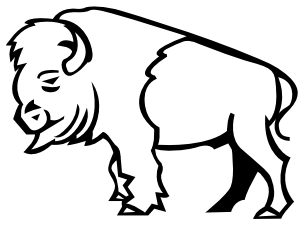
BISEN: Biochemical Simulation Environment

User's Manual
Version 1.0.6



http://bbc.mcw.edu/BISEN

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Chapter 1

Introduction

The Biochemical Simulation Environment (BISEN) software package is a suite of tools for generating equations and associated computer programs for simulating biochemical systems. The Version 1.0 package can be used to generate appropriate systems of differential equations for user-specified multicompartment systems of enzymes and transporters accounting for detailed biochemical thermodynamics, rapid equilibria of multiple biochemical species, and dynamic proton and metal ion buffering. The basic theory and methodology associated with this tool is outlined briefly in chapter 6, but described in some detail in Vinnakota et al. [7]. Biochemical system components are specified using a human read/writeable biochemical scripting language; model outputs are in the form of a MATLAB M-file that computes the differential equations for the systems.

The package was constructed and is maintained and distributed by the computational biology group in the Biotechnology and Bioengineering Center at the Medical College of Wisconsin. The package, updates, and other information can be obtained at the URL http://bbc.mcw.edu/Bisen. When reporting on work that makes use of this package, please cite the following reference:

Vanlier J, Wu F, Qi F, Vinnakota KC, Han Y, Dash RK, Yang F and Beard DA. BISEN: Biochemical Simulation Environment. *Bioinformatics* 25(6):836-837, 2009.

The following sections of this manual provide step-by-step instructions on how to use the package based on tutorial examples at different levels of complexity, and further information on how the package is maintained and updated.

1.1 MATLAB Programming Environment

The current version of the BISEN Package utilizes the propriety MATLAB (Mathworks Inc.) programming environment. Running the package requires that the user have MATLAB installed with a current license. The BISEN package has been tested on MATLAB Release R2008a.

1.2 Setting Paths

All of the files associated with the package must be in the MATLAB path or in the current working directory in order for the model building engine to work. In the archive in which the package is distributed, the package is organized to include seven subdirectories 'Transporters' and 'BiochemicalReactions', in which model modules are stored, 'Warnings', in which error warnings are stored, 'Databases', in which reaction,

transport and reactant databases are stored, 'BuilderFiles', in which builder files are stored, 'ExperimentalData', in which experimental data required by computational simulation are stored,'Examples', in which all the examples are stored. If this file structure is maintained and all files and directories exist in the current working directory, then paths can be set in MATLAB with the following commands.

For Windows-Based Systems:

```
path(path,'..\Warnings');
path(path,'..\BiochemicalReactions');
path(path,'..\Transporters');
path(path,'..\Databases');
path(path,'..\BuilderFiles');
path(path,'..\ExperimentalData');
path(path,'..\Examples');
```

For Linux-Based Systems:

```
path(path,'.\Warnings');
path(path,'.\BiochemicalReactions');
path(path,'.\Transporters');
path(path,'.\Databases');
path(path,'.\BuilderFiles');
path(path,'.\ExperimentalData');
path(path,'.\Examples');
```

Chapter 2

Overview of Package and Description of Files

The BISEN package uses a text parsing algorithm to translate a simple list of biochemical system components into a set of differential equations in a MATLAB M-file. This section provides a reference list of file types used and constructed by the BISEN package. User-constructed input files make use of a Biochemical Scripting Language (BSL), described below in Section 3 along with detailed instructions on how to construct models using the package.

2.1 Biochemical Thermodynamic Data and Reaction and Transporter Stoichiometry

Physicochemical data on biochemical reactants and reactions are stored in the three Excel spreadsheet files listed below. These files provide data on more than 50 reactions and transport processes and over 100 associated biochemical reactants along with their respective references denoted in the cell comments. The reactants and reactions included in these databases define the scope of possible models that can be constructed with the package. Users may add additional reactants and reactions to these databases in order to be able to expand the package's capabilities for their specific applications. When doing so, users are urged to proceed with appropriate care and caution.

ReactantDatabase.xls. This file specifies the basic thermodynamic and ion binding data for biochemical reactants. For each reactant entry the following information is provided:

1. Detailed name of reactant.
2. Abbreviation used for model description.
3. Gibbs free energy of formation for reference species associated with reactant.
4. Enthalpy of formation for reference species.
5. Charge of references species.
6. Number of protons in reference species.
7. First proton dissociation constant, given as pK, the negative of the base-10 logarithm of the dissociation constant.

8. Reaction enthalpy of the first proton dissociation
9. First potassium ion dissociation constant, given as pK.
10. Reaction enthalpy of the first potassium dissociation
11. First magnesium ion dissociation constant, given as pK.
12. Reaction enthalpy of the first magnesium dissociation

The symbol '#' is used to indicate lack of data for a particular entry. When not specified, the pK's are assumed to be infinite (no binding) with corresponding dissociation constants equal to zero. Gibbs energies, enthalpies and dissociation constants are tabulated at 298.15 K, while reactants are tabulated at 0 M ionic strength and dissociation constants at 0.1 M ionic strength. The sources of the constants are given in the cell comments.

ReactionDatabase.xls. This file specifies the reference stoichiometry associated with chemical reactions available in the database. For each reaction, the following information is provided:

1. Detailed name of reaction.
2. Abbreviation used for model description.
3. Stoichiometry of reaction.
4. E. C. Number.

It is important to note three important features regarding the reaction stoichiometries provided in this file: (1.) the specification is for reference reactions, in terms of the reference species defined in the reactant database (ReactantDatabase.xls); (2.) protons (designated by 'H' in the reaction equations) appear explicitly as chemical species in reference reactions; (3.) the reactions use the abbreviations defined in the reactant database (ReactantDatabase.xls). Mismatches between abbreviations used here and those used in the reactant database will lead to errors; (4.) when specifying the reference reaction one should be careful that all the operators, coefficients and reactants are separated by spaces.

TransportDatabase.xls. This file specifies the reference stoichiometry associated with chemical transporters available in the database. For each reaction, the following information is provided:

1. Detailed name of reaction.
2. Abbreviation used for model description.
3. Stoichiometry of reaction.
4. Charge translocated by the transporter.
5. E. C. Number.

Each reference transport reaction involves two compartments. The two compartments are specified in the stoichiometric equation using the identifiers '(1)' and '(2)' following each species abbreviation. For example, the stoichiometric equation

$$\mathrm{ATP(1)} = \mathrm{ATP(2)}$$

indicates that ATP is transported from the first to the second compartment. The charge entry indicates how many charges are transported from compartment 2 to compartment 1. Therefore, for example, the stoichiometric equation

$$\text{ATP}(2) + \text{ADP}(1) = \text{ATP}(1) + \text{ADP}(2)$$

has associated with it a charge translocation value of -1, meaning that one charge is translocated from compartment 1 to 2 every time this transport reaction turns over. This reaction actually involves exchanging an ATP (with charge -4) for an ADP (with charge -3), for an overall transfer of -1 from compartment 2 to 1. Please note that when a transport process occurs at a porous membrane, which is highly permeable to small-size ions, the charge entry for that transport process is adjusted to 0.

When a transporter transports a reactant that is bound to an ion use square brackets to include the ion transport. An example of this is the proton transported along with glutamate in the glutamate/aspartate anti-transporter.

$$[\text{H}](2) + \text{glutamate}(2) + \text{aspartate}(1) = [\text{H}](1) + \text{aspartate}(2) + \text{glutamate}(1)$$

2.2 Model Component Modules for Enzymes and Transporters

The directories 'BiochemicalReactions' and 'Transporters' contain files that provide computational models to simulate the kinetics of individual enzymes and transporters. The format and specifications of these files are described below.

Biochemical Reactions

This directory contains a file for each reaction specified in the reaction database ReactantDatabase.xls. The file names are the reaction abbreviations with the file extension '.txt'. For example, the kinetic model module for the creatine kinase reaction is provided in the text file 'CK.txt'.

Each of these reaction files list the equations required to simulate the kinetics of the enzyme. To account for multiple model versions and allow for nonreacting allosteric species to influence reaction kinetics, the model syntax makes use of a number of keywords. These keywords are listed below:

Keyword	Meaning
model	This keyword indicates the beginning of a new model version for a particular enzyme or transporter. The keyword 'model' is followed by an identifier associated with the model. A few lines of text can optionally be specified to describe the model, as long as they do not contain any keywords.
allosteric_reactants	Reactants that do not appear in the biochemical reaction but may influence the reaction's kinetics must be listed as allosteric reactants following this keyword.
equations	The model equations, using matlab syntax are listed following this keyword.
EOF	Indicates the end of the file

In addition the protected variable names used in the model equations are listed below:

Protected Variable	Meaning
J	This variable is use to specify the flux through the reaction. Each submodel must specify the resulting flux.
Keq	This variable references the apparent equilibrium constant for the reaction or transporter.
unspecified	This keyword, when appearing in the model equations, indicates the introduction of an unspecified model parameter.
Ky_x	This variable indicates the dissociation constant of species X with ion Y. For example Kh_ATP will refer to the proton dissociation constant of ATP.
P_x	This variable indicates the binding polynomial of reactant x. The binding polynomial can be used to compute a specific species concentration.
h, m, k	These variables reference the proton, magnesium and potassium concentration.
V	The volume of this compartment
Reactant Abbreviations	Each reactant concentration is referenced by its abbreviation.

To illustrate how these keywords and protected variable names are applied, consider the following examples.

The file 'CK.txt' contains the following text:

```
model E.CK.0

  equations

    k1_CK = unspecified;
    J = k1_CK*(phosphocreatine*ADP - creatine*ATP/Keq);

model E.CK.1

  equations

    x_CK = 1e7;
    CRtot = 42.7e-3;
    K_CK = exp(50.78/RT);
    Cr_c   = CRtot - phosphocreatine;
    ATP_c1 = ATP * 1/P_ATP; % Mg2+ unbound species;
    ADP_c1 = ADP * 1/P_ADP; % Mg2+ unbound species;
    J  = x_CK * (K_CK*ADP_c1*phosphocreatine*h - ATP_c1*Cr_c );

EOF
```

In this case, there are two possible models of this enzyme, E.CK.0 and E.CK.1, that may be invoked when building a model including creatine kinase. The first one is a simple mass action model, while the second model invokes more complex reaction kinetics.

In model E.CK.0 the apparent rate constant for the reaction 'k1_CK' is a parameter with an unspecified value. Model E.CK.1 invokes no unspecified parameters.

The E.CK.0 model invokes the protected variable name Keq, which the model-builder will recognize as the apparent equilibrium constant for the CK reaction. That means that the variable Keq in the flux expression will be replaced with the apparent equilibrium constant, computed as a function of ionic strength, temperature, pH, and metal ion concentrations in the kinetic model. The second model uses a user-specified equilibrium constant variable K_CK, which is set to a constant value, and therefore not computed using the built-in thermodynamic database or adjusted for the possibly variable biochemical state of the model.

The dissociation constants and binding polynomials can be used to compute specific species. For example, the magnesium bound ATP concentration can be calculated as follows:

```
MgATP = (ATP/P_ATP)*(m/10^(-Km_ATP))).
```

Transporters

The transporter files are similar in terms of structure and keywords. However, in the case of a transporter, each state variable name is followed by a compartment number. In these equations, the compartment number is defined so that (1) refers to the from compartment and (2) refers to the destination compartment.

An example of this is a model for the permeation of glutamate.

```
model T.GLUPERM.0

    equations

        x_GLUPERM = unspecified;
        J = gamma * x_GLUPERM * (glutamate(1) - glutamate(2));

EOF
```

2.3 MATLAB Scripts for Generating Models

The M-files included with the BISEN model-building package are listed in Table 2.1. The file names in italics are files required by the builder. These files are organized in sub-folders in the package distribution and should be in the current path wherever the user chooses to work.

File Name	Description
BuildDXDT.m	This is the main program for parsing a model described in the modular Biochemical Scripting Language (BSL) into a set of differential equations coded in a MATLAB M-file function. The syntax for running this program and the syntax and features of the input and output files are described in Section 3.
setParameter.m	This file can be used for adjusting model constants and setting initial conditions by name rather than index.
generateParameterset.m	This file can be used to generate a human-readable m-file that returns the supplied parameter vector.
validateParameters.m	This file can be used to check a parameter or initial condition vector for validity.
checkPools.m	This file is used for checking the conserved pools of a model.
validateParameters.m	Checks whether the current parameter set is sufficient for the model. It will throw an error when something is missing and identify what is missing.
IndexPHMK.m	Used by the builder to replace binding polynomials and dissociation constants with the correct variables when parsing submodels.
conservationMatrix.m	Computes the conservation matrix
readReactionEntry.m	Reads a reaction submodel and parses the content replacing the equilibrium constant keyword with the equilibrium constant variable name specific for the current reaction. Also substitutes the flux and $\delta\Psi$ variables in a similar manner.
readTransportEntry.m	Similar to readReactionEntry, parses transporter submodel however.
str2array.m	Convert a string into a cell array.
IonTempCorr.m	Corrects Gibbs energy change of reaction for temperature and ionic strength.
convertKi.m	Computes the equilibrium constant from pKa taking into account the desired ionic strength and temperature.
getISCF.m	Computes the factors needed for IonTempCorr.m and computeKi.m
BISENlogo.m	Used for displaying the BISEN logo
appendCompName.m	Used by the builder to append the compartment names to state variables in equations.

Table 2.1: M-files included in the BISEN package

Chapter 3

Creating Biochemical Systems Models

Systems models are specified using the Biochemical Scripting Language (BSL), which facilitates simple flexible and modular specification of complex models.

A BSL file lists the compartments that make up a model, along with the enzymes present in each compartment and transporters that transport mass between compartments. BSL files use several keywords that demarcate different input sections of the file:

Keyword	Meaning
ionic_strength	This keyword is followed by a value which specifies the ionic strength of the solution. If omitted an ionic strength of 0.17 M (estimated from a muscle cell) is assumed. Note that this optional keyword has to be specified at the start of the bsl file.
temperature	This keyword is followed by a value which specifies the experimental temperature in Celsius. If omitted a temperature of 37.0^oC (i.e. 298.15^oK) is assumed. Note that this optional keyword has to be specified at the start of the bsl file.
globals	The first lines after the optional ionic strength and/or temperature setting can be used for parameters that can be accessed by all submodels. Regular matlab code is acceptable in this first section. Here the keyword unspecified, specifies that value will become a model parameter (e.g. Ca = unspecified;).
export	The keyword export can be used at the end of the section where globals are specified to export any internal variables. The model will only export the value that was assigned to the variable last.
compartment	Entries following this keyword define a compartment name and volume followed by a list of the enzymes to be included in the specified compartment.
transport	Entries following this keyword define two linked compartments and list the transporters to be included in the model. Note that all compartments have to be defined before defining transporters.
clamped	After specifying the list of reactions or transporters, in a compartment or transport block certain concentrations can be specified as clamped using this keyword. Clamping a variable means manually setting the time derivative to zero. In the case of a transport field, this will clamp the state variable in both compartments. If the parser is unable to find the value that needs to be clamped it will throw a warning, but continue parsing nontheless.
permeate	This keyword is used to specify species or ions that passively permeate across compartment barriers. These species are not affected by the compartimentalization.
EOF	This keyword indicates the end of a file.

3.1 Example 1: ATP Hydrolysis

As a fist simple example, consider building a one-compartment model including the ATP hydrolysis reaction. A model of this system is specified in the BSL model file 'Example1.bsl', which is distributed with the BISEN Package. This file contains the following entries:

```
compartment  A  1.0 1.0
  ATPASE E.ATPASE.0
EOF
```

Since no commands appear between the start of the file and 'compartment', no global model variables are declared. The 'compartment' command declares a compartment, labeled 'A', with water content 1.0 and fractional region volume 1.0. Following the compartment declaration, one reaction (ATPase) is listed, with the kinetic model E.ATPASE.0 specified.

The ATPASE reaction is found in the Reaction Database, and the model E.ATPASE.0 is specified in the biochemical reaction module ATPASE.txt. The model generating engine is called to parse this BSL

model into a MATLAB ordinary differential equation script using the following syntax.

```
modelInfo = BuildDXDT('Example1.bsl','dXdT.m');
```

The program *BuildDXDT.m* is passed two input strings, specifying the input BSL file and the name of the output M-file that is to be built. In this case, the automatically constructed output M-file *dXdT.m* has the following contents.

```
% function [f,J,] = dXdT(t,x,T,BX,K_BX,par)
%
%
%
% Output parameters:
%   f     time derivatives of the model
%   J     flux
%
%
% Mandatory input parameters:
%   t     time
%   x     state variables at t=0
%   T     temperature in degreesCelcius
%   BX    buffer sizes
%   K_BX  proton buffer dissociation constants ( A )
%   par   parameter vector for the free parameters
%
%
% State Variables:
% [ATP_A , ADP_A , Pi_A ]
%
%
% Free Parameters:
% [k1_ATPASE ]

function [f,J,varargout] = dXdT(t,x,T,BX,K_BX,par)
%% GLOBAL VARIABLES
%% LIST OF STATE VARIABLES
% 1 ATP_A
% 2 ADP_A
% 3 Pi_A
% 4 h_A
% 5 m_A
% 6 k_A

% PARTIAL VOLUME FRACTIONS
VWater_A = 1; % [=] l water (l region)^{-1}, Vinnakota and Bassingthwaighte, AJP, 2004
VRegion_A = 1; % [=] l region (l tissue)^{-1}, Vinnakota and Bassingthwaighte, AJP, 2004
```

```
%% THERMODYNAMIC DATA
RT = 8.314*(T+273.15)/1e3; % kJ  mol^{-1}
F = 0.096484; % kJ mol^{-1} mV^{-1}

%% STATE VARIABLES

% Concentrations of Reference Species
ATP_A = x(1);
ADP_A = x(2);
Pi_A = x(3);
% Concentrations of H, Mg, and K
h_A = x(4);
m_A = x(5);
k_A = x(6);

% Membrane potentials

%% DISSOCIATION CONSTANTS
% ATP_A
Kh(1) = 2.7990983755242071e-007;
Km(1) = 0.00010815244062499601;
Kk(1) = 0.097055055483606045;
% ADP_A
Kh(2) = 4.1856568564882887e-007;
Km(2) = 0.00088211913575503352;
Kk(2) = 0.13114858875318428;
% Pi_A
Kh(3) = 2.1306351186738022e-007;
Km(3) = 0.032137949367841569;
Kk(3) = 0.37888645618434613;

%% BINDING POLYNOMIALS
P( 1 ) = 1  + h_A/Kh(1) + m_A/Km(1) + k_A/Kk(1);
P( 2 ) = 1  + h_A/Kh(2) + m_A/Km(2) + k_A/Kk(2);
P( 3 ) = 1  + h_A/Kh(3) + m_A/Km(3) + k_A/Kk(3);

%% THERMODYNAMIC EQUATIONS
DGro_ATPASE =4.5083;

Keq_ATPASE_A = exp(-DGro_ATPASE/RT)/P(1)*P(2)*P(3)/h_A;

%% FLUX EQUATIONS
%ATPASE_A
k1_ATPASE=par(1);
J_ATPASE_A=k1_ATPASE*(ATP_A-ADP_A*Pi_A/Keq_ATPASE_A);

%% REACTANT TIME DERIVATIVES
```

```
f(1,:) = ( 0  - 1*J_ATPASE_A ) / VWater_A; % ATP_A
f(2,:) = ( 0  + 1*J_ATPASE_A ) / VWater_A; % ADP_A
f(3,:) = ( 0  + 1*J_ATPASE_A ) / VWater_A; % Pi_A

%% ION EQUATIONS
% COMPARTMENT A:
ii = [1  2  3]; % Indices of SVs in compartment A
% PARTIAL DERIVATIVES
pHBpM = -sum( (h_A*x(ii)'./Kh(ii))./(Km(ii).*P(ii).^2) );
pHBpK = -sum( (h_A*x(ii)'./Kh(ii))./(Kk(ii).*P(ii).^2) );
pHBpH = +sum( (1+m_A./Km(ii)+k_A./Kk(ii)).*x(ii)'./(Kh(ii).*P(ii).^2) );
pMBpH = -sum( (m_A*x(ii)'./Km(ii))./(Kh(ii).*P(ii).^2) );
pMBpK = -sum( (m_A*x(ii)'./Km(ii))./(Kk(ii).*P(ii).^2) );
pMBpM = +sum( (1+h_A./Kh(ii)+k_A./Kk(ii)).*x(ii)'./(Km(ii).*P(ii).^2) );
pKBpH = -sum( (k_A*x(ii)'./Kk(ii))./(Kh(ii).*P(ii).^2) );
pKBpM = -sum( (k_A*x(ii)'./Kk(ii))./(Km(ii).*P(ii).^2) );
pKBpK = +sum( (1+h_A./Kh(ii)+m_A./Km(ii)).*x(ii)'./(Kk(ii).*P(ii).^2) );
% PHIs
J_H = (0 + 1*J_ATPASE_A) / VWater_A;
J_M = (0) / VWater_A;
J_K = (0) / VWater_A;
Phi_H = J_H - sum( h_A*f(ii)'./(Kh(ii).*P(ii)) );
Phi_M = -sum( m_A*f(ii)'./(Km(ii).*P(ii)) );
Phi_K = J_K -sum( k_A*f(ii)'./(Kk(ii).*P(ii)) );
% ALPHAs
aH = 1 + pHBpH;
aM = 1 + pMBpM;
aK = 1 + pKBpK;
% ADDITIONAL BUFFER for [H+]
aH = 1 + pHBpH + BX(1)/K_BX(1)/(1+h_A/K_BX(1))^2; % M
% DENOMINATOR
D = aH*pKBpM*pMBpK + aK*pHBpM*pMBpH + aM*pHBpK*pKBpH - ...
    aM*aK*aH - pHBpK*pKBpM*pMBpH - pHBpM*pMBpK*pKBpH;
% DERIVATIVES for H,Mg,K
f(4,:) =  ( (pKBpM*pMBpK - aM*aK)*Phi_H + ...
            (aK*pHBpM - pHBpK*pKBpM)*Phi_M + ...
            (aM*pHBpK - pHBpM*pMBpK)*Phi_K ) / D;
f(5,:) =  ( (aK*pMBpH - pKBpH*pMBpK)*Phi_H + ...
            (pKBpH*pHBpK - aH*aK)*Phi_M + ...
            (aH*pMBpK - pHBpK*pMBpH)*Phi_K ) / D;
f(6,:) =  ( (aM*pKBpH - pKBpM*pMBpH)*Phi_H + ...
            (aH*pKBpM - pKBpH*pHBpM)*Phi_M + ...
            (pMBpH*pHBpM - aH*aM)*Phi_K ) / D;

%% ELECTROPHYS EQUATIONS
%% FLUX VECTOR:
J = [ J_ATPASE_A];
```

This file describes a complete model to simulate ATP hydrolysis in a well-mixed aqueous solution. The function accepts several input variables and outputs the time derivatives of the state variables and the flux for the chemical reaction, as detailed in the function header. For this example BISEN model builder creates a model with six state variables: the three reactant concentrations, ATP, ADP, and Pi, and the three ion concentrations, $[H^+]$, $[Mg^{2+}]$, and $[K^+]$. These state variables and there indices are listed in the comments generated for the model file. (See 'LIST OF STATE VARIABLES'.)

Using one of the built-in ordinary differential equation solvers in MATLAB, it is fairly straightforward to simulate this model. The steps are: (1.) Set buffering constant and biochemical state parameters; (2.) Specify initial values for the state variables; (3.) Run the simulation; and (4.) Plot the results. For this model, to generate the simulations results illustrated in [6], the following commands are used.

```
%% Setting up and running Example 1
% (1.) Setting parameter values (no buffering)
B_X   = 0;      % Additional proton buffering capacity
K_BX = 1e-7;    % Dissociation constant of additional proton buffer
par(1) = 0.1;   % k (kinetic constant for ATP hydrolysis)
T = 25;         % Temperature
% (2.) Setting initial values
xo(1) = 10e-3;  % [ATP] (M)
xo(2) = 0;      % [ADP] (M)
xo(3) = 0;      % [Pi] (M)
xo(4) = 1e-7;   % [H] (M)
xo(5) = 1e-3;   % [Mg] (M)
xo(6) = 150e-3; % [K] (M)
% (3.) Running for 15-second time course
[t,x] = ode45(@dXdT,[0 15],xo,[],T,B_X,K_BX,par);
% (4.) Plotting results
figure(1); p = plot(t,x(:,1)*1e3,'k-',t,x(:,2)*1e3,'k-',t,x(:,5)*1e3,'k-');
set(p,'linewidth',1.5);
text(2,9,'[ATP]','Fontsize',14);
text(2,3.5,'[ADP]','Fontsize',14);
text(5,2.3,'[Mg^{2+}]','Fontsize',14);
ylabel('Concentration (mM)','Fontsize',14);
xlabel('Time (sec)','Fontsize',14);
set(gca,'Fontsize',14);
figure(2); plot(t,-log10(x(:,4)),'k-','linewidth',1.5);
ylabel('pH','Fontsize',14);
xlabel('Time (sec)','Fontsize',14);
set(gca,'Fontsize',14);
```

Note that this script uses the built-in MATLAB ODE integrator function *ode45*, which is reasonable suited to this application. Other integrator functions are available in MATLAB and from third-party sources. More information can be found, for example, by typing *doc ode45* at the MATLAB command prompt.

For debugging purposes, a file with the extension .log is also generated, which describes the computation of the Gibbs energies of reaction and the applied temperature and ionic strength corrections. Here, the values of the indicated variables are denoted between the square brackets and the stoichiometry is

shown between parentheses. This can be helpful when problems arise when new data is added to the reactant database. The initial lines show the untransformed Gibbs energies of formation and the associated stoichiometries of the reactions. These are followed by the ionic strength correction on both the enthalpy change of the reaction and the Gibbs energy change of the reaction. Finally temperature correction is done using the transformed enthalpy.

```
----------------
GIBBS ENERGIES
----------------

Reaction: ATPASE
  0 + (-1)dG_ATP[-2768.100] + (-1)dG_H2O[-237.190] + (1)dG_ADP[-1906.130] +
  (1)dG_Pi[-1096.100] + (1)dG_H[0.000]
  0 + (-1)dH_ATP[-3619.210] + (-1)dH_H2O[-285.830] + (1)dH_ADP[-2626.540] +
  (1)dH_Pi[-1299.000] + (1)dH_H[0.000]
  Ionic strength correction: DrG[3.060000] - beta_G[0.724132] * Q[-2.000000]
                             = 4.508263
  Ionic strength correction: DrH[-21.973592] - beta_H[0.368398] * Q[-2.000000]
                             = -21.236796
  Temp correction: drG = (1-Tn[298.15]/To[298.15]) * drH[-21.2368]
                                                + (Tn[298.15]/To[298.15]) * drG = 4.508263
```

This section is followed by a section outlining the temperature and ionic strength corrections on each of the pK values.

```
-----------------
PKA CORRECTIONS
-----------------

 ATP
   pKH
     Ionic strength correction: pKa_in[6.710] + beta_K[-0.020] * z[8.000] = 6.553
     Ionic strength correction: dHr[-2.000] + beta_H2[0.057] * z[8.000] = -1.544
     Temperature correction: pKa + (1/T_new[298.150]-1/T_old[298.150]) *
                                   drH[-1.544]/2.3026*R = 6.553

     pK = 0.000
   pKM
     Ionic strength correction: pKa_in[4.280] + beta_K[-0.020] * z[16.000] = 3.966
     Ionic strength correction: dHr[-18.000] + beta_H2[0.057] * z[16.000] = -17.088
     Temperature correction: pKa + (1/T_new[298.150]-1/T_old[298.150]) *
                                   drH[-17.088]/2.3026*R = 3.966

     pK = 0.000
   pKK
     Ionic strength correction: pKa_in[1.170] + beta_K[-0.020] * z[8.000] = 1.013
```

```
Ionic strength correction: dHr[-1.000] + beta_H2[0.057] * z[8.000] = -0.544
Temperature correction: pKa + (1/T_new[298.150]-1/T_old[298.150]) *
                              drH[-0.544]/2.3026*R = 1.013

pK = 0.097

...
```

Methods behind these calculations are explained in section 6.5.

Chapter 4

More Examples

This chapter presents two more examples of increasing complexity. This first is a two-compartment systems with two enzymes and two transporters; the second is the large-scale model of mitochondrial metabolism of Wu et al. [9].

4.1 Example 2: A Two-Compartment System with a Membrane Potential

In this section we detail how to build and simulate the model illustrated in Figure 4.1 described in [6].

This model includes two compartments: Two reactions occur in the top compartment, ATP hydrolysis and the creatine kinase (CK) reaction. Protons (H^+) and adenine nucleotides (ATP^{4-} and ADP^{3-}) are transported between the two compartments via the F_1F_0-ATPase and the adenine nucleotide translocase (ANT), which are described in the transporter database.

To build the model, we construct the following BSL file ('Example2.bsl') that defines two compartments, labeled cytoplasm and matrix:

```
compartment  cytoplasm  0.8425 0.4970
  ATPASE  E.ATPASE.0
  CK  E.CK.0

compartment  matrix  0.6514 0.2106

transport   cytoplasm   matrix
  ANT     T.ANT.1
  F1F0ATPASE  T.F1F0ATPASE.0

EOF
```

Again, there are no global model parameters. The first line defines a compartment called 'cytoplasm' with water content 0.8425 mL water/(mL cytoplasm) and fractional volume 0.4970 mL cytoplasm/(mL cardiac tissue). Please note that the users may define water content and volume fraction values in an alternative way, as long as it is consistent with the units of reaction/transport fluxes and reactant concentrations in different compartments. The enzymes ATPASE and CK are specified in this compartment. A second compartment called 'matrix' is defined after the list of enzymes belonging to the compartment

'cytoplasm'. There are no non-transport reactions defined in this compartment. The transport line in the BSL file defines a list of transporters between the cytoplasm and matrix compartments.

```
transport   cytoplasm   matrix
```

The list of transporters that follow the transport declaration defines the ANT and F1F0ATPASE transporters, with kinetic modules T.ANT.1 and T.F1F0ATPASE.0, respectively.

The BSL file can be used to generate an M-file model:

```
ModelInfo = BuildDXDT('Example2.bsl','dXdT.m');
```

The constructed M-file ($dXdT.m$), which is not listed here, has 265 lines. Thus even for a relatively simple model, the utility generating complex code from relatively simple BSL-based declaration is apparent. The generated model can be simulated using the following commands.

```
%% Simulating Example 2
% (1.) Setting parameter values (no buffering)
B_X(1:2)  = 1;      % Buffer capacity in both compartments
K_BX(1:2) = 1e-7;   % Buffer dissoc. constant in both compartments
T = 25;             % Temperature
% Enzyme and transporter activities:
par(1) = 0.1;       % k_ATPase (cytoplasm)
par(2) = 100.0;     % k_CK (cytoplasm)
par(3) = 1e-4;      % k_ANT
par(4) = 1e-3;      % F1F0 ATPase
par(5) = 1e-5;      % membrane capacitance
% (2.) Setting initial values
xo(1)  = 10e-3; % ATP_cytoplasm
xo(2)  = 0; % ADP_cytoplasm
xo(3)  = 0; % Pi_cytoplasm
xo(4)  = 10e-3; % phosphocreatine_cytoplasm
xo(5)  = 10e-3; % creatine_cytoplasm
xo(6)  = 0;     % ATP_matrix
xo(7)  = 10e-3; % ADP_matrix
xo(8)  = 5e-3; % Pi_matrix
xo(9)  = 1e-7; % h_cytoplasm
xo(10) = 1e-3; % m_cytoplasm
xo(11) = 100e-3; % k_cytoplasm
xo(12) = 1e-7; % h_matrix
xo(13) = 1e-3; % m_matrix
xo(14) = 100e-3; % k_matrix
xo(15) = 0;  % DPsi_cytoplasm_to_matrix
% (3.) Running for 15-second time course
[t,x] = ode23s(@dXdT,[0 60],xo,[],T,B_X,K_BX,par);
figure(1); plot(t,x(:,15))
```

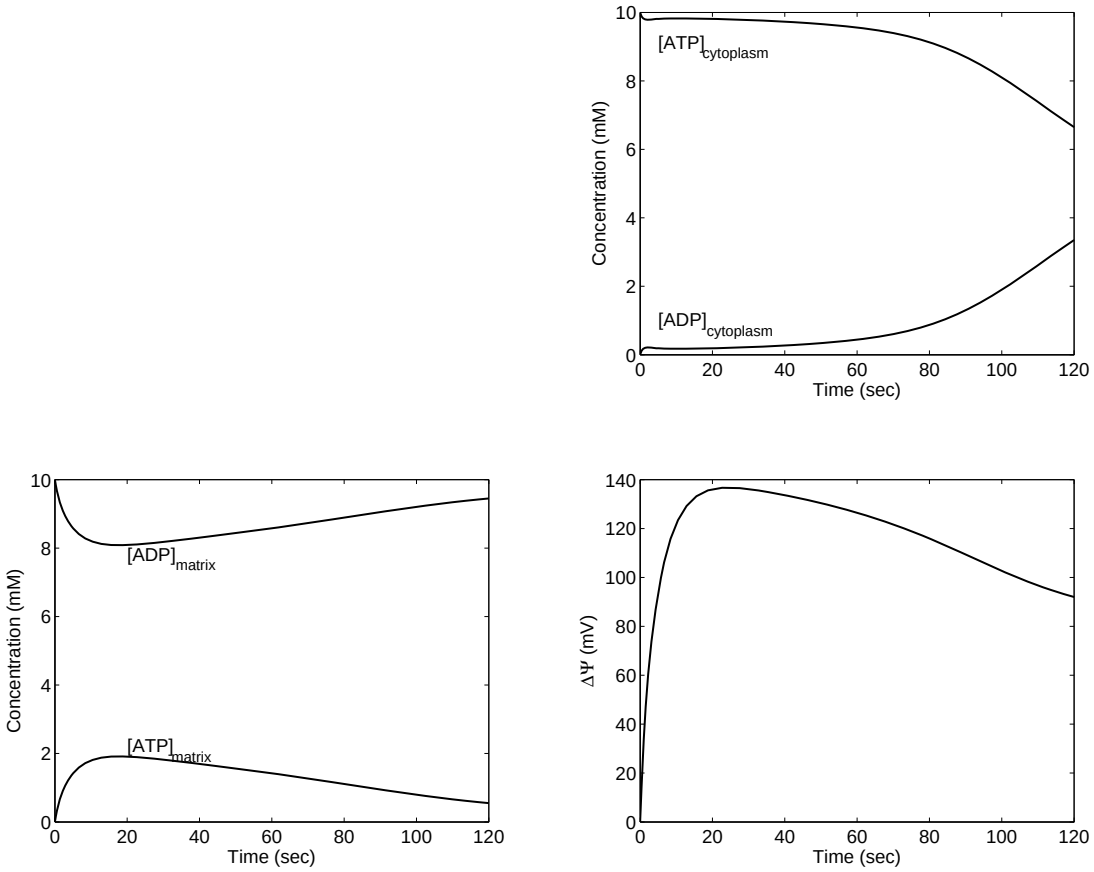

Figure 4.1: Example 2: Top left shows the two-compartment system with ATP hydrolysis, F_1F_0-ATPase, adenine nucleotide translocase (ANT), and creatine kinase (CK). This model is built from the BSL file 'Example2.bls', as described in Section 4.1. Top right and bottom left show the time courses of ADP and ATP in the matrix and cytoplasm respectively. Bottom right shows the time course of the membrane potential.

The simulated timecourses of several of the variables are illustrated in figure 4.1. In the initial state, there is a concentration gradient driving ATP into the matrix and ADP out. An exchange of cytoplasmic ATP for matrix ADP is associated with the reverse operation of the ANT transporter. Subsequent build-up of ATP in the matrix leads to reverse operation of the F1F0-ATPase transporter. (The forward operation direction is defined by the arrow directions in the model diagram in the top left panel of 4.1). ANT and F1F0-ATPase operating in reverse mode both lead to a net transfer of positive charge from the matrix side to the cytoplasmic side of the membrane, resulting in an increase in $\Delta\Psi$, which is defined as the potential difference, cytoplasm potential minus matrix potential. Eventually, as ATP is consumed in the cytoplasm, the membrane potential begins to diminish.

4.2 Example 3: Computer Model of Mitochondrial TCA Cycle and Oxidative Phosphorylation

In this example the BISEN implementation of the large scale computational model of mitochondrial oxidative phosphorylation by Wu et al. [9] is presented. Kinetic modules based on the original paper were constructed and the reaction, reactant and transport databases were set up using thermodynamic data from [9] and [1].

The model contains the following compartments and associated water contents and fractional volumes as shown in Table 4.1. W_c is the ratio of buffer volume to mitochondrial volume and equal to 80 for the LaNoue experiments and 800 for the Bose experiments. An overview of the included reactions is

Compartment	*Water content*	*Fractional volume*
Matrix (matrix)	0.6514 ($\frac{mL_{water}}{mL_{mito}}$)	$1/(1+W_c)$ ($\frac{mL_{mito}}{mL_{total}}$)
Intermembrane Space (IM)	0.0724 ($\frac{mL_{water}}{mL_{mito}}$)	$1/(1+W_c)$ ($\frac{mL_{mito}}{mL_{total}}$)
Cytoplasm/Buffer (cytoplasm)	1.0 ($\frac{mL_{water}}{mL_{buffer}}$)	$W_c/(1+W_c)$ ($\frac{mL_{mito}}{mL_{total}}$)

Table 4.1: Model compartments, water contents and fractional volumes

given in table 4.2, while the transporters are given in tables 4.3 and 4.4. Aside from these reactions, the intermembrane space is permeable to ADP, ATP, AMP, Pi, pyruvate, citrate, alpha-ketoglutarate, malate, succinate, aspartate and glutamate. Since these permeable species all depend on the same mitochondrial membrane area per cell volume ratio, this was set to be a global model parameter in the model. Depending on the actual experimental conditions, the ionic strength and temperature are set at the start of the model file.

Reaction	*Abbrev*	*Stoichiometry*
Pyruvate Dehydrogenase	PDH	pyruvate + COAS + NAD + H2O ⇌ CO2tot + acetylcoA + NADH + H
Citrate Synthase	CTS	acetylcoA + oxaloacetate + H2O ⇌ COAS + citrate + 2 H
Aconitatse	ACON	citrate ⇌ isocitrate
Isocitrate Dehydrogenase	IDH	isocitrate + NAD + H2O ⇌ ketoglutarate + NADH + CO2tot + 2 H
α ketoglutarate dehydrogenase	AKGDH	ketoglutarate + COAS + NAD + H2O ⇌ CO2tot + succinylcoA + NADH + H
Succinyl-CoA synthetase	SCS	succinylcoA + GDP + Pi ⇌ succinate + GTP + COAS + H
Succinate dehydrogenase	SDH	succinate + coQ ⇌ coQH2 + fumarate
Fumarase	FUM	fumarate + H2O ⇌ malate
Malate Dehydrogenase	MDH	malate + NAD ⇌ oxaloacetate + NADH + H
Nucleoside Diphosphokinase	NDK	GTP + ADP ⇌ GDP + ATP
Aspartate Aminotransferase	AAT	ketoglutarate + aspartate ⇌ oxaloacetate + glutamate
Hexokinase	HK	glucose + ATP ⇌ glucose6phos + H
Adenylate kinase	AK	2 ADP ⇌ ATP + AMP

Table 4.2: The chemical reactions

The membrane between intermembrane space and buffer is fully permeable to protons, potassium and magnesium. CO_2 is clamped in the matrix. Magnesium and potassium are clamped in the buffer, which causes them to be clamped in the intermembrane space as well (due to the permeate command). In a similar manner, oxygen is clamped in the entire model.

This leads to the following BSL file for the LaNoue experiments ('Example3_LaNoue.bsl'):

```
temperature 28

  % Global model parameters
```

Name	*Abbreviation*	*Out*	*In*
Pyruvate/H+ co-transporter	PYRH	pyruvate + H	
Glutamate/H+ co-transporter	GLUH	glutamate + H	
Citrate/Malate anti-transporter	CITMAL	citrase	malate
Ketoglutarate/Malate anti-transporter	AKGMAL	malate	ketoglutarate
Malate/Pi MALPI anti-transporter	MALPI	malate	pi
Aspartate/H-Glutamate anti-transporter	ASPGLU	glutamate	aspartate
Succinate/Malate anti-transporter	SUCMAL	succinate	malate
Adenine Nucleoside Translocate	ANT	ADP	ATP

Table 4.3: Transporters that merely transport and do not alter the transported species

Name	*Abbreviation*	*Transport*
Complex I	ETC1	$NADH_{in} + Q_{in} + 5H_{in} \rightleftharpoons NAD_{in} + QH_{2,in} + 4H_{out}$
Complex III	ETC2	$QH_{2,in} + 2cytotox_{out} + 2H_{in} \rightleftharpoons Q_{in} + 2cytocred_{out} + 4H_{out}$
Complex IV	ETC3	$2cytocred_{out} + 0.5O_{2,aq,in} + 4H_{in} \rightleftharpoons 2cytored_{out} + H_2O + 2H_{out}$
F1F0 ATPase	F1F0 ATPase	$ADP_{in} + Pi_{in} + 3H^+_{out} + H_{in} \rightleftharpoons ATP_{in} + H_2O_{out} + 3H_{in}$

Table 4.4: Transporters that do alter the species involved

```
    % mito membrane area per cell volume micron^{-1};
    gamma  = 5.99;

  % minimal parameter value
    MinCon = 0;

compartment     matrix   0.6514 1/81

    PDH   E.PDH.1
    CTS     E.CTS.0
    ACON    E.ACON.0
    IDH     E.IDH.0
    AKGDH   E.AKGDH.0
    SCS     E.SCS.0
    SDH     E.SDH.0
    FUM     E.FUM.0
    MDH     E.MDH.0
    NDK     E.NDK.0
    AAT     E.AAT.0

    clamped CO2tot

compartment     cytoplasm 1 80/81

    HK  E.HK.1

    clamped M
    clamped K
```

```
compartment     im 0.0724   1/81

transport   im matrix

    F1F0ATPASE      T.F1F0ATPASE.0
    PYRH            T.PYRH.0
    GLUH            T.GLUH.0
    CITMAL          T.CITMAL.0
    AKGMAL          T.AKGMAL.0
    MALPI           T.MALPI.0
    SUCMAL          T.SUCMAL.0
    ASPGLU          T.ASPGLU.0
    ETC1            T.ETC1.0
    ETC3            T.ETC3.0
    ETC4            T.ETC4.0
    PIH             T.PIH.1
    KH              T.KH.0
    ANT             T.ANT.0
    HLEAK           T.HLEAK.0

    clamped O2aq

transport   cytoplasm im

    PYRPERM     T.PYRPERM.0
    CITPERM     T.CITPERM.0
    MALPERM     T.MALPERM.0
    AKGPERM     T.AKGPERM.0
    SUCPERM     T.SUCPERM.0
    GLUPERM     T.GLUPERM.0
    ASPPERM     T.ASPPERM.0
    FUMPERM     T.FUMPERM.0
    ICITPERM    T.ICITPERM.0
    ADPPERM     T.ADPPERM.0
    ATPPERM     T.ATPPERM.0
    AMPPERM     T.AMPPERM.0
    PIPERM      T.PIPERM.0

    permeate H
    permeate K
    permeate M

EOF
```

Analogous to [9] datasets from two independent experiments were used to determine the unknown model parameters. The LaNoue et al. [4] data were measured from isolated rat heart mitochondria in

resting (state 2) and active state (state 3) with pyruvate and malate or only pyruvate as substrates. The Bose et al. [3] data were measured from isolated pig heart mitochondria in state 2 and 3, using glutamate and malate as substrates. The temperature and buffer volume are not the same in both experiments therefore both experiments need separate BSL files ('Example3_LaNoue.bsl' and 'Example3_Bose.bsl', respectively). One can build and carry out both models by running the matlab files *Example3_LaNoue_state2.m*, *Exmaple3_LaNoue_state3.m*, and *Example3_Bose_state23.m*.

4.2.1 Interacting with large scale models

Since large scale models tend to involve many parameters, it is wise to use the routines for managing parameters supplied with the BISEN package. When a model is built a MATLAB mat file is also generated. This file contains a structure named modelInfo. This structure relates internal parameters, state variables and flux indices to their respective names in the model by storing their indices. Using the routine *setParameter* enables the user to set and reference model parameters by name rather than index. When the number of parameters of a submodel change, users will not need to shift parameters or initial conditions around since the modelInfo structure changes accordingly.

For example, setting the pyruvate concentration in the buffer to 2 mM can be done as follows:

```
x0 = setParameter( x0, modelInfo.SVarID, 'pyruvate_cytoplasm' ,   2e-3 );
```

Unspecified parameters can be set in an analogous manner. The activity of hexokinase is an unspecified parameter named x_HK in the hexokinase submodel. The following line of code turns off hexokinase activity:

```
params = setParameter( params, modelInfo.ParID, 'x_HK', 0 );
```

If the parameter does not exist, this function will display a warning, but not break execution. Obtaining the value of a specific parameter, flux or state variable can be done manually using the modelInfo structure. For example, obtaining the pyruvate concentration in the cytoplasm from a solution vector x can be done as follows:

```
pyr = x( :, modelInfo.SVarID.pyruvate_cytoplasm );
```

If one wishes to store a whole set of parameters in the form of a human readable M-file the routine *generateParameterSet* can be used. This uses the modelInfo structure, and a user supplied vector to generate a m-file which returns the parameter or initial condition vector as output.

To obtain fluxes one needs to simulate the system in a first step and then use the result of such a simulation to compute the internal fluxes. When additional export parameters are specified in the BSL file, then the values of these during simulation can be acquired in a similar manner. The following code shows how to obtain the complex IV flux. Here t and x are the time vector and state variable matrix (the solution matrix).

```
for a = 1 : length( t )
  [y,J(a,:)] = dXdT( t(a), x(a,:).', T, BX, K_BX, params );
  J_ETC4(a) = J( a, modelInfo.FluxID.ETC4_im_to_matrix );
end
```

The '.mat' file is useful in the sense that one does not need to rebuild the model every time MATLAB is restarted. Simply loading the '.mat' file will supply the user with the modelInfo structure.

4.2.2 Simulating the experiments

To be able to simulate the experiment, the simulation files used in the original paper were ported to the new framework. These are heavily commented and available in the files *Example3_LaNoue_state2.m*, *Exmaple3_LaNoue_state3.m*, and *Example3_Bose_state23.m*. As shown in 4.2, 4.3 and 4.4, the model simulations reproduce both sets of experiments quite well.

Aside from the differences in physicochemical constants, a few notable differences between the two model implementations are listed in table 4.5.

BISEN implementation	**Implementation according to [8]**
pH held constant by large proton buffer	pH clamped
Temperature correction of metabolite Gibbs energy of formation and pKa's done separately for both experimental temperatures	No temperature correction of the metabolite Gibbs energy of formation and pKa's for different experimental temperatures.
Redundant state variables present	Some state variables derived off of total concentration pools.

Table 4.5: Differences between implementations of the model of the TCA cycle and oxidative phosphorylation

4.3 Conserved pools

Reaction stoichiometry imposes a constraint on the system, namely that of conserved pools. Conserved pools always appear as linearly dependent rows in the stoichiometry matrix. Checking the conserved pools can be beneficial to diagnose problems with the model. The absence of expected conserved pools can indicate a problem, while fluctuations of pools that should have been conserved can indicate unadequate solver accuracy.

To be able to check the conserved pools, the conservation matrix of the system is computed in the builder according to the method proposed by [5]. To be able to use this matrix one can use the command *checkPools*. If supplied with the modelInfo matrix, this command gives a list of conserved pools. Supplying the routine with a solution matrix results in an actual estimation of the conserved pool concentration, based on the summation of its constituents.

```
checkPools( modelInfo );
```

For example, after running example 2, one can use the following line of code to inspect the list of conserved pools and their respective time course (which should be constant).

```
checkPools( ModelInfo, x, 1, t );
```

The command, aside from the graphs produced outputs a list of pools and their respective concentration. As shown below, these concentrations correspond to the initial pool concentrations.

```
Pool 1 (Avg value: 0.02, Max change: 3.469e-018)
      phosphocreatine_cytoplasm
      creatine_cytoplasm
Pool 2 (Avg value: 0.01, Max change: 1.735e-018)
      ATP_cytoplasm
      ADP_cytoplasm
Pool 3 (Avg value: 0.01, Max change: 1.735e-018)
      ATP_matrix
      ADP_matrix
Pool 4 (Avg value: 0.025, Max change: 6.536e-015)
      ATP_cytoplasm
```

```
      ATP_matrix
      Pi_cytoplasm
      phosphocreatine_cytoplasm
      Pi_matrix
Max pool change: 6.53644e-015
```

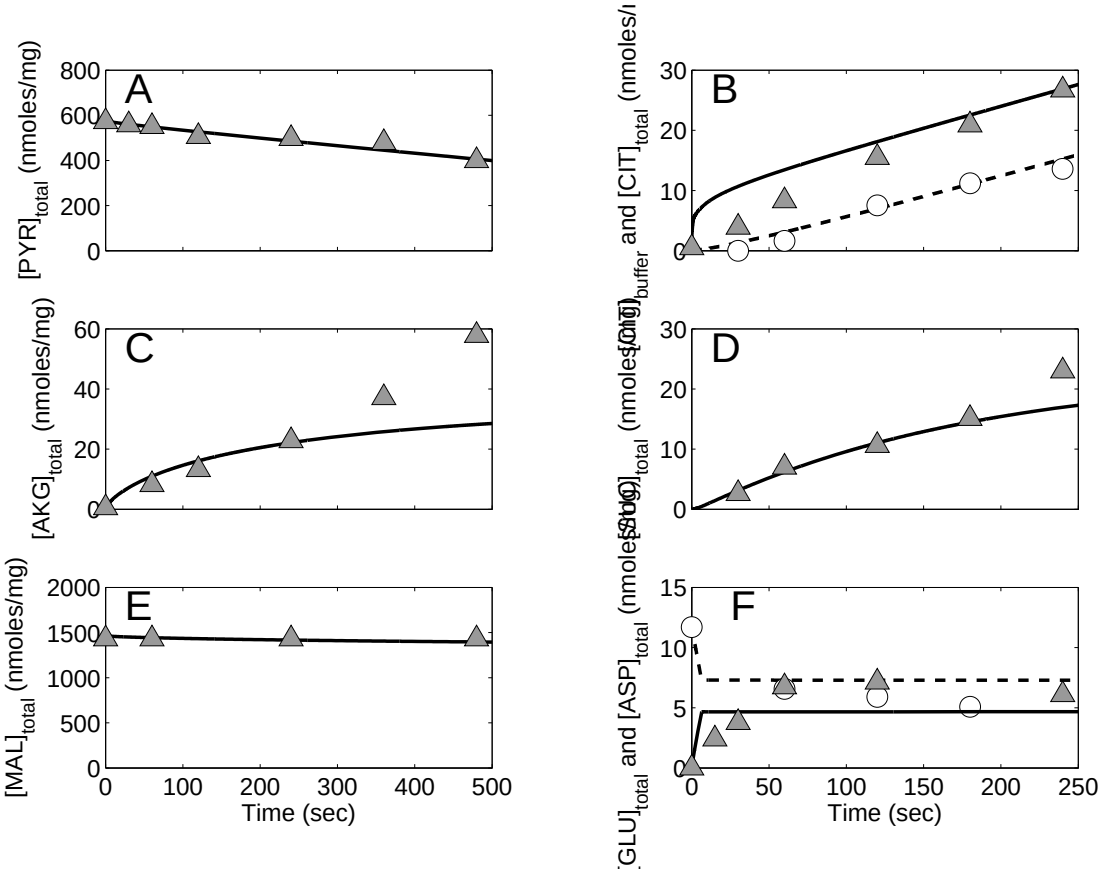

Figure 4.2: **Comparison of model simulations to state-2 kinetic data.** Here measured data points are shown along with model simulations (solid lines).

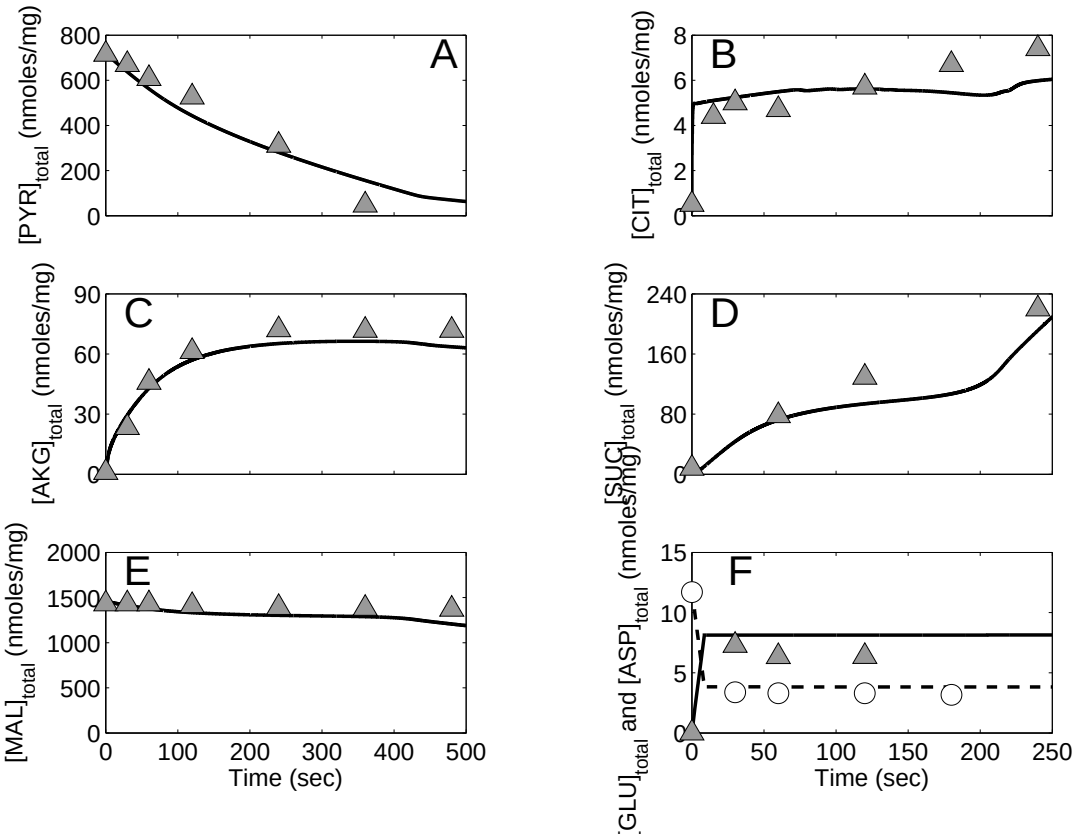

Figure 4.3: **Comparison of model simulations to state-3 kinetic data.** Here measured data points are shown along with model simulations (solid lines).

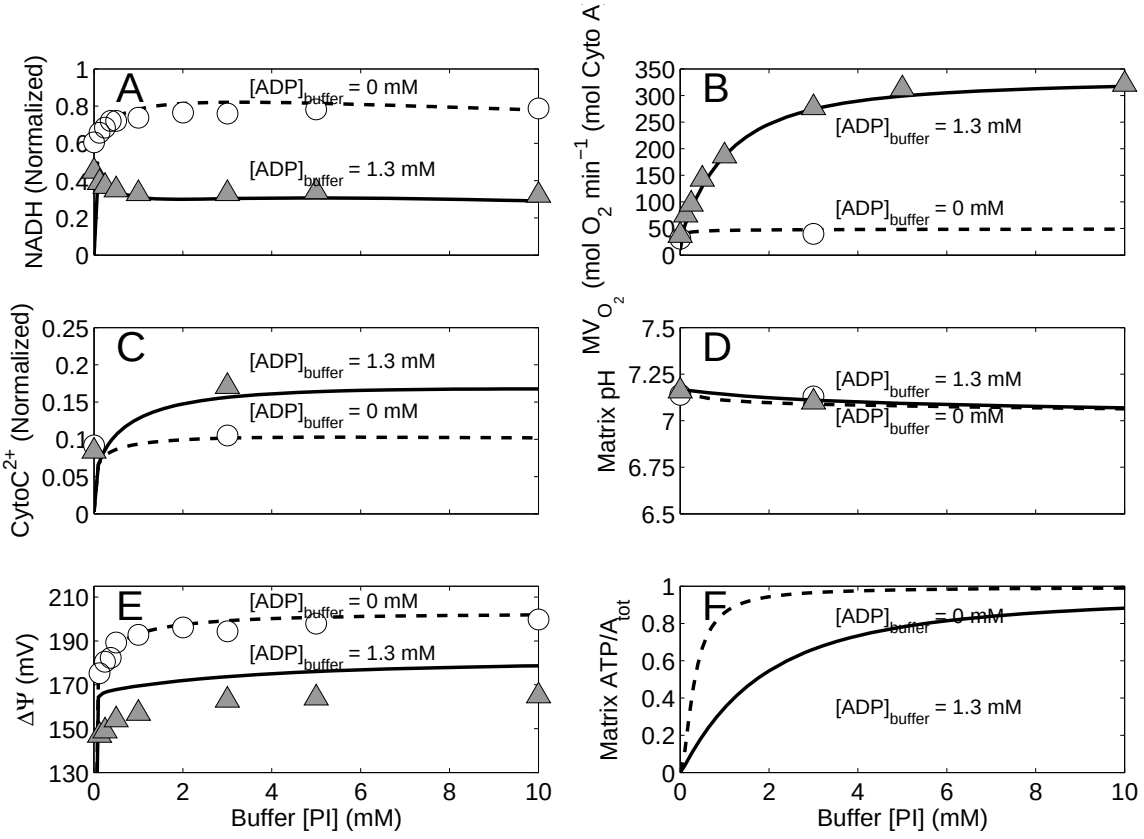

Figure 4.4: **Comparison of model simulations with experimental data on NADH, MV_{O2}, cytochrome c redox state, matrix pH and membrane potential.** Here measured data points are shown along with model simulations (solid lines).

Chapter 5

Further Information

5.1 Where to Get BISEN

BISEN is available from the following location: http://bbc.mcw.edu/BISEN

5.2 Bug Reporting and Tracking

Please report bugs at the following location: http://bbc.mcw.edu/BISEN

5.3 Future Plans

This version of the builder is a first step in the direction of a thermodynamic framework for simulating biochemical reactions. However, there is some work left to be done.

5.3.1 Additional ion binding

Currently, the framework is set up for 3 ions namely protons, magnesium and potassium. In the current version the solution of the linear system of equations for computing the time derivatives of these ions is hard coded. In the future, it could be possible to incorporate an automatic solver for these equations in the builder to automatically compute the ion equations for 4 or more ions. This way Ca^{2+} and Na^{+} could be taken into account. Additional ion binding could be implemented by using the MATLAB symbolic toolbox to generate the actual expressions or by using an implementation of Cramer's rule.

5.3.2 Model reduction

Many models contain conserved pools or moieties. A conserved pool effectively means that one of the variables of the pool is redundant and can be replaced by a linear combination of the other variables. It is worth investigating whether such a reduction in state variables improves computational performance of the generated models.

Furthermore, the 'permeate' command specifies the permeation of two compartments. When this occurs, only one of the state variables actually has a value associated with it. In the current version the redundant state variable is not removed from the state variable vector.

Chapter 6

Thermodynamics of biochemical reactions

This section gives a very brief overview of the thermodynamics involved in biochemical reactions. It is based on the theory in [2], [1] and [7].

6.1 Definitions

To be able to treat the theory unambiguously, the following definitions are used throughout this report. A *reactant* (e.g. ATP) is basically a compound that can be subdivided into *species* with a specific binding (e.g. $MgATP^{2-}$, ATP^{4-}, $HATP^{3-}$ etc). A *biochemical reaction* is a reaction mechanism that is not stoichiometrically balanced in terms of mass or charge (6.1).

$$ATP \rightleftharpoons ADP + PI \tag{6.1}$$

A *reference reaction* however is stoichiometrically balanced (6.2).

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + HPO_4^{2-} + H^+ \tag{6.2}$$

6.2 NPT Ensemble

Biochemical reactions in a biological system are best understood in a constant pressure setting, hence the choice of the NPT ensemble is made. The relative probability of a state in the NPT system is expressed in terms of enthalpy (6.3). Here H represents the enthalpy, T the temperature, P the pressure and μ the chemical potential.

$$dH = TdS - VdP + \mu dN \tag{6.3}$$

The probability law for an NPT system is given by (6.4).

$$P = \frac{e^{-\beta H}}{Z} \tag{6.4}$$

$$Z = \sum_i e^{-\beta H_i} \tag{6.5}$$

The average enthalpy of the NPT system is computed as (6.6). And the Gibbs free energy is defined as G=E-TS+PV or G=H-TS.

$$H = \frac{\sum_i H_i e^{-\beta H_i}}{\sum_i e^{-\beta H_i}} = -\frac{\delta}{\delta\beta} ln[\sum_i e^{-\beta H_i}] = -\frac{\delta}{\delta\beta} lnZ. \tag{6.6}$$

$$H = G - T(\frac{\partial G}{\partial T})_{N,V} = -T^2[\frac{\partial}{\partial T}(\frac{G}{T})]_{N,V} = [\frac{\partial(G/T)}{\partial(1/T)}]_{N,V} \tag{6.7}$$

Using the manipulation briefly outlined in (6.7) it can be derived that the Gibbs free energy can be expressed as (6.8). Hence a system that minimizes the Gibbs free energy, maximizes Z (probability weighted sum of states). NPT systems thrive to decrease G.

$$G = -k_B T lnZ \tag{6.8}$$

A general chemical reaction can be expressed in terms of species A_i and stoichiometric coefficients ν_i. Negative stoichiometric coefficients refer to reactants, while positive ones refer to products. For example for (6.9), the stoichiometric coefficients would be $\nu_1 = -5$, $\nu_2 = 4$ and $\nu_3 = 2$.

$$5A_1 \rightleftharpoons 4A_2 + 2A_3 \tag{6.9}$$

Hence the associated change in Gibbs free energy at constant temperature and pressure would be (6.10) where ϕ represents the number of times the reaction has progressed. Based on this equation, definition (6.11) is made.

$$(dG)_{P,T} = \sum_{i=1}^{N_s} \nu_i \mu_i d\phi \tag{6.10}$$

$$\Delta_r G = (\frac{dG}{d\phi})_{P,T} = \sum_{i=1}^{N_s} \nu_i \mu_i \tag{6.11}$$

If one assumes that the molecules do not interact in such a way that the energy associated with a given conformation of one molecule is influenced by the conformation of another molecule in the system then the following expression can be derived for μ (6.12) leading to the expression shown in (6.13). Using Avogadro's constant this can be expressed as (6.14) where $\Delta_r G^0$ is given by (6.15) where $\Delta_f G_i^0$ is the free energy of formation of species i at a specific temperature, pressure and ionic strength.

$$\mu = \mu^0 + k_B T ln\frac{[C]}{[C_0]} \tag{6.12}$$

$$\Delta_r G = \Delta_r G^0 + \sum_{i=1}^{N_s} \nu_i k_B T ln\frac{[C]_i}{[C]_0} \tag{6.13}$$

$$\Delta_r G = \Delta_r G^0 + \sum_{i=1}^{N_s} \nu_i R T ln\frac{[C]_i}{[C]_0} \tag{6.14}$$

$$\Delta_r G^0 = \sum_{i=1}^{N_s} \nu_i \Delta_f G_i^0 \tag{6.15}$$

At equilibrium $\Delta_r G$ equates to zero, which results in the well known expression (6.16) for the equilibrium constant.

$$K_{eq} = e^{-\Delta_r G^0/RT} = \prod_{i=1}^{N_s} (\frac{[C]_i}{[C]_0})^{\nu_i} \tag{6.16}$$

Fluxes obey the relationship given in (6.17).

$$\frac{J^+}{J^-} = e^{\frac{\Delta G}{RT}} \tag{6.17}$$

6.3 Electrophysiology

Another important phenomenon in biological reactions is the electrostatic potential across cell membranes. The thermodynamic potential of a chemical process with movement of charge across a membrane is given by (6.18). Here z_i denotes the valence of the charged species, ν_i the number of ions transported and $\Delta\Psi$ the electrostatic potential difference between the two compartments separated by the membrane. Furthermore, N_c and k_B are the Coulomb and Boltzmann constants.

$$\Delta\mu = \Delta\mu^0 + \frac{\Delta\Psi}{N_c} \sum_{i \in inside} \nu_i z_i + k_B T \sum \nu_i ln[C]_i \tag{6.18}$$

Similar to the derivation of (6.14), the equation for the Gibbs free energy can be written as (6.19) and the apparent equilibrium becomes (6.20). In this equation F is the Faraday constant which is defined as the Avogadro constant divided by the Coulomb constant.

$$\Delta_r G = \Delta_r G^0 + \sum_{i=1}^{N_s} \nu_i RT ln \frac{[C]_i}{[C]_0} + F\Delta\Psi \sum_{i \in inside} \nu_i z_i \tag{6.19}$$

$$K_{eq} = e^{-\Delta_r G^0/RT - \frac{F\Delta\Psi}{RT}} = \prod_{i=1}^{N_s} (\frac{[C]_i}{[C]_0})^{\nu_i} \tag{6.20}$$

The biological membrane itself is treated as a capacitor with constant capacitance, leading to the following expression for the membrane potential ($\Delta\Psi$) (6.21). This equation assumes a linear current/voltage relationship and can be used to calculate the change in membrane potential using the charge fluxes across the membrane.

$$C_m \frac{d\Delta\Psi}{dt} = -\sum I_{outward} \tag{6.21}$$

6.4 Proton and ion binding

Tabulated versions of the equilibrium constant of a reaction are valid for a specific defined state ($[H_2O = 55.5M$ and $pH = 7]$. These concentrations are implicitly incorporated in the standard Gibbs energies. In an in vitro system however, pH is often not constant. Nor are ion concentrations in the solution. Therefore the framework needs to take proton and ion binding into account.

The state with i protons bound to reactant L shall be referred to as $[LH_i]$. Considering only proton binding, the total concentration of L is given by (6.22) and the concentration of L with just one protonation is given by (6.23). Here K_1 denotes the dissociation constant.

$$[L] = \sum_{i=0}^{N} [LH_i] \tag{6.22}$$

$$[LH_1] = [LH_0] \frac{[H^+]}{K_1} \tag{6.23}$$

Iteratively accounting for N protonations leads to the general equation (6.24).

$$[LH_i] = [LH_0]\frac{[H^+]^i}{\prod_{j\leq i} K_j} \tag{6.24}$$

The total concentration is then given by (6.25), which relates the total concentration with the least protonated form of the reactant. This conversion is known as the binding polynomial. In this framework, the model state variables correspond to the *reactants*, the sum of all species. Therefore the amount of proton-bound ATP can be computed as (6.26), where $[ATP]$ corresponds to the model state variable.

$$[L] = [LH_0](1 + \sum_{i=1}^{N} \frac{[H^+]^i}{\prod_{j\leq i} K_j}) = [LH_0]P_L([H^+]) \tag{6.25}$$

$$[HATP^{3-}] = \frac{[ATP]}{P_{ATP}([H^+])}\frac{[H^+]}{K_H^{ATP}} \tag{6.26}$$

Calculating the K_{eq} for the reaction shown in (6.27) results in expression (6.28) with $\Delta_r G^0$ the Gibbs free energy change for the reaction.

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + HPO_4^{2-} + H^+ \tag{6.27}$$

$$K_{eq} = e^{\frac{-\Delta_r G^0}{RT}} = (\frac{[ADP^{3-}][HPO_4^{2-}][H^+]}{[ATP^{4-}]})_{eq} = (\frac{[ADP][PI][H+]}{[ATP]})_{eq}\frac{P_{ATP}([H^+])}{P_{ADP}([H^+])P_{PI}([H^+])} \tag{6.28}$$

This expression can be written in terms of reactant concentrations (in this case $[ATP]$, $[PI]$ and $[ATP]$, which are state variables) using the binding polynomials. Equation (6.17) can then be used to relate the forward to the backward flux.

$$K_{eq}^{'} = (\frac{[ADP][PI]}{[ATP]})_{eq} = K_{eq}\frac{P_{ADP}([H^+])P_{PI}([H^+])}{[H^+]P_{ATP}([H^+])} \tag{6.29}$$

This method can be extended to account for multiple ion binding by generalizing the binding polynomial to the expression given in (6.30). Here $[M_l^{z_l+}]$ corresponds to any ion.

$$P([H^+], [M_j^{z_j+}]) = 1 + \sum_{i=1}^{N_H} \frac{[H^+]^i}{\prod_{j\leq i} K_j} + \sum_{l}^{n_{ions}} \sum_{i=1}^{N_{M_l}} \frac{[M_l^{z_l+}]^i}{\prod_{j\leq i} K_{M_l,j}} \tag{6.30}$$

To be able to account for proton and ion binding, one needs to have their respective concentrations. To be able to obtain an expression for this concentration one needs to consider mass conservation. The conservation of metal ions can be written as (6.31).

$$[M_{j,total}^{z_j+}] = [M_{j,free}^{z_j+}] + [M_{j,bound}^{z_j+}] + [M_{j,flux}^{z_j+}] \tag{6.31}$$

While the conservation of protons can be expressed as (6.32). Here $[H^+]_{reference}$ denotes the protons present in the reference species and $[H_{flux}^+]$ the flux into the compartment.

$$[H_{total}^+] = [H_{free}^+] + [H_{bound}^+] + [H_{reference}^+] + [H_{flux}^+] \tag{6.32}$$

Differentiation with respect to time results in equation (6.33).

$$0 = \frac{d[H_{free}^+]}{dt} + \frac{d[H_{bound}^+]}{dt} + \frac{d[H_{reference}^+]}{dt} + \frac{d[H_{flux}^+]}{dt} \tag{6.33}$$

Using the chain rule, the second term can be expanded into (6.34) leading to an equation depending on both the cation as well as the species derivatives.

$$\frac{d[H^+_{bound}]}{dt} = \frac{\partial[H^+_{bound}]}{\partial[H^+]}\frac{d[H^+]}{dt} + \sum_j \frac{\partial[H^+_{bound}]}{\partial[M_j^{z_j+}]}\frac{d[M_j^{z_j+}]}{dt} + \sum_i \frac{\partial[H^+_{bound}]}{\partial[L_i]}\frac{d[L_i]}{dt} \tag{6.34}$$

The reference term is based on the proton stoichiometry of the individual reactions and the respective flux of that reaction (6.35).

$$\frac{d[H^+_{reference}]}{dt} = -\sum_{k=1}^{N_{reactions}} n_k J_k \tag{6.35}$$

The flux term corresponds to the proton flux into the current compartment J_t^H. Substituting the expanded terms and rearranging equation (6.33) results in differential equations for the proton concentration (6.36). A similar approach can be used to obtain the differential equations governing the ion concentration (6.37).

$$\frac{d[H^+]}{dt} = \frac{-\sum_j^{N_{ions}} \frac{\partial[H^+_{bound}]}{\partial[M_j^{z_j+}]}\frac{d[M_j^{z_j+}]}{dt} - \sum_i^{N_{species}} \frac{\partial[H^+_{bound}]}{\partial[L_i]}\frac{d[L_i]}{dt} + \sum_{k=1}^{N_{reactions}} n_k J_k + J_t^H}{1 + \frac{\partial[H^+_{bound}]}{\delta[H^+]}} \tag{6.36}$$

$$\frac{d[M_j^{z_j+}]}{dt} = \frac{-\sum_l^{N_{ions}} \frac{\partial[M^{z_j+}_{j,bound}]}{\partial[M_l^{z_j+}]}\frac{d[M_l^{z_j+}]}{dt} - \sum_i^{N_{species}} \frac{\partial[M^{z_j+}_{j,bound}]}{\partial[L_i]}\frac{d[L_i]}{dt} + J_t^H}{1 + \frac{\partial[M^{z_j+}_{j,bound}]}{\delta[M_j^{z_j+}]}} \tag{6.37}$$

If all the terms are known, then these equations can be solved as a linear system of equations to obtain the uncoupled time derivatives of the protons and ions. Under the assumption that ion binding is a rapid equilibrium process one can write the terms in front of the time derivatives in terms of dissociation constants, reactant concentrations and binding polynomials. If one assumes higher order binding negligible by choosing the reference state appropriately for the physiological range then the resulting terms will become (6.38) to (6.42). Here $[M_j^{z_j+}]$ corresponds to the concentration of ion M^{z_j+}.

$$\frac{\partial[H^+_{bound}]}{\partial[M_j^{z_j+}]} = -\sum_{i=1}^{N_{reactants}} \frac{\frac{[L_i][H^+]}{K_i^H}}{K_i^{M_j}(P_i([H^+],[M_x^{z_x+}]))^2} \tag{6.38}$$

$$\frac{\partial[H^+_{bound}]}{\partial[H^+]} = -\sum_{i=1}^{N_{reactants}} \frac{[L_i](1+\sum_{j=1}^{n_{ions}} \frac{[M_j^{z_j+}]}{K_i^{M_j}})}{K_i^H(P_i([H^+],[M_x^{z_x+}]))^2} \tag{6.39}$$

$$\frac{\partial[M^{z_j+}_{j,bound}]}{\partial[H^+]} = -\sum_{i=1}^{N_{reactants}} \frac{\frac{[L_i][M_j^{z_j+}]}{K_i^{M_j}}}{K_i^H(P_i([H^+],[M_x^{z_x+}]))^2} \tag{6.40}$$

$$\frac{\partial[M^{z_j+}_{j,bound}]}{\partial[M_k^{z_k+}]} = -\sum_{i=1}^{N_{reactants}} \frac{\frac{[L_i][M_j^{z_j+}]}{K_i^{M_j}}}{K_i^{M_k}(P_i([H^+],[M_x^{z_x}]))^2} \tag{6.41}$$

$$\frac{\partial[M_{j,bound}^{z_j+}]}{\partial[M_j^{z_j+}]} = -\sum_{i=1}^{N_{reactants}} \frac{[L_i](1+\sum_{l\neq j}^{n_{ions}} \frac{[M_l^{z_l+}]}{K_{M_l}})}{K_l^H (P_i([H^+],[M_x^{z_x+}]))^2} \tag{6.42}$$

Where the binding polynomial accounting for first order bindings only (6.43) is based on the general expression (6.30).

$$P([H^+],[M_x^{z_x+}]) = 1 + \frac{[H^+]}{K_i^H} + \sum_j \frac{M_j^{z_j+}}{K_i^{M_j}}. \tag{6.43}$$

In the present work, the system is set up for 3 ions namely H^+, K^+ and Mg^{2+}.

6.5 Temperature and ionic strength dependence

As mentioned earlier, the change in Gibbs energy of a reaction can be calculated according to (6.44). Similarly the change in enthalpy can be calculated according to (6.45).

$$\Delta_r G^0 = \sum_{i=1}^{N_s} \nu_i \Delta_f G_i^0 \tag{6.44}$$

$$\Delta_r H = \sum_{i=1}^{N_s} \nu_i \Delta_f H_i \tag{6.45}$$

Assuming that the enthalpy is independent of temperature (which is reasonable over the physiological range [1]), then the binding affinities can be temperature corrected by means of the enthalpy of the involved reactants (6.46).

$$ln\frac{K_{T_2}}{K_{T_1}} = \frac{\Delta_r H^0 (T_2 - T_1)}{R T_1 T_2} \tag{6.46}$$

Using the definition of pKa (6.47) and the fact that $\frac{1}{log_{10}(e)} = 2.3026$, this equation can be rearranged into (6.48).

$$pKa = -log_{10}(K_a) \tag{6.47}$$

$$pKa_{T_2} = pKa_{T_1} + (\frac{1}{T_2} - \frac{1}{T_1})\frac{\Delta_r H^0}{2.3026R} \tag{6.48}$$

The effects of ionic strength on pK can be approximated using the relation (6.49). Here $\sum \nu_i z_i^2$ refers to the change in z_i^2 due to the dissociation.

$$pKa_{I_2} = pKa_{I_1} + \frac{\alpha_K}{2.303}(\frac{I_1^{\frac{1}{2}}}{1+1.6I_1^{\frac{1}{2}}} - \frac{I_2^{\frac{1}{2}}}{1+1.6I_2^{\frac{1}{2}}})\sum \nu_i z_i^2 \tag{6.49}$$

$$\alpha_K = 1.10708 - 1.54508 * 10^{-3} T + 5.95584 * 10^{-6} T^2 \tag{6.50}$$

The enthalpy itself is also a function of the ionic strength of the solution. The effect of ionic strength can be described by the following empirical equation (6.51).

$$\Delta_r H^0 = \Delta_r H^0(I=0) + \frac{\alpha_H I^{\frac{1}{2}} \sum v_i z_i^2}{1+1.6I^{\frac{1}{2}}} = \Delta_r H^0(I=0) + \beta_H(T,I)\sum v_i z_i^2 \tag{6.51}$$

$$\alpha_H = -1.28466 * 10^{-5} T^2 + 9.90399 * 10^{-8} T^3 \tag{6.52}$$

$$\beta_H = \frac{\alpha_H(T) I^{\frac{1}{2}}}{1 + 1.6 I^{\frac{1}{2}}} \tag{6.53}$$

The free energy of formation also depends on ionic strength. This dependence is approximated by (6.54).

$$\Delta_r G^0 = \Delta_r G^0(I = 0) - \frac{\alpha_G I^{\frac{1}{2}} \sum \nu_i z_i^2}{1 + 1.6 I^{\frac{1}{2}}} = \Delta_r G^0(I = 0) - \beta_G(T, I) \sum \nu_i z_i^2 \tag{6.54}$$

$$\alpha_G = RT\alpha_K = 9.20483 * 10^{-3} T - 1.28467 * 10^{-5} T^2 + 4.95199 * 10^{-8} * T^3 \tag{6.55}$$

$$\beta_G = \frac{\alpha_G(T) I^{\frac{1}{2}}}{1 + 1.6 I^{\frac{1}{2}}} \tag{6.56}$$

These empirical formulas are based on the assumption that the heat capacities are zero [1].

The effect of temperature on the Gibbs energy, assuming that the enthalpies are approximately constant is given by (6.57) [2]. Due to the linearity, one can substitute the reaction enthalpy and reaction Gibbs energy change in this equation.

$$\Delta_f G_i^0(T_2) = \left(1 - \frac{T_2}{T_1}\right) \Delta_f H_i^0 + \frac{T_2}{T_1} \Delta_f G_i^0(T_1) \tag{6.57}$$

6.6 Carbon dioxide

Carbon dioxide does not significantly bind to metal ions or protons itself, but can be hydrolyzed to H_2CO_3 via the reaction (6.58). Protons can dissociate from H_2CO_3.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \tag{6.58}$$

It is convenient to express apparent thermodynamic properties in terms of total CO_2, which is defined as (6.59)

$$[\sum CO_2] = [CO_2] + [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \tag{6.59}$$

Writing down the expression containing the binding polynomial for carbon dioxide leads to (6.60), where it can be seen that $\sum CO_2$ is expressed in terms of $[CO_3^{2-}]$.

$$[\sum CO_2] = [CO_3^{2-}](1 + \frac{[H^+]}{K_1} + \frac{[H^+]^2}{K_1 K_2} + \frac{[H^+]^2}{K_h K_1 K_2}) \tag{6.60}$$

Here K_h refers to the formation of H_2CO_3 from water and carbon dioxide. Because of the fact that the total carbon dioxide is now expressed in terms of $[CO_3^{2-}]$, the water and protons involved need to be taken into account in terms of stoichiometry.

For the reaction (6.61) this would lead to the sum of (6.62) and (6.63), which is given by (6.64).

$$\sum A \rightleftharpoons \sum B + \sum CO_2 \tag{6.61}$$

$$A \rightleftharpoons B + CO_2 \tag{6.62}$$

$$CO_2 + H_2O \rightleftharpoons CO_3^{2-} + 2H^+ \tag{6.63}$$

$$A + H_2O \rightleftharpoons B + CO_3^{2-} + 2H^+ \tag{6.64}$$

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