology file to form a single topology file. Use the editor to enter the number of water molecules in the merged file.
(For information on using this tool, see "mergetpl" in "B Utilities".)

In this example, a new topology file "lys_w.tpl" is created by merging the protein "lys_1.tpl" and water "tip3p.tpl" files, and the number of water molecules is entered in the "MOLECULES" section of "lys_w.tpl".

■Entering the number of water molecules in the topology file "lys_w.tpl"

```
TPL> TITLE
LYSOZYME test

TPL> MOLECULES
LYZ                                    1
WAT                                 4592       ; Number of water molecules
  :
 (Omitted)
```

(4) How to use SHAKE

To execute shake, you must provide myPresto with a SHAKE file describing the SHAKE conditions. The SHAKE file designates the atoms to be restrained, and the restraint distance between them. These are designated as follows.
(For details, see the section on SHAKE files in "A Input/Output files".)

- Molecule name
- Number of atoms subject to SHAKE, relative atom no. in molecule of atoms subject to SHAKE, distance between atoms

■SHAKE file (lys_w.shk)

```
SHAKE> SHAKE
LYZ                                          ; Molecule name
4    1    2    3    4 ->                     ; Number of SHAKE atoms, SHAKE atom number
1.01000   1.64962   1.01000   1.01000   1.64962   1.64962    ; Distance between atoms

2    6    5 ->                                ; Number of SHAKE atoms, SHAKE atom number
1.0901                                       ; Distance between atoms

3    8    7    9 ->                          ; Number of SHAKE atoms, SHAKE atom number
1.0905   1.0917   1.7691                      ; Distance between atoms
  :

  :

SHAKE> SHAKE
WAT                                          ; Molecule name
3    1    2    3       ->                    ; Number of SHAKE atoms, SHAKE atom number
0.95720   1.51360   0.95720                  ; Distance between atoms
```

The first section above designates SHAKE for 4 atoms. This comprises a tetrahedron, so the settings designate the distances between 6 atoms. The designation sequence of distances between atoms is 1-2, 2-3, 3-1 (up to this point, settings are the same as for 3 atoms), 1-4, 2-4 and 3-4. In order to execute SHAKE designated with this SHAKE file, designate "SETSHAKE=READ NAMESH=lys_w.shk" and "SHAKEM=HBON" in the control file.

【Note】

- Within each line, the part after "; " is a comment. "->" indicates that the line continues.
- The number of atoms comprising SHAKE is set to 2, 3 or 4.
- SHAKE between multiple molecules cannot be designated.

(5) Energy minimization

The following example shows the case where CAP water is used.

■Control file (min_wat.inp)

```
    EXE> INPUT
         TOPOLOGY=   FORM     NAMETO=    lys_w.tpl
         COORDINA=   PDB      NAMECO=    lys_w.pdb
         REFCOORD=   PDB      NAMERE=    lys_w.pdb
         SETBOU=     READ     NAMEBO=    lys_w.capbc
         SETSHAKE=   READ     NAMESH=    lys_w.shk
         QUIT
    EXE> MINI
         METHOD=  STEEP       CPUTIM=     360000.0
         LOOPLI=  4000        UPDATE=     20
         MONITO=     5        CONVGR=     0.2D0
         CUTMET=  RESA        CUTLEN=     8.0D0
         DIEFUN=  CONS        DIEVAL=     1.0D0
         BESTFI=  YES

;  in case of SPHERE boundary
;        BOUNDARY=   SPHERE
;        RADIUS=     35.0     SETCEN=  YES
;
;  in case of CAP boundary
         CALCAP=     CALC     FUNCAP=  HARMonic
         RADCAP=     34.0     FORCAP=  100.0
         SETCEN=     YES
         SHAKEM=     HBON
         QUIT
    EXE> OUTPUT
         COORDINATE=  PDB     NAMECO=   lys_w_min.pdb
         QUIT
    EXE> END
```

【Note】 In boundary conditions for a sphere or ellipsoid, you cannot designate a sphere region smaller than the atom coordinates included in the input PDB. If there are atoms outside the boundary conditions, the program will issue a warning and stop.

(6) MD calculation in water

This indicates input for NVT calculation using Gaussian constraint under the restraint given by the CAP constraint and SHAKE. Equilibrium has not been reached in the first step of MD, so designate "NAMERO=lys_w_md.res" in the control file, output a restart file and quit.

■Control file (md_wat.inp)

```
    EXE> INPUT
         TOPOLOGY=   FORM      NAMETO=  lys_w.tpl
         COORDINA=   PDB       NAMECO=  lys_w_min.pdb
         OUTMONIT=   READ      NAMEMO=  lys.mntinp
         REFCOORD=   PDB       NAMERE=  lys_w.pdb
         SETBOU=     READ      NAMEBO=  lys_w.capbc
         SETSHAKE=   READ      NAMESH=  lys_w.shk
         QUIT
    EXE> MD
         LOOPLI=     2000
         SETTIM=     500.0D0       CPUTIM= 3600000.0D0
         UPDATE=     20
         TIMEST=     2.0D0
         OUTTRJ=     100
         OUTLOG=     10
         LOGFOR=     DETA          STOPCE= BOTH

         METHOD=     CANONICAL     THERMO= CONS
         SETTEM=     300.0D0
         INITIA=     SET
         STARTT=     300.0D0
         RANDOM=     654321
         NAMERO=     lys_w_md.res
;  in case of SPHERE boundary
;        BOUNDARY=   SPHERE
;        RADIUS=     35.0          SETCEN=  YES
;  in case of CAP boundary
         CALCAP=     CALC          FUNCAP=  HARMonic
         RADCAP=      34.0         FORCAP=  100.0
         SETCEN=     YES

         NAMETR= lys_wat.mnt       MNTRTR=  ASCI
         BESTFI=     YES
         CUTMET=     RESA          CUTLEN=  10.0D0
         DIEFUN=     CONS          DIEVAL=  1.0D0
         SHAKEM=     HBON
         CALV15=     CALC            CALV5N=     NOCALC
         CALE15=     CALC            CALE5N=     NOCALC
         CALHYD=     NOCALC          CALH5N=     NOCALC
         QUIT
    EXE> OUTPUT
         COORDINATE=  PDB      NAMECO=    lys_w_md_1.pdb
         QUIT
    EXE> END
```

(7) Restarting

In MD for performing sampling, the usual approach is to restart from the previous MD results. The coordinates and velocities used for restarting are designated with "RESTARt=YES" and "NAMERI=lys_w_md.res". Atom names are not written in the restart file, so the coordinates matching the atom names in the INPUT phase are designated as "COORDINA=PDB NAMECO=lys_w_min.pdb".

■Control file (md_wat2.inp)

```
    EXE> INPUT
         TOPOLOGY=   FORM      NAMETO=  lys_w.tpl
         COORDINA=   PDB       NAMECO=  lys_w_min.pdb
         OUTMONIT=   READ      NAMEMO=  lys.mntinp
         REFCOORD=   PDB       NAMERE=  lys_w.pdb
         SETBOU=     READ      NAMEBO=  lys_w.capbc
         SETSHAKE=   READ      NAMESH=  lys_w.shk
         QUIT
    EXE> MD
         LOOPLI=     100
         SETTIM=     500.0D0   CPUTIM= 3600000.0D0
         UPDATE=     20
         TIMEST=     2.0D0
         OUTTRJ=     100
         OUTLOG=     10
         LOGFOR=     DETA      STOPCE= BOTH

         RESTARt=    YES       NAMERI=     lys_w_md.res
         METHOD=     CANONICAL THERMO=     CONS
         SETTEM=     300.0D0
         INITIA=     SET
         STARTT=     300.0D0
         RANDOM=     654321
         NAMERO=     lys_w_md_2.res
;  in case of CAP boundary
         CALCAP=     CALC      FUNCAP=  HARMonic
         RADCAP=      34.0     FORCAP=  100.0
         SETCEN=     YES

         NAMETR=   lys_wat2.mnt  MNTRTR=  ASCI
         BESTFI=     YES
         CUTMET=     RESA      CUTLEN=  10.0D0
         DIEFUN=     CONS      DIEVAL=  1.0D0
         SHAKEM=     HBON
         CALV15=     CALC          CALV5N=     NOCALC
         CALE15=     CALC          CALE5N=     NOCALC
         CALHYD=     NOCALC        CALH5N=     NOCALC
         QUIT
    EXE> OUTPUT
         COORDINATE=  PDB      NAMECO=    lys_w_md_2.pdb
         QUIT
    EXE> END
```

(8) Log output

Sample output of the calculation log is indicated below.

■Log output

```
*****************************************************************************

 MD LOOP NUMBER        :              20 TIME (PSEC)           :         0.01000
 LAP CPU TIME  (SEC)   :      39.5810551

 TEMPERATURE (K)       :     299.9925948 SCALING FACTOR        :   0.0000000E+00
 TOTAL      (KCAL/MOL) : -0.2936448E+05 POTENTIAL  (KCAL/MOL) : -0.4029400E+05
 KINETIC    (KCAL/MOL) :  0.1092952E+05
 HAMILTONIAN(NOSE)     : -0.2936448E+05                                        -(1)
  TOTAL  :  -0.4029400E+05  BOND   :   0.2342204E+03  ANGLE  :   0.5456552E+03
  TORS.  :   0.7281127E+03  IMPRO. :   0.5763917E+02  VDW14  :   0.4271405E+03
  ELE14  :   0.3779113E+04  VDW15  :   0.3002577E+04  ELE15  :  -0.4906846E+05
  DHR.   :   0.0000000E+00  REP.   :   0.0000000E+00  CAP.   :   0.0000000E+00

 R.M.S.F. (KCAL/MOL*A) :   0.1761278E+02 RMSD (ANGSTROMS)      :   0.2545461E+00  -(2)

    NAME OF MOLECULE       MAX FORCE      TEMPERATURE    CAP ENERGY    ERROR     -(3)
     LYZ                   0.6555757E+02  0.1755462E+03  0.0000000E+00
     WAT                   0.7155075E+02  0.3165385E+03  0.0000000E+00

 INFO>CUTOFF: 1-5 VDW & 1-5 HYD.BOND :     4877928 &           0

 *****************************************************************************
```

(1) Value of the virtual Hamiltonian, which is a conserved quantity in the Nose-Hoover method.
(However, this is output only when "THERMO=NOSE" is designated.)

(2) RMSD is calculated for atoms in the 1st chain.
In this example, this is RMSD for lysozyme.

(3) The maximum force value, temperature and CAP potential values are displayed for each molecule.

【Note】 Be careful of the designation of "CALHYD=NOCALC" for different force fields. With a force field like Amber91 which openly includes the LJ12-10 type potential of hydrogen bonds, this is set to "CALHYD=CALC". With Amber94/96/99, hydrogen bonds are included in the Coulomb force, so this is set to "CALHYD=NOCALC".

5.4 Sample-4: Expanded ensemble (Force-biased McMD) —Calculation of Alanine peptide—

(1) Force-Biased Multicanonical MD calculation

cosgene can be used to perform Force-Biased Multicanonical MD(F.B.McMD) calculation. Prepare the protein coordinates and topology file as usual. Using Ace-Ala-Ala-Nme as an example, the dihedral angle input ala-ala.dih has been prepared as the initial input. First use tplgene to prepare the initial coordinates ala-ala.pdb and topology file ala-ala.tpl. F.B.McMD is an MD method, and thus cannot be used for energy minimization. Perform energy minimization in the normal way.

In F.B.McMD, specify expanded ensemble (METHOD= EXPA) and F.B.McMD (EXPAND= FORC). Next, set the temperature range of the multicanonical distribution that you wish to produce. The range TEMMIN= 250 K to TEMMAX= 700 K is specified in the example. The weight factor for scaling is created from the energy histogram in F.B.McMD. The range over which the energy histogram is created (ENEMIN to ENEMAX) must be sufficient to cover the energy distribution of canonical distribution at TEMMIN and the energy range of canonical distribution at TEMMAX, and thus NVT calculation should be previously performed at TEMMIN and TEMMAX to obtain approximate values for ENEMIN and ENEMAX.In the example, the energy distribution at T = 250 K is 20 to 30 kcal/mol, and the energy distribution at T = 700 K is 50 to 70 kcal/mol, and thus ENEMIN = -100 kcal/mol, ENEMAX = 400 kcal/mol are specified to provide sufficient leeway. Specify the bin size (BINSIZ) used when the histogram is created. 2 kcal/mol is specified in the example. The temperature for MD simulation (TO= SETTEM) is the temperature used when the kinetic equation is solved, and is set such that TEMMIN < SETTEM < TEMMAX. Specify the number of loops (RESETC) per F.B.McMD iteration and the number of data discard dummy loops (DUMMYL), as well as the overall number of loops (LOOPLI). LOOPLI should be an integer multiple of RESETC.

■Control file

```
;fbmcmd_vac.inp
    EXE> INPUT
         TOPOLOGY=   FORM     NAMETO=  ala_ala.tpl
         COORDINA=   PDB      NAMECO=  ala_min.pdb
         OUTMONIT=   READ     NAMEMO=  ala.mntinp
         REFCOORD=   PDB      NAMERE=  ala_min.pdb
         QUIT
    EXE> MD
         LOOPLI=     30000000
         SETTIM=     50000.0D0    CPUTIM= 3600000.0D0
         UPDATE=     20
         TIMEST=     0.5D0
```

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```
        OUTTRJ=     1000
        OUTCOO=     1000
        OUTLOG=     2000
        LOGFOR=     DETA       STOPCE= BOTH

        METHOD=     EXPA       EXPAND= FORC
        RESETC=     300000     DUMMYL= 1000
        TEMMAX=     700.0      TEMMIN= 250.0
        ENEMIN=    -100.0      ENEMAX= 400.0
        BINSIZ=       2.0
        SETTEM=     600.0
        LIMITS=     0.001      LIMITC=  0.001

        INITIA=     SET
        STARTT=     600.0D0
        RANDOM=     654321

        NAMETR= ala_vac.mnt  MNTRTR=  ASCI
        NAMERO= ala_vac.rst
        NAMECO= ala_vac.cor  MNTRCO=  SING
        NAMETO= ala_vac.eto  MNTRTO=  ASCI
        BESTFI=     YES

        CUTMET=     RESA     CUTLEN=  99.0D0
        DIEFUN=     DIST     DIEVAL=  4.0D0

        CALV15=     CALC
        CALE15=     CALC
        CALHYD=     NOCALC
        CALV5N=     NOCALC
        CALE5N=     NOCALC
        CALH5N=     NOCALC
        QUIT
    EXE> OUTPUT
        COORDINATE=  PDB     NAMECO=   ala_md.pdb
        QUIT
    EXE> END
```

When F.B.McMD ends, the expand.energy file, expand.prob file, and expand.scale file are output along with the coordinate trajectory file. Reweighting analysis for the purpose of reproducing the canonical distribution is performed using the reweightFB tool (see "B. Utilities" at the end of this manual) and the expand.scale output file.

(2) Reconfiguration of canonical distribution

Prepare the reweightFB analysis tool. Compile as shown below. Analysis is completed in a short time and thus optimization options are normally not necessary.

```
% f90  reweightFB.f90  -o reweightFB.x
```

Prepare the input file inp_MUCA as shown below. Among the files, the ttt3 file is the most important for reproduction of the canonical distribution.

```
F.B.scale
ttt1 ttt2 ttt3 ttt4
2.0 251  10 100
600 260 700 20 1.d-05
```

1st line : Input filename (expand.scale in default)
2nd line : Output file name
- 1st column : Distribution coefficient
- 2nd column : Energy, density of state index, entire probability
- 3rd column : Energy, probability of reweighted canonical distribution
- 4th column : Temperature, mean energy, square of mean energy, specific heat

3rd line :
- 1st column : bin size specified in F.B.McMD (=BINSIZ)
- 2nd column : Data number of histogram (=(ENEMAX-ENEMIN)/BINSIZ + 1)
- 3rd column : Iteration number at which you wish to begin reweight
- 4th column : Iteration number at which you wish to end reweight
  Normally used until the final iteration (= LOOPLI/RESETC)

4th line :
- 1st column : TO(SETTEM) used in MD simulation
- 2nd column : Lower limit of temperature of canonical distribution that you wish to generate (> TEMMIN)
- 3rd column : Upper limit of temperature of canonical distribution that you wish to generate (> TEMMAM)
- 4th column : Temperature gradations. "Upper temperature limit - lower temperature limit" is divided by this number.
- 5th column : Threshold of WHAM analysis. Set to 10^{-5} or less.

Using the prepared analysis tool and input file, execute with

```
% reweightFB.x  < inp_MUCA
```

The output file ttt3 is as shown below. The example indicates that a canonical distribution at 260 K is reproduced by sampling the structure output on the trajectory at the probability in the second column in the energy range 21 kcal/mol to 61 kcal/mol.

```
0.260000E+03
0.210000E+02 0.480194E-04
0.230000E+02 0.122335E-02
0.250000E+02 0.917415E-02
0.270000E+02 0.322814E-01
0.290000E+02 0.732955E-01
0.310000E+02 0.126560E+00
0.330000E+02 0.173496E+00
0.350000E+02 0.193453E+00
0.370000E+02 0.173022E+00
0.390000E+02 0.116629E+00
0.410000E+02 0.614765E-01
0.430000E+02 0.263826E-01
0.450000E+02 0.928163E-02
0.470000E+02 0.277370E-02
0.490000E+02 0.708900E-03
0.510000E+02 0.158080E-03
0.530000E+02 0.312139E-04
0.550000E+02 0.549539E-05
0.570000E+02 0.875144E-06
0.590000E+02 0.126766E-06
0.610000E+02 0.167411E-07
```

Sample 6 explains a method for outputting a representative structure by extracting the structure from the coordinate trajectory according to this probability distribution.
Cut out only the part for the temperature that you wish to reproduce from the output file ttt3 so as to make a two-column energy and probability distribution file (pdf260_fb) as shown below.

```
0.210000E+02 0.480194E-04
0.230000E+02 0.122335E-02
0.250000E+02 0.917415E-02
 (Middle omitted)
0.510000E+02 0.158080E-03
0.530000E+02 0.312139E-04
0.550000E+02 0.549539E-05
0.570000E+02 0.875144E-06
0.590000E+02 0.126766E-06
0.610000E+02 0.167411E-07
```

5.5 Sample-5 : Expanded ensemble (Simulated Tempering McMD) —Alanine peptide calculation—

(1) Simulated Tempering Multicanonical MD calculation

cosgene can be used to perform Simulated Tempering Multicanonical MD (S.T.McMD) calculation. The following explanation uses the same system as in sample 4. Follow the procedure in sample 4 to perform the calculations through energy minimization.

To perform S.T.McMD, specify expanded ensemble (METHOD= EXPA) and S.T.McMD (EXPAND= SIMU) . Next, set the temperature range of the multicanonical distribution that you wish to obtain. The range TEMMIN= 250 K to TEMMAX= 700 K is specified in the example. Perform NVT calculation ahead of time at TEMMIN and TEMMAX in the same way as for F.B.McMD to determine the ENEMIN, ENEMAX, and BINSIZ parameters for the energy histogram.

The temperature transition width ΔT will be TEMMAX (maximum temperature) - TEMMIN (minimum temperature) divided by the temperature division number (STTNUM). The temperature division number (STTNUM) can be made larger to decrease ΔT and obtain a natural temperature transition, however, the number of calculations required to sample all temperatures will increase.

The implementation of S.T.McMD in cosgene uses the guide coefficient $(E-E_0)/k_BT^2$ to calculate the temperature transition probability. Use the same method as for ENEMIN to obtain an approximate value for the minimum energy E_0 and set the parameter STEBAS to this value. The temperature for performing MD simulation (TO= SETTEM) is the temperature used when solving the kinetic equation, and is set such that TEMMIN < SETTEM < TEMMAX. Set the number of loops (RESETC) per S.T.McMD iteration and the number of data discard dummy loops (DUMMYL), as well as the overall number of loops (LOOPLI). LOOPLI should be an integer multiple of RESETC.

■Control file

```
;stmcmd_vac.inp
    EXE> INPUT
         TOPOLOGY=   FORM     NAMETO=  ala_ala.tpl
         COORDINA=   PDB      NAMECO=  ala_min.pdb
         OUTMONIT=   READ     NAMEMO=  ala.mntinp
         REFCOORD=   PDB      NAMERE=  ala_min.pdb
         QUIT
    EXE> MD
         LOOPLI=     30000000
         SETTIM=     50000.0D0    CPUTIM= 3600000.0D0
         UPDATE=     20
         TIMEST=     0.5D0
```

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```
        OUTTRJ=     1000
        OUTCOO=     1000
        OUTLOG=     2000
        LOGFOR=     DETA       STOPCE= BOTH

        METHOD=     EXPA       EXPAND= SIMU
        RESETC=     300000     DUMMYL= 1000
        TEMMAX=     700.0      TEMMIN= 250.0
        ENEMIN=    -100.0      ENEMAX= 400.0
        BINSIZ=       2.0
        SETTEM=     600.0
        LIMITS=     0.001      LIMITC=  0.001
        STTNUM=     100
        STEBAS=    -100.0

        INITIA=     SET
        STARTT=     600.0D0
        RANDOM=     654321

        NAMETR= ala_st.mnt  MNTRTR=  ASCI
        NAMERO= ala_st.rst
        NAMECO= ala_st.cor  MNTRCO=  SING
        NAMETO= ala_st.eto  MNTRTO=  ASCI
        BESTFI=     YES

        CUTMET=     RESA     CUTLEN=  99.0D0
        DIEFUN=     DIST     DIEVAL=  4.0D0

        CALV15=     CALC
        CALE15=     CALC
        CALHYD=     NOCALC
        CALV5N=     NOCALC
        CALE5N=     NOCALC
        CALH5N=     NOCALC
        QUIT
    EXE> OUTPUT
        COORDINATE=  PDB     NAMECO=   ala_st.pdb
        QUIT
    EXE> END
```

When S.T.McMD ends, the expand.energy file, expand.prob file, and expand.scale file are output in addition to the coordinate trajectory file. The reference temperature is output in the log file, and thus the temperature transition state references the S.T. energy (1st column: energy, 2nd column: temperature). Reweighting analysis for the purpose of reproducing the canonical distribution is performed by means of the reweightST tool (see "B. Utilities" at the end of this manual) and the expand.energy output file.

(2) Reconstitution of the canonical distribution

Prepare the reweightST analysis tool. Compile as shown below. Analysis is completed in a short time and thus optimization options are normally not necessary.

```
% f90  reweightST.f90  -o reweightST.x
```

Prepare the input file inp_ST as shown below.
This is the most important canonical file for reproduction of the canonical distribution.

```
S.T.energy
average canonical
2.0 250.0 700.0 9.0
1 29999000
600.0
```

1st column : Input file name (expand.energy in defalt)
2nd column : Output file name
  1st column : Mean energy
  2nd column : Probability of reweighted canonical distribution
3rd column : 1st column : bin size specified in S.T.McMD (=BINSIZ)
  2nd column : Lower limit of distribution output temperature (> TEMMIN)
  3rd column : Upper limit of distribution output temperature (< TEMMAX)
  4th column : Temperature division number. "Upper temperature limit - lower temperature limit" is divided by this number, calculated, and output.
4th column : 1st column : Start of sampling interval (counted from the end of the dummy loop)
  2nd column : End of sampling interval (counted from the end of the dummy loop)
5th column : 1st column : Reference temperature, SETTEM specified in S.T.McMD

Using the prepared analysis tool and input file, execute with

```
% reweightST.x  < inp_ST
```

The output file canonical is as shown below. This was created from the energy histogram sampled at 362.5K (334.4 K to 390.6K), and indicates that the distribution is reproduced by sampling the structures output on the trajectory at the probability in the second column in the energy range 17 kcal/mol to 73 kcal/mol.

```
 (Omitted)
362.5000
  0.170000E+02 0.482616E-06
  0.190000E+02 0.144785E-05
  0.210000E+02 0.231656E-04
  0.230000E+02 0.209455E-03
  0.250000E+02 0.121378E-02
  0.270000E+02 0.491255E-02
 (Omitted)
  0.550000E+02 0.655827E-02
  0.570000E+02 0.320747E-02
  0.590000E+02 0.141262E-02
  0.610000E+02 0.610027E-03
  0.630000E+02 0.256269E-03
  0.650000E+02 0.112932E-03
  0.670000E+02 0.448833E-04
  0.690000E+02 0.164089E-04
  0.710000E+02 0.337831E-05
  0.730000E+02 0.144785E-05
 (Omitted)
```

As with F.B.McMD in Sample 4, cut out only the part for the temperature that you wish to reproduce from this probability distribution file, and use it in the structure extraction and clustering of Sample 6 (pdf363_st) .

5.6 Sample-6：Extend ensemble (Generalized ST McMD)—Calculation of Alanine peptide—

(1) Generalized Simulated Tempering Multicanonical MD calculation

Cosgene can calculate Generalized Simulated Tempering Multicanonical MD (G.S.T.McMD). This function can investigate the structure of a wide range of energy distributions by automatically and optimally shifting between subensembles in accordance with Tsallis distribution. The system used in sample 4 will be explained. Calculate until energy is minimized in accordance with the procedure of sample 4.

For G.S.T.McMD, designate Extend ensemble (METHOD= EXPA) and designate G.S.T.McMD (EXPAND= GST).

The most important parameters for G.S.T.McMD are GSTBAS, GSTETA, GSTMIN, GSTMAX and GSTNUM. Setting these determines the number of sampled energy ranges and used subensembles. Determine these numbers taking into consideration the temperature range to be re-configured later. At first, determine the safe upper limit temperature (Tmax) and lower limit temperature (Tmin) so that the temperature range to be reconfigured is included. Obtain in advance the average potential energy by NVT calculation in the upper limit and lower limit temperatures.

The multiple subensemble index λ is the discrete value of GSTMIN～GSTMAX divided by the number of GSTNUM. E_0：GSTBAS is for guaranteeing $E-E_0>0$ during calculation. Determine this based on the minimum value of the potential energy in NVT calculation of Tmin.

If T is effective temperature, T_0 is temperature designated by SETTEMP (reference temperature), η is GSTETA, E_0 is GSTBAS, and E is potential energy, the following relationship holds:

$$T/T_0=\eta+\lambda(E-E_0)$$

Designate parameters so that the equation provides a value from $Tmin/T_0$ to $Tmax/T_0$. Set η to approximately $Tmin/T_0$. Set GSTMAX so that $\eta+\lambda(Emax-E_0)$ exceeds $Tmax/T0$ λ and is the upper limit GSTMAX (Emax is determined based on the average potential energy of NVT calculation in Tmax). Set GSTNUM so that transition between subensembles becomes smooth for the system.

ENEMIN, ENEMAX and BINSIZ are for preparing a histogram of potential energy. Determine these so that they do not exceed the range from ENEMIN to ENEMAX during calculation, as the case of F.B.McMD based on the result of NVT calculation at Tmin and Tmax.

In order to optimally transfer index λ, the weight of λ is re-calculated by RESETC. It is required to set a sufficient size for RESETC depending on the system. GSTUPD

becomes the step interval for attempting the transition between subensembles.

■Control file

```
;gstmcmd_vac.inp
    EXE> INPUT
        TOPOLOGY= FORM NAMETO= ala_ala.tpl
        COORDINA= PDB NAMECO= ala_min.pdb
        OUTMONIT= READ NAMEMO= ala.mntinp
        REFCOORD= PDB NAMERE= ala_min.pdb
        QUIT
    EXE> MD
        LOOPLI= 20000000
        SETTIM= 50000.0D0 CPUTIM=3600000.0D0
        UPDATE= 20
        TIMEST= 0.5D0
        OUTTRJ= 1000
        OUTCOO= 1000
        OUTLOG= 2000
        LOGFOR= DETA STOPCE= BOTH

        METHOD= EXPA EXPAND= GST
        SETTEM= 600.0

        RESETC=100000
        GSTCON=10000000
        GSTBAS=0.0d0
        GSTETA=0.3d0
        GSTSAM=50000
        MNTRXE=ASCI
        ENEMIN=0.0
        ENEMAX=500.0
        BINSIZ=0.5
        GSTUPD=100
        GSTMIN=0.001d0
        GSTMAX=0.015d0
        GSTNUM=40

        INITIA= SET
        STARTT= 600.0D0
        RANDOM= 654321

        NAMETR= ala_gst.mnt MNTRTR= ASCI
        NAMERO= ala_gst.rst
        NAMECO= ala_gst.cor MNTRCO= SING
        NAMETO= ala_gst.eto MNTRTO= ASCI
        BESTFI= YES

        CUTMET= RESA   CUTLEN= 99.0D0
        DIEFUN= DIST   DIEVAL= 4.0D0
```

(Continued)

(Continued)

```
        CALV15= CALC
        CALE15= CALC
        CALHYD= NOCALC
        CALV5N= NOCALC
        CALE5N= NOCALC
        CALH5N= NOCALC
        QUIT
    EXE>OUTPUT
        COORDINATE=PDB NAMECO=ala_gst.pdb
        QUIT

    EXE>END
```

When G.S.T.McMD is completed, the coordinate trajectory file, expand.energy, and expand.scale file are output. The change in potential energy can be verified in the first column of expand.energy. The state of transition of λ can be verified in the third column. Analysis by Reweighting for reproducing the Canonical distribution is performed by tool reweight GST (refer to the end of the manual "B utility" for details) and the output file expand.energy.

(2) Reconstruction of canonical distribution

Prepare analysis tool reweight GST. Compile as follows. Analysis is completed in a short time. Generally, optimization options are not required.

```
% f90  reweightGST.f90  -o reweightGST.x
```

Prepare input file inp_GST as shown below.
The ttt3 file is the most important for reproducing canonical distribution.

```
expand.energy
lambda-pdf lambda-ene-pdf
ttt1 ttt2 ttt3 ttt4
0.5 0.001d0 0.015d0 40 0.0d0 0.3d0
1 18000000
250.0 700.0 50.0 600
```

1st line: Input filename (default: expand.energy)
2nd line : Output filename
1st column : λ distribution
2nd column : Energy distribution by λ
3rd line : Output filename
1st column : Partition function
2nd column: Exponent and total probability of energy and density of state,
3rd column: Probability of energy and reweighed canonical distribution
4th column : Square and specific heat of temperature, average energy, square of average energy, and specific heat
4th line : 1st column : Bin size designated by G.S.T.McMD (=BINSIZ)
2nd column : Lower limit of λ (=GSTMIN)
3rd column : Upper limit of λ (=GSTMAX)
4th column : Number of partitions of λ (=GSTNUM)
5th column : Base energy (=GSTBAS)
6th column : η (=GSTETA)
5th line : 1st column : Beginning of sampling interval
2nd column : End of sampling interval (<LOOPLI-GSTNUM*GSTSAM)
For performing preparatory sampling of GSTNUM*GSTSAM step portion.
6th line : 1st column : Lower limit of temperature of canonical distribution to be generated
2nd column : Upper limit of temperature of canonical distribution to be generated

3rd column : temperature interval

4th column : TO(SETTEM) used for simulation MD

Execute using the prepared analysis tool and input file.

```
% reweightGST.x  < inp_GST
```

The output file (ttt3 in this example) is as follows: Sampling the energy structure shown in the first column from the structure trajectory by the probability in the second column shows the reproduction of the canonical distribution of 300k.

```
(Previous lines omitted)
 0.300000E+03
 0.137500E+02 0.234968E-07
 0.142500E+02 0.836684E-07
(Middle lines omitted)
 0.577500E+02 0.658355E-07
 0.582500E+02 0.430977E-07
 0.587500E+02 0.279324E-07
 0.592500E+02 0.181532E-07
(Following lines omitted)
```

As in the case of F.B.McMD of Sample 4, only the temperature l portion to be reproduced (which is cut out from the probability distribution file) can be used for extracting the structure and clustering of sample 7.

5.7 Sample-7：Expanded sampling—Structure extraction and clustering

(1) Structure extraction using the reconstituted canonical distribution

Energy distributions at any temperature were calculated by reweighting in Sample 4 (F.B.McMD) and Sample 5 (S.T.McMD). In the following, coordinates are extracted from the trajectory file so as to fill those energy distributions.
The selection analysis tool is used for structure extraction (for detailed information, see "B. Utilities" at the end of this manual). Prepare the following input file.

```
pdf363_st
ala_st.cor
S
1000
300000000
0.5
ala_st_363.cor
32
```

On the 1st line, specify the energy probability distribution file created at the end of Sample 4 or Sample 5.

On the 2nd line, specify the trajectory file output at the time of MD execution.

The 3rd line is the trajectory file type (Single | Double). This must match the specification at the time of MD execution.

On the 4th and 5th lines, specify the sampling interval.

On the 6th line, specify the percentage of the structures to be extracted. The percentage specified here of structures in the sampling interval are output.

On the 7th line, specify the output trajectory file name.

On the 8th line, specify the number of atoms.

Execute with

```
% selection  < select.inp
```

(2) Structure clustering

Next, the procedure for using cluster analysis to extract representative structures from the many structures extracted to constitute the canonical distribution in (1) is explained. Clustering is performed using inter-structure RMSD.

The clustering analysis tool is used for cluster analysis (for detailed information, see "B. Utilities" at the end of this manual).

First prepare a control file (clustering.inp) similar to the following:

```
ala_ala.tpl
y
ala_ala.fit
y
ala_ala.rmsd
400
10
1
500
ala_st_363.cor
S
average
ala_st_363.cls
ala_st_363.tree
```

On the 1st line, specify the topology file.

On the 2nd line, specify whether or not bestfit is applied when RMSD calculation is performed (y | n).

If bestfit is applied, enter the name of the file that specifies the atoms used on the next line.

Here a file with the following content is prepared as ala_ala.fit. In this example, the hydrogen of residue 1-4 of chain 1 is not used for bestfit. This format is the same as that of the cosgene file that specifies alignment of the system center of mass. For detailed information, see A.2.11.

```
SETBST> LIST
FIX 1 1 1 4 H* YES;
```

On the 4th line, indicate (y | n) whether or not there is a specification of atoms used in RMSD calculation. If there is, on the next line indicate the name of the file that specifies the atoms used in RMSD calculation. In this example, the file ala_ala.rmsd, which has the same content as ala_ala.fit, is used. The atoms used in bestfit and RMSD can be checked in the execution log.

On the 6th line, specify the number of structures used for clustering. This will depend on the system, however, specify a number under 1000. On the 7th line, specify the final number of clusters.

On the 8th and 9th lines, specify the start and end positions of the coordinate trajectory range to be used.

Specify this trajectory range so that the number of structures specified on the 6th line can be obtained. In addition, the range should be within the range of the number of structures extracted with the select tool as described above.

On the 10th line, specify the name of the coordinate trajectory file. In this example, the trajectory file used to reproduce the canonical distribution with the select tool is used.

On the 11th line, specify the format of the trajectory file (S | D) .

On the 12th line, enter the clustering method ("nearest" | "furthest" | "median" | "centroid" | "average" | "flexible" | "ward") .

If "flexible" is specified on this line, set the β value on the next line.

On the 13th line, specify the first name of the output PDB file.

On the 14th line, specify the name of the output dendrogram file.

```
% clustering  < clustering.inp
```

Execute with the above command. Among the log output items, the number of structures output per cluster and the mean RMSD express the cluster characteristics well.

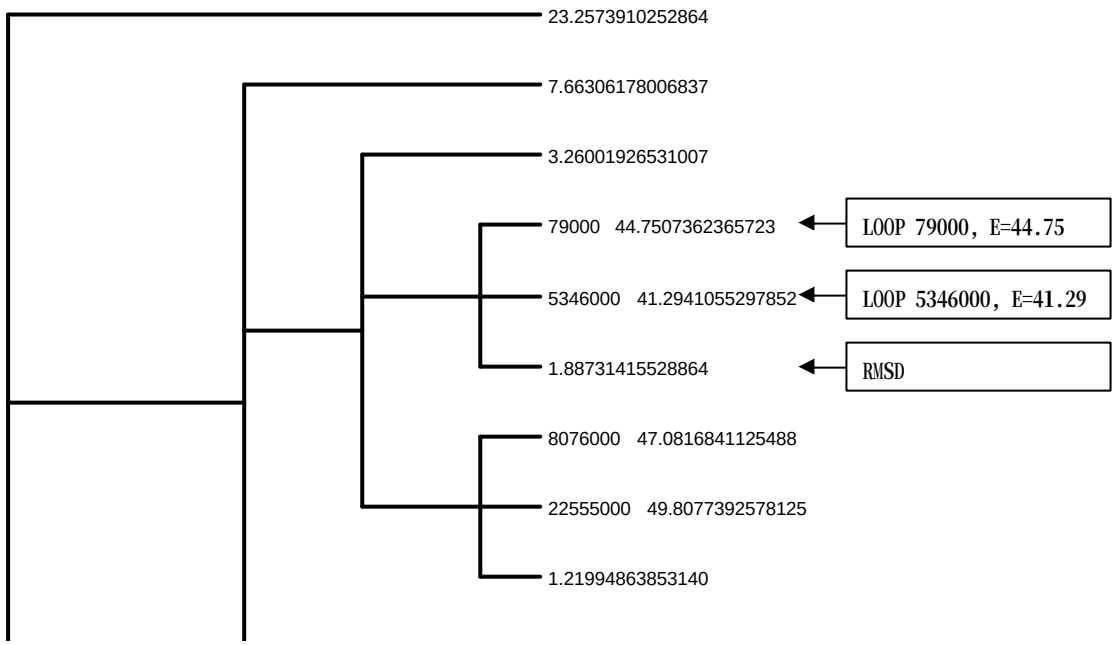
```
        (Previous lines omitted)

 CLUSTER ID      :           1
 STRUCTURE COUNT :          32
 LOOP NUMBER     :    16969000
 RMSD OF AVERAGE :   1.44551613437161
 OUTPUT PDB FILE :ala_st_363.cls.16969000

 CLUSTER ID      :           2
 STRUCTURE COUNT :          32
 LOOP NUMBER     :     8055000
 RMSD OF AVERAGE :   1.87505849035797
 OUTPUT PDB FILE :ala_st_363.cls.8055000

       (Following lines omitted)
```

In addition to the structures of the specified number of clusters (PBX format), a dendrogram file (PHYLIP format) is output and can be displayed using general tools. The dendrogram is displayed in three branches: two structures (or clusters) and the RMSD between them.



5.8 Sample-8：Generation of low molecule topology —Calculation of Methanol—

(1) Preparation of calculation input file

Topology files of proteins and nucleotides are created with tplgene. By contrast, tplgeneL is used to create topology files of low-molecule compounds.

There are two general methods for creating topology files of low molecules.

Method 1 : Create "tplgeneL original format files" based on the charge, bonding order, and coordinate information obtained by quantum chemistry calculations, and use those files for calculation.

Method 2 : Prepare a "Sybyl mol2" file, enter values for the charge information items in the file, and use the resulting file for calculation.

In the explanation that follows, the environment variables of tplgeneL are set as shown below (for the setting procedure, see "3.2.6 Environment variables").

```
TPLL_INPUT_PATH    ⇒ ./          Current directory
TPLL_OUTPUT_PATH   ⇒ ./          Current directory
TPLL_DB_PATH       ⇒ /home/user01/myPresto/tplgeneL/DB
                                 Directory for tplgeneL force field parameter DB
```

【Note】 There are two types of tools for creating tplgeneL original format files: one for GAMESS output and one for Gaussian output.
(For detailed information, see "B.8 Gamess2tplinp" and "B.9 Gauss2tplinp".)

(2) Generating a topology file from a tplgeneL original format file

The procedure for creating a topology file based on the calculation results of the quantum chemistry calculation program GAMESS is explained below.

■Execution method

① First perform quantum chemistry calculation of the methanol molecule. Use GAMESS to perform the calculation, and specify "methnol.log" for the result output file.

② Use the Gamess2tplinp tool to create a tplgeneL original format file from the "methanol.log" file obtained in ①.

In the execution directory, type in "Gamess2tplinp methanol.log". In this case, the following three files will be created: "methanol.charge", "methanol.bond", and

"methanol.zmat".

```
%Gamess2tplinp  methanol.log
```

③ Copy the files obtained in ② to the input file directory.

④ Execute tplgeneL. Type "tplgeneL" without any arguments and start the program interactively.

```
%tplgeneL
```

⑤ Successively specify the file format, input file name, compensation method for missing parameters, name of the force field parameter DB, and whether or not the fragment DB will be used.

■Execution example

```
Please select Input File Format by the next number!
   1 : tplgeneL original (*.bond,*.charge,*.zmat)
   2 : Sybyl mol2 (*.mol2)
1
Please select Input File Name!
./
ala.bond     ala.zmat          methanol.mol2       phenylalanine.charge  xylitol.charge
ala.charge   methanol.bond     methanol.zmat       phenylalanine.zmat    xylitol.zmat
ala.mol2     methanol.charge   phenylalanine.bond  xylitol.bond
methanol

 What processing do you do if there is a missing parameter?
 Please select 1 or 2! (default : 1)
   1 :  use default parameters.
   2 :  calculate parameters.
   3 :  use default parameters when default parameters exist.
        use calculated parameters when default parameters don't exist.

1
Please select Input DB Name(prm_gaff.db/prm_amber99.db)!
   (default : prm_gaff.db)
/home/user01/myPresto/tplgeneL/DB
angle.prm             bond.prm          nonbond_amber99.db  prm_gaff.db
atomtype_amber99.db   frg_amber99.db    nonbond_gaff.db
atomtype_gaff.db      frg_gaff.db       prm_amber99.db
prm_gaff.db
Do you want to use FragmentDB ? (yes(y)/no(n) default : no)
no

 %% Program is done. %%
 %% This program ended normally. %%
```

(3) Creating a topology file from a Sybyl mol2 file

The procedure for creating a topology file from the Sybyl mol2 file of the desired low-molecule compound is explained below.

■Execution method

① Prepare a Sybyl mol2 file for the low-molecule compound for which you wish to create a topology file. Here, this will be the mol2 file ""methanol.mol2" for methanol. Save "methanol.mol2" in the input file directory specified in the environment variable.

② Execute tplgeneL. Type "tplgeneL" without any arguments and start the program interactively.

```
%tplgeneL
```

③ Successively specify the file format, input file name, compensation method for missing parameters, name of the force field parameter DB, and whether or not the fragment DB will be used.

【Note】 tplgeneL does not have a function for adding missing hydrogen atoms. The mol2 file that is prepared must include the hydrogen atom information.

■Execution example

```
Please select Input File Format by the next number!
   1 : tplgeneL original (*.bond,*.charge,*.zmat)
   2 : Sybyl mol2 (*.mol2)
2
Please select Input File Name!
./
ala.bond     ala.zmat          methanol.mol2       phenylalanine.charge  xylitol.charge
ala.charge   methanol.bond     methanol.zmat       phenylalanine.zmat    xylitol.zmat
ala.mol2     methanol.charge   phenylalanine.bond  xylitol.bond
methanol

 What processing do you do if there is a missing parameter?
 Please select 1 or 2! (default : 1)
   1 :  use default parameters.
   2 :  calculate parameters.
   3 :  use default parameters when default parameters exist.
        use calculated parameters when default parameters don't exist.

1
Please select Input DB Name(prm_gaff.db/prm_amber99.db)!
   (default : prm_gaff.db)
/home/user01/myPresto/tplgeneL/DB
angle.prm             bond.prm          nonbond_amber99.db  prm_gaff.db
atomtype_amber99.db   frg_amber99.db    nonbond_gaff.db
atomtype_gaff.db      frg_gaff.db       prm_amber99.db
prm_gaff.db
Do you want to use FragmentDB ? (yes(y)/no(n) default : no)
no

 %% Program is done. %%
 %% This program ended normally. %%
```

5.9 Sample-9 : Free energy calculation (Filling Potential method) –Calculation of methane in water–

(1) Procedure for calculating free energy using the Filling Potential method

In Filling Potential calculation, the stable state where two target molecules are bound together is taken as the initial structure. An umbrella potential is generated for one of the molecules in this bound state, causing molecular motion. When this motion occurs, the locus of the atom designated as the landmark is stored, and at the next calculation, a repulsive potential is generated around the previous locus. This allows the establishment of a new locus, not a previous locus, in the next calculation, enabling calculation that overcomes the potential barrier.

These steps are repeated until the molecules are in a completely dissociated state. The loci traced up to this point are analyzed by WHAM analysis, allowing the free energy difference to be obtained.

① MD calculation with an added umbrella potential is performed.
② The umbrella potential of the next MD calculation is generated around the locus.
③ The locus of the landmark atom is extracted and saved.
④ Steps ① to ③ are repeated until a dissociated state is obtained.
⑤ WHAM analysis is performed on the loci obtained to this point.

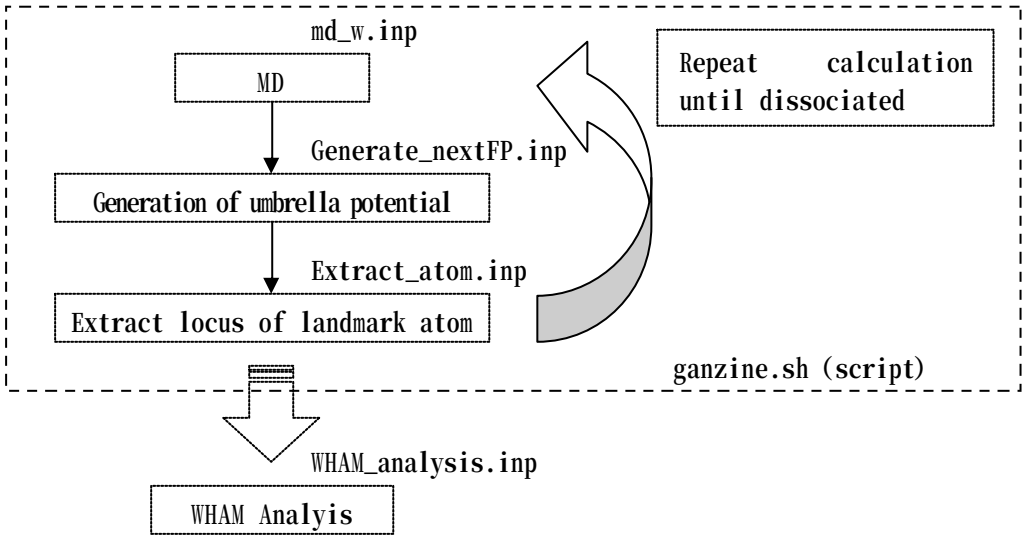


Figure. Procedure for Free Energy Calculation Using the Filling Potential Method

(2) Explanation of sample input file

・Initial coordinate structure **(ch42w.pdb)**	: Prepare the initial structure of the system in PDB format.
・MD calculation input **(md_w.inp)**	: Input for MD calculation by means of Filling Potential.
・Input for umbrella potential update program **(Generate_nextFP.inp)**	: Input file of program that updates the umbrella potential.
・Input for landmark atom locus extraction program **(Extract_atom.inp)**	: Input file of program that extracts the locus of the landmark atom from the entire locus file.
・Umbrella potential file **(newopt_fp, newopt_fp_ini)**	: Umbrella potential position information file. Updated as calculation progresses.
・Script for execution of entire calculation **(ganzene.sh)**	: Script that executes the 3 Filling Potential programs in succession.
・WHAM analysis program input **(WHAM_analysis.head)**	: Form of input file for program that analyzes the locus file calculated by script using the WHAM method

【Note】 The parts that must be changed at the least to calculate another system are shown in **bold** in the input file example.

(3) MD calculation with added umbrella potential (cosgene)

To perform MD calculation with an umbrella potential added, specify "CALUMB=CALC" and "NAMEUM=newopt_fp" in the EXE> MD group of the cosgene control file.

(4) Generate the umbrella potential (Generate_nextFP tool)

Enter the initial center coordinates of the umbrella potential in "newopt_fp_ini". The umbrella potential coordinates while calculation is in progress and the dimensions at that time are entered in "newopt_fp". The "newopt_fp" file is also used as input for the next MD calculations.

■Umbrella potential file

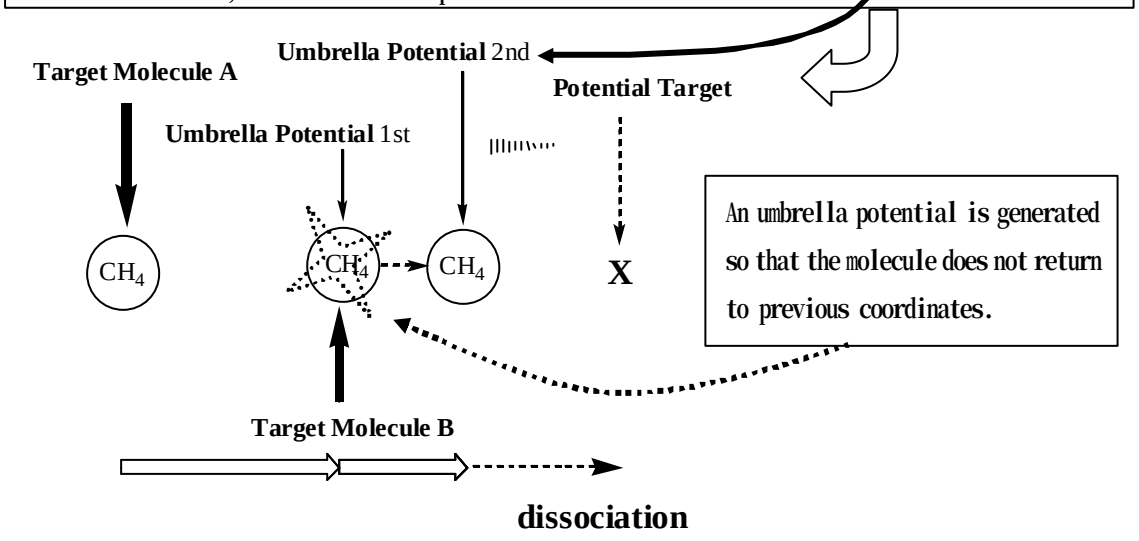
```
FILL.>GAUS        ;  Specification of unbrella potential shape (GAUS only)
1  1              ;  Number of nest and target atom for umbrella potential
6                 ;  Atom number of landmark atom
0.000             ;  Height of Gause potential
0.0300            ;  Width of Gauss potential
ATOM  6  CA  ACE  2  0.000  0.000  −2.000      ;  Coordinates of center of
potential
```

【Note】Do not enter anything after the coordinates of the center of the potential or a calculation error will result.

The umbrella potential file for the next MD calculation can be generated from the locus file of the immediately previous MD calculation results using the Generate_nextFP tool. This tool generates the next umbrella potential so that the molecule does not return to the coordinate points of the previous locus. It also sets the coordinates of the final umbrella potential at the same time.

■Input of Generate_nextFP tool (Generate_nextFP.inp)

```
newopt_fp       ; Umbrella potential file
newopt_fp2      ; Next umbrella potential file
initial.pdb     ; Initial coordinates
xx_traject.cor  ; Trajectory file name
-1000           ; Number of trajectory read skips
1000            ; Number of trajectory loadings
s               ; Coordinate trajectory file format ( "s"ingle or "d"ouble )
n               ; Screen display of PDB file
HAR1            ; Shape of centripetal coefficient
300.0           ; Applicable temperature
0.5             ; Height of Gauss repulsion factor
2.5  6.0        ; Parameter for adjusting movement distance by RMSD
3.000           ; Width of Gauss repulsion factor
5.0             ; Height of centripetal coefficient
1.0             ; Width of centripetal coefficient
ATOM   4131  O  WAT   839    0.000  0.000  -8.000  16.00  -0.83
                ; Target coordinates for center of umbrella potential (PDB format)
1 50            ; Number of sweep starts and ends
```



(5) Extraction of locus of landmark atom (Extract_Atom tool)

The Extract_Atom tool is used to extract the locus of the atom that will be the landmark from the umbrella potential description file and locus file output during MD calculation.

■Input of Extract_Atom tool (Extract_Atom.inp)

```
newopt_fp
1033        ; Number of all atoms (check initial structure file with "tail" or other
command)
1           ; Number of atoms extracted
xx_traject.cor   xx_cont.cor  ; Loaded trajectory name__Output trajectory name
0           ; Number of coordinate read skips
2000        ; Number of coordinate loadings
s           ; Format of coordinate trajectory file ( “s”ingle or “d”ouble )
```

【Note】If a large value is used for the number of coordinate loadings, an error will occur. If an error occurs, try changing this value. The value depends on the entries in the MD calculation settings file.

(6) Script for executing the entire calculation (ganzene.sh)

This script repeatedly runs the three Filling Potential programs (MD calculation, umbrella potential generation, and landmark atom extraction). Verify that the file names are consistent with other input.

■Script (ganzene.sh)

```
#!/bin/csh
# program directory
set cosgene=/user1/myPresto/cosgene/bin/cosgene
                ;  Change this section as appropriate for your environment.
cp ch42w.pdb initial.pdb                ;  Initial structure settings
      :
rm xx_traject.cor

@ cycle1 = 60                           ;  Number of calculation cycles

@ counter = 1                           ;  Initialization of counter

cp newopt_fp_ini newopt_fp              ;  Initial umbrella file settings
```

(Continued on next page)

(Continued from previous page)

```
# program directory
set cosgene=/user1/myPresto/cosgene/bin/cosgene
                              ; Please change this part for your environment

while ($counter < $cycle1)                ; Loop setting

$cosgene  <  md_w.inp  >  output          ; Execution command for MD calculation

while (! -e final.pdb)                    ; Set to not proceed to the next step
sleep 10                                  ; until the MD calculation loop ends.
end                                       ;
sleep 30                                  ;

cp final.pdb         w_$counter.pdb       ; Copy final structure
mv output            w_$counter.output    ; Copy MD calculation output

$GeneFPx  <  $GeneFPinp  >  GeneFP.out    ; Update umbrella potential

while (! -e newopt_fp2)
sleep 2
end

mv newopt_fp2 newopt_fp                   ; Copy output as initial coordinate file
                                          ; of umbrella potential.
$Extractx  <  $Extractinp  >  Extract.out ; Extract landmark atom

while (! -e xx_cont.cor)
sleep 3
end
sleep 2

mv xx_cont.cor    w_$counter.cor          ; Save extracted trajectory

mv final.pdb initial.pdb                  ; Copy final structure as initial
                                          ; structure of next calculation
```

(7) WHAM Analysis (WHAM_analysis tool)

This tool analyzes the locus files of the extracted landmark atom and calculates free energy using the WHAM method. Note that if there is insufficient locus file overlapping, the output values may not be produced. The radius for calculating the mean energy will also need to be adjusted as appropriate for the size of the system.

■Input of WHAM_analysis tool (WHAM_analysis.inp)

```
newopt_fp     ;  Umbrella potential file for the last MD calculation
0             ;  Number of file read skips
2000          ;  Number of samplings (depends on MD calculation and number of extracts)
s             ;  Precision of loaded file ( "s"ingle or "d"ouble )
1.0           ;  Radius for calculating mean energy
1000          ;  Number of WHAM analysis iterations
300.0         ;  Calculation temperature
m             ;  Select whether priority is given to memory or speed.
60            ;  Number of trajectory files
w_1.cor  w_1.option ;  Trajectory file name and Umbrella potential file name
w_2.cor  w_2.option ;  Enter all file names one-by-one.
 :
w_60.cor w_60.option
```

The free energy calculation calculates the data point within the radius for averaging the energy, which is the second value setting from the bottom of the input file. Adjust the value setting as appropriate for the desired range. The analysis results are output in the last section of the OUT file.

■Example output file

```
        INFORMATION> WHAM ANALYSIS RESULT
    EXP-ID    R.M.S.D(A)        AVERAGE              FREE-ENERGY
         1      0.000000       0.000009425        0.712812629E+01
         2      0.500101       0.000002134        0.804317891E+01
         3      0.410962       0.000006086        0.739754038E+01
         4      0.859058       0.000002663        0.790654353E+01
                  :                :                    :
                  :                :                    :
          (Structure shear value) (Existence probability distribution(P(ξ)) (Free
energy value)
```

The final free energy values that are output are plotted and the PMF is indicated. The free energy is calculated based on the following equation:

$$F(\xi) = -kT\ln P(\xi)$$

k and T are constants that depend on the calculation settings. The P(ξ) value changes each calculation cycle. If the existence probability is not sufficient, the free energy calculation will not be performed and an error will be displayed: ' ---------- ' . In this case, it will be necessary to redo the entire calculation so that sufficient overlapping is obtained, taking such measures as widening the energy calculation region.

【Note】 The WHAM analysis program uses a large amount of memory. Limitations such as the *StackSize* may be configured in some calculation environments. Set as large a *StackSize* as possible before executing calculation.

【Note】 In WHAM analysis of sample 9, please use Wham_AnalysisSample.f90 instead of Wham_Analysis.f90 to define the distance between the molecules as reaction coordinates.

5.10 Sample-10：RESPA method

MD simulation using multi-time step (RESPA) is explained in the following. First, set the integration method to multi-time step (INTEGR= MTS). Normally a time-step (= TIMEST) of about 0.5 fsec is used. This will be the short time-step of the most inner side. Multi-time step is a 3-step operation, integrating over the time step TIMEST in the most inner loop, the time step TIMEST ×FREQME in the second loop, and the time step TIMEST×FREQME×FREQLO in the most outer loop. The interaction is updated such that the item specified in CALC is updated during the most inner loop, the item specified in MEDI is updated during the second loop, and the item specified in LONG is updated during the most outer loop. Normally it is effective to integrate the bonding/angle parts in the short step (CALBON= CALC, CALANG= CALC), dihedral angle items in the middle step (CALTOR= MEDI, CALIMP= MEDI), and electrostatic / van der Waals interaction in the slow step (CALV14= LONG, CALE14= LONG, CALV15= LONG, CALE15= LONG).

【Note】 RESPA is a multistep method of solving the kinetic equation, and thus cannot be used for energy minimization. In addition, it cannot be used together with SHAKE or the rigid body model.

■Control file

```
EXE> INPUT
     TOPOLOGY=   FORM     NAMETO=  vas-dih.tpl
     COORDINA=   PDB      NAMECO=  vas_min.pdb
     OUTMONIT=   READ     NAMEMO=  vas.mntinp
     REFCOORD=   PDB      NAMERE=  vas_min.pdb
     QUIT
EXE> MD
     LOOPLI=     2000
     INTEGR=     MTS            ; Specify RESPA method
     TIMEST=     0.5D0          ; Time step of most inner side
     FREQME=     2              ; Most inner side × 2
     FREQLO=     2              ; Most inner side ×2×2

     SETTIM=     500.0D0   CPUTIM= 3600000.0D0
     UPDATE=     20
     OUTTRJ=     100
     OUTLOG=     200
     LOGFOR=     DETA      STOPCE= BOTH

     METHOD=     CANONICAL
     SETTEM=     300.0D0
     INITIA=     SET
     STARTT=     300.0D0
     RANDOM=     654321
```

(Continued on next page)

(Continued from previous page)

```
        NAMETR= vas_vac.mnt  MNTRTR=  ASCI
        BESTFI=      YES
        CUTMET=      RESA    CUTLEN=  99.0D0
        DIEFUN=      DIST    DIEVAL=  4.0D0

        CALBON=      CALC        ; Short
        CALANG=      CALC        ; Short
        CALTOR=      MEDI        ; Middle
        CALIMP=      MEDI        ; Middle
        CALV14=      LONG        ; Long
        CALE14=      LONG        ; Long
        CALV15=      LONG        ; Long
        CALE15=      LONG        ; Long

        CALHYD=      NOCALC
        CALV5N=      NOCALC
        CALE5N=      NOCALC
        CALH5N=      NOCALC
        QUIT

    EXE> OUTPUT
        COORDINATE=  PDB     NAMECO=   vas_md.pdb
        QUIT
    EXE> END
```

5.11 Sample-11 : RATTLE —Calculation of indometacin in water—

It is possible to restrain both the position coordinates and velocity in myPresto by simultaneously specifying the Velocity-Verlet method and the SHAKE method to execute the RATTLE method.

In this sample, MD calculation of an indometacin-in-water system is performed by means of the Velocity-Verlet method, which applies the RATTLE method to water molecules and indometacin.

(1) Preparation of molecules

Use another molecule construction program to create the structure of indometacin. In the sample, the three-dimensional structure of indometacine has been previously prepared in PDB format as indo.pdb. In addition, the results of calculation of indometacin by Gaussian98 have been prepared in indo_g98.out.

First, prepare indo.charge/indo.bond using Gauss2tplinp, and calculate the RESP charge using the RESP specification file resp.in. The RESP charge calculation tool can be obtained from the AMBER home page (http://amber.scripps.edu/). Calculate the RESP charge and then modify the value of the charge of indo.charge or substitute the RESP charge in the charge item of the mol2 file. When finished, create a topology file using tplgeneL. Name the indometacin coordinate file, topology file, and mol2 file thus created "indo.pdb", "indo.tpl", and "INDO.mol2".

Next, use the water addition tool setwater to add TIP3P as CAPwater at a radius of 12 Å centered on the center of mass of indometacin, and name the PDB file "indo_w.pdb". The topology file of TIP3P is tip3p.tpl, so use the attachment tool mergetpl to merge the topology file with indo.tpl and thereby create the topology file "indo_w.tpl" of the indometacin + TIP3P water system.

In addition, for in-water calculation, prepare the file indo_w.capbc which sets the boundary conditions, and enter the CAP restraint conditions in the control file.

(2) Specification of restraint target

As with the SHAKE method, use the SHAKE file to specify the target of restraint of the RATTLE method. Use the attachment tool SHAKEinp to create the SHAKE restraint file indo_w.shk from indo_w.pdb and indo_w.tpl.

(For detailed information on SHAKE files, see the SHAKE file section of "A. Input Files".)

■SHAKE file (indo_w.shk)

```
SHAKE> SHAKE
indo
      4      2      3      4      5 ->;  C   LGD  H   LGD  H   LGD  H   LGD
   1.1126   1.8434   1.1126   1.1135   1.8025   1.8038

      2      7      8 ->;  C   LGD  H   LGD
   1.1034

      2      9     10 ->;  C   LGD  H   LGD
   1.0998

      2     16     17 ->;  C   LGD  H   LGD
   1.1015

      2     18     19 ->;  C   LGD  H   LGD
   1.1023

      2     22     23 ->;  C   LGD  H   LGD
   1.1026

      2     24     25 ->;  C   LGD  H   LGD
   1.1020

      4     27     28     29     30 ->;  C   LGD  H   LGD  H   LGD  H   LGD
   1.1123   1.7800   1.1127   1.1123   1.8098   1.8049

      2     33     34 ->;  C   LGD  H   LGD
   1.0977

      3     35     36     37 ->;  C   LGD  H   LGD  H   LGD
   1.1123   1.7944   1.1137

SHAKE> SHAKE
WAT
      3      1      2      3       ->  ; O       H1       H2                WAT
   0.95720  1.51360  0.95720
```

(3) Control file

To use the RATTLE method, specify the Velocity-Verlet method and SHAKE method simultaneously. If RATTLE will be applied to all H atoms, set the time step (TIMEST) to 0.5 to 2.0 fsec.

■Control file (rattle.inp)

```
EXE> INPUT
     TOPOLOGY=   FORM      NAMETO=     indo_w.tpl
     COORDINA=   PDB       NAMECO=     indo_w.pdb
     SETBOU=     READ      NAMEBO=     indo_w.capbc
     SETSHAKE=   READ          ; SHAKE file load command
     NAMESH=     indo_w.shk    ; SHAKE file name specification
     QUIT
EXE> MD
     INTEGR=     VELO      ; Specify Velocity-Verlet method
     SHAKEM=     ALLB      ; Specify SHAKE method
     LOOPLI=     10000
     SETTIM=     5000.0D0
     CPUTIM=     3600000.0D0
     UPDATE=     20
     TIMEST=     2.0D0
     OUTCOO=     1000
     OUTTRJ=     1000
     OUTLOG=     1000
     LOGFOR=     DETA
     STOPCE=     TRAN
     METHOD=     CANONICAL
     THERMO=     NOSE
     SETTEM=     300.0D0
     INITIA=     SET
     STARTT=     300.0D0
     RANDOM=     654321

     CALCAP=      CALC
     RADCAP=      10.0
     FORCAP=      50.0
     FUNCAP=      HARMonic
     SETCEN=      YES

     CUTMET=     RESA      CUTLEN=     12.0D0
     DIEFUN=     CONS      DIEVAL=     1.0D0
     CALV15=     CALC      CALE15=     CALC
     CALHYD=     NOCALC    CALV5N=     NOCALC
     CALE5N=     NOCALC    CALH5N=     NOCALC
     QUIT
EXE> OUTPUT
     COORDINATE=  PDB      NAMECO=     indo_wmd.pdb
     QUIT
EXE> END
```

5.12 Sample-12：Rigid —Calculation of indometacin in water—

In this sample, MD calculation of an indometacin-in-water system is performed by applying the rigid body model to water molecules. Note that the rigid body model can only be applied to water molecules; currently the TIP3P model and TIP4P model can be calculated. Other molecules are calculated by calculating all atoms as usual. In the TIP4P model, the mass of the charge point is zero, and thus cannot be handled by models other than the rigid body model.

【Note】The rigid body model is only effective when the Velocity-Verlet method is used for calculation.

(1) File preparation

Follow the same steps as in Sample 11 to prepare the input file. Generation of TIP4P water can be performed using the water molecule addition tool setwater. Insert the water molecule topology file tip4p.tpl into the topology file using mergetpl. Parameters for the force field in the water molecule are entered in TIP4P, however, these are not used in the rigid body model.

(2) Specification of rigid body model

The rigid body model file is used to specify the restraint targets of the rigid body model. (For detailed information on the rigid body model file, see the rigid body model file section of "A. Input Files".)

■Rigid body model file (indo_w4.rig)

```
RIGID> NUMBER
indo
   4    2    3    4    5
   2    7    8
   2    9   10
   2   16   17
   2   18   19
   2   22   23
   2   24   25
   4   27   28   29   30
   2   33   34
   3   35   36   37

RIGID> COOR
WAT
 4 ->
 1     0.000    0.000    0.000 ->
 2     0.757    0.586    0.000 ->
 3    -0.757    0.586    0.000 ->
 4     0.000    0.150    0.000
```

(3) Control file

To use the rigid body model, use of the rigid body model and the rigid body model file name must be specified in the MD phase of the control file. If H atoms that have not been made rigid remain in the system, set the time step (TIMEST) to about 0.5 fsec.

■Control file (rigid.inp)

```
EXE> INPUT
     TOPOLOGY=   FORM      NAMETO=    indo_w4.tpl
     COORDINA=   PDB       NAMECO=    indo_w4.pdb
     SETBOU=     READ      NAMEBO=    indo_w4.capbc
     QUIT
EXE> MD
     RIGIDM=     YES                  ; Specify use of rigid body model
     NAMERM=     indo_w4.rig          ; Specify rigid body model file name
     INTEGR=     VELO                 ; Specify Velocity-Verlet method
     LOOPLI=     10000
     SETTIM=     5000.0D0
     CPUTIM=     3600000.0D0
     UPDATE=     20
     TIMEST=     2.0D0
     OUTCOO=     1000
     OUTTRJ=     1000
     OUTLOG=     1000
     LOGFOR=     DETA
     METHOD=     CANONICAL
     SETTEM=     300.0D0
     INITIA=     SET
     STARTT=     300.0D0
     RANDOM=     654321

     CALCAP=      CALC
     RADCAP=      10.0
     FORCAP=      50.0
     FUNCAP=      HARMonic
     SETCEN=      YES

     CUTMET=     RESA      CUTLEN=     12.0D0
     DIEFUN=     CONS      DIEVAL=     1.0D0
     CALV15=     CALC      CALE15=     CALC
     CALHYD=     NOCALC    CALV5N=     NOCALC
     CALE5N=     NOCALC    CALH5N=     NOCALC
     QUIT
EXE> OUTPUT
     COORDINATE=  PDB      NAMECO=     indo_w4md.pdb
     QUIT
EXE> END
```

5.13 Sample-13 : Calculation of periodic systems using NPT and PME —Calculation of methane in water—

【Andersen method】

In this sample the NPT ensemble and Andersen methods are used to calculate a periodic boundary system consisting of two methane molecules in a unit cell filled with water.

The system is created by generating water molecules around the two methane molecules using the set_water tool. Each side of the three-dimensional unit cell is 18.64775 Å.

As a basic principle, SHAKE cannot be used for NPT calculation. Here calculation is performed with All Atom. Specify "METHOD=NPT", and specify the Velocity-Verlet method ("INTEGR=VELO") for the integrator. The Andersen method is used, and thus "BAROST=ANDE" is specified. In the Andersen method, only isotropic cells are transformed. Specify "MODIFI=ISOT" so that the option for specifying the cell shape is always isotropic cell transformation with one degree of freedom. As calculation will be performed using the boundary condition of a periodic system, specify "BOUNDA=PERI" for the boundary condition setting.

The target pressure "SETPRE" is 1 atmosphere in this sample.

The relaxation time setting depends on the system, however, it is generally best to set the relaxation time of temperature control "COUPHB" to about 100 to 500 fsec, and the relaxation time of pressure control "COUPPI" to several times the relaxation time of temperature control, or about 1000 to 5000 fsec.

1-5 electrostatic interaction is calculated using the Particle-Mesh-Ewald (PME) method. For the PME settings, "CALPME=CALC" is specified, and "CUTLEN" is set so as to openly calculate the electric field, requiring a value of about 6 to 8 Å, or less than half the length of a side of the unit cell. The number of mesh nodes are set in "MESHLX", "MESHLY","MESHLZ" for the X, Y, and Z axes. A value of 1 to 3 Å is reasonable for the mesh width, as a short width increases precision but also lengthens the calculation time. In the sample, "MESHLX=18" is specified (same for "MESHLY" and "MESHLZ") so as to partition the side length of 18.64775 Å into mesh nodes of about 1 Å. The Ewald parameter "EWAPRM" may affect precision depending on the system, and a value of 0.35 is generally set. Specifying a low value for the order "PMEORD" of the polynomial for interpolation from the grid potential will make calculation quicker but decrease precision, while specifying a high value will increase precision but decrease speed. This value is set to about 3 to 6. PME is a no-cutoff method, however, this does not mean that calculation is performed without creating an atom interaction table; an atom interaction table is created to calculate nearby electrostatic interaction and thus "CALE15=CALC", "CALE5N=NOCALC",

"CALV15=CALC", "CALV5N=NOCALC", and "CALH5N=NOCALC" are specified.

■Control file

```
    EXE> INPUT
         TOPOLOGY=   FORM     NAMETO=  initial.tpl
         COORDINA=   PDB      NAMECO=  initial.pdb
         REFCOORD=   PDB      NAMERE=  initial.pdb
         QUIT
    EXE> MD
         LOOPLI=     2000000    ;1000 psec
         SETTIM=     5000.0D0      CPUTIM=    3600000.0D0
         UPDATE=     10
         TIMEST=     0.5D0
         OUTLOG=     1000          LOGFOR=    DETA
         STOPCE=     NO

         METHOD=     NPT           ; NPT
         INTEGR=     VELO          ; Velocity-Verlet
         BAROST=     ANDErsen      ; Andersen
         MODIFI=     ISOT
         SETPRE=     1.0
         COUPHB=     100.0
         COUPPI=     2000.0

         SETTEM=     300.0D0
         INITIA=     SET         RANDOM=     654321
         STARTT=     300.0D0
         CUTMET=     RESC        CUTLEN=     8.0D0
         DIEFUN=     CONS        DIEVAL=     1.0D0

         BOUNDA=     PERI
         LXCELL=     18.64775    LYCELL=     18.64775    LZCELL=     18.64775
         SETCEN=     NO
         CENTRX=     0.0         CENTRY=     0.0         CENTRZ=     0.0
; for PME
         CALPME=     CALC        PMESPD=     HIGH
         EWAPRM=     0.35
         MESHLX=     18          MESHLY=     18          MESHLZ=     18
         PMEORD=     6

         CALV15=     CALC        CALV5N=     NOCALC
         CALE15=     CALC        CALE5N=     NOCALC
         CALHYD=     NOCALC      CALH5N=     NOCALC
         QUIT

    EXE> OUTPUT
         COORDINATE=  PDB      NAMECO= final.pdb
         QUIT
    EXE> END
```

【Parrinello-Rahmann method】

In this sample the NPT ensemble and Parrinello-Rahmann methods are used to calculate a periodic boundary system consisting of two methane molecules in a unit cell filled with water.

The system is created by generating water molecules around the two methane molecules using the set_water tool. Each side of the three-dimensional unit cell is 18.64775 Å.

As a basic principle, SHAKE cannot be used for NPT calculation. Here calculation is performed with All Atom. Specify "METHOD=NPT", and specify the Velocity-Verlet method ("INTEGR=VELO") for the integrator. The Parrinello-Rahmann method is used, and thus "BAROST=PARA" is specified. In the Parrinello-Rahmann method, transformation of anisotropic cells is allowed. The five selections below are available for the option "MODIFI", which specifies the cell shape.

```
MODIFI = FLEX              ;6 degrees of freedom, rhombic cell (default)
       = MONOckinic        ;4 degrees of freedom, extension/contraction in 3 directions,
                             angle γ of base cell (angle between a and b axes) changes
       = ORTHorhombic      ;3 degrees of freedom, extension/contraction in 3 directions
       = ISOTropic         ;1 degree of freedom, isotropic change. Resembles Andersen method.
       = SINGle_direction  ;1 degree of freedom, change only in direction of z axis
```

In the Parrinello-Rahmann method, the boundary condition setting "BOUNDA" is always set to "BOUNDA=HEXA", regardless of the "MODIFI" specification.

【Note】Set the periodic boundary condition to "BOUNDA=HEXA", not "BOUNDA=PERI".

The target pressure "SETPRE" is 1 atmosphere in this sample.

The relaxation time setting depends on the system, however like the Andersen method, it is generally best to set the relaxation time of temperature control "COUPHB" to about 100 to 500 fsec, and the relaxation time of pressure control "COUPPI" to several times the relaxation time of temperature control, or about 1000 to 5000 fsec.

PME is specified in the same way as for the Andersen method in the previous section. The cell shape changes during NPT, and thus in particular when "MODIFI=FLEX" or "MODIFI=MONO", the interaction cutoff length "CUTLEN" may become longer than half the cell side length as simulation progresses. Considering the possibility that the cell shape may distort to a hexagonal close-packed lattice, the cutoff length should be set to a length sufficiently shorter than half the initial cell side length, or a sufficiently large cell should be prepared.

■Control file

```
    EXE> INPUT
         TOPOLOGY=   FORM      NAMETO=  initial.tpl
         COORDINA=   PDB       NAMECO=  initial.pdb
         REFCOORD=   PDB       NAMERE=  initial.pdb
         QUIT
    EXE> MD
         LOOPLI=     2000000     ;1000 psec
         SETTIM=     5000.0D0       CPUTIM=     3600000.0D0
         UPDATE=     10
         TIMEST=     0.5D0
         OUTLOG=     1000           LOGFOR=     DETA
         STOPCE=     NO

         METHOD=     NPT            ; NPT
         INTEGR=     VELO           ; Velocity-Verlet
         BAROST=     PARA           ; Parrinello-Rahmann
         MODIFI=     ISOT
         SETPRE=     1.0
         COUPHB=     100.0
         COUPPI=     2000.0

         SETTEM=     300.0D0
         INITIA=     SET          RANDOM=     654321
         STARTT=     300.0D0
         CUTMET=     RESC         CUTLEN=     8.0D0
         DIEFUN=     CONS         DIEVAL=     1.0D0

         BOUNDA=     HEXA           ; Parrinello-Rahmann
         LXCELL=     18.64775     LYCELL=     18.64775     LZCELL=     18.64775
         SETCEN=     NO
         CENTRX=     0.0          CENTRY=     0.0          CENTRZ=     0.0
; for PME
         CALPME=     CALC         PMESPD=     HIGH
         EWAPRM=     0.35
         MESHLX=     18           MESHLY=     18           MESHLZ=     18
         PMEORD=     6

         CALV15=     CALC         CALV5N=     NOCALC
         CALE15=     CALC         CALE5N=     NOCALC
         CALHYD=     NOCALC       CALH5N=     NOCALC
         QUIT

    EXE> OUTPUT
         COORDINATE=  PDB      NAMECO= final.pdb
         QUIT
    EXE> END
```

5.14 Sample-14 : Fast Multipole Method —MD calculation using counter ions—

The Fast Multipole Method (FMM) and MD calculation using counter ions are explained below using zinc finger protein (PDB code: 1A1H) as an example.

(1) Creating a topology file of the protein and DNA complex

First, separate the protein section and the DNA section of the 1A1H file into separate files and generate separate topology files for each. This is necessary because different databases are used to generate the topology files of the peptide chain and nucleic acid in tplgene and simultaneous processing is not possible. In the example, the hydrogen-added protein part is "zif1.pdb" and the topology file is "zif1.tpl". The hydrogen-added DNA part is "zif2.pdb" and the topology file is "zif2.tpl".

(2) Counter ion addition

Counter ion addition is performed by adding solvent water molecules to the system and then replacing suitable water molecules with Na+ and Cl− ions. For this reason, solvent water molecules must first be generated as explained in sample-3.

First, merge the two PDB files "zif1.pdb" and "zif2.pdb" and create the PDB file "zifcmp.pdb_vac" as a complex that includes no solvents. Use the setwater tool to add solvent water as explained in sample-3. Add the solvent water at a radius of 40Å as in the input file "setwater.inp". Prepare the coordinates and topology file including solvent water as "zifcmp.pdb" and "zifcmp.tpl".

At this point, a calculation of the charge of the entire system of the prepared data results in -8, and thus a positive charge of 8 must be added to make the system neutral. There are 8186 solvent water molecules, and thus we replace about 1% with NaCl, adding 80 Na+ ions and 72 Cl- ions as counter ions. Addition of the counter ions is performed with the "add_ion.f" tool. "add_ion" calculates the electric field created by the solute (molecules other than solvent water molecules) at the coordinates of each solvent water molecule using the distance-dependant dielectric ($\varepsilon \propto r$), and replaces the watermolecules at the highest and lowest potentials with counter ions. This process of calculation and replacement is repeated until the specified number of counter ions have all been placed. Each counter ion is placed a fixed distance (or more) away from the previously placed counter ions.

Compiling method :

```
% f90 add_ion.f -o add_ion
```

Method of use :

Type "add_ion". Input from standard input. The input example is "ion.input".

```
% add_ion < ion.input
```

Input example :

```
zifcmp.pdb
protein.pdb
ion.pdb
wat.pdb
80
72
6.0
```

1st line : Input file name : Name of coordinate file for entire system to which solvent water molecules are added.

2nd line : Output file name : Name of coordinate file for entire system.

3rd line : Output file name : Name of coordinate file for counter ions.

4th line : Output file name : Name of coordinate file for solvent water molecules replaced by counter ions.

5th line : How to calculate the number of counter ions.

1=the number of counter ions indicated directly.

2=the minimum number of counter ions is automatically calculated to neutralize the system.

3= the density of the counter ions is indicated. The number of counter ions is automatically calculated to neutralize the system.

In case of 1 in the 5th line,

6th line : Number of Na+ ions

7th line : Number of Cl- ions

8th line : When counter ions are successively added, new counters are not added within a fixed distance (radius) of previously added counter ions. This is that fixed radius (Å).

In case of 2 in the 5th line,

6th line : When counter ions are successively added, new counters are not added within a fixed distance (radius) of previously added counter ions. This is that fixed radius (Å).

In case of 3 in the 5th line,

6th line : density of the counter ion (mol of ions/mol of water)

7th line : When counter ions are successively added, new counters are not added within a fixed distance (radius) of previously added counter ions. This is that fixed radius (Å).

Add the generated counter ion coordinates to the overall system.

```
% cp protein.pdb zifcmx.pdb
% cat ion.pdb | grep NA+ >> zifcmx.pdb
% cat ion.pdb | grep CL- >> zifcmx.pdb
```

Add the counter ions to the topology file. When doing so, the order of the protein, DNA, solvent water, and counter ions must be the same as that in the MOLECULES column of the topology file and in PDB.

The arrangement of counter ions added with add_ion is not stable in terms of energy. For this reason, before proceeding to MD calculation of the entire system, perform MD calculation only on the solvent parts (solvent water and counter ion) with the protein andDNA coordinates fixed, so as to bring the solvent parts sufficiently closer to an equilibrium state.

<min_wat.inp>

```
      EXE> INPUT
           TOPOLOGY=   FORM      NAMETO=    zifcmx.tpl
           COORDINA=   PDB       NAMECO=    zifcmx.pdb
           REFCOORD=   PDB       NAMERE=    zifcmx.pdb
           POSITION=   READ      NAMEPO=     M_all.res

           SETBOU=     READ      NAMEBO=    zifcmx.capbc
           QUIT
      EXE> MINI
           METHOD=  STEEP        CPUTIM=     360000.0
           LOOPLI=  5000         UPDATE=     10
           MONITO=   100         CONVGR=     0.2D0
           CUTMET=  RESA         CUTLEN=     8.0D0
           DIEFUN=  CONS         DIEVAL=     1.0D0
           BESTFI=  YES

   ;  in case of CAP boundary
           CALCAP=     CALC      FUNCAP=  HARMonic
           RADCAP=     40.0      FORCAP=  100.0
           SETCEN=     YES

           CALPSR=     CALC
           WETPSR=    10.00
           QUIT
      EXE> OUTPUT
           COORDINATE=  PDB      NAMECO=  zifcmx_min.pdb
           QUIT
      EXE> END
```

The coordinates of the protein and DNA are fixed with the "M_all.res" command.

<M_all.res>

```
   GROUP> LIST
        1    1    1   85    *    *     1.0    MASS   YES
        2    3    1   11    *    *     1.0    MASS   YES
   END
   GROUP> STOP
```

Perform MD calculation only on the solvent parts (solvent water and counter ions) to bring the solvent parts sufficiently closer to equilibrium.

<md_wat.inp>

```
;
; md input for lysozyme
;
    EXE> INPUT
         TOPOLOGY=   FORM     NAMETO=  zifcmx.tpl
         COORDINA=   PDB      NAMECO=  zifcmx_min.pdb
         REFCOORD=   PDB      NAMERE=  zifcmx.pdb
         SETBOU=     READ     NAMEBO=  zifcmx.capbc
         POSITION=   READ     NAMEPO=  M_all.res
         QUIT
    EXE> MD
         LOOPLI=     100000
         SETTIM=     500.0D0   CPUTIM= 3600000.0D0
         UPDATE=     40
         TIMEST=     0.5D0
         OUTTRJ=     1000
         OUTLOG=     1000
         LOGFOR=     DETA      STOPCE= BOTH

         METHOD=     CANONICAL
         SETTEM=     310.0D0
         INITIA=     SET
         STARTT=     310.0D0
         RANDOM=     654321

;  in case of CAP boundary
         CALCAP=     CALC     FUNCAP=  HARMonic
         RADCAP=      40.0    FORCAP=  100.0
         SETCEN=     YES
         BESTFI=     YES
; position restraint
         CALPSR=     CALC
         WETPSR=    10.00
; FMM
         USEFMM=     YES      FMMSPD=  HIGH
         FMTREE=     4
         FMPOLE=     6

         CUTMET=     RESA     CUTLEN=   8.0D0
         DIEFUN=     CONS     DIEVAL=  1.0D0
         CALV15=     CALC
         CALE15=     CALC
         CALHYD=     NOCALC   CALV5N=     NOCALC
         CALE5N=     NOCALC   CALH5N=     NOCALC
         QUIT
    EXE> OUTPUT
         COORDINATE=  PDB     NAMECO=   zifcmx_md.pdb
         QUIT
    EXE> END
```

When the solvent parts are sufficiently close to equilibrium, perform MD calculation on the entire system, including the protein and DNA. If FMM overflows, set a larger value for FMTREE.

<md_wat2.inp>

```
;
; md input for lysozyme
;
    EXE> INPUT
         TOPOLOGY=   FORM     NAMETO=  zifcmx.tpl
         COORDINA=   PDB      NAMECO=  zifcmx_md.pdb
         REFCOORD=   PDB      NAMERE=  zifcmx.pdb
         SETBOU=     READ     NAMEBO=  zifcmx.capbc
         QUIT
    EXE> MD
         LOOPLI=     100000
         SETTIM=     500.0D0    CPUTIM= 3600000.0D0
         UPDATE=     20
         TIMEST=     0.5D0
         OUTTRJ=     1000
         OUTLOG=     1000
         LOGFOR=     DETA       STOPCE= BOTH

         METHOD=     CANONICAL
         SETTEM=     310.0D0
         INITIA=     SET
         STARTT=     310.0D0
         RANDOM=     654321

;  in case of CAP boundary
         CALCAP=     CALC     FUNCAP=  HARMonic
         RADCAP=      40.0    FORCAP=  100.0
         SETCEN=     YES
         BESTFI=     YES
; FMM
         USEFMM=     YES      FMMSPD=  HIGH
         FMTREE=     4
         FMPOLE=     8

         CUTMET=     RESA     CUTLEN=   8.0D0
         DIEFUN=     CONS     DIEVAL=  1.0D0
         CALV15=     CALC
         CALE15=     CALC
         CALHYD=     NOCALC   CALV5N=     NOCALC
         CALE5N=     NOCALC   CALH5N=     NOCALC
         QUIT
    EXE> OUTPUT
         COORDINATE=  PDB     NAMECO=   zifcmx_res.pdb
         QUIT
    EXE> END
```

5.15 Sample-15：GB/SA —Calculation of Vassopressin—

(1) Preparation of parameter file for GB/SA calculation

Calculation using the Generalized Born/Surface Area (GB/SA) model and the Accessible Surface Area (ASA) model is possible in cosgene. Prepare the protein coordinates and topology file in the usual way. In this example using Vassopressin, dihedral angle input "vas.dih" has been prepared for the initial input. First use tplgene to prepare the initial coordinates vas-dih.pdb and the topology file vas-dih.tpl.

The GB/SA model requires that a GB/SA parameter file be prepared for the molecule to be calculated. For the protein, the parameter file creation tool mkGBSAin.pl and the GB/SA parameter database gb_sa.db are used.

The ASA model also requires the creation of a GB/SA parameter file for the molecule to be calculated. The parameter file is the same as that for GB/SA.

■Execution method

① Prepare an initial coordinate file (vas-dih.pdb), topology file (vas-dih.tpl), and gd_sa.db in the directory where mkGBSAin.pl will be executed.

② Execute mkGBSAin.pl. Type "mkGBSAin.pl". Follow the instructions in the program to specify the topology file name and the parameter file name.

■Execution example

```
% mkGBSAin.pl
%% INPUT DB FILE NAME. %%
gb_sa.db
%% SELECT INPUT FILE BY THE NEXT NUMBER.  %%
   1 : PDB FILE
   2 : TPL FILE
2
%% INPUT FILE NAME.  %%
vas-dih.tpl
%% INPUT OUTPUT FILE NAME.  %%
vas-dih.sol
```

(2) Energy minimization

Energy minimization is possible in the GB/SA model. Specify vas-dih.sol in the GB/SA parameter file that you created.

Calculation using the GB/SA model and ASA model is possible in cosgene. Calculation will be performed as follows based on the specification method:

- When CAL-GB= CALC, CALASA= CALC : GB/SA model
- When CAL-GB= NOCALC, CALASA= CALC : ASA model

■Control file (min_vac.inp)

```
EXE> INPUT
     TOPOLOGY=   FORM     NAMETO=    vas-dih.tpl
     COORDINA=   PDB      NAMECO=    vas-dih.pdb
     REFCOORD=   PDB      NAMERE=    vas-dih.pdb
     ASAREA=     READ     NAMESA=    vas-dih.sol   ;GB/SA parameter file
     QUIT
EXE> MINI
     METHOD=  CONJ        CPUTIM=     360000.0

     LOOPLI=  1000        UPDATE=     20
     MONITO=    5         CONVGR=     0.1D0

     CAL-GB=     CALC   ;GB specification
     CALASA=     CALC   ;SA specification

     CUTMET=  RESA        CUTLEN=    30.0D0
     DIEFUN=  CONS        DIEVAL=     1.0D0
     QUIT
EXE> OUTPUT
     COORDINATE=  PDB     NAMECO=   vas_min.pdb
     QUIT
EXE> END
```

(3) MD simulation

The GB/SA and ASA models can also be used in MD simulation. Options are the same as for energy minimization. In the GB/SA model, a long energy cutoff length (CUTLEN) must be specified or energy will not be stored well. Set the energy cutoff length to at least 20 Å, or if possible to more than 30 Å.

■Control file (md_vac.inp)

```
EXE> INPUT
     TOPOLOGY=   FORM     NAMETO=  vas-dih.tpl
     COORDINA=   PDB      NAMECO=  vas_min.pdb
     OUTMONIT=   READ     NAMEMO=  vas.mntinp
     REFCOORD=   PDB      NAMERE=  vas_min.pdb
     ASAREA=     READ     NAMESA=  vas-dih.sol   ;GB/SA parameter file
     QUIT
EXE> MD
     LOOPLI=     2000
     SETTIM=     500.0D0   CPUTIM= 3600000.0D0
     UPDATE=     20
     TIMEST=     0.5D0
     OUTTRJ=     100
     OUTLOG=     200
     LOGFOR=     DETA      STOPCE= BOTH

     METHOD=     CANO      INTEGR= VELO
     SETTEM=     300.0D0
     INITIA=     SET
     STARTT=     300.0D0   RANDOM=  654321
     NAMETR=  vas_vac.mnt  MNTRTR=  ASCI
     BESTFI=     YES

     CAL-GB=     CALC   ;GB specification
     CALASA=     CALC   ;SA specification
     GBDELT=      0.0   ;Born radius correction value
     GBOFFS=     0.09   ;vdW radius correction value

     CUTMET=     RESA     CUTLEN=  99.0D0
     DIEFUN=     CONS     DIEVAL=  1.0D0

     CALV15=     CALC          CALV5N=     NOCALC
     CALE15=     CALC          CALE5N=     NOCALC
     CALHYD=     NOCALC        CALH5N=     NOCALC
     QUIT
EXE> OUTPUT
     COORDINATE=  PDB     NAMECO=   vas_md.pdb
     QUIT
EXE> END
```

A Input/Output files

A.1 Input/Output files of cosgene

cosgene performs file input/output for the following purposes:

(1) Designating simulation conditions

(2) Saving the simulation state

(3) Outputting simulation results

These files are referred to generically as input/output files.

A.1.1 Explanation of phase

cosgene performs file input/output in the following phases.

(1) INPUT phase

Input of files for system topology, coordinates and simulation conditions etc.

(2) OUTPUT phase

File output of topology and coordinates of the system after simulation.

(3) MINimize phase

Minimization of potential energy of system.

(4) MD phase

Molecule dynamics simulation of system.

A.2 Input Files

Input files for the structure search engine are shown below.

Item no.	File name	Applicable phase	Use
#1	Control file	-	Control of structure search engine
#2	Topology file	All phases	Specifies topology of system to be simulated
#3	Coordinate file	All phases	Specifies coordinates of atoms in system to be simulated
#4	SHAKE file	MIN, MD	Specifies SHAKE atoms and restraint distance
#5	Fixed atom / free atom specification file	MIN, MD	Specifies free/fixed atoms.
#6	CAP specification file	All phases	Specifies cell shape and CAP restraint
#7	Ex CAP specification file	All phases	Specifies cell shape and Ex CAP restraint
#8	Position restraint file	All phases	Specifies position restraint
#9	Distance restraint file	All phases	Specifies distance restraint
#10	Dihedral angle restraint file	All phases	Specifies dihedral angle restraint
#11	Monitor specification file	MD	Specifies items to be monitored
#12	System center of mass alignment specification file	MIN, MD	Specifies center of mass alignment atoms
#13	GB/SA and ASA parameter specification file	MIN, MD	Specifies GB/SA and ASA parameters
#14	Restart file	MD	Specifies restart information
#15	Rigid body model file	MD	Specifies rigid body model atoms

A.2.1 Control file

Applicable phases: All phases

Applications: This designates execution phase control for cosgene, and parameters designated for each phase.

Note:
The character indicating the exponent of a real number must be "D".

```
Example of real number exponent designation)
CPUTIM = 60.0D0
```

Syntax:

```
[Line designating execution phase [Line designating parameters in phase]… Parameter end line]…
```

Line designating execution phase: The phase to be executed is designated with the following character strings.

INPUT phase=	“EXE> INPUT”
OUTPUT phase=	“EXE> OUTPUT”
MINimize phase=	“EXE> MIN”
MD phase=	“EXE> MD”
Execution end phase=	“EXE> END”

Parameter end line: The end of the parameter designation line in each phase is designated in the following format:

“QUIT”

Parameter designation in each phase: The parameters in each phase are designated using the following format.

```
Keyword “=” Value
```

※Keywords are comprised of 6 alphanumeric characters, and there are four types of values, depending on the keyword: enumerated, real, integer and character.

```
Keyword designation examples)
UNITAN = 30              ;   Integer parameter
NAMEAN = aa.ana          ;   Character parameter
BINCLO = NO              ;   Enumerated parameter
CPUTIM = 60.0D0          ;   Real parameter
```

The following indicates keywords and values for each phase. Only the part indicated with uppercase letters is used.
The part in parentheses in the "Description" column indicates the following, depending on the type in the "Value" column

- Enumerated type : Uppercase letters indicate the designated part, and the underlined part is the default value.
- Integer type, Real type, Character type : Default value

A.2.1.1 INPUT phase

Number	Item	Keyword	Value	Description
#1	Input topology	TOPOLOgy	Enumerated	File loading and format (NOREad | FORMatted | BINAry)
#2		UNITTOpology	Integer	Unit no. (10)
#3		NAMETOpology	Character	File name ("")
#4	Input atom coordinates	COORDInate	Enumerated	File loading and format (NOREad | PDB | BINAry)
#5		UNITCOordinate	Integer	Unit no. (11)
#6		NAMECOordinate	Character	File name ("")
#7	Input SHAKE information	SETSHAke	Enumerated	File loading (NOREad | READ)
#8		UNITSHake	Integer	Unit no. (12)
#9		NAMESHake	Character	File name ("")
#10	Output SHAKE automatic preparation information	DBGSHA	Selection	File write (NOWRite | ASCIi)

#11		UNITDS	Integer	Device number (84)
#12		NAMERS	Character	filename("")
#13	Input fixed atom information	SETVARiables	Enumerated	File loading (NOREad | READ)
#14		UNITVAribles	Integer	Unit no. (13)
#15		NAMEVAriables	Character	File name ("")
#16	Input boundary conditions	SETBOUndary	Enumerated	File loading (NOREad | READ)
#17		UNITBOundary	Integer	Unit no. (14)
#18		NAMEBOundary	Character	File name ("")
#19	inputExtendCAP information	SETEXC	Selection	File read (NOREad | READ)
#20		UNITEC	Integer	Device number (23)
#21		NAMEEC	Character	filename("")
#22	Input position restraint coordinates	REFCOOrdinate	Selection	File read and format (NOREad | PDB)
#23		UNITREfcoordinate	Integer	Device number (15)
#24		NAMEREFcoordinate	Text	File name
#25	Input position restraint information	POSITIonrestrain	Selection	File read (NOREad | READ)
#26		UNITPOsition	Integer	Device number (16)
#27		NAMEPOsition	Text	File name ("")
#28	Input distance restraint information	DISTANcerestrain	Selection	File read (NOREad | READ)
#29		UNITDIstance	Integer	Device number (17)
#30		NAMEDIstance	Text	File name ("")
#31	Input dihedral angle restraint information	DIHEDRalrestrain	Selection	File read (NOREad | READ)
#32		UNITDH	Integer	Device number (18)
#33		NAMEDH	Text	File name ("")
#34	Input monitor item structure information	OUTMONitoritems	Selection	File read (NOREad | READ)
#35		UNITMOnitoritems	Integer	Device number (19)
#36		NAMEMOnitoritems	Text	File name ("")
#37	Input GB/SA and ASA parameter information	ASAREA	Selection	File read (NOREad | READ)
#38		UNITSA	Integer	Device number (77)

#39		**NAMESA**	Text	File name ("")
#40	Input UMBRELLA restraint information	**UMBREL**	Selection	File read (**NOREad** | **READ**)
#41		**UNITUI**	Integer	Device number (22)
#42		**NAMEUI**	Text	File name ("")
#43	Centering of system center of mass	**SETORIgin**	Selection	Centering specification (**NO** | **YES**)

A.2.1.2 OUTPUT phase

Number	Item	Keyword	Value	Description
#1	Output topology	**TOPOLOgy**	Enumerated	**File loading and format** (**NOWRite** | **FORMatted** | **BINAry**)
#2		**UNITTOpology**	Integer	Unit no. (90)
#3		**NAMETOpology**	Character	File name ("")
#4	Output atom coordinates	**COORDInate**	Enumerated	**File loading and format** (**NOWRite** | **PDB** | **BINAry**)
#5		**UNITCOordiante**	Integer	Unit no. (91)
#6		**NAMECOordinate**	Character	File name ("")

A.2.1.3 MINimize phase

Number	Item	Keyword	Value	Description
#1	MIN energy data	**UNITAN**	Integer	Unit no.(30)
#2		**NAMEAN**	Character	File name("")
#3	Job control	**CPUTIM**elimit	Real	CPU time upper limit (60.0)
#4		**MONITO**rinterval	Integer	Log output interval (10)
#5		**LOGFOR**mat	Enumerated	Log output format (**SHOR**t | **DETA**il)
#6		**BESTFI**tmini	Enumerated	Display of (least square fitting) value (**NO** | **YES**)
#7	Energy minimization	**METHOD**ofmini	Enumerated	Calculation method (**STEE**pest | **CONJ**ugate)
#8		**LOOPLI**mit	Integer	Number of MINIMIZE loop iterations (0)
#9		**CONVGR**adient	Real	Convergence RMSF value (0.1)
#10		**ISTEPL**ength	Real	Initial step length (0.01)
#11		**UPRATE**	Real	Step length upward multiplier for STEEP method (1.2)
#12		**DOWNRA**te	Real	Step length downward multiplier for STEEP method (0.6)
#13		**LINESE**archlimit	Integer	Number of loop iterations for CONJUGATE method (10)
#14		**CONVLI**nesearch	Real	CONJUGATE method convergence conditions (0.1)
#15	Interaction calculation	**CALBON**d	Enumerated	Bond force calculation (**CALC** | **NOCA**lc)
#16		**CALANG**le	Enumerated	Angle force calculation (**CALC** | **NOCA**lc)
#17		**CALTOR**sion	Enumerated	Torsion force calculation (**CALC** | **NOCA**lc)
#18		**CALIMP**roper	Enumerated	Improper force calculation (**CALC** | **NOCA**lc)
#19		**CALV14**	Enumerated	1-4 van der Waals force calculation (**CALC** | **NOCA**lc)
#20		**CALE14**	Enumerated	1-4 electrostatic force calculation (**CALC** | **NOCA**lc)
#21		**CUTMET**hod	Enumerated	Cutoff method (**RESC** | **ATOM** | **RESA**)

#22		**CUTLEN**gth	Real	Cutoff length (8.0)
#23		**USESPL**	Selection	Application of spline interpolation (**NO** | **YES**)
#24		**CUT-ON**	Real	Spline start interval (6.0)
#25		**UPDATE**interval	Integer	Interaction table update interval (20)
#26		**CALV15**	Enumerated	1-5 vdw force calculation with cutoff (**CALC** | **NOCA**lc)
#27		**CALE15**	Enumerated	1-5 electrostatic force calculation with cutoff (**CALC** | **NOCA**lc)
#28		**CALHYD**	Enumerated	1-5 hydrogen force calculation with cutoff (**CALC** | **NOCA**lc)
#29		**CALV5N**	Enumerated	1-5 vdw force calculation with no cutoff (**CALC** | **NOCA**lc)
#30		**CALE5N**	Enumerated	1-5 electrostatic force calculation with no cutoff (**CALC** | **NOCA**lc)
#31		**CALH5N**	Enumerated	1-5 hydrogen force calculation with no cutoff (**CALC** | **NOCA**lc)
#32		**DIEFUN**ction	Enumerated	Distance dependence of electrostatics (**CONS** | **DIST**)
#33		**DIEVAL**ue	Real	Electrostatic coefficient (1.0)
#34	PME method, Ewald method	**CALPME**	Enumerated	Execute PME method (**NO** | **YES**)
#35		**CALEWA**ld	Enumerated	Execute Ewald method (**NO** | **YES**)
#36		**PMESPD**	Selection	Adjustment of calculation interval in PME method (**NORM** | **HIGH**)
#37		**PMEUPD**	Selection	Calculation method for PMESPD= HIGH (**CUT** | **RECI**)
#38		**REATOL**	Real	Permissible tolerance in Ewald method (1.0d-19)

#39		**EWAPAR**	Real	Inverse space calculation coefficient in PME method and Ewald method (0.35)
#40		**PMEORDer**	Integer	PME method multipolar expansion order (5)
#41		**MESHLX**	Integer	PME method x-axis mesh count (16)
#42		**MESHLY**	Integer	PME method y-axis mesh count (16)
#43		**MESHLZ**	Integer	PME method z-axis mesh count (16)
#44	FMM method	**USEFMM**	Selection	Execute FMM method (**NO** | **YES**)
#45		**FMMSPD**	Selection	Adjustment of calculation interval in FMM method (**NORM** | **HIGH**)
#46		**FMTREE**	Integer	FMM method tree size (3)
#47		**FMPOLE**	Integer	FMM method multipolar expansion order (8)
#48		**FMNUMA**	Integer	FMM method maximum atom number in cell(1000)
#49	Restraint force calculation	**CALPSR**	Selection	Position restraint calculation (**CALC** | **NOCA**lc)
#50		**WETPSR**	Real	Position restraint weight (5.0)
#51		**CALDSR**	Selection	Distance restraint force calculation (**CALC** | **NOCA**lc)
#52		**WETDSR**	Real	Distance restraint weight (1.0)
#53		**CALDHR**	Selection	Dihedral angle restraint force calculation (**CALC** | **NOCA**lc)
#54		**WETDHR**	Real	Dihedral angle restraint weight (10.0)
#55		**CALREP**ulsion	Selection	Repulsion force calculation (**CALC** | **NOCA**lc)
#56		**WETREP**ulsion	Real	Repulsion weight (1.0)
#57		**REPSCA**le	Real	Repulsion scale factor (1.0)
#58		**REPDEL**ta	Real	Repulsion permissible tolerance (1.0)
#59		**CALCAP**	Selection	Cap restraint force calculation (**CALC** | **NOCA**lc)
#60		**EXTCAP**	Selection	Extend cap restraint calculation (**CALC** | **NOCA**lc)

#61		**RADCAP**	Real	Cap radius (20.0)
#62		**FORCAP**	Real	Cap coefficient (150.0)
#63		**FUNCAP**	Selection	Cap type (**HARM**onic | **BIQU**adratic)
#64		**TEMPER**ature	Real	Convergence temperature (300.0)
#65		**SHAKEM**ethod	Selection	Shake type (**NOSH**ake | **HBON**d | **ALLB**ond)
#66		**COVSHK**	Real	Shake convergence value (1.0D-6)
#67		**LIMSHK**	Integer	Shake loop upper limit (1000)
#68	GB calculation	**CAL-GB**	Selection	GB calculation (**CALC** | **NOCA**lc)
#69		**GBWELE**	Real	Conductivity of water (78.3)
#70		**GBMELE**	Real	Conductivity of protein (1.0)
#71		**GBDELT**	Real	Born radius correction value (0.0)
#72		**GBLAMB**	Real	Capacity correction value (1.0)
#73		**GBOFFS**	Real	van der Waals radius correction value (0.09)
#74	ASA calculation	**CALASA**	Selection	ASA calculation (**CALC** | **NOCA**lc)
#75		**ASAPRO**	Real	PROBE radius (1.4)
#76		**ASAWEI**	Real	ASA weight (1.0)
#77		**ASACUT**	Real	ASA cutoff length 4.5)
#78	Boundary conditions	**BOUNDA**ry	Selection	Base cell shape (**NO** | **PERI** | **ELLIPS**oid | **SPHERE**)
#79		**SETCEN**ter	Selection	Set center of mass of starting molecule to cell center (**NO** | **YES**)
#80		**CENTRX**	Real	x coordinate of cell center(0)
#81		**CENTRY**	Real	y coordinate of cell center (0)
#82		**CENTRZ**	Real	z coordinate of cell center (0)
#83		**LXCELL**	Real	x-axis length of cubic cell(40)
#84		**LYCELL**	Real	y-axis length of cubic cell(40)
#85		**LZCELL**	Real	z-axis length of cubic cell(40)
#86		**ELLIPA**	Real	Ellipsoid cell radius (30)
#87		**ELLIPB**	Real	Ellipsoid cell radius (30)
#88		**ELLIPC**	Real	Ellipsoid cell radius (30)
#89		**RADIUS**	Real	SPHERE cell radius (30)

#90		**REPLAC**	Selection	Coordinate pull back method (**ATOM** | **RESI** | **CHAI**)

A.2.1.4 MD phase

Number	Item	Keyword	Value	Description
#1	Restart file input	UNITRI	Integer	Unit no.(40)
#2		NAMERI	Character	File name("")
#3	Restart file output	UNITRO	Integer	Unit no.(41)
#4		NAMERO	Character	File name("")
#5	Atom velocity output	MNTRVElocity	Enumerated	Format (NO | ASCIi | SINGle | DOUBle)
#6		UNITVElocity	Integer	Unit no.(43)
#7		NAMEVElocity	Character	File name("")
#8		OUTVELocity	Integer	Output interval (0)
#9	Energy information output	MNTRENergy	Enumerated	Format (NO | ASCIi | SINGle | DOUBle)
#10		UNITENergy	Integer	Unit no.(44)
#11		NAMEENergy	Character	File name("")
#12		OUTENErgy	Integer	Output interval (0)
#13	Atom position output	MNTRCOordinate	Enumerated	Format (NO | ASCIi | SINGle | DOUBle)
#14		UNITCOordinate	Integer	Unit no.(42)
#15		NAMECOordinate	Character	File name("")
#16		OUTCOOrdinate	Integer	Output interval(0)
#17	Trajectory output	MNTRTRajectory	Enumerated	Format (NO | ASCIi | SINGle | DOUBle)
#18		UNITTRajectory	Integer	Unit no.(50)
#19		NAMETRajectory	Character	File name("")
#20		OUTTRJ	Integer	Output interval(0)
#21	Total energy (total potential energy) output	MNTRTOtalenergy	Enumerated	Format (NO | ASCIi | SINGle | DOUBle)
#22		UNITTOtalenergy	Integer	Unit no.(59)
#23		NAMETOtalenergy	Character	File name("")
#24	Job control	CONTINuousjob	Enumerated	Inherit the result physical quantities between MD calculations within the same job (NO | YES)
#25		RESTARt	Enumerated	Restart (NO | YES)
#26		OUTRST	Real	Auto restart file output interval in seconds (0.0) 0 or less: no output

#27		**OUTRSL**	Integer	Auto restart file output loop interval (0) 0 or less: no output
#28		**NAMETI**	Text	Input coordinate trajectory file name ("")
#29		**NUMTRJ**	Integer	Position of coordinate trajectory (0)
#30		**CPUTIM**elimit	Real	Upper limit of CPU time (60.0)
#31		**OUTLOG**	Integer	Log output interval (1)
#32		**LOGFOR**mat	Selection	Log output format (**SHOR**t | **DETA**il)
#33		**BESTFI**t	Selection	Execute best fit (**NO** | **YES**)
#34	MD method	**LOOPLI**mit	Integer	MD loop iterations (0)
#35		**TIMEST**ep	Real	MD time increment (1.0)
#36		**SETTIM**elimit	Real	Simulation time (5.0)
#37		**HEATLO**op	Integer	Heat loop iterations (0)
#38		**STARTT**empearture	Real	Initial temperature (300.0)
#39		**INITIA**lvelocity	Selection	Initial velocity specification (**ZERO** | **SET** | RESEt)
#40		**RANDOM**seed	Integer	Random seed number for setting initial velocity (584287)
#41		**SETTEM**perature	Real	Simulation temperature (300.0)
#42		**TEMPCO**ntrol2	Selection	Temperature control type (**NO** | **YES**)
#43		**STOPCE**nterofmass	Selection	Stop rotation/movement of total system (**NO** | **TRAN**slate | **ROTA**tion | **BOTH**)
#44		**METHOD**	Selection	Ensemble type (**MICR**ocanonical| **CANO**nical | **NPT** | **EXPA**nded)
#45		**THERMO**stat	Selection	Canonical ensemble temperature control type (**CONS**tant| **NOSE**-hoover)
#46		**COUPLI**ngtime	Real	Coupling time in Nose-Hoover method (100.0)
#47		**BAROST**at	Selection	NPT ensemble pressure control type (**ANDE**rsen | **PARA**)

#48		**MODIFI**	Selection	Cell shape change condition in Parrinello Rahman method (**FLEX** | MONO | ORTH | **SI**NG | **ISOT**)
#49		**SETPRE**	Real	Pressure setting (1.0)
#50		**COUPHB**	Real	Temperature control coupling time scale (1000.0)
#51		**COUPPI**	Real	Pressure control coupling time scale (1000.0)
#52		**INTEGR**ation	Selection	Integrator type (**LEAP**-flog | **VELO**city)
#53		**FREQME**	Integer	Calculation frequency (medium) (1)
#54		**FREQLO**	Integer	Calculation frequency (long) (1)
#55	Expanded ensemble	**EXPAND**-ensemble	Selection	Expanded ensemble type (**FORC**e-bias | **SI**MUlated tempering)
#56		**DUMMYL**oop	Integer	Number of dummy loop iterations (1)
#57		**RESETC**	Integer	Initial value of histogram update interval (300000)
#58		**BINSIZ**e	Real	Histogram bin size (5.0)
#59		**ENEMIN**	Real	Minimum energy (-10000.0)
#60		**ENEMAX**	Real	Maximum energy (10000.0)
#61		**TEMMIN**	Real	Minimum temperature (250.0)
#62		**TEMMAX**	Real	Maximum temperature (700.0)
#63		**LIMITS**	Real	Minimum search threshold value (0.001)
#64		**LIMITC**	Real	Probability density function calculation threshold value (0.001)
#65		**STTNUM**	Integer	Temperature division number(100)
#66		**STEBAS**	Real	Minimum energy (base energy) (0.0)
#67		**GSTMIN**	Real	Lower limit of parameter λ (0.001d0)
#68		**GSTMAX**	Real	Upper limit of parameter λ (0.006d0)
#69		**GSTNUM**	Integer	Number of parameters λ (20)
#70		**GSTUPD**	Integer	Update interval of parameter λ (100)

#71		**GSTCON**	Integer	Convergence MD number of parameterλ (10000000)
#72		**GSTSAM**	Integer	Sampling number before scaling parameterλ (50000)
#73		**GSTBAS**	Real	Minimum energy (base energy) (25.5)
#74		**GSTETA**	Real	(0.5)
#75		**FBRSTO**	Selection	FB distribution data file output format (**NOWR** | **ASCI** | **DOUB**)
#76		**NAMEFO**	Character	Output FB distribution data filename ("")
#77		**FBRSTI**	Selection	FB distribution data file input format (**NORE** | **ASCI** | **DOUB**)
#78		**NAMEFI**	Character	Input FB distribution data filename ("")
#79		**UNITFR**	Integer	FB distribution data file device number (85)
#80		**UNITEP**	Integer	Device number of probability data file (78)
#81		**NAMEEP**	Character	Probability data filename ("expand.prob")
#82		**UNITES**	Integer	Device number of scale factor data file (77)
#83		**NAMEES**	Character	Scale factor data filename ("expand.scale")
#84		**UNITEE**	Integer	Device number of energy data file (79)
#85		**NAMEEE**	Character	Energy data filename ("expand.energy")
#86	Tsallis Dynamics	**ELOWER**	Real	Low energy side threshold (0.0d0)
#87		**EUPPER**	Real	High-energy side threshold (0.0d0)

#88		ROF1DR	Real	Condition of ρ1 parameter d
#89		ROF2XI	Real	Value of ρ2 parameter ξ
#90		ROF2VX	Real	Condition of ρ2 parameter δ
#91		ROF2VY	Real	Condition of ρ2 parameter γ
#92		UNITZT	Integer	Zeta value monitor file device number(80)
#93		NAMEZT	Character	Zeta value monitor filename ("zeta_TD.dat")
#94		OUTZET	Integer	Zeta value monitor file output interval(1)
#95		MNTRZT	Selection	Zeta value monitor file output format (NO | ASCI | SING | DOUB)
#96		UNITCK	Integer	Tsallis integral check value monitor file device number (75)
#97		NAMECK	Character	Tsallis integral checkvaluemonitor filename ("check_TD.dat")
#98		OUTCHK	Integer	Tsallis integral check value monitor file output interval (1)
#99		MNTRCK	Selection	Tsallis integral check value monitor file output format (NO | ASCI | SING | DOUB)
#100	Monitor output of distribution density function	UNITDD	Integer	Tsallis distribution density function monitor file device number(83)
#101		NAMEDD	Character	Tsallis distribution density function monitor filename ("")
#102		OUTDDF	Integer	Tsallis distribution density function monitor file output interval (1)

#103		MNTRDD	Selection	Tsallis distribution density function monitor file output format (NO | ASCI | SING | DOUB)
#104	Monitoroutput ofenergy	UNITPK	Integer	Device number(81)
#105		NAMEPK	Character	Filename ("")
#106		OUTPKT	Integer	Output interval(0)
#107		MNTRPK	Selection	Output format(NO | ASCI | SING | DOUB)
#108		FLGPKT	Selection	Output item(---, --+, -+-, -++, +--, +-+, ++-, +++) Potential, kinetic, total are expressed in three characters. "+" is output target.
#109	Total physical quantity monitor output (barycentre, total momentum, total angular momentum, total force, total torque, rmsd)	UNITQU	Integer	Device number(82)
#110		NAMEQU	Character	Filename("")
#111		OUTQUA	Integer	Output interval(1)
#112		MNTRQU	Selection	Output format (NO | ASCI | SING | DOUB)
#113	Interaction calculation	CALBONd	Selection	Bond force calculation (CALC | NOCAlc | MEDIum | LONG)
#114		CALANGle	Selection	Angle force calculation (CALC | NOCAlc | MEDIum | LONG)
#115		CALTORsion	Enumerated	Torsion force calculation (CALC | NOCAlc | MEDIum | LONG)
#116		CALIMProper	Enumerated	Improper force calculation (CALC | NOCAlc | MEDIum | LONG)

#117		**CALV14**	Enumerated	1-4 van der Waals force calculation (**CALC** | NOCAlc | **MEDI**um | **LONG**)
#118		**CALE14**	Enumerated	1-4 electrostatic force calculation (**CALC** | NOCAlc | **MEDI**um | **LONG**)
#119		**CUTMET**hod	Enumerated	Cutoff method (**RESC** | **ATOM** | **RESA**)
#120		**CUTLEN**gth	Real	Cut off length (8.0)
#121		**UPDATE**interval	Integer	Interaction table update interval (20)
#122		**USESPL**	Selection	Application of spline interpolation (**NO** | **YES**)
#123		**CUT-ON**	Real	Spline start interval (6.0)
#124		**CALV15**	Enumerated	1-5 vdw force calculation with cutoff (**CALC** | NOCAlc | **MEDI**um | **LONG**)
#125		**CALE15**	Enumerated	1-5 electrostatic force calculation with cutoff (**CALC** | NOCAlc | **MEDI**um | **LONG**)
#126		**CALHYD**	Enumerated	1-5 hydrogen force calculation with cutoff (**CALC** | NOCAlc | **MEDI**um | **LONG**)
#127		**CALV5N**	Enumerated	1-5 vdw calculation without cutoff (CALC | **NOCA**lc | **MEDI**um | **LONG**)
#128		**CALE5N**	Enumerated	1-5 electrostatic force calculation without cutoff (CALC | **NOCA**lc | **MEDI**um | **LONG**)
#129		**CALH5N**	Enumerated	1-5 hydrogen force calculation without cutoff (CALC | **NOCA**lc | **MEDI**um | **LONG**)
#130		**DIEFUN**ction	Enumerated	Distance dependence of electrostatics (**CONS** | **DIST**)
#131		**DIEVAL**ue	Real	Electrostatic coefficient (1.0)
#132	PME method, Ewald method	**CALPME**	Enumerated	Execute PME method (**NO** | **YES**)
#133		**CALEWA**ld	Enumerated	Execute Ewald method (**NO** | **YES**)

#134		**PMESPD**	Enumerated	Adjustment of calculation interval in PME method (**NORM** | **HIGH**)
#135		**PMEUPD**	Selection	Calculation method for PMESPD=HIGH (**CUT** | **RECI**)
#136		**REATOL**	Real	Permissible tolerance with Ewald method (1.0d-19)
#137		**EWAPAR**	Real	Inverse space calculation coefficient in PME method and Ewald method (0.35)
#138		**PMEORDer**	Integer	PME method multipole expansion order (5)
#139		**MESHLX**	Integer	PME method x-axis mesh count (16)
#140		**MESHLY**	Integer	PME method y-axis mesh count (16)
#141		**MESHLZ**	Integer	PME method z-axis mesh count (16)
#142	FMM method	**USEFMM**	Selection	Execute FMM method (**NO** | **YES**)
#143		**FMMSPD**	Selection	Adjustment of calculation interval in FMM method (**NORM** | **HIGH**)
#145		**FMTREE**	Integer	FMM method tree size (3)
#146		**FMPOLE**	Integer	FMM method multipole expansion order (8)
#147		**FMNUMA**	Integer	Maximum cell number in FMM cell (1000)
#148	Restraint force calculation	**CALPSR**	Selection	Position restraint calculation (**CALC** | **NOCAlc** | **MEDIum** | **LONG**)
#149		**WETPSR**	Real	Position restraint weight (5.0)
#150		**CALDSR**	Selection	Distance restraint force calculation (**CALC** | **NOCAlc** | **MEDIum** | **LONG**)
#151		**WETDSR**	Real	Distance restraint weight (1.0)
#152		**CALDHR**	Selection	Dihedral angle restraint force calculation (**CALC** | **NOCAlc** | **MEDIum** | **LONG**)
#153		**WETDHR**	Real	Dihedral angle restraint weight (10.0)
#154		**CALREPulsion**	Selection	Repulsion force calculation (**CALC** | **NOCAlc** | **MEDIum** | **LONG**)

#155		**WETREP**ulsion	Real	Repulsion weight (1.0)
#156		**REPSCA**le	Real	Repulsion scale factor (1.0)
#157		**REPDEL**ta	Real	Repulsion permissible tolerance (1.0)
#158		**CALCAP**	Selection	Cap restraint force calculation (**CALC** | **NOCA**lc | **MEDI**um | **LONG**)
#159		**RADCAP**	Real	Cap radius (20.0)
#160		**FORCAP**	Real	Cap coefficient (150.0)
#161		**FUNCAP**	Selection	Cap type (**HARM**onic | **BIQU**adratic)
#162		**EXTCAP**	Selection	Extend cap restraint force calculation (**CALC** | **NOCA**lc)
#163		**TEMPER**ature	Real	Restraint temperature (300.0)
#164		**SHAKEM**ethod	Selection	Shake method (**NOSH**ake | **HBON**d | **ALLB**ond)
#165		**COVSHK**	Real	Shake convergence value (1.0D-6)
#166		**LIMSHK**	Integer	Shake loop upper limit (1000)
#167	Rigid body model	**RIGIDM**odel	Selection	Rigid body model specification (**NO** | **YES**)
#168		**UNITRM**	Integer	Device number (56)
#169		**NAMERM**	Text	File name ("")
#170		**DBGRIG**	Selection	Rigid-body automatic generation information output designation (**NOWR** | **ASCI**)
#171		**UNITDR**	Integer	Device number (84)
#172		**NAMEDR**	Character	Filename ("")
#173	GB calculation	**CAL-GB**	Selection	GB calculation (**CALC** | **NOCA**lc| **MEDI**um | **LONG**)
#174		**GBWELE**	Real	Conductivity of water (78.3)
#175		**GBMELE**	Real	Conductivity of protein (1.0)
#176		**GBDELT**	Real	Born radius correction value (0.0)
#177		**GBLAMB**	Real	Capacity correction value (1.0)
#178		**GBOFFS**	Real	van der Waals radius correction value (0.09)
#179	ASA calculation	**CALASA**	Selection	ASA calculation (**CALC** | **NOCA**lc| **MEDI**um | **LONG**)

#180		**ASAPRO**	Real	PROBE probe (1.4)
#181		**ASAWEI**	Real	ASA weight (1.0)
#182		**ASACUT**	Real	ASA cutoff length (4.5)
#183	UMBRELLA restraint calculation	**CALUMB**	Selection	UMBRELLA potential calculation (**NOCA**lc | **CALC** | **MEDI**um | **LONG**)
#184	Boundary conditions	**BOUNDA**ry	Selection	Base cell shape (**NO** | **PERI** | **ELLIPS**oid | **SPHERE**)
#185		**SETCEN**ter	Selection	Set center of mass of starting molecule to center of cell (**NO** | **YES**)
#186		**CENTRX**	Real	x coordinate of cell center (0.0)
#187		**CENTRY**	Real	y coordinate of cell center (0.0)
#188		**CENTRZ**	Real	z coordinate of cell center (0.0)
#189		**LXCELL**	Real	x-axis length of cubic cell(40.0)
#190		**LYCELL**	Real	y-axis length of cubic cell(40.0)
#191		**LZCELL**	Real	z-axis length of cubic cell (40.0)
#192		**ANGLBC**	Real	Angle between LYCELL and LZCELL (90.0)
#193		**ANGLCA**	Real	Angle between LZCELL and LXCELL (90.0)
#194		**ANGLAB**	Real	Angle between LXCELL and LYCELL (90.0)
#195		**ELLIPA**	Real	Ellipsoid cell radius (30.0)
#196		**ELLIPB**	Real	Ellipsoid cell radius (30.0)
#197		**ELLIPC**	Real	Ellipsoid cell radius (30.0)
#198		**RADIUS**	Real	SPHERE cell radius (30.0)
#199		**REPLAC**	Selection	Coordinate pull back method (**ATOM** | **RESI** | **CHAI**)

A.2.2 Topology files

Applicable phase: All phases

Application: This designates the topology of the system to be simulated.

Note:
You can designate whether to set the file in ASCII or binary (8 byte real number format designation).

Syntax: The following two types are provided, depending on the output designation.

(1) ASCII format designation (TOPOLO=FORM)

Note:
Within each line, the part after "; " is a comment. "->" indicates that the line continues.
Each part of the designation must be written according to the sequence given in the molecule designation part.
Keywords are recognized as the 4 characters following "TPL>".

Syntax: A topology file is comprised of the following parts.

```
Title  Molecule designation  Atom designation  [BOND designation]  [ANGLE designation]
[TORSION designation]  [IMPROPER designation]  Potential function designation
Non-bond interaction designation
```

(1-1) Title

Write the title of the pertinent topology. It must not exceed 10 lines.

```
"TPL>TITL" line [Title line…]
```

TPL>TITL" line [Title line…])

```
TPL> TITLE
ALA-DIMER (AMBER UNITED ATOM)
```

(1-2) Molecule designation

Write the molecules of the pertinent molecule.

"TPL> MOLE"line [Molecule name line…]

A molecule name line is comprised of the following parts.

Molecule name Number of molecules

Molecule name: Write an alphabetic number of less than 40 characters

Number of molecules: Write the quantity of the pertinent molecule.

Example)

```
TPL> MOLECULE
ALA-DIMER-1                                    1
WATER(TIP3P-MODEL)-2                         449
```

(1-3) Atom designation

Following the sequence of the molecule designation, write information on the atoms comprising that molecule.

"TPL>ATOM" line Molecule name line [Atom description line…]

The atom description line is comprised of the following parts.

Atom name Atom type Interaction type Residue name Residue no. Mass Van der Waals radius Charge Number of 1-2 bond atoms Number of 1-3 bond atoms Number of 1-4 bond atoms [1-2 bond atom partner…] [1-3 bond atom partner…] [1-4 bond atom partner…] [Internal coordinate description (z-matrix)]

Atom name : Write the atom name in 8 characters or less.
Atom type : Write the atom type in 4 characters or less.
Interaction type : Write the interaction type as an integer.
Residue name : Write the residue name in 8 characters or less.
Residue no. : Write the relative no. of the residue in the molecule

Mass : Write the mass of the atom per 1mol in g units.
Van der Waals radius : Write the Van der Waals radius in Å units.
Number of 1-2 bond atoms : These are atoms with a 1-2 bond. Write the number of atoms coming after the pertinent atom.
Number of 1-3 bond atoms : These are atoms with a 1-3 bond. Write the number of atoms coming after the pertinent atom.
1-4 bond atom partner : Write the relative position of atoms with a 1-4 bond, for just the number of 1-4 bond atoms.
1-2 bond atom partner : Write the relative position of atoms with a 1-2 bond, for just the number of 1-2 bond atoms.
1-3 bond atom partner : Write the relative position of atoms with a 1-3 bond, for just the number of 1-3 bond atoms.
1-4 bond atom partner : Write the relative position of atoms with a 1-4 bond, for just the number of 1-4 bond atoms.

Internal coordinate description (z-matrix): Description is as follows.

- 1-2 bond atom partner: Relative position of atom with 1-2 bond.
- 1-3 bond atom partner: Relative position of atom with 1-3 bond.
- 1-4 bond atom partner: Relative position of atom with 1-4 bond.
- Phase reference atom: Relative position of atom to serve as the phase reference.
- Equilibrium distance: Write the equilibrium distance in Å units.
- Equilibrium angle: Write the equilibrium angle in degree units.
- Phase: Write the initial phase in degree units.

Example)

```
TPL> ATOM
ALA-DIMER-1                    ;  Molecule name line
N       N3     14          ->  ;  Atom name, Atom type, Interaction type
ALA     1                  ->  ;  Residue name, Residue no.
14.010  1.850  -0.263      ->  ;  Mass, Van der Waals radius, Charge
4       2      2           ->  ;  Number of 1-2 bond atoms, Number of 1-3 bond atoms,
                               ;  Number of 1-4 atoms
1       2      3      4    ->  ;  1-2 bond partner atoms
5       6                  ->  ;  1-3 bond partner atoms
7       8                  ->  ;  1-4 bond partner atoms
0  0  0  0  0.0000  0.0000  0.0000  -> ; Internal coordinates (z-matrix)
```

(1-4) BOND designation

Following the molecule designation sequence, write information on bonds existing in the molecule.

```
"TPL> BOND" line   Molecule name line  [BOND description line…]
```

The BOND description line is comprised of the following parts.

BOND component atom 1	BOND component atom 2	Coefficient	Equilibrium distance

BOND component atoms 1 and 2 : Write the atoms comprising the BOND as integers.
Coefficient : Write the BOND force coefficient in KCAL(MOL*Å2 units).
Equilibrium distance : Write the equilibrium distance in Å units.

Example)

```
TPL> BOND
ALA-DIMER-1                    ; Molecule name line
1    2    434.00   1.010       ; Component atom 1  Component atom 2  Coefficient
                                ; Equilibrium distance
```

(1-5) ANGLE designation

Following the molecule designation sequence, write information on ANGLEs existing in the molecule.

"TPL> ANGL"line Molecule name line [ANGLE description line…]

The ANGLE description line is comprised of the following parts.

ANGLE component atom 1 ANGLE component atom 2 ANGLE component atom 3 Coefficient Equilibrium angle

ANGLE component atoms 1, 2, 3 : Write the atoms comprising the ANGLE as integers. 2 is the center of the angle.
Coefficient : Write the ANGLE force coefficient in KCAL(MOL*θ2 units).
Equilibrium distance : Write the equilibrium angle in degree units.

Example)

```
TPL> ANGLE
ALA-DIMER-1                    ; Molecule name line
2   1   3   35.000   109.50    ; Component atom 1    Component atom 2
                                ; Coefficient  Equilibrium angle
```

(1-6) TORSION designation

Following the molecule designation sequence, write information on TORSION existing

in the molecule.

```
""TPL>TORS" line  Molecule name line  [TORSION description line…]
```

The TORSION description line is comprised of the following parts.

```
TORSION component atom 1  TORSION component atom 2  TORSION component atom 3
TORSION component atom 4  Coefficient  Number of overlapping TORSIONs
Applicability  Initial phase  1-4 interaction calculation flag
```

TORSION component atom 1, 2, 3, 4 : Write the atoms comprising the TORSION as integers. Atoms 1, 2 and 3, and atoms 2,3 and 4, comprise a plane.

Coefficient : Write the TORSION force coefficient in KCAL/MOL units.

Number of overlapping TORSIONs: Write the number of TORSIONs overlapping with the pertinent TORSION.

Applicability : Write the applicability (reciprocal of period) of the pertinent TORSION.

Phase : Write the initial phase of the pertinent TORSION in degree units.

1-4 interaction calculation flag: To calculate electrostatic and Van der Waals force between atoms 1 and 4 of the pertinent TORSION, write 1. Otherwise, write 0.

Example)

```
TPL> TORSION
ALA-DIMER-1                     ;  Molecule name line
2     1     5     6      ->     ;  Component atom  Component atom 2
                                 ;  Component atom 3  Component atom 4
1.4   6     3     0.0    ->     ;  Coefficient  Number of overlapping TORSIONs
                                 ;  Applicability  Initial phase
1                                ;  1-4 interaction calculation flag
```

(1-7) IMPROPER-TORSION designation

Following the molecule designation sequence, write information on IMPROPER-TORSION existing in the molecule.

```
"TPL>IMPR" line  Molecule name line  [IMPROPER description line…]
```

The IMPROPER description line is comprised of the following parts.

```
IMPROPER component atom 1  IMPROPER component atom 2  IMPROPER component atom 3
IMPROPER component atom  Coefficient  Number of overlapping IMPROPERs
Applicability  Initial phase  1-4 interaction calculation flag
```

IMPROPER component atom 1, 2, 3, 4: Write the atoms comprising the IMPROPER as integers. Atoms 1, 2 and 3, and atoms 2,3 and 4, comprise a plane.
Coefficient: Write the IMPROPER force coefficient in KCAL/MOL units.
Number of overlapping IMPROPERs: Write the number of IMPROPERs overlapping with the pertinent IMPROPER.
Applicability: Enter the applicability (reciprocal of period) of the relevant IMPROPER.
Phase: Indicate the initial phase of the relevant IMPROPER in degrees.
1-4 interaction calculation flag: To calculate electrostatic and Van der Waals force between atoms 1 and 4 of the relevant IMPROPER, write 1. Otherwise, write 0.

Example)

```
TPL> IMPROPER
ALA-DIMER-1                    ;  Molecule name line
6     5     1     7      ->     ;  Component atom 1  Component atom 2  Component
atom 3  Component atom 4
14.0  1     3    180.0    ->     ;  Coefficient  Number of overlapping IMPROPERS
Applicability  Initial phase
0                              ;  1-4 interaction calculation flag
```

(1-8) Potential function specification

Specify system potential function information.

```
"TPL> FUNC" line  [Potential function specification line  ...]
```

The potential function specification line consists of the following parts.

```
Number  Number of coefficients  Name of potential function
```

Number : When writing multiple lines, number each line by an integer in ascending order starting from 1.
Number of coefficients: Write the number of coefficients of the potential function.
Potential function name: Write the name of the potential function.
(AMBER | OPLS | ECEPP | CHARM)

Example)

```
TPL> FUNCTION
1          4          AMBER    ;  Number  Number of coefficients  Potential function
```

(1-9)Non-bond interaction specification

Specify non-bond interaction information for the system.

```
"TPL> NONB" line  [Non-bonding interaction specification line ...]
```

The syntax of the non-bond interaction description line varies depending on the potential function ((A), (B)).

(A) van der Waals case

Write the following in the non-bond interaction description line.

```
Interaction type 1  Interaction type 2  Function type  vanderWaals radius
vanderWaals depth  1-4 vanderWaals coefficient  1-4 electrostatic coefficient
```

Interaction type 1: Write the interaction type of the atom.
Interaction type 2: Write "0".
Function type: Write "1".
vanderWaals radius: Write the van der Waals radius of the pertinent atom in Å units.
vanderWaals depth: Write van der Waals energy depth in KCAL/MOL units.
1-4 vanderWaals coefficient: Write the coefficient used in 1-4 van der Waals energy calculation.
1-4 electrostatic coefficient: Write the coefficient used in 1-4 electrostatic energy calculation.

(B) Hydrogen bond calculation

```
Interaction type 1  Interaction type 2  Function type
12th degree coefficient  10th degree coefficient
```

Interaction type 1 : Write the interaction type of the atom.
Interaction type 2 : Write the interaction type of the atom.
Function type : Write "2".
12th degree coefficient: Write the coefficient of the 12th degree term between the pertinent atoms in KCAL/(MOL*Å12) units.
10th degree coefficient: Write the coefficient of the 12th degree term between the pertinent atoms in KCAL/(MOL*Å10) units.

Example)

```
TPL> NONBOND
; van der Waals case
1    0   1        ->   ;  Atom type 1  Atom type 2 ("0")   Function type ("1")
1.9080   0.0860  ->   ;  vanderWaals radius  vanderWaals depth
0.8333   0.5          ;  1-4vanderWaals coefficient  1-4 electrostatic coefficient

; Hydrogen bond case
14   14   2        ->   ;  Atom type 1  Atom type 2  Function type ("2")
1.8000   0.2420       ;  12th degree coefficient  10th degree coefficient
```

(2) Real number format designation (TOPOLO=BINA)

Title Molecule Atom Residue Chain Extension/Shrinkage Deformation angle Dihedral angle Improper Number of potential functions Atom type

Title :

Number of lines	INTEGER×1
Title statement	CHARACTER(80)×Number of molecules

Molecule :

Number of molecules	INTEGER×1
Molecule name	CHARACTER(40)×Number of molecules
Number of belonging chains	INTEGER×Number of molecules

Atom :

Number of atoms	INTEGER×1
Belonging molecules	INTEGER×Number of atoms
Belonging chains	INTEGER×Number of atoms
Belonging residues	INTEGER×Number of atoms
Non-linked interaction type	INTEGER×Number of atoms
Number of 1-2 interactions	INTEGER×Number of atoms
Number of 1-3 interactions	INTEGER×Number of atoms
Number of 1-4 interactions	INTEGER×Number of atoms
Electrostatic charge	INTEGER×Number of atoms
Mass	INTEGER×Number of atoms
VanDerWaals radius	INTEGER×Number of atoms
Atom name	CHARACTER(8)×Number of atoms
Type name	CHARACTER(4)×Number of atoms
Belonging residue name	CHARACTER(8)×Number of atoms
1-2 interaction table no.	INTEGER×Number of atoms×Number of 1-2 interactions
1-3 interaction table no.	INTEGER×Number of atoms×Number of 1-3 interactions
1-4 interaction table no.	INTEGER×Number of atoms×Number of 1-4 interactions

Residue：

Number of residues	INTEGER×1
Start atom no.	INTEGER×Number of residues
End atom no.	INTEGER×Number of residues

Chain：

Number of chains	INTEGER×1
Final atom no.	INTEGER×Number of chains

Extension/Shrinkage：

Number of extension/shrinkage	INTEGER×1
Component atoms	INTEGER×Number of extension/shrinkage×2
Coefficient	REAL*8×Number of extension/shrinkage
Minimum energy distance	REAL*8×Number of extension/shrinkage

Deformation angle：

Number of deformation angles	INTEGER×1
Component atoms	INTEGER×Number of deformation angles×3
Coefficient	REAL*8×Number of deformation angles
Minimum energy angle	REAL*8×Number of deformation angles

Dihedral angles：

Number of dihedral angles	INTEGER×1
Component atoms	INTEGER×Number of dihedral angles×4
Number of assumable angles	INTEGER×Number of dihedral angles
Coefficient	REAL*8×Number of dihedral angles
Symmetry	REAL*8×Number of dihedral angles
Phase	REAL*8×Number of dihedral angles
1-4VanDerWaals coefficient	REAL*8×Number of dihedral angles
1-4 electrostatic coefficient	REAL*8×Number of dihedral angles

Improper：

Number of impropers	INTEGER×1
Component atoms	INTEGER×Number of impropers×4
Number of assumable angles	INTEGER×Number of impropers
Coefficient	REAL*8×Number of impropers
Symmetry	REAL*8×Number of impropers
Phase	REAL*8×Number of impropers
1-4VanDerWaals coefficient	REAL*8×Number of impropers
1-4 electrostatic coefficient	REAL*8×Number of impropers

Number of potential functions：

umber of potential functions	INTEGER×1
Function type	INTEGER×Number of potential functions
Function name	CHARACTER(40)×Number of potential functions

Atom type :

Number of atom types	INTEGER×1
Potential function type (FUNC_VDW, FUNC_HYD)	INTEGER×Number of atom types×Number of atom types
1/Distance**6 VanDerWaals	REAL*8×Number of atom types×Number of atom types
1/Distance**12 VanDerWaals	REAL*8×Number of atom types×Number of atom types
1/Distance**10 hydrogen bond	REAL*8×Number of atom types×Number of atom types
1/Distance**12 hydrogen bond	REAL*8×Number of atom types×Number of atom types
Vdw minimum radius	REAL*8×Number of atom types×Number of atom types
Depth of well type potential	REAL*8×Number of atom types×Number of atom types
1-4vdw scale factor	REAL*8×Number of atom types×Number of atom types
1-4 electrostatic scale factor	REAL*8×Number of atom types×Number of atom types

A.2.3 Coordinate file

Applicable phases: All phases

Application: This designates the atom coordinates of the system to be simulated.

Note:
You can designate whether to set the file in ASCII or binary (8 byte real number format designation).

Syntax: The following two types are provided, depending on the output designation.

(1) ASCII format designation (COORDI= PDB)
Follow PDB format specifications.

(2) Real number format designation (COORDI= BINA)

Date	CHARACTER(80)×1
User name	CHARACTER(80)×1
Number of atoms	INTEGER×1
x coordinate	REAL*8×Number of atoms
y coordinate	REAL*8×Number of atoms
z coordinate	REAL*8×Number of atoms

A.2.4 SHAKE file

Applicable phases: MIN, MD phases

Application: This designates the atoms subject to SHAKE and the restraint distance.

Notes:
-Within each line, the part after "; " is a comment. "->" indicates that the line continues.
-The number of atoms comprising SHAKE is set to 2, 3 or 4.
-SHAKE between multiple molecules cannot be designated.

Syntax: SHAKE designation is comprised of the following lines.

```
[SHAKE information start line  Molecule designation line  SHAKE restraint information line…]...
```

SHAKE information start line: Line where first character string is "SHAKE> SHAKE"

Molecule designation line: Line where molecule name is written

SHAKE restraint information line: This line describes the number of atoms subject to SHAKE, the relative atom no. within the molecule of atoms subject to SHAKE, and the SHAKE restraint distance. The file is written as follows depending on the number of atoms comprising SHAKE.

For 2 atom SHAKE:

```
"2"  Atom 1 no.  Atom 2 no.  Distance between atom 1- atom 2
```

For 3 atom SHAKE:

```
"3"  Atom 1 no.  Atom 2 no.  Atom 3 no.
Distance between atom 1 and atom 2  Distance between atom 2 and atom 3
Distance between atom 3 and atom 1
```

For 4 atom SHAKE:

```
"4"  Atom 1 no.  Atom 2 no.  Atom 3 no.  Atom 4 no.
Distance between atom 1 and atom 2   Distance between atom 2 and atom 3
Distance between atom 3 and atom 1   Distance between atom 1 and atom 4
Distance between atom 3 and atom 4   Distance between atom 2 and atom 4
```

```
Example)

SHAKE> SHAKE                        ;  Start of SHAKE information
ALA-DIMER-1                         ;  Molecule name
4                   ->              ;  Number of SHAKE atoms=4
1    2    3    4   ->              ;  Nos. of atoms subject to SHAKE
1.01000  1.64962  1.01000 ->
1.01000  1.64962  1.64962           ;  Distance between atoms
2                   ->              ;  Number of SHAKE atoms=2
9   10              ->              ;  Nos. of atoms subject to SHAKE
1.01000                             ;  Distance between atoms

SHAKE> SHAKE                        ;  Start of SHAKE information
WATER(TIP3P-MODEL)-2                ;  Molecule name
3                   ->              ;  Number of SHAKE atoms =3
1    2    3         ->              ;  Nos. of atoms subject to SHAKE
0.95720   1.51360   0.95720         ;  Distance between atoms
```

A.2.5 Fixed atom and free atom designation file

Applicable phase: MIN, MD phase

Application: This designates fixed atoms and free atoms.

Notes:
Within each line, the part after ";" is a comment.
Items in parentheses can be omitted.

Syntax: Designation of free/fixed atoms is comprised of the following lines.

```
[Free/Fixed atom information start line  Free/Fixed atom information line…]…
```

Free/Fixed atom information start line: Designate using the following character strings.
(1) Atom list designation = "SETVAR> LIST"
(2) Atom range designation = "SETVAR> RADIUS"

Free/Fixed atom information: Write as follows, in accordance with the start line.

(1) "SETVAR> LIST" case:

```
Format  Chain start no.  Chain end no.  Residue start no.  Residue end no.
Atom name designation (List output)
```

(2) "SETVAR> RADIUS" case: Write as follows, in accordance with the designation method.

Center atom designation:

```
Atom no.  Radius lower limit  Radius upper limit  Atom name designation  (List output)
```

Center coordinate designation:

```
Format "COOR"  x coordinate  y coordinate  z coordinate
Radius lower limit  Radius upper limit  Atom name designation  (List output)
```

※The items above are comprised of the following values

List output ::= "YES" | "NO" (Default is "NO")

Format ::= "FREE" | "FIX"

Atom name designation ::= A wild card (*) can be designated.

```
Example)
SETVAR> LIST                                          ;  Atom list designation start
line
FIX   1     1     1     130      *     YES           ;  Atom information
FREE  2     10000 1     1        0*                  ;  Atom information

SETVAR> RADIUS                                        ;  Atom range designation start
line
FIX   ATOM  100           10.0   20.0    C*    YES ;  Center atom designation
FREE  COOR  0.0 10.0 2.0  10.0   20.0    *         ;  Center coordinate designation
```

A.2.6 CAP designation file

Applicable phases: All phases

Applications: This designates cell form and CAP restraint.

Notes:
Within each line, the part after "; " is a comment.
Items in parentheses can be omitted.

Syntax: The boundary designation is comprised of the following lines.

```
[Boundary information start line  Boundary information line…]…
```

Boundary information start line: Designate using the following character strings.
(1) Designation of object of CAP calculation= "BOUND> INCLUDE"
(2) Designation of CAP center= "BOUND> CENTER"
(3) Cubic cell size designation= "BOUND> BOX"
(4) CAP radius designation= "BOUND> RADIUS"

Boundary information: Write as follows, in accordance with the start line.
(1) "BOUND> INCLUDE" case:

```
Molecule name  Chain start name  Chain end no.  (List output)
```

(2) "BOUND> CENTER" case: There are three types of designation, and they are each described as follows.

Chain center of mass designation:

```
"CHAI"  Chain no.
```

Atom designation:

```
"ATOM"  Chain no. to which center atom belongs
Residue no. to which center atom belongs  Atom name
```

Coordinate designation:

```
"COOR"  x coordinate  y coordinate  z coordinate
```

(3) "BOUND> BOX" case:

```
x component  y component  z component
```

(4) "BOUND> RADIUS" case:

```
Radius value
```

※The above items are comprised of the following values.
List output ::= "YES" | "NO" (Default is "NO")

```
Example)
BOUND> INCLUDE                          ;  Start line for designating object of
                                        ;  CAP calculation
WATER     1      200  YES               ;  CAP calculation object designation
BOUND> CENTER                           ;  CAP center designation start line
CHAIN          1                        ;  Chain center of mass designation
ATOM           1      1      CA         ;  Atom designation
COORDINATEs    0.0D0  0.0D0  0.0D0      ;  Coordinate designation
BOUND> BOX                              ;  Cubic cell size designation start line
0.0D0  0.0D0  0.0D0                     ;  Cubic cell size designation
BOUND> RADIUS                           ;  CAP radius designation start line
30.0D0                                  ;  CAP radius designation
```

A.2.7 ExtendCAP designation file

Target phase : All phases

Application : To designate Extend CAP restraint target and restraint condition.

Special instruction :

The portion in a line after " ; " is a comment.

Format : Designation of ExtendCAPrestraint is configured with the following lines.

```
[ " EXTCAP>CONDition " line     Restraint condition specification line
  " EXTCAP>INCLude "  line     Restraint target specification line... ] ...
```

As shown below, designate following the restraint condition designation line(" EXTCAP>CONDition " line).

Designate the center, radius and force coefficient of the spherical body spherical bodyCAPrestraint:

```
" SPHEre "  x coordinate of center  y coordinate of center  z coordinate of center
  Radius  Force coefficient  [List output]
```

Designate the focus, sum of distances from focus, and force coefficient of the ellipsoidal body CAPrestraint:

```
" GEometric ELlipsoid "
x coordinate of Focus A  y coordinate of Focus A  z coordinate of Focus A
x coordinate of Focus B  y coordinate of Focus B  z coordinate of Focus B
Sum of distances from focus / 2.0 d0  Force coefficient  [List output]
```

Designate the center and radius in the direction o f the x, y and z axis of the ellipsoidal body CAP restraint:

```
" ALgebraic ELipsoid "
x coordinate of center  y coordinate of center  z coordinate of center
Radius in x direction  Radius in y direction  Radius in z direction
Force coefficient  [List output]
```

As shown below, designate following the ExtendCAPrestraintcalculation target designatation line (“EXTCAP>INCLude”).

Designation by chain:

“CHAI” Molecule name First chain number Last chain number [List output]

Designation by residue:

“RESI” First chain number Last chain number First residual group number Last residual group number Residual group name [List output]

Designation by atom:

“ATOM” First chain number Last chain number First residual group number Last residual group number Atom name Residual group name [List output]

※The items shown above are configured with the following values.

List output ::= "YES" | "NO"

(Example of designating two CAP restraint)

```
EXTCAP> COND                                    ; Specify restraint conditions of
                                                  CAP restraint 1
;SPHERE 39.0 26.0 -4.0 27.0 100.0 YES           ;  (Specification by sphere)
GEEL 86.0 2.0 -1.0 10.0 58.0 1.0 48.5 150.0 YES ;  (Specification by ellipsoid)
;ALEL 0.0 0.0 0.0 12.0 12.0 12.0 150.0 YES      ;  (Specification by ellipsoid)

EXTCAP> INCL                                    ; Specify restraint target of CAP
                                                    restraint 1
;CHAI WAT 1 216 YES                             ;  (Specification by chain)
;RESI 1 1 1 3 HYD YES                           ;  (Specification by residual)
ATOM 9 9 1 5 * * YES                            ;  (Specification by atom)


EXTCAP> COND                                     ; Specify restraint conditions
                                                   of CAP restraint 2
;SPHERE 39.0 26.0 -4.0 27.0 100.0 YES           ;  (Specification by sphere)
GEEL 86.0 2.0 -1.0 10.0 58.0 1.0 49.5 150.0 YES ;  (Specification by ellipsoid)
;ALEL 0.0 0.0 0.0 12.0 12.0 12.0 150.0 YES      ;  (Specification by ellipsoid)

EXTCAP> INCL                                    ; Specify restraint target of CAP
                                                  restraint 2
;CHAI WAT 1 216 YES                             ;  (Specification by chain)
;RESI 1 1 1 3 HYD YES                           ;  (Specification by residual)
ATOM 10 1365 1 1 * WAT YES                      ;  (Specification by atom)
```

A.2.8 Position restraint file

Applicable phases: All phases

Applications: This designates position restraint.

Notes:
Within each line, the part after ";" is a comment.
Items in parentheses can be omitted.

Syntax: Position restraint designation is comprised of the following lines.

```
[Position restraint information start line  Position restrain information line…
 Parameter end line  End information line]…
```

Position restraint information start line: Designate using the following character strings.
(1) Restraint list designation = "GROUP> LIST"
(2) Restraint range designation = "GROUP> RADIUS"

Parameter end line: Designate the end of the parameter designation line in the following format.

End information line: Line where first character string is "GROUP> STOP"

Position restraint information: Write as follows, in accordance with the start line.
(1) "GROUP> LIST" case:

```
Chain start no.  Chain end no.  Residue start no.  Residue end no.
Atom name designation  Residue name designation  Coefficient  "MASS"  (List output)
```

(2) "GROUP> RADIUS" case:

```
Center chain no.  Center residue no.  Center atom name  Radius lower limit
Radius upper limit  Atom name designation  Coefficient  "MASS" (List output)
```

※The items above are comprised of the following values

List output ::= "YES" | "NO" (Default is "NO")

Designate "MASS" to make restraint force proportional to atom mass.

```
Example)
GROUP> LIST                                          ;Restraint list designation start
                                                     ; line
1    1    5   13    CA   *     1.0    MASS   YES    ; Position restraint information
1    1    1   67    N*  ARG    1.0    MASS          ; Position restraint information
END
GROUP> STOP

GROUP> RADIUS                                        ; Restraint range designation
                                                     ; start line
1   10   CA   0.0   5.0  C*   1.0   MASS   YES      ; Position restraint information
1   10   CA   0.0   5.0  N*   1.0   MASS   YES      ; Position restraint information
1   10   CA   0.0   5.0  O*   1.0   MASS   YES      ; Position restraint information
1   10   CA   0.0   5.0  S*   1.0   MASS   YES      ; Position restraint information
END
GROUP> STOP
```

A.2.9 Distance restraint file

Applicable phases: All phases

Applications: This designates distance restraint.

Notes:
Within each line, the part after "; " is a comment.
Items in parentheses can be omitted.

Syntax: Designation of distance restraint is comprised of the following lines.

```
[Distance restraint information start line  Distance restraint information…
Parameter end line  End information line]…
```

Distance restraint information start line: Designate using the following character string.
(1) Restraint list designation = "RDDSTC> LIST"

Parameter end line: Designate the end of the parameter designation line in the following format.
"END"

End information line: Line where first character string is "RDDSTC> STOP"

Distance restraint information: Write as follows, in accordance with the start line.

(1) "RDDSTC> LIST" case:

```
Atom designation 1  Atom designation 2  Lower bound coefficient
Upper bound coefficient  Lower bound distance  Upper bound distance (List output)
```

※The items above are comprised of the following values
Atom designation ::= Belonging chain no. Belonging residue no.
Belonging residue name Atom name
List output ::= "YES" | "NO" (Default is "NO")

```
Example)
RDDSTC> LIST                                    ;  Restraint list designation start line
1   1 ILE  HA   1    2
ALA  HN     1.00  1.00  1.95  2.50  YES        ;  Distance restraint information
1   2 ALA  HA   1   65 VAL  CG* ->
    1.00  1.00  2.70  4.90  YES                ;  Distance restraint information
1   8 HIS  HD2  1   19 THR  HG2* ->
    1.00  1.00  2.00  5.00                     ;  Distance restraint information
1   4 PRO  HB*  1    5 ALA  HN ->
    1.00  1.00  1.95  3.50                     ;  Distance restraint information
1   4 PRO  HB*  1    6 CYS  HN ->
    1.00  1.00  1.95  3.50                     ;  Distance restraint information
1   4 PRO  HD*  1   66 LEU  CD* ->
    1.00  1.00  2.70  6.40                     ;  Distance restraint information
1  20 ASN  HB*  1   43 ALA  HB* ->
    1.00  1.00  2.00  4.50                     ;  Distance restraint information
1   5 ALA  HB*  1    7 VAL  HN ->
    1.00  1.00  1.95  5.00                     ;  Distance restraint information
1  11 ALA  HB*  1   61 VAL  CG* ->
    1.00  1.00  2.70  6.40                     ;  Distance restraint information
1  29 THR  HA   1   51 PHE  CZ ->
    1.00  1.00  2.70  6.40                     ;  Distance restraint information
1  89 ILE  HN   1   76 ARG+ O ->
    0.50  0.50  1.70  2.30 HBOND               ;  Distance restraint information
END
RDDSTC> STOP
```

A.2.10 Dihedral angle restraint file

Applicable phases: All phases

Application: This designates dihedral angle restraint.

Notes:
Within each line, the part after ";" is a comment.
Items in parentheses can be omitted.

Syntax: Designation of dihedral angle restraint is comprised of the following lines.

```
[Dihedral angle restraint information start line  Dihedral angle restraint information line…
Parameter end line  End information line]…
```

Dihedral angle restraint information start line: Designate using the following character strings.
(1) Restraint list designation= "CDIHE> LIST"
(2) Restraint no. designation = "CDIHE> NUMBER"

Parameter end line: Designate the end of the parameter designation line in the following format.
"END"

End information line: Line where first character string is "CDIHE> STOP"

Dihedral angle restraint information: Write as follows, in accordance with the start line.

(1) "CDIHE> LIST" case:

```
Belonging chain no.  Atom designation 1  Atom designation 2  Atom designation 3
Atom designation 4  Lower bound coefficient  Upper bound coefficient
Lower bound angle  Upper bound angle  (List output)
```

(2) "CDIHE> NUMBER" case:

```
Atom no. 1  Atom no. 2  Atom no. 3  Atom no. 4  Lower bound coefficient
Upper bound coefficient  Lower bound angle  Upper bound angle  (List output)
```

※The items above are comprised of the following values

Atom designation ::= Belonging residue no. Atom name

List output ::= "YES" | "NO" (Default is "NO")

```
Example)
CDIHE> LIST                              ;  Restraint list designation start
1   11 CA   11 C   12 N   12 CA ->
1.0  1.0   175.0 -175.0   Y             ;  Dihedral angle restraint information
1   16 CA   16 C   17 N   17 CA ->
1.0  1.0   175.0 -175.0   Y             ;  Dihedral angle restraint information
END
CDIHE> STOP

CDIHE> NUMBER                            ;  Restraint list designation start
77    79    81    93 ->
2.0  2.0  -90.0  -40.0    Y             ;  Dihedral angle restraint information
END
CDIHE> STOP
```

A.2.11 Monitor designation file

Applicable phases: MD

Application: This designates the object of monitoring.

Notes:
Within each line, the part after ";" is a comment.
Items in parentheses can be omitted.
For the keyword, the system recognizes the 4 characters after "MONI>".

Syntax: Monitor designation is comprised of the following lines.

```
[Monitor designation information start line  Monitor designation information line…]…
```

Monitor designation information start line: Designate using the following character strings.

(1) Atom position designation="MONI> COOR"
(2) Distance designation = "MONI> DIST"
(3) Deformation angle designation = "MONI> ANGL"
(4) Dihedral angle designation = "MONI> TORS"

Monitor designation information line: Write as follows, in accordance with the start line.

(1) "MONI> COOR" case:

```
Atom designation 1  (List output)
```

(2) "MONI> DIST" case:

```
Atom designation 1  Atom designation 2  (List output)
```

(3) "MONI> ANGL" case:

```
Atom designation 1  Atom designation 2  Atom designation 3 (List output)
```

(4) "MONI> TORS" case:

```
Atom designation 1  Atom designation 2
Atom designation 3  Atom designation 4  (List output)
```

※The items above are comprised of the following values.

Atom designation ::= Chain no. Residue no. Atom name

List output ::= "YES" | "NO" (Default is "NO")

```
Example)
MONI> COORDINATE                          ; Atom position designation start line
1 3 CA   YES                              ; Monitor designation information
1 3 C    YES                              ; Monitor designation information

MONI> DISTANCE                            ; Distance designation start line
1 1 O   1 4 H   YES                       ; Monitor designation information

MONI> ANGLE                               ; Deformation angle designation start line
1 1 C    1 1 O 1 2 N    YES               ; Monitor designation information

MONI> TORSION                             ; Dihedral angle designation start line
1 1 C   1 2 N   1 2 CA  1 2 C   YES       ; Monitor designation information
1 3 N   1 3 CA  1 3 C   1 4 N   YES       ; Monitor designation information
```

A.2.12 File for designating center of mass alignment of system

Applicable phases: MIN, MD

Application: This designates atoms subject to center of mass alignment.

Notes:
Within each line, the part after ";" is a comment.
Items in parentheses can be omitted.

Syntax: Designation of atoms subject to center of mass alignment is comprised of the following lines.

```
[Start line for information on atoms subject to BESTFIT
 Line of information on atoms subject to BESTFIT... ]...
```

Start line for information on atoms subject to center of mass alignment: Designate using the following character strings.
(1) Atom list designation = "SETBST> LIST"
(2) Atom range designation = "SETBST> RADIUS"

Information on atoms subject to center of mass alignment: Write as follows, in accordance with the start line.
(1) "SETBST> LIST" case:

```
Format  Chain start no.  Chain end no.  Residue start no.  Residue end no.
Atom name designation (List output)
```

(2) "SETBST> RADIUS" case: Write as follows, in accordance with the designation method.
Center atom designation:

```
Format  "ATOM"  Atom no.  Radius lower limit  Radius upper limit
Atom name designation  (List output)
```

Center coordinate designation :

```
Format  "COOR"  x coordinate  y coordinate  z coordinate
Radius lower limit  Radius upper limit  Atom name designation  (List output)
```

※The items above are comprised of the following values

List output ::= "YES" | "NO" (Default is "NO")

Format ::= "FREE" | "FIX"

Atom name designation ::= A wild card (*) can be designated.

```
Example)
SETBST> LIST                              ;  Atom list designation start line
FIX   1      1         1        130        *       YES      ; Atom information
FREE  2     10000      1        1          0*               ; Atom information

SETBST> RADIUS                            ;  Atom range designation start line
FIX   ATOM  100           10.0   20.0    C*    YES          ;Center atom designation
FREE  COOR  0.0 10.0 2.0  10.0   20.0    *                  ; Center coordinate
                                                              designation
```

A.2.13 System GB/SA and ASA parameter specification file

Applicable phases : MIN, MD

Use : Specifies GB/SA and ASA parameters

Items that require special mention :

The result of calculation cannot be guaranteed unless the data of all atoms are set in this file.

Text following ";" on a line is a comment.

Format : GB/SA atom information consists of the following lines.

```
[First GB/SA information line    GB/SA information line...  ]...
```

First GB/SA information line : Specify with the following text strings.

(1) Atom list specification = " SOL> LIST "

(2) Generic name specification = " SOL> ATOM "

GB/SA information line : Write as indicated below depending on the first line.

(1) When " SOL> LIST " is specified :

Specify GB/SA information in a one-to-one list for the input topology.

```
[Unused 20 characters  Hydrogen flag  ASA vdW radius (Å)
ASA atomic solvation parameter (kcal/mol/Å²)
GB/SA atomic solvation parameter (cal/mol/Å²)
[GB vdW radius (Å)  GB scale factor]]...
```

(2) When " SOL> ATOM " is specified :

Set GB/SA information for the specified atom. Wild cards can be used in the atom and residual names.

```
[Atom name  Residual name  Hydrogen flag  ASA vdW radius (Å)
ASA atomic solvation parameter(kcal/mol/Å²)
GB/SA atomic solvation parameter (cal/mol/Å²)
[GB vdW radius (Å)  GB scale factor]]...
```

```
Example)
SOL>LIST
SOL       1   N  LYS1   1.550000 -0.132000  5.400000  1.625000  0.790000
SOL       2   H1 LYS0   0.000000  0.000000  5.400000  1.150000  0.850000
SOL>ATOM
H*  LYS0  0.000000  0.000000  5.400000  1.150000  0.850000
```

A.2.14 Umbrella restraint file

Applicable phase : MD

Use : Specifies Umbrella Potential conditions.

Items requiring special mention :
Text following ";" on a line is a comment.

Format : The Umbrella Potential specification consists of the following lines.

[First Umbrella Potential information line Umbrella Potential information line...]

First Umbrella information line : Specify the potential type with the following text strings.

(1) Filling Potential-Potential (Gauss function)	"FILL> GAUS"
(2) Filling Potential-Potential (single center harmonic oscillator)	"FILL> HAR1"
(3) Filling Potential-Potential (single center linear)	"FILL> LIN1"
(4) Filling Potential-Potential (two center harmonic oscillator)	"FILL> HAR2"
(5) Filling Potential-Potential (two center linear)	"FILL> LIN2"

Umbrella Potential information line :

Function number and applied atom number specification line Atom specification line... Specification lines of structure trajectories...

(1) Function number and applied atom number specification line : Specify the number of functions and number of applied atoms.

Number of functions (central structure) Number of applied atoms

(2)Atom specification line : Write the PDB atom IDs in ascending order.

Atom ID

(3)Structure trajectory specification lines :
The specification of the trajectory of each structure consists of the following

lines.

```
[Potential height line  [Potential spread line]..  [Structure 1 line  [Structure 2 line]]..]..
```

(3-1) Potential height line :

Specify the force constant or height of the potential that is centered on the relevant structure.

```
Force constant (umb%coef) or Gauss function height (umb%weight)
```

(3-2) Potential spread line : Write the spread of the potential that is centered on the relevant structure.

```
CAP radius (ε:epsilon)  or Gauss function width (EllipCoef)
```

(3-3a) Structure coordinate line : Structure 1 line

Write atom coordinates in this structure in PDB format.

(x coordinate = 31 - 38 columns, y coordinate = 39 - 46 columns, z coordinate = 47 - 54 columns)

```
...    x coordinate  y coordinate  z coordinate ...
```

(3-3b) Structure coordinate line : Structure 2 line (using HAR2/LIN2)

Write atom coordinates in this structure in PDB format.

(x coordinate = 31 - 38 columns, y coordinate = 39 - 46 columns, z coordinate = 47 - 54 columns)

```
...    x coordinate  y coordinate  z coordinate ...
```

```
Example)
FILL> GAUS                              ;  Function specification
3  2                                    ;  Number of functions and number of atoms used
86                                      ;  Atom specification line
102
4.00000000000000                        ;  Height centered on structure 1
0.0300000                               ;  Width
0.0300000
ATOM     86  N   VAL A  22     -6.505   3.453   0.990 16.00 -0.38  ;Coordinates of structure 1
ATOM    102  N   VAL A  22     -7.661   3.818  -1.008 16.00 -0.65
5.00000000000000                        ;  Height centered on structure 2
0.0300000                               ;  Width
0.0300000
ATOM     86  N   VAL A  22     -6.405   3.443   0.980 16.00 -0.38  ;Coordinates of structure  2
ATOM    102  N   VAL A  22     -7.561   3.828  -1.018 16.00 -0.65
5.00000000000000                        ;  Height centered on structure 3
0.0300000                               ;  Width
0.0300000
ATOM     86  N   VAL A  22     -6.405   3.443   0.980 16.00 -0.38  ;Coordinates of structure 3
ATOM    102  N   VAL A  22     -7.561   3.828  -1.018 16.00 -0.65
FILL> HAR1                              ;  Function specification
2  1                                    ;  Number of functions and applied number of atoms
86                                      ;  Atom specification line
50.0                                    ;  Force constant centered on structure 1
0.5                                     ;  CAP radius of structure 1
ATOM     86  N   VAL A  22     -6.505   3.453   0.990 16.00 -0.38  ;Coordinates of structure 1
20.0                                    ;  Force constant centered on structure 2
1.0                                     ;  CAP radius of structure 2
ATOM     86  N   VAL A  22     -6.405   3.443   0.980 16.00 -0.38  ;Coordinates of structure 2
FILL> HAR2                              ;  Function specification
1  1                                    ;  Number of functions and applied number of atoms
86                                      ;  Atom specification line
50.0                                    ;  Force constant centered on structures 1 and 2
0.5                                     ;  CAP radius centered on structures 1 and 2
ATOM     86  N   VAL A  22     -6.505   3.453   0.990 16.00 -0.38  ;Coordinates of structure 1
ATOM     86  N   VAL A  22     -6.405   3.443   0.980 16.00 -0.38  ;Coordinates of structure 2
```

A.2.15 Restart file

Applicable phase : MD

Use : Specifies restart information. The auto restart file has the same format as the restart file, is output at the specified times, and is used for rollback when a problem occurs.

Items requiring special mention :

The restart file is a binary file.

The restart file consists of the following lines. The total amount of data varies depending on the calculation conditions.

Format :

Title line (same for all calculation conditions) Atom number line (same for all calculation conditions)
Energy information line (same for all calculation conditions) Atom information line (same for all calculation conditions)
[Atom force information line (only when using Velocity Verlet method or RESPA method)]
[Nose-Hoover method information line (only when using Nose-Hoover method)]
[Rigid body model information line (only when using rigid body model)]
[NPT ensemble information line (only when using NPT ensemble)]
[Multicanonical ensemble information line (only when using multicanonical ensemble)]

Title line : Same for all calculation conditions

Title	: character*80×1

Atom number line : Same for all calculation conditions

Number of atoms	: integer*4×1
Number of free atoms	: integer*4×1

Energy information line: Same for all calculation conditions

Number of loops	: integer*4×1
Simulation time	: real*8×1
Total energy	: real*8×1
Kinetic energy	: real*8×1
Potential energy	: real*8×1

Atom information line : Same for all calculation conditions

Atom coordinates	: real*8×3× Number of atoms
Free atom velocity	: real*8×3× Number of free atoms

Atom force information line : Only when using Velocity Verlet method or RESPA method

Using Velocity Verlet method :

Gradient	: real*8×3× Number of atoms

Using RESPA method :

Gradient (Short)	: real*8×3× Number of atoms
Gradient (Medium)	: real*8×3× Number of atoms
Gradient (Long)	: real*8×3× Number of atoms

Nose-Hoover method information line : Only when using Nose-Hoover method

Coordinates of virtual system	: real*8×3× Number of molecule types
Momentum of virtual system	: real*8×3× Number of molecule types
Velocity of virtual system	: real*8×3× Number of molecule types

Rigid body model information line : Only when rigid body model is used

Rigid body velocity	: real*8×3× Number of rigid bodies	
Rigid body coordinates	: real*8×3× Number of rigid bodies	
Rigid body quaternion	: real*8×3× Number of rigid bodies	
Rigid body angular momentum	: real*8×3× Number of rigid bodies	
Rigid body translational force	: real*8×3× Number of rigid bodies	
Rigid body torque	: real*8×3× Number of rigid bodies	
Rigid body translational force (Short)	: real*8×3× Number of rigid bodies	※
Rigid body translational force (Medium)	: real*8×3× Number of rigid bodies	※
Rigid body translational force (Long)	: real*8×3× Number of rigid bodies	※
Rigid body torque (Short)	: real*8×3× Number of rigid bodies	※
Rigid body torque (Medium)	: real*8×3× Number of rigid bodies	※
Rigid body torque (Long)	: real*8×3× Number of rigid bodies	※

※Only when RESPA method is used.

NPT ensemble information line : Only when using the Andersen method or Parrinello-Rahman method

Using the Andersen method :

Cell size	: real*8 ×1
Piston information	: real*8 ×4
Heat bath information 1	: real*8 ×4
Heat bath information 2	: real*8 ×4

Using the Parrinello-Rahman method :

Cell matrix	: real*8 ×1
Inverse matrix of cell matrix	: real*8 ×1
Format of cell matrix	: real*8 ×1
Piston information	: real*8 ×20
Heat bath information 1	: real*8 ×4
Heat bath information 2	: real*8 ×4

Using the Andersen method or Parrinello-Rahman method :

Virial matrix	: real*8 ×3×3× number of energy types
Virial matrix (Short)	: real*8 ×3×3×number of energy types ※
Virial matrix (Medium)	: real*8 ×3×3×number of energy types ※
Virial matrix (Long)	: real*8 ×3×3×number of energy types ※

※Only when using RESPA method.

Multicanonical ensemble information line: Only when using multicanonical ensemble

Energy of relevant step	: real*8 ×1
Number of loops in sampling interval	: integer*4 ×1
Total number of loops	: integer*4 ×1
Energy histogram of sampling interval	: real*8× number of samples
Total energy histogram	: real*8× number of samples
Scaling factor	: real*8× number of samples
Lower limit of energy range after relevant step	: integer*4 ×1
Upper limit of energy range after relevant step	: integer*4 ×1
Lower limit of energy range of relevant step	: integer*4 ×1
Upper limit of energy range of relevant step	: integer*4 ×1

A.2.16 Rigid body model file

Applicable phase : MD

Use : Specifies molecules and atoms of the rigid body model

Items requiring special mention :

Text following ";" on a line is a comment.
The rigid body model is only effective when Velocity-Verlet is specified ("INTEGR=VELO").
A structure specified in SHAKE/RATTLE cannot be specified as a rigid body model.
The same atoms cannot be specified in multiple rigid body models.
The rigid body model is effective in the MD phase (it cannot be used during Minimize).

Syntax : The rigid body model atom specification consists of the following lines.

[First line of rigid body model [Rigid body molecule specification line Rigid body atom information line]…]…

First line of rigid body model : Specify using a text string as follows.

(1) Specify rigid body atom list = "RIGID> NUM"
(2) Specify rigid body atom list + Model coordinates = "RIGID> COO"

Rigid body molecule specification line : Specify the molecule name

Rigid body atom information line : Write as follows depending on the first line.

(1) For "RIGID> NUM" :

Number of atoms [Relative atom ID...]

(2) For "RIGID> COO" :

Number of atoms [Relative atom ID x coordinate y coordinate z coordinate…

```
Example)
RIGID> NUM                          ;  Specification of rigid body atom list
WAT                                 ;  Rigid body molecule name line
3   1 2 3                           ;  Rigid body atom information line

RIGID> COO                          ;  Rigid body atom list + coordinate specification
WAT                                 ;  Rigid body molecule name line
3       1   0.0 0.1 0.0 ->          ;  Rigid body atom information lines
        2  -0.8 0.4 0.0 ->
        3   0.8 0.4 0.0
```

A.3 Output files

The output files of the structure search engine are shown below.

Item no.	File name	Output phase	Use
#1	MIN energy trajectory	MIN	Energy trajectory in energy minimization
#2	MD energy trajectory	MD	Energy trajectory in MD calculation
#3	Monitor specification trajectory	MD	Trajectory for content of monitor specification file
#4	Total energy data	MD	Total energy (total potential energy)
#5	Coordinate trajectory	MD	Trajectory of atom coordinates
#6	Velocity trajectory	MD	Trajectory of atom velocity

A.3.1 MIN energy trajectory

Output phase: MIN

Description: Energy trajectory with energy minimization

Note:
The MIN energy data file is an 8-bit binary file.

Syntax:

[MIN information + MIN energy information]×Number of output iterations

MIN information :

Number of pertinent loop iterations	: integer*4×1
WORK1	: real*8×1
CPU time	: real*8×1

MIN energy information :

Step length	: real*8×1
Root mean square change	: real*8×1
WORK2	: real*8×1
Energy detailed information※	: real*8×20 (20 : Number of energy types)
Root mean square force	: real*8×1
Number of 1-5 van der Waals	: integer*4×1
Number of 1-5 hydrogen bonds	: integer*4×1
Root mean square deviation	: real*8×1

※ The "WORK1" and "WORK2" fields are currently unused. It is set to 0.0d0.

※ The content of each field of "Energy detailed information" is as indicated on the following page.

Breakdown of energy detailed information:
(1) Potential energy
(2) Bond
(3) Angle
(4) Torsion
(5) Improper torsion
(6) 1-4 van der Waals
(7) 1-4 electrostatic
(8) 1-5 van der Waals
(9) 1-5 electrostatic
(10) 1-5 hydrogen bond
(11) 1-5 van der Waals (No cutoff)
(12) 1-5 electrostatic (No cutoff)
(13) 1-5 hydrogen bond (No cutoff)
(14) Position restraint
(15) Distance restraint
(16) Dihedral angle restraint
(17) Repulsion
(18) CAP restraint
(19) Unused 1
(20) Unused 2
(21) Unused 3
(22) Unused 4
(23) Generalized Born
(24) Accessible Surface Area
(25) Unused 5
(26) Unused 6
(26) Unused 7
(26) Unused 8
(26) Unused 9

A.3.2 MD energy trajectory

Output phase: MD

Description: Energy trajectory with MD calculation

Note:
You can designate whether to set the file in ASCII or binary (4 byte real number format designation or 8 byte real number format designation).

Syntax: The following 3 types are provided depending on the output designation.

(1) ASCII format designation (MNTREN= ASCII)

[MD information line MD energy information line]×Number of output iterations

MD information line:

Number of pertinent loop iterations Simulation time CPU time

MD information line:

Total energy Kinetic energy Temperature Energy detailed information*
"Root mean square force" "Number of 1-5 van der Waals"
"Number of 1-5 hydrogen bonds" "Root mean square deviation"

※ The content of each field of "Energy detailed information" is as indicated on the previous page.

(2) 4 byte real number format designation (MNTREN= SINGLE)

[MD information + MD energy information]×Number of output iterations

MD information :

Number of pertinent loop iterations	: integer*4×1
Simulation time	: real*4×1
CPU time	: real*4×1

MD energy information:

Total energy	: real*4×1
Kinetic energy	: real*4×1
Temperature	: real*4×1
Energy detailed information	: real*4×29 (29 : Number of energy types)
Root mean square force	: real*4×1
Number of 1-5 van der Waals	: integer*4×1
Number of 1-5 hydrogen bonds	: integer*4×1
Root mean square deviation	: real*4×1

(3) 8 byte real number format designation (MNTREN= DOUBLE)

[MD information + MD energy information]×Number of output iterations

MD information:

Number of pertinent loop iterations	: integer*4×1
Simulation time	: real*8×1
CPU time	: real*8×1

MD energy information:

Total energy	: real*8×1
Kinetic energy	: real*8×1
Temperature	: real*8×1
Energy detailed information	: real*8×29 (29 : Number of energy types)
Root mean square force	: real*8×1
Number of 1-5 van der Waals	: integer*4×1
Number of 1-5 hydrogen bonds	: integer*4×1
Root mean square deviation	: real*8×1

A.3.3 Monitor designation trajectory

Output phases: MD

Description: Trajectory for content of monitor designation file

Note:
You can designate whether to set the file in ASCII or binary (4 byte real number format designation or 8 byte real number format designation).

Syntax: The following 3 types are provided depending on the output designation.

(1) ASCII format designation (MNTRTR= ASCII)

[Trajectory information line Data line] × Number of output iterations

Trajectory information line :

Number of pertinent loop iterations Number of atom positions Number of distances between two atoms Number of distortion angles Number of dihedral angles Number of pertinent data items

※ Number of pertinent data items = 3 × Number of atom positions + Number of distances between two atoms + Number of distortion angles + Number of dihedral angles

Data line :

Real number value × Number of pertinent data items

(2) 4 byte real number format designation(MNTRTR= SINGLE)

[Trajectory information + Data] × Number of output iterations

Trajectory information：

Number of pertinent loop iterations	: integer*4×1
Number of atom positions	: integer*4×1
Number of distances between two atoms	: integer*4×1
Number of distortion angles	: integer*4×1
Number of dihedral angles	: integer*4×1
Number of pertinent data items	: integer*4×1

Data：

Data	: real*4×（Number of pertinent data items）

※ Number of pertinent data items＝3×Number of atom positions＋Number of distances between two atoms＋Number of distortion angles＋Number of dihedral angles

(3) 8 byte real number format designation(MNTRTR= DOUBLE)

[Trajectory information＋Data]×Number of output iterations

Trajectory information：

Number of pertinent loop iterations	: integer*4×1
Number of atom positions	: integer*4×1
Number of distances between two atoms	: integer*4×1
Number of distortion angles	: integer*4×1
Number of dihedral angles	: integer*4×1
Number of pertinent data items	: integer*4×1

Data：

Data	: real*8×（Number of pertinent data items）

※ Number of pertinent data items＝3×Number of atom positions＋Number of distances between two atoms＋Number of distortion angles＋Number of dihedral angles

A.3.4 Total energy data

Output phases: MD

Description: Total energy data

Note:
You can designate whether to set the file in ASCII or binary (4 byte real number format designation or 8 byte real number format designation).

Syntax: The following 3 types are provided depending on the output designation.

(1) ASCII format designation (MNTRTO= ASCII)

[Total energy data information line]×Number of output iterations

Total energy information line :

Total energy data

(2) 4 byte real number format designation(MNTRTO= SINGLE)

[Total energy information]×Number of output iterations

Total energy information :

Total energy : real*4×1

(3) 8 byte real number format designation(MNTRCO= DOUBLE)

[Total energy information]×Number of output iterations

Total energy information :

Total energy : real*8×1

A.3.5 Coordinate trajectory

Output phases: MD

Description: Atom coordinate trajectory

Note:
You can designate whether to set the file in ASCII or binary (4 byte real number format designation or 8 byte real number format designation).

Syntax: The following 3 types are provided depending on the output designation.

(1) ASCII format designation (MNTRCO= ASCII)

[Energy information line Atom coordinate information line] × Number of output iterations

Energy information line :

Number of pertinent loop iterations Simulation time CPU TIME Total energy Kinetic energy Temperature Potential energy " Root mean square force " " Number of 1-5 van der Waals "" Number of 1-5 hydrogen bonds " " Root mean square deviation "

Atom coordinate information line :

[x coordinate y coordinate z coordinate] × Number of free atoms

(2) 4 byte real number format designation(MNTRCO= SINGLE)

[Energy information + Atom coordinate information] × Number of output iterations

Energy information :

Number of pertinent loop iterations	: integer*4×1
Simulation time	: real*4×1
CPU TIME	: real*4×1
Total energy	: real*4×1
Kinetic energy	: real*4×1
Temperature	: real*4×1
Potential energy	: real*4×1
Root mean square force	: real*4×1
Number of 1-5 van der Waals	: integer*4×1
Number of 1-5 hydrogen bonds	: integer*4×1
root mean square deviation	: real*4×1

Atom coordinate information :

[Energy information＋Atom coordinate information]×Number of output iterations

(3) 8 byte real number format designation(MNTRCO= DOUBLE)

[Energy information＋Atom coordinate information]×Number of output iterations

Energy information :

Number of pertinent loop iterations	: integer*4×1
Simulation time	: real*8×1
CPU TIME	: real*8×1
Total energy	: real*8×1
Kinetic energy	: real*8×1
Temperature	: real*8×1
Potential energy	: real*8×1
root-mean square force	: real*8×1
Number of 1-5 van der Waals	: integer*4×1
Number of 1-5 hydrogen bonds	: integer*4×1
root-mean square deviation	: real*8×1

Atom coordinate information :

Atom coordinates	: real*8×3×Number of free atoms

A.3.6 Velocity trajectory

Output phases: MD

Description: Atom velocity trajectory

Note:
You can designate whether to set the file in ASCII or binary (4 byte real number format designation or 8 byte real number format designation).

Syntax: The following 3 types are provided depending on the output designation.

(1) ASCII format designation (MNTRVE= ASCII)

```
[ Energy information line  Energy information line]×Number of output iterations
```

Energy information line :

```
Number of pertinent loop iterations  Simulation time  CPU TIME
Total energy  Kinetic energy  TemperaturePotential energy
“Root mean square force”  “Number of 1-5 van der Waals”
“Number of 1-5 hydrogen bonds”  “Root mean square deviation”
```

Energy information line :

```
[x component  y component  z component]×Number of free atoms
```

(2) 4 byte real number format designation(MNTRVE= SINGLE)

```
[ Energy information＋Atom velocity information ]×Number of output iterations
```

Energy information :

Number of pertinent loop iterations	: integer*4×1
Simulation time	: real*4×1
CPU TIME	: real*4×1
Total energy	: real*4×1
Kinetic energy	: real*4×1
Temperature	: real*4×1
Potential energy	: real*4×1
root-mean square force	: real*4×1
Number of 1-5 van der Waals	: integer*4×1
Number of 1-5 hydrogen bonds	: integer*4×1
root mean square deviations	: real*4×1

Atom velocity information :

Atom velocity	: real*4×3×Number of free atoms

(3) 8 byte real number format designation(MNTRVE= DOUBLE)

[Energy information＋Atom velocity information]×Number of output iterations

Energy information :

Number of pertinent loop iterations	: integer*4×1
Simulation time	: real*8×1
CPU TIME	: real*8×1
Total energy	: real*8×1
Kinetic energy	: real*8×1
Temperature	: real*8×1
Potential energy	: real*8×1
root-mean square force	: real*8×1
Number of 1-5 van der Waals	: integer*4×1
Number of 1-5 hydrogen bonds	: integer*4×1
root-mean square deviation	: real*8×1

Atom velocity information :

Atom velocity	: real*8×3×Number of free atoms

B Utilities

B.1 setwater

This adds water to proteins and other systems. Water molecule models that can be created are TIP3P and TIP4P. Before using the tool, copy the water molecule coordinate data (system attachment: tools/setwater/tip3_base.pdb and tip4_base.pdb) to the work directory.

This tool can also add crystal water. To add crystal water, prepare coordinates in PDB format for the extracted crystal water only. For the syntax, use the record name "HETATM" and the residual name "HOH".

Example of crystal water

```
HETATM    1  O   HOH     1       -7.948  -7.948  -7.948
HETATM    2  O   HOH     2       -7.948  -7.948  -4.844
HETATM    3  O   HOH     3       -7.948  -7.948  -1.741
```

【Note】 setwater generates coordinates for solvent water based on the coordinates of water previously brought to equilibrium using periodic boundary conditions, a temperature of 300 K, and a density of 1 g/cm^3. Before using this tool, copy the water molecule coordinate data (system attachment: tools/tip3_base.pdb and tip4_base.pdb) into the work directory.

【Note】 Crystal water is assumed to have hydrogen omitted (oxygen only) in the PDB. If hydrogen is added to oxygen when adding crystal water, the hydrogen is oriented in a fixed direction only.

Input data

(1) Name of PDB file of protein or other system to which water is to be added
(2) Whether or not PDB file for crystal water is used
(3) Name of crystal water PDB file (when crystal water PDB file is used)
(4) Name of PDB output file for results
(5) Shape of cell to which water is added

(6) Radius or length of side of cell to which water is added
(7) Type of center of cell towhich water is added (specify using center of system or any coordinate system)
(8) Coordinates of center of cell to which water is added (when cell center is specified by coordinates)
(9) Density coefficient of water molecules to be added (normally 1.0)
(10) Damping factor of van der Waals radius (normally 1.0)
(11) Water molecule model (TIP3P or TIP4P)

■Usage example

```
% setwater
 --- setwater ---
Input file name (PDB of target molecule) ?
protein.pdb                                                    (1)
  -> none.pdb
Do you use crystal water file (Y or N) ?
Y                                                              (2)
Input file name (PDB of crystal water) ?
crystal_water.pdb                                              (3)
  -> box.pdb
Input file name (output) ?
new_water.pdb                                                  (4)
  -> res
Input cell type (sphere="S", ellipsoid="E", cube="C", parallelepiped="P") ?
E                                                              (5)
Input length (A,B,C) ?
10.0 20.0 30.0                                                 (6)
  -> ellipsoid :   10.0000000000  20.0000000000  30.0000000000
Input center of water (mass center="C", 3D-coordinate="D") ?
D                                                              (7)
Input coordinate (X,Y,Z) ?
10.0 0.0 0.0                                                   (8)
  -> coodinate :   10.0000000000   0.0000000000   0.0000000000
Input density of water (usually 1.0) ?
1.0                                                            (9)
  ->    1.0000000000
Input vdW damping factor (usually 1.0) ?
1.0                                                            (10)
  ->    1.0000000000
Input water model (TIP3P="3", TIP4P="4") ?
3                                                              (11)
  ->    TIP3P
%
```

B.2 mergetpl

This merges multiple topology files into a single topology file.

Input data

(1) Name of topology file to be merged (1st file)
(2) Name of topology file to be merged (2nd file)
(3) Name of topology file to be merged (3rd to 10th file)
(4) Name of resulting topology file to be output

【Note】 If the potential coefficient specifications ("TPL> FUNC" line) and the non-bond interaction specifications ("TPL> NONBOND" line) are different, the resulting merged topology file will not be correct. The contents of the "TPL> FUNC" and "TPL> NONBOND" lines of the topology files initially specified in input are output in the resulting topology file.

【Note】 The above problem frequently occurs when a protein topology file created with tplgene is merged with a low molecule topology file created with tplgeneL, or when topology files for TIP4P model water molecules treated as rigid bodies are merged, or when handling topology files that have been manually modified, and thus Note is particularly required in these cases.

■Usage example

```
% mergetpl
--- mergetpl ---
  Input file name ? ( end: RETURN )
aa.tpl                                          (1)
  Input file name ? ( end: RETURN )
bb.tpl                                          (2)
  Input file name ? ( end: RETURN )
cc.tpl                                          (3)
  Input file name ? ( end: RETURN )

  Output file name ?
output.tpl                                      (4)
 --- done ---
%
```

◆Handling water molecule topology files

The TIP3P model and TIP4P model are provided with the system as water molecule topology files (tools/common/tip3p.tpl and tip4p.tpl). The content of "interaction type" in the non-bond interaction specification ("TPL> NONBOND" line), which corresponds to the "OW" atom, differs between the TIP3P topology file and the TIP4P topology file.

【TIP3P】

```
 18   0   1   1.76830   0.152000   0.833333   0.500
```

【TIP4P】

```
 18   0   1   1.7699    0.155000   0.833333   0.500
```

As such, when using a TIP4P topology file, the value of the part corresponding to "OW" on the "TPL> NONBOND" line must be changed for TIP4P use when merging files (to prevent mixing with TIP3P).

```
  (Omitted)
     :
TPL> NONBONDS
;NUMBER OF TYPE=    39
     1     0  1   1.90800   0.086000   0.8333333   0.500; c
     2     0  1   1.90800   0.109400   0.8333333   0.500; c3
     3     0  1   0.60000   0.015700   0.8333333   0.500; h
     4     0  1   0.00000   0.000000   0.8333333   0.500; ho
     5     0  1   0.60000   0.015700   0.8333333   0.500; hs
     6     0  1   1.48700   0.015700   0.8333333   0.500; hc
     7     0  1   1.38700   0.015700   0.8333333   0.500; h1
     8     0  1   1.28700   0.015700   0.8333333   0.500; h2
     9     0  1   1.18700   0.015700   0.8333333   0.500; h3
    10     0  1   1.10000   0.015700   0.8333333   0.500; hx
    11     0  1   1.45900   0.015000   0.8333333   0.500; ha
    12     0  1   1.40900   0.015000   0.8333333   0.500; h4
    13     0  1   1.35900   0.015000   0.8333333   0.500; h5
    14     0  1   0.00000   0.000000   0.8333333   0.500; hw
    15     0  1   1.82400   0.170000   0.8333333   0.500; n
    16     0  1   1.66120   0.210000   0.8333333   0.500; o
    17     0  1   1.66120   0.210000   0.8333333   0.500; o2
    18     0  1   1.76990   0.155000   0.8333333   0.500; ow  TIP4P
     :
  (Omitted)
```

B.3 SHAKEinp

This creates a SHAKE file that specifies the atom number and restraint distance of the target atom based on a topology file and a PDB file. To specify the TIP3P water molecule model with this tool, the SHAKE file of the TIP3P model (system attachment: tools/SHAKEinp/tip3_shk.model) is required. Before use, copy this file to the work directory. If the SHAKE file does not exist in the work directory, system data is used to output TIP3P information.

Input data

(1) Name of topology file of system for which SHAKE file is to be created
(2) PDB name of system for which SHAKE file is to be created
(3) Name of output SHAKE file
(4) Whether or not TIP3P water molecule model is to be used (only when water molecules are included)

Options

-itpl	<tpl_file>	Specify topology file name in <tpl_file>.
-ipdb	<pdb_file>	Specify PDB file name in <pdb_file>.
-oshk	<shk_file>	Specify SHAKE file name in <shk_file>.
-h		Display method of use of SHAKEinp.

Items specified using command line options are skipped during interactive input. Only items not specified using options are specified by interactive input.

■Usage example

```
% SHAKEinp
Please input TPL filename.
indo_tip3p.tpl                                    (1)
Please input PDB filename.
indo_tip3p.pdb                                    (2)
Please input SHAKE filename.
indo_tip3p.shk                                    (3)

INFORMATION>
        H2O was detected.
        Do you want to use TIP3P model?[yes/no]
yes                                               (4)

INFORMATION> toolWriteTip3p
        The file "tip3_shk.model" is found.
        Information given by this file is used for the Tip3p model.

%% Program is done. %%
%% This program is normal end. %%
```

B.4 RIGIDinp

This creates a specification file for a rigid body model from a topology file. This program obtains information on atoms bonding with hydrogen from the topology file, and performs restraint treating the bonded atom group as a rigid body.

If the TIP3P or TIP4P water molecule model is to be specified as the rigid body, the rigid body specification file for the TIP3P or TIP4P model (system attachment: tools/RIGIDinp/tip3_rig.model or tip4_rig.model) is necessary. Before executing the program, copy this file to the work directory. If restraint is to be performed on any fragment, the fragment information must be entered in the fragment DB file (system attachment: tools/RIGIDinp/fragment.db). Before executing the program, copy this file into the work directory.

Input data

(1) Name of topology file of system for which rigid body model specification file is to be created.

(2) Specification level of rigid body model

(i) Specification only of bonds with hydrogen as a rigid body.

(ii) In addition to (i), specification of any fragment as a rigid body

Options

-i <tpl_file>

Specify the topology file name in <tpl_file>

-l [allH | fr]

Specify the specification level of the rigid body model

(i) Restrain only bonds with hydrogen ⇒ allH

(ii) (i) + fragment restraint ⇒ fr

※If the option " -l" is not specified, only bonds with hydrogen will be restrained.

【Note】 The name of the output rigid body model file will be XXX.rig
(where "XXX" is the name of the topology file without the extension).

■Usage example

```
% RIGIDinp -i indo.tpl
```

The procedure for using RIGIDinp can be viewed by specifying the option "-h" or "-help".

```
% RIGIDinp -h
  or
% RIGIDinp -help
```

B.5 GBSAinp

This creates a GB/SA parameter specification file from a topology file. To create a GB/SA parameter specification file with this utility, the GB/SA parameter DB file is required (system attachment: tools/GBSAinp/gb_sa.db). Before use, copy this file to the work directory.

Input data

（1）Name of GB/SA parameter DB file (system attachment: tools/GBSAinp/gb_sa.db)
（2）Name of topology file of system for which the GB/SA parameter file is to be created.
（3）Name of output GB/SA parameter specification file

■Usage example

```
% mkGBSAin.pl
%% INPUT DB FILE NAME. %%
gb_sa.db
%% SELECT INPUT FILE BY THE NEXT NUMBER.  %%
   1 : PDB FILE
   2 : TPL FILE
2
%% INPUT FILE NAME.  %%
vas-dih.tpl
%% INPUT OUTPUT FILE NAME.  %%
vas-dih.sol
```

B.6 Free energy calculation (Filling potential method + WHAM method) analysis

This creates an Umbrella Potential file that indicates the repulsive potential and centripetal potential in Filled Potential calculation, and analyzes the histogram of the free energy from the coordinate trajectory group and Umbrella Potential analysis results.

B.6.1 Generate_NextFP

This reads the MD coordinate trajectory, the Umbrella Potential file at that MD, and the user specifications, and creates a new Umbrella Potential file.

Input data

(1) Control file
- (1－1) Name of Umbrella Potential specification file at previous MD.
- (1－2) Name of output Umbrella Potential specification file
- (1－3) Initial coordinate PDB file name
- (1－4) Name of coordinate trajectory file at previous MD
- (1－5) Number of coordinate trajectory read skips
- (1－6) Number of coordinate trajectory loadings
- (1－7) Coordinate trajectory file format ("s"ingle | "d"ouble)
- (1－8) PDB file screen display option ("y"es | "n"o)
- (1－9) Centripetal coefficient type
- (1－10) Temperature
- (1－11) Height of Gauss repulsive coefficient
- (1－12) Control range of update interval of center coordinates of Gauss potential
- (1－13) Width of Gauss repulsive coefficient
- (1－14) Height of Centripetal coefficient
- (1－15) Width of Centripetal coefficient
- (1－16) Final target coordinates
- (1－17) Sweep start number, end number

(2) Initial coordinate PDB file

(3) Coordinate trajectory file at previous MD

(4) Umbrella Potential specification file of previous MD input

■Usage example

```
% Generate_NextFP  <  genefp.inp
```

■Example of control file

```
newopt_fp        ; Input Umbrella Potential specification file
newopt_fp2       ; Output Umbrella Potential specification file
initial.pdb      ; Initial coordinates
xx_traject.cor   ; Previous MD trajectory
-1000            ; Number of trajectory read skips
2000             ; Number of trajectory loadings
s                ; Trajectory file format
y                ; PDB file atom display
HAR2             ; Centripetal coefficient type (HAR1 | HAR2 | LIN1 | LIN2)
300.0            ; Applicable temperature
0.5              ; Height of Gauss repulsive coefficient
2.5 6.0          ; Control range of update interval of center coordinates of Gauss
3.0              ; Width of Gauss repulsive coefficient
5.0              ; Height of centripetal coefficient
1.0              ; Width of centripetal coefficient
ATOM  4131  O  WAT  839   0.000  0.000  -8.000 15.00 -0.83 ; Target focus coordinates 1
ATOM  4131  O  WAT  839   0.000  0.000  -8.000 17.00 -0.83 ; Target focus coordinates 2
1 50             ; Sweep start number, end number
```

Output data

(1) Umbrella Potential file

■Example of output Umbrella Potential file

```
FILL> GAUS
       2       1   ; DIMENSION     NUMBER OF ATOMS
       6           ; ATOM ID

      0.0000000    ; WEIGHT DIM=     1
      0.0300000    ; RADIUS DIM=     1

ATOM                            0.000   0.000  -2.000 ; CENTER-1 ATOM=     6


      0.5000000    ; WEIGHT DIM=     2
      3.0000000    ; RADIUS DIM=     2

ATOM                            0.283   0.269  -2.312 ; CENTER-1 ATOM=     6


FILL> HAR1
       1       1   ; DIMENSION     NUMBER OF ATOMS
       6           ; ATOM ID

      0.5000000    ; WEIGHT DIM=     1
      3.0000000    ; RADIUS DIM=     1

ATOM                            0.283   0.269  -2.312 ; CENTER-1 ATOM=     6
```

B.6.2 Extract_Atom

The tool reads the MD coordinate trajectory, the Umbrella Potential file at that MD, and the user specifications, and creates a trajectory file that extracts only the coordinates of the atom subjected to the Umbrella Potential.

Input data

(1) Control file
  (1－1) Name of Umbrella Potential specification file
  (1－2) Number of coordinate trajectory file atoms
  (1－3) Number of coordinate trajectory files
  (1－4) Name of input/output coordinate trajectory file
  (1－5) Number of coordinate trajectory read skips
  (1－6) Number of coordinate trajectory loadings
  (1－7) Format of coordinate trajectory file ("s"ingle | "d"ouble)
(2) Coordinate trajectory file group
(3) Umbrella Potential file of previous MD input

■Example of control file

```
FILL> GAUS
       2       1   ; DIMENSION     NUMBER OF ATOMS
       6           ; ATOM ID

      0.0000000    ; WEIGHT DIM=     1
      0.0300000    ; RADIUS DIM=     1

newopt_fp                       ; Input Umbrella Potential specification file
1023                            ; Number of trajectory file atoms
4                               ; Number of input trajectory files
xx_trj1.cor      w_1.cor        ; Name of input/output trajectory file  - 1
xx_trj2.cor      w_2.cor        ; Name of input/output trajectory file  - 2
xx_trj3.cor      w_3.cor        ; Name of input/output trajectory file  - 3
xx_trj4.cor      w_4.cor        ; Name of input/output trajectory file  - 4
-1000                           ; Number of trajectory read skips
2000                            ; Number of trajectory loadings
s                               ; Trajectory file format
```

■Usage example

```
% Extract_Atom  <  extract.inp
```

B.6.3 Wham_Analysis

This utility calculates the free energy at each trajectory from multiple MD coordinate trajectories and the Umbrella Potential specification file.

Input data

(1) Control file
  (1－1) Name of Umbrella Potential specification file for the last MD
  (1－2) Number of file read skips
  (1－3) Number of samplings (depends on MD calculation and number of extracts)
  (1－4) Precision of loaded file ("s"ingle or "d"ouble)
  (1－5) Radius for calculating mean energy
  (1－6) Number of WHAM analysis iterations
  (1－7) Calculation temperature
  (1－8) Select whether priority is given to memory or speed.
  (1－9) Number of trajectory files
  (1－10) Trajectory file name and Umbrella potential file name
(2) Coordinate trajectory file of each MD
(3) Umbrella Potential file of each MD input

■Example of control file

```
w_4.option  ; Name of Umbrella Potential specification file for the last MD
0           ; Number of file read skips
2000        ; Number of samplings
s           ; Precision of loaded file
0.5         ; Radius for calculating mean energy
1000        ; Number of WHAM analysis iterations
310         ; Calculation temperature
m           ; Select whether priority is given to memory or speed.
4           ; Number of trajectory files
w_1.cor w_1.option  ; trajetcory file and umbrella potential file for the 1st MD
w_2.cor w_2.option  ; trajetcory file and umbrella potential file for the 2nd MD
w_3.cor w_3.option  ; trajetcory file and umbrella potential file for the 3rd MD
w_4.cor w_4.option  ; trajetcory file and umbrella potential file for the 4th MD
```

■Usage example

```
% Wham_Analysis  <  wham.inp
```

Output data

(1) Results of free energy calculation

At the end of standard output, a table showing the trajectory number, RMSD, and free energy is output as shown below. If coordinates do not exist in the specified range, "--------" appears in the free energy column.

■Example of output file

```
FILL> GAUS
       2       1   ; DIMENSION     NUMBER OF ATOMS
       6           ; ATOM ID

INFORMATION> WHAM ANALYSIS RESULT
          EXP-ID R.M.S.D(A)  AVERAGE         FREE-ENERGY
               1  4.000000    0.000000115    0.984069810E+01
               2  4.513558    0.000000103    0.990723128E+01
               3  4.090083    0.000000228    0.942155513E+01
               4  3.778652    0.000000000    -----------
```

B.7 Expanded ensemble analysis tools

These tools are used to analyze the expanded ensemble result.

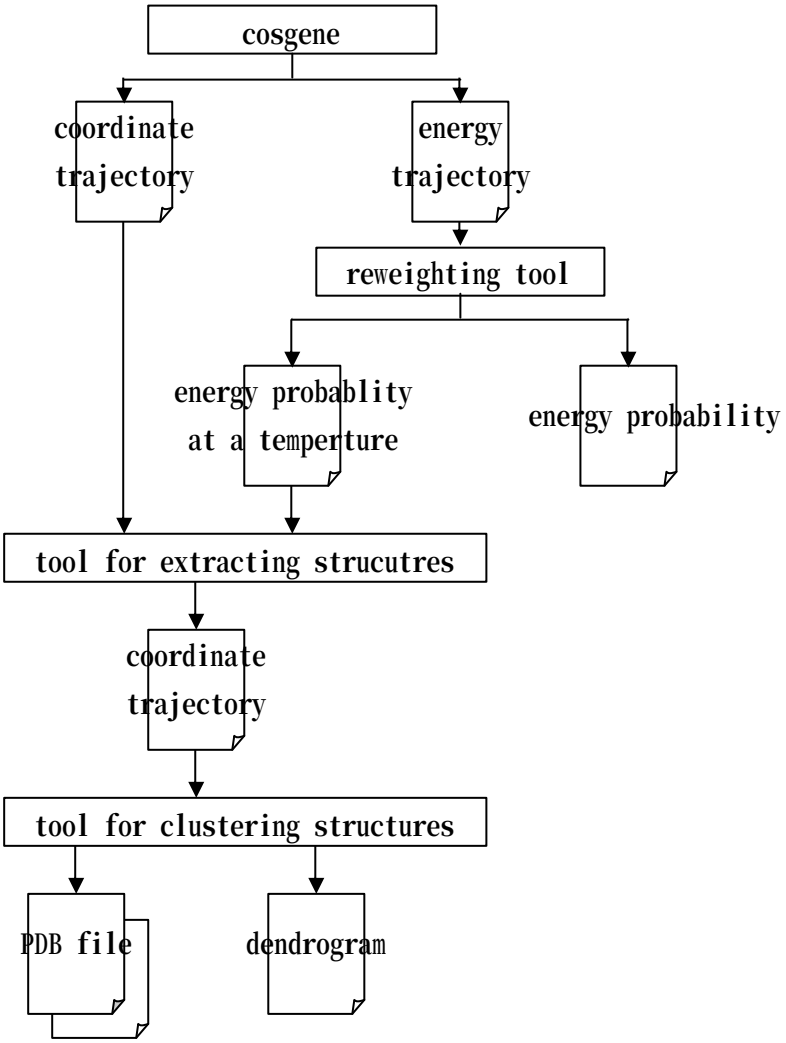
They perform clustering analysis for structures sampled during McMD and output representative structures as PDB format.

These tools consist of a tool for extracting structures and a tool for clustering structures.

(1)tool for extracting structures

This tool extracts strucures from coordinate trajectory file according to energy probability determined by reweighting tool.

(2)tool for clustering structures



B.7.1 reweightFB

This tool reads the force-biased McMD energy trajectory file and user specifications, and creates a file that indicates a new canonical distribution.

Input data

(1) Control file
  (1－1) Name of MD energy trajectory file in force-biased McMD method
  (1－2) Output file name
    (a) Number of loop iterations for convergence and value of relative partition function
    (b) Energy, density, and combined probability
    (c) Canonical distribution of energy at each temperature
    (d) Temperature, mean energy
  (1－3) Histogram parameters
    (a) bin size ((KCAL/MOL)(same as MD value)
    (b) bin number (same as MD value)
    (c) Lower limit of sampling range of energy trajectory
    (d) Upper limit of sampling range of energy trajectory
  (1－4) Temperature and probability density coefficient parameters
    (a) Temperature setting for simulation
    (b) Lower temperature limit (K) of canonical distribution to be output
    (c) Upper temperature limit (K) of canonical distribution to be output
    (d) Interval (K) of canonical distribution to be output
    (e) Lower limit of probability density coefficient for which canonical distribution is to be obtained
(2) MD energy trajectory file in force-biased McMD method

■Example of control file

```
F.B.scale              ; Name of force-biased McMD energy trajectory file
function.dat dencity.dat canonical.dat temperature.dat     ; Output file names
1.0 351 1 44           ; bin size  bin number
                       ; Lower limit of sampling range of energy trajectory
                       ; Upper limit of sampling range of energy trajectory
600 200 800 10 1.d-05  ; Temperature  Lower temperature limit  Upper temperature limit
Temperature step  Lower limit of probability density coefficient
```

■Usage example

```
% reweightFB  <  rew.inp
```

B.7.2 reweightST

This tool reads the Simulated-Tempering MCMD energy trajectory file and the user specifications, and creates a file showing the new canonical distribution.

Input data

(1) Control file

(1－1) Name of MD energy trajectory file in the Simulated-Tempering method.

(1－2) Output file name

(a) Canonical distribution of energy at each temperature

(b) Average energy of each temperature

(1－3) Histogram parameters

(a) bin size of potential (KCAL/MOL) (must be the same as the executed MD value)

(b) bin size of temperature

(c) Lower limit of temperature at which distribution is output

(d) Upper limit of temperature at which distribution is output

(e) Number of temperature divisions

(1－4) Sampling interval

(a) Start of sampling interval

(b) End of sampling interval

(1－5) Initial temperature

(2) MD energy trajectory file in the Simulated-Tempering method ("S.T.energy")

■Example of control file

```
S.T.energy                ; Simulated-Tempering energy trajectory
canonical average         ; Output file name
1.0 100.0 200.0 700.0 6.0 ; Potential bin size  Temperature bin size
                          ; Lower temperature limit Upper temperature limit Number of temperature divisions
0 800                     ; Sampling interval start  Sampling interval end
600.0                     ; Initial temperature
```

Output data

(1) Canonical distribution of energy at each temperature

(2) Mean energy at each temperature

B.7.3 reweightGST

This tool reads the energy trajectory file and user designation of Generalized Simulated-Tempering MCMD and prepares a new file indicating the canonical distribution.

Input data

(1) Control file

(1-1) Filename of MD energy trajectory obtained by Generalized Simulated-Tempering method

(1-2) Output filename

(a) Loop number of convergence loop and partition function of potential

(b) Energy, density and total probability

(c) Canonical distribution of energy by temperature

(d) Temperature, average energy

(1-3) Histogram parameters

(a) Bin size of potential (KCAL/MOL)(combined with executed MD)

(b) Lower limit of λ (combined with executed MD value)

(c) Upper limit of λ (combined with executed MD value)

(d) Number of divisions of λ

(e) Energy reference value by G.S.T. (combined with executed MD value)

(f) η value (combined with executed MD value)

(1-4) Sampling interval

(a) Beginning of sampling interval

(b) End of sampling interval

(1-5) Temperature

(a) Lower limit of temperature for output distribution

(b) Upper limit of temperature for output distribution

(c) Initial temperature

(2) Energy trajectory file by G.S.T.method ("G.S.T.energy")

■Example of control file

```
G.S.T.energy                    ; Energy trajectory by Generalized Simulated Tempering method
partition dencity canonical average   ; Name of output file
1.0 0.001 0.006 10 0.0 4.5      ; binsize, lower limit of λ, upper limit of λ, number of λ bins,
reference energy, η value
200 800 100                     ; Lower temperature limit Upper temperature limit Number of temperature divisions
```

B.7.4 selection

This tool extracts structures from coordinate trajectory file according to energy probability determined by reweighting tool and reconstruct coordinate trajectory at any temperature.

Input data

Input data for this tool are listed below.

(1) coordinate trajectory
coordinate trajectory file of cosgene

(2)Energy probability file
output file of reweighting tool

(3)control file of this tool
(3-1)name of energy probability file
(3-2)name of coordinate trajectory file
(3-3)type of trajectory file(Single | Double)
(3-4)starting point for sampling
(3-5)ending point for sampling
(3-6)coefficient (number of structures extracted is proportional to this)
(3-7)name of output trajectory file
(3-8)number of atoms

Example of control file)

```
pdf.total
ala8.cor_ST
S
0
10000000
100.0
select.cor
32
```

Example of standard output)

```
***** COORDINATE TRAJCETORY SELECT TOOL FOR COSGENE (2005/08/31) *****
   FUNCTION : SELECT TRAJECTORY AND OUTPUT TRAJECTORY FILE

   INPUT :
     (1) ENERGY PROBABILITY DENCTY FUNCTION FILE NAME
     (2) COSGENE TRAJCECTORY FILE NAME
     (3) TRAJECTORY FORMAT
     (4) START LOOP NUMBER
     (5) END LOOP NUMBER
     (6) SELECTION RATE
     (7) OUTPUT TRAJECTORY FILE NAME
   OUTPUT :
     (1)SELECTED TRAJECTORY
 ************************************************************

 INPUT ENERGY PROBABILITY DENCITY FUNCTION FILE NAME
 INPUT TRAJECTORY FILE NAME
 INPUT COORDINATE TRAJECTORY FORMAT ("S"ingle | "D"ouble)
 INPUT START LOOP NUMBER
 INPUT END LOOP NUMBER
 SELECTION RATE (0.0 < RATE
 OUTPUT NEW TRAJECTORY FILE NAME
 ***** SELECT TRAJECTORY RESULT                          *****
  1) DISTRIBUTION
   POTENTIAL-ENERGY  PROBABILITY(%)  TRAJECTORIES   SAMPLES  SAMPLE-RATE(%)
     -0.55000E+01           0.933              0         0   -----
     -0.45000E+01           1.000              1         1  100.000
     -0.35000E+01           0.987              0         0   -----
     -0.25000E+01           0.970              0         0   -----
     -0.15000E+01           1.007              0         0   -----
     -0.50000E+00           1.023              4         4  100.000
      0.50000E+00           1.039              2         2  100.000
      0.15000E+01           1.031              4         4  100.000
      0.25000E+01           1.022             10        10  100.000
      0.35000E+01           0.952             13        12   92.308
      0.45000E+01           0.905             10        10  100.000
      0.55000E+01           0.837             12         9   75.000
      0.65000E+01           0.796             15        11   73.333
      0.75000E+01           0.776             13        11   84.615
    P.D.F. SUM=   99.9852000000000
    STRUCT SUM=         2000
    SAMPLE SUM=         1056

  2) INPUT FILES
       TRAJECTORY FILE        :
         ala8.cor_ST

  3) SELECTION
      TOTAL TRAJECTORY NUMBER :         2000
      SAMPLING BOUND          :            0 -     10000000
      SAMPLING NUMBER         :         2000
      RATE                    :   100.000000000000
      OUTPUT NUMBER           :         1056
      TRAJECTORY FILE         :
         select.cor
 ************************************************************
```

B.7.5 clustering

This tool performs clustering of structures sampled in various expanded ensemble calculations and outputs representative structures as a PDB file.

Input data

(1) Control file
  (1－1) Topology file name
  (1－2) Application of best fit ("Y" | "N")
    (1－2－1) Name of file specifying best fit atoms
  (1－3) Specification of RMSD calculation atoms ("Y" | "N")
    (1－3－1) Name of file specifying RMSD calculation atoms
  (1－4) Number of samples
  (1－5) Number of clusters
  (1－6) Start of sampling interval
  (1－7) End of sampling interval
  (1－8) Input trajectory file name
  (1－9) Trajectory file model ("S" | "D")
  (1－10) Clustering method ("nearest " |"furthest" | "median " | "centroid"
                | "average " | "flexible" | "ward ")
    (1－10－1) β value when flexible is specified
  (1－11) Start of output PDB name
  (1－12) Dendrogram file name
(2) Input topology file for cosgene
(3) cosgene output trajectory file
(4) Best fit atom specification file (when best fit atoms are specified)
    Specify best fit atoms in the same format as the "File for designating center of mass alignment of system" of cosgene. (See "A.2.11 File for designating center of mass alignment of system" on page 161.)
(5) RMSD calculation atom specification file (when RMSD calculation atoms are specified)
    Specify RMSD calculation atoms in the same format as the "File for designating center of mass alignment of system" of cosgene. (See "A.2.11 File for designating center of mass alignment of system" on page 161.)

【Note】

To avoid the need for too much memory, it is best to keep the number of structures sampled to under 1000.

【Note】

It is possible to specify more than 1000 structures for sampling. If insufficient memory is available, the following message will be output and the program will stop.

"CANNOT ALLOCATE MEMORY, DECREASE SAMPLING NUMBER"

■Example of control file

```
ala8.tpl       ; Topology file name
n              ; Use best fit
n              ; Specify RMSD calculation atoms
10             ; Number of samples
10             ; Number of clusters
10             ; Start of sampling interval
40             ; End of sampling interval
ala8.cor_ST    ; Name of input trajectory file
S              ; Trajectory file model
nearest        ; Clustering method
ala8.cls       ; Start of output PDB name
ala8.tree      ; Dendrogram file name
```

■Example of best fit atom specification file

(Example of best fit only on protein atoms excluding hydrogen)

```
SETBST> LIST
 FIX 1    1  1 32 H*  YES  ; "H*" of residuals 1 to 32 of protein (chain 1) excluded from best fit
 FIX 2    2  1  1 *   YES  ; All atoms of ligand (chain 2) excluded from best fit
 FIX 3 1000  1  1 *   YES  ; All atoms of water molecules (chains 3 to 1000) excluded from best fit
```

■RMSD calculation atom specification file

(Example of RMSD calculation only on ligand atoms other than hydrogen)

```
SETBST> LIST
 FIX 1    1  1 32  * YES ; All atoms of residuals 1 to 32 of protein (chain 1) excluded from best fit
 FIX 2    2  1  1 H*  YES ; "H*" of ligand (chain 2) excluded from best fit
 FIX 3 1000  1  1  *  YES ; All atoms of water molecules (chains 3 to 1000) excluded from best fit
```

Output data

(1) Log (output in standard output)
- (1－1) Methods for using tools
- (1－2) Data input inquiries
- (1－3) Clustering conditions
- (1－4) Input topology file information
- (1－5) List of best fit atoms (if specified for display in the best fit atom specification file)
- (1－6) List of RMSD calculation atoms (if specified for display in the RMSD calculation atom specification file)
- (1－7) Status of clustering progress
- (1－8) Output PDB file name

(2) PDB file of representative structures

The cluster number, structure number, energy, and number of loop iterations are output as remarks, and atom information is output. The output file name is "'start of output PDB name'+ '.' + number of loop iterations".

(3) Dendrogram file

Outputs a dendrogram with the number of loop iterations and potential as leaf names.

■Example of PDB file of representative structures

```
REMARK     CLUSTER   :          1
REMARK     STRUCTURE NUMBER:          140
REMARK     LOOP      :       10000
REMARK     POTENTIAL :   176.955627441406
ATOM      1  CA  ACE     1       2.508   1.314  -3.948 12
ATOM      2 HH31 ACE     1       2.771   1.634  -4.954  1.01  0.11
ATOM      3 HH32 ACE     1       2.166   0.280  -3.974  1.01  0.11
ATOM      4 HH33 ACE     1       1.718   1.947  -3.546  1.01  0.11
ATOM      5  C   ACE     1       3.771   1.408  -3.102 12.01  0.60
```

■Example of dendrogram file

```
(
(
"10000 176.96 KCAL/MOL"
,
(
"13000 174.52 KCAL/MOL "
,
"16000 184.61 KCAL/MOL "
)
```

(Continued on next page)

(Continued from previous page)

```
)
,
(
(
"19000 163.18 KCAL/MOL "
,

(
"22000 162.05 KCAL/MOL "
,
"28000 147.56 KCAL/MOL "
)
)
,
"25000 146.70 KCAL/MOL "
)
)
;
```

B.8 Existing probability (Potential Mean Force) analysis tool

The PMF analysis tool inputs one-dimensional or two dimensional data and existing probabilities, and outputs the probability of existence of the data. This tool supports Canonical ensemble and multi canonical ensemble calculation.

The results of PMF tool analysis of two-dimensional data can be processed into a topographical plot for Excel using the topography preparation tool.

B.8.1 pmf

This inputs the monitor designation trajectory file, energy trajectory file, energy probability distribution file (indispensable for multi canonical ensemble) and user designations, and outputs the existence probabilities of the data.

Input data

(1) Control file
- (1－1) MD format ("C"anonical | "M"ulti-canonical)
- (1－2) Number of dimensions of data (1 | 2)
- (1－3) Trajectory data type ("S"ingle | "D"ouble)
- (1－4) Monitor designation trajectory filename
- (1－5) Energy trajectory filename
- (1－6) Upper limit and lower limit of data (total of four for two dimensions)
- (1－7) Number of data bins (total of two for two dimensions)
- (1－8) Output file format ("N" | "S" | "C")
  - N : Total histogram data
  - S : Scatter plot format
  - C : Topography data
- (1－9) Output filename
- (1－10) Energy probability distribution file (indispensable if (1－1) is "M")
- (1－11) Number of samples of scatter plot (indispensable if (1－8) is "S")
- (1－12) Output data type ("P"robability | "E"nergy)
- (1－13) Temperature for converting energy (indispensable if (1－12) is "E")

(2) Monitor designation trajectory file

(3) Energy trajectory file

(4) Energy probability distribution file (indispensable if (1－1) is "M")

■Example of control file

```
C              ; Type of executed MD "C"anonical | "M"ulti-canonical
2              ; Number of structures (1 or 2)
S              ; Monitor specification・Energy trajectory type ("S"ingle | "D"ouble)
aa.tra         ; Monitor specification trajectory filename
aa.ene         ; Energy trajectory filename
-180.0 180.0 -180.0 180.0  ; Upper/lower limit of structure 1  Upper/lower limit of
structure 2
30 30          ; Number of structure divisions
C              ; Output format ("N"ormal | "S"catter-plot | "C"ontour-map)
cont.data      ; Output filename
P              ; Output of probability
```

Output data

If the designation of input data (1－8) is "N", "S" or "C", the following data is respectively output.

(1) In the case of "N"

Prepares histogram in accordance with input data. Prepares histogram data in csv file format.

Each line is configuredwith " lower limit of structure 1, [lower limit of structure 2], probability".

■Example of output

```
 -0.1800000E+03, -0.1800000E+03,  0.2000000E-02
 -0.1680000E+03, -0.1800000E+03,  0.5200000E-02
 -0.1560000E+03, -0.1800000E+03,  0.1160000E-01
```

(2) In the case of "S"

Representative points in the number specified by the user are prepared in accordance with the probability distribution.

Data format is csv file format, as in (1).

（3）In the case of "C"

Prepares data in csv file format with the leading line containing the data matrix number, data lower limit, data range, maximum value, and monitor designation trajectory name. The next line and following lines the express the probability distribution in the form of a matrix.

These lines can be converted into topographical data with the topography preparation tool.

■Output example

```
SIZE= 8 8  LOWER= -180.0 -180.0 BOUND= 360.0 360.0 MAX =  0.312E-01 FILE=aa.tra
0.200E-02,0.520E-02,0.116E-01,0.144E-01,0.240E-02,0.160E-02,0.320E-02,0.200E-02
0.120E-02,0.120E-02,0.200E-02,0.400E-02,0.360E-02,0.800E-03,0.800E-03,0.120E-02
0.800E-03,0.400E-03,0.360E-02,0.400E-02,0.280E-02,0.800E-03,0.800E-03,0.400E-03
```

B.8.2 contour

Inputs the topographical data file prepared with the pmf tool and prepares topographical data in CSV file format.

input data

（1）Control file

（1－1）Input topographical data filename

（1－2）Number of topographies

（1－3）Value of topographies

（1－4）Output topography filename

Output data

（1）Topography file

B.9 pca

Performs clustering of coordinates of specified atoms by principal component analysis and outputs typical structure and principal component analysis results.

input data

(1) Control file
- (1－1) Topology filename
- (1－2) Application of overlap of structure ("Y" | "N")
- (1－3) Overlapped target atom designation filename (indispensable if (1－2) is "Y")
- (1－4) Identification of RMSD calculation target atom ("Y" | "N")
- (1－5) RMSD calculation target atom designation file (indispensable if (1－4) is "Y")
- (1－6) Number of structures to be sampled
- (1－7) Number of clusters
- (1－8) Start of sampling
- (1－9) End of sampling
- (1－10) Coordinate trajectory filename
- (1－11) Coordinate trajectory file type ("S" | "D")
- (1－12) Clustering method ("nearest" | "furthest" | "median" | "centroid" | "average" | "flexible" | "ward")
- (1－13) β value for flexible method (indispensable if (1－12) is "flexible")
- (1－14) Scaling application of principal component ("Y" | "N")
- (1－15) One-axis plot
- (1－16) Two-axis plot
- (1－17) kmax value
- (1－18) Plot data filename

(2) Superposition target atom designation file (when (1－2) is "Y")
(3) RMSD calculation target atom designation file (when (1－4) is "Y")
(4) coordinate trajectory file

output data

(1) Plot data file
(2) Tree diagram file (filename is "pca.tree")
(3) Representative structure PDB file (filename is "pca.*")

■Example of control file

```
ala_ala.tpl    ; topology file name
y              ; use bestfit ("y" | "n")
ala_ala.bst    ; bestfit file name, when use bestfit
y              ; restrict rmsd target  ("y" | "n")
ala_ala.rmsd   ; rmsd target file name, when restrict rmsd target
200            ; sampling number of coordinate
4              ; delegate structure count
0              ; sampling start number
1000           ; sampling last number
select.cor     ; coordinate traJctory file name
s              ; coordinate traJctory file format ( "s" | "d" )
average        ; clustering method name
pca            ; result pdb file prefix
n              ; scale principle comnponent ("y" | "n")
2              ; 1-axis plot data dimension
3              ; 2-axis plot data dimension
30             ; number of clustering elements
pca.plot       ; plot data
```

B.10 Gamess2tplinp

This utility creates input files for tplgeneL from the output file of the GAMESS quantum chemistry calculation program.

Input data

（1）Name of GAMESS output file

Output data

（1）Charge information file (XXX.charge)

（2）Bond order information file (XXX.bond)

（3）Z-matrix information file (XXX.zmat)

【Note】"XXX" in the above is the name of the GAMESS output file with the extension removed.

■Usage method

```
% Gamess2tplinp   methanol.log
```

B.11 Gauss2tplinp

This utility creates input files for tplgeneL from the output file of the quantum chemistry calculation program Gaussian98.

Input data

（1）Name of Gaussian98 output file

Output data

（1）Charge information file (XXX.charge)

（2）Bond order information file (XXX.bond)

（3）Z-matrix information file (XXX.zmat)

【Note】"XXX" in the above is the name of the Gaussian98 output file with the extension removed.

■Usage method

```
% Gauss2tplinp   methanol.out
```

B.12 tpl2mol2

This utility creates and outputs an MDL mol or Sybyl mol2 format file from a topology file and a PDB file. The following options are used to specify the format of the input and output files.

Options

-ipdb <pdbfile>
Specifies the PDB file <pdbfile> as an input file.

-itpl <tplfile>
Specifies the topology file <tplfile> as an input file.

-omol2 <mol2file>
Specifies that the output file will be the Sybyl mol2 file <mol2file>.

-omdl <mdlfile>
Specifies that the output file will be the SD file <mdlfile>.

-h, -help
Displays a help message.

【Note】 The topology file and PDB file specifications are mandatory (options -ipdb and -itpl)

■Usage method

```
% tpl2mol2  -ipdb 2ala.pdb  -itpl 2ala.tpl  -omol2 2ala.mol2  -omdl 2ala.mol
```

B.13 add_ion

This utility calculates the electric field created by the solute (molecules other than solvent water molecules) at the coordinates of each solvent water molecule using the distance-dependant dielectric ($\varepsilon \propto r$), and replaces the water molecules at the highest and lowest potentials with counter ions. This process of calculation and replacement is repeated until the specified number of counter ions have all been placed. Each counter ion is placed a fixed distance (or more) away from the previously placed counter ions.

Input data

1st line : Input file name : Name of coordinate file of entire system to which solvent water molecules have been added.
2nd line : Output file name : Name of coordinate file of entire system
3rd line : Output file name : Name of counter ion coordinate file
4th line : Output file name : Name of coordinate file of solvent water molecules replaced by counter ions.
5th line : Number of Na^+ ions
6th line : Number of Cl^- ions
7th line : When counter ions are successively added, new counter ions are not added within a fixed distance (radius) of previously added counter ions. This is that fixed radius (Å).

■Input example

```
zifcmp.pdb_vac
protein.pdb
ion.pdb
wat.pdb
80
72
6.0
```

■Usage example

Type "add_ion" . Input is from standard input. The input example is ion.input.

```
% add_ion < ion.input
```

【Note】 The arrangement of counter ions added with add_ion is not stable in terms of energy. For this reason, before proceeding to MD calculation of the entire system, perform MD calculation only on the solvent parts (solvent water and counter ion) with the protein and DNA coordinates fixed, so as to bring the solvent parts sufficiently closer to an equilibrium state.

【Note】 The order of the protein, DNA, solvent water, and counter ions must be the same as that in the MOLECULES column of the topology file and in PDB.

B.14 confgene

This utility generates conformers of the input molecule entered in the Sybyl mol2 format file, and creates and outputs PDB files, a Z-matrix file, a RESP input file, and Gaussian input files. Conformers are generated only for parts other than rings by random search. Charge information can be specified by user manual input, or by automatic calculation.

【Note】 Automatic calculation of the charge is not complete. In addition, if the bond order information is not correct, the charge will not be calculated correctly. The order of the atoms can be changed. The order of the atoms will be output without mutual contradiction in the PDB output, RESP output, Gaussian input, and other output files.

Input data

(1) Name of mol2 file of molecule for which you wish to generate conformers.

(2) Total number of conformers that you wish to generate.
If the specified number of conformers does not theoretically exist, or conformers without collisions between atoms cannot be obtained during the fixed number of trials of the random search, the number of conformers generated may be less than the specified number.

(3) Specification of rotation angle (N)
Conformers are generated by rotating rotatable dihedral angles through angles of (360 ÷ N) degrees each.

(4) Total charge of atom.
To calculate automatically, enter "a". If entering a number, enter from the left side with no spaces in front of the number.

(5) Number of atom that will be the starting point for changing the order of the atoms.
Any number less than or equal to the number of atoms included in the molecule. Normally "1" can be used. With this atom as the starting point, the order of the atoms will be changed such that smaller numbers are assigned to closer atoms on the graph.

Output files

(In the following, "N" conformers are generated.)

(1) conf1.pdb to confN.pdb :
Generated conformers. "conf1.pdb" is the same as the input coordinates. A rotation of equivalent atoms such as a methyl group will be counted as a different conformer, and thus

conformers that are chemically the same may be included.

(2) conf1.com to confN.com :

Gaussian input files corresponding to the generated conformers (conf1.pdb to confN.pdb). The execution option is AM1 structure optimization, however, the option for electric field grid generation for RESP calculation is indicated in the comments.

(3) resp.in :

RESP input file

(4) qin :

Initial charge file for RESP. All charges are set to "0".

(5) zmat.dat :

Z-matrix of the initial conformer of the molecule.

■Usage method

```
% confgene
 Input File name (mol2 file)
ligand.mol2                                    (1)
 File =ligand.mol2
 Input number of conformers
2                                              (2)
 no_conf= 2
 Input number of rotation phase(=6:60 deg,=3:120 deg)
3                                              (3)
 no_phase= 3
 Input total charge of the molecule(a=auto calc)
-2                                             (4)
 charge  =-2
 readmol3=ligand.mol2
 numatom 41, 40
 Input start atom number
1                                              (5)
```

B.15 confgenec

Generates the conformation of the input molecule described by a file in Sybyl mol2 format. Prepares and outputs files in Sybyl mol2, MDL mol, or PDB format. Generates the ring structure portion with four or more member rings of conformation. Generates and outputs optical isomers at the same time if a chiral center exists in the molecule.

Input data

Indispensable items

(1) Filename of molecule whose conformation is to be generated

(2) File format of input molecule (1 : Sybyl mol2, 2 : MDL mol, 3 : PDB)

(3) Total number of conformations to be generated

Designate "a" to generate all conformations that can be obtained.

If no conformation number exists in principle or no conformations with interatomic collisions are obtained when conformations are generated, the generated number of conformations may be smaller than designated. If "a" is designated and there are many isomeric forms, a maximum of 999 data items are output.

Options (cannot be specified in interactive format)

(4) Designation of rotation angle (N)

Conformations are generated by rotating a dihedral that can be rotated by (360 ÷ N) degrees. If no designation is made, the process is performed with N=6.

(5) Interatomic check option

When preparing a structure with small interatomic distances, use this option to adjust the coordinates so that no structural overlapping occurs.

Output files

(When N conformations are generated and Sybyl mol2 is designated as the output file format)

(1) confXXX.mol2 : XXX indicates a three digit numerical value from 1 to N.

Generate conformation coordinate output. If MDL mol or PDB is designated for the output file format, the extension will be mol or pdb.

(2) confXXXc.mol2 : XXX is a three digit numerical value from 1 to N.

If c follows the numerical value in the filename, it is an optical isomer. If

MDL mol or PDB is designated for the output file format, the extension will be mol or pdb.

■Instructions for use

```
% confgeneC
 Please select Input File Format by the next number!
   1 : Sybyl mol2 (*.mol2)
   2 : MDL mol (*.mol)
1                                                    (1)

 INFORMATION> toolGetFilename
      Sybyl mol2 input file was selected.

 Please select Output File Format by the next number!
   1 : Sybyl mol2 (*.mol2)
   2 : MDL mol (*.mol)
   3 : PDB pdb (*.pdb)
1                                                    (2)

 INFORMATION> toolGetFilename
      Sybyl mol2 output file was selected.

 Please select Input File Name!
sample.mol2                                          (3)
 Please input number of conformers(a=all pattern).
a                                                    (4)
  input file = sample.mol2

 number of conformers that want to be created = 999
 num of rotation phase = 6

 INFORMATION> toolSetChiralFlg
       This molecule has 3 chiral center(s).

 INFORMATION> toolCountCirc
       Circular structure(s) have found.
 INFORMATION> toolCreateChiralMol
      New coordinates are generated for chiral center "C(3)"
 INFORMATION> toolCreateChiralMol
      New coordinates are generated for chiral center "C(5)"
 INFORMATION> toolCreateChiralMol
      New coordinates are generated for chiral center "C(6)"

 This program creates 11 conformers.

 Program is done normally.
```

B.16 Free energy perturbative method (under development)

Free energy is calculated by the free energy perturbative method with the functions (1) to (3) shown below. (1) is the cosgene function. (2) and (3) are tools.

(1) "vdWparameter and electrical charge" performs scaling of vdWparameter and electrical charge and adds these to output topology data newly generated by scaling.
(2) "Analyzetool" inputs the topology file and coordinate trajectory file in cosgene format and calculates energy by step by using these topology data and coordinate data.
(3) "FEPtool" inputs two energy trajectory files in cosgene format and calculates free energy from these energy data.

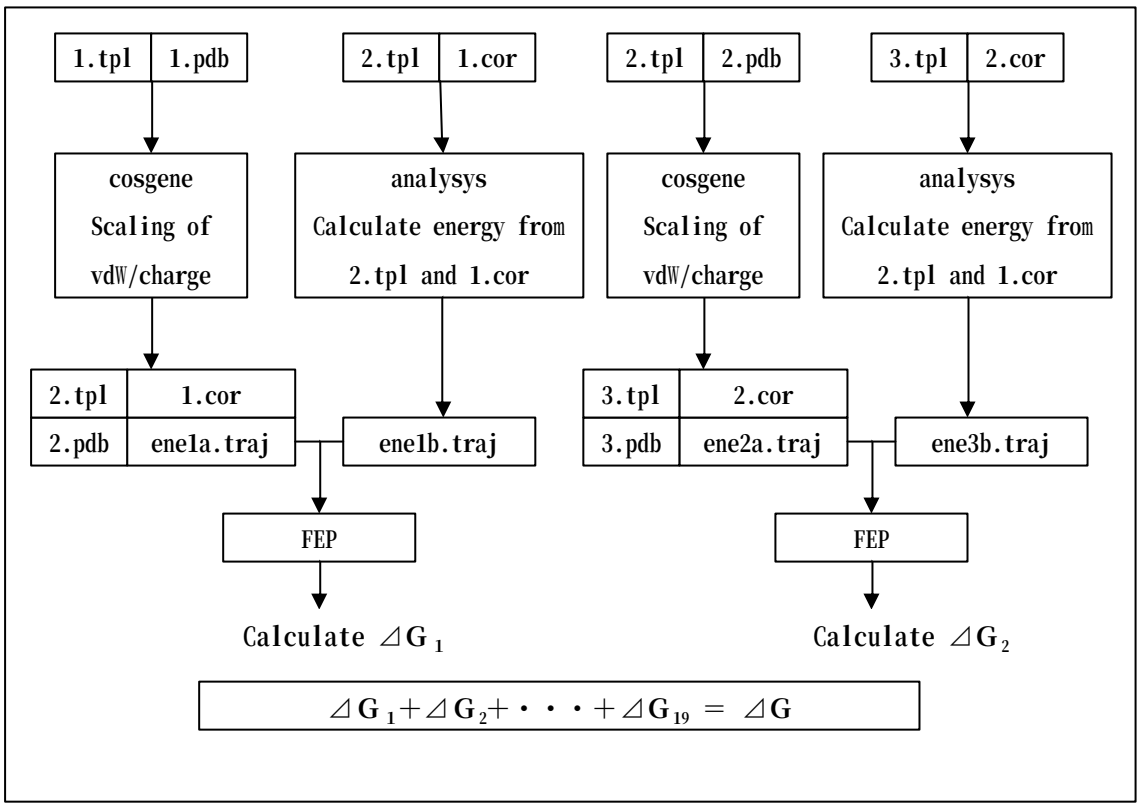
【Note】 The free energy perturbative-related tools are under development. The calculation results are not guaranteed.

B.16.1 Calculation method

Free energy is calculated by the free energy perturbative method using steps (1) to (4) below.

(1) MD is calculated by cosgene. The PDB file, coordinate trajectory file, energy trajectory file, and scaled topology file are output.
(2) Based on the topology file output of (1), energy is calculated in terms of the coordinates in the coordinate trajectory file output for the energy trajectory in (1).
A file is output.
(3) Free energy is calculated using the energy trajectory file obtained in (1) and (2).
(4) Steps (1) to (3) are repeated. Free energy is calculated using the free energy perturbative method by calculating the total sum of each free energy.

【Schematic diagram of free energy perturbative method】



Free energy⊿G is calculated.

B.16.2 vdw parameter and electrical charge scaling function (cosgene)

File-inputs scaling target atom, scaling factor of vdWparameter and scaling factor of electrical charge to cosgene. Performs scaling of vdW parameter and electrical charge of designated atom. Topology data newly generated by scaling is added to the output topology data.

Instructions for use

Designate scaling in the OUTPUT phase of the control file. Enter input instructions for "scaling file" in the INPUT phase. Designate the target atom and scaling factor with "scaling file".

(1) Scaling designation (OUTPUT phase)

Item number	Item	Keyword	Value	Description
#1	Scaling designation	**TPLSCL**	Selection	Scaling of VdW parameter and electrical charge (**NO** | YES)

(2) "Scaling file" input designation (INPUT phase)

Item number	Item	Keyword	Value	Description
#1	Scaling file designation	**SCALIN**	Selection	"Scaling file" designation (**NORE** | FORM)
#2		**UNITSC**	Integer	Device number (**28**)
#3		**NAMTSC**	String	Filename (" ")

(3) "Scaling file" format

Target phase: OUTPUT phase

Application : Designate scaling target atom and scaling factor of VdW parameter and electrical charge.

Format : The scaling file is configured using the following lines.

```
[ Target atom ID  vdW radius scaling factor  Charge scaling factor ]...
```

■Example of use

```
1       0.95d0          0.95d0
6       0.90d0          0.90d0
```

B.16.3 Analyze

Inputs a topology file and coordinate trajectory file to calculate energy in each step. Calculation results are output as a log in cosgene format and as a file in the energy trajectory file format of cosgene.

Input data

(1) Control files

Control files consist of the following groups. Each group ends by "QUIT".

- **EXE> INPUT** group : Indicates input filenames.
- **EXE> MD** group : Indicates energy calculation conditions.

※ The control file format is the same as for the control files of cosgene.

※ Control files of cosgene include the **EXE> MIN** group, the **EXE> ANALYZE** group and the **EXE> OUTPUT** group. If the control file of this tool specifies this information, it will be omitted (error handling is not performed).

(2) Topology file (only for ASCII format)

(3) Coordinate trajectory file (ASCII, SINGLE and DOUBLE are supported)

Specify the input coordinate trajectory file in the INPUT phase of control file as follows:

Item number	Item	Keyword	Value	Description
#1	Coordinate trajectory file designation	**CRDTRJ**	Selection	Coordinate trajectory file designation (**NORE** | ASCI | SING | DOUB)
#2		**UNITCT**	Integer	Device number (29)
#3		**NAMTCT**	String	Filename ("")

Output data

(1) Log (output to standard output in cosgene log format)

(2) Energy trajectory file (ASCII, SINGLE, DOUBLE are supported)

■Instructions for use

```
% analyze < analysis.inp > analysis.log
```

B.16.4 FEP

Inputs two energy trajectory files to calculate free energy from the energy data and outputs the results to standard output.

Input data

(1) Control file
  (1－1) Set the temperature [K]
  (1－2) Threshold of temperature [K]
  (1－3) Free energy calculation loop initial value
  (1－4) Trajectory filename (string, 80 characters or less)
  (1－5) File format of file designated by (1-4)
    ("A"scii | "S"ingle | "D"ouble)
  (1－6) Trajectory filename (80 characters or less)
  (1－7) File format of file designated in (1-6)
    ("A"scii | "S"ingle | "D"ouble)
(2) Energy trajectory file output by cosgene
    (ASCII, SINGLE, DOUBLE are supported)
(3) Energy trajectory file output by analyze
    (ASCII, SINGLE, DOUBLE are supported)

Output data

(1) Free energy (output to standard output)

■Instructions for use

```
% analyze < analysis.inp > analysis.log
```

■Example of FEP tool control file

```
300.0          ; Temperature setting
5.0            ; Threshold value
1              ; Initial loop value
ini.trj        ; Trajectory file name
D              ; File format of ini.trj ("A"scii | "S"ingle | "D"ouble )
fin.trj        ; Trajectory file name
D              ; File format of fin.trj ("A"scii | "S"ingle | "D"ouble )
```

B.17 Hgene

This tool can add H atoms to an input molecule, remove H atoms from the input molecule, calculate Gasteiger charges, and etc. The input/output file format is PDB, MDL mol, Sybyl mol2, and MOPAC dat file format.
The options can be shown by typing "Hgene ?H".

Input file option (*.mdl, *.mol2/*.sm2, *.pdb)

-imdl, -imol2, -ipdb

Output file option

-omdl, -omol2, -opdb, -omopcrt

Options for functions

-h(--hydrogen)
Adding H atoms

-d(--delete-hydrogen)
Removing H atoms

-ch(--charge) [value]
Charge for Mopac dat file

-dc(--default-charge)
Default atomic charge is used

-p(--ph)
Dominant ion form (-COOH → -COO , etc)

-co(--correct-bondtype)
Transforming bond order "ar" to "1" and/or "2"

-ct(--check-totalelec)
Checking the total number of electrons. If the molecule is not closed shell, this tool warns and stops calculation.

■Example of use

```
% Hgene -ipdb vas-dih.pdb  -h -ct -omol2 vas-dih.mol2
% Hgene ?imol2 vas-dih.sm2  -d -omol2 vas-dih.mol2
```

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