Kinetic Modeling as a Tool to Integrate Multilevel Dynamic Experimental Data

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Chapter 11

Kinetic Modeling as a Tool to Integrate Multilevel Dynamic Experimental Data

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Abstract

The metabolic networks are the most well-studied biochemical systems, with an abundance of in vitro and in vivo data available for quantitative estimation of its kinetic parameters. In this chapter, we present our approach to developing mathematical description of metabolic pathways. The model-based integration of reaction kinetics and the utilization of different types of experimental data including temporal dependencies have been described in detail. Software package DBSolve7 which allows us to develop kinetic model of the biochemical system and integrate experimental data has been presented.

Key words: Kinetic modeling, metabolic pathways, DBSolve7, integration of experimental data.

1. Introduction

In this chapter we are presenting the kinetic modeling (KM) approach (an extension of traditional network tools and simulators with combination of novel model construction techniques) to the collection, integration, and analysis of biological data. The method fully enables integration, mainly on account of the heterogeneous nature of the **time series** data being generated, and identifies the key underlying **kinetic** biological mechanisms, and thus allows researchers to generate robust hypotheses for **consequences** of external perturbations.

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The appearance of new modern measuring techniques, such as HPLC, MS, capillary electrophoresis, and DNA arrays (1–3), makes it possible to detect biologically significant species in small quantities with appropriate range of accuracy. The assays based on these techniques have enabled measuring the level of expression of various genes, the concentration of different proteins, and intermediates of intracellular metabolic and signaling pathways. Such an avalanche-like increase in biological information has posed a new problem for biologists: How to analyze and to interpret the ever-growing body of experimental data?

A large body of experimental data has stimulated the development of various quantitative approaches to their analysis and interpretation and led to appearance of cross-disciplinary scientific directions at the interface of biology, physics, chemistry, and mathematics, such as bioinformatics and systems biology. The subject of systems biology encompasses, on the one hand, any biological systems, the characteristics of which can be quantitatively measured and, on the other hand, the mathematical models that mimic the behavior of these biological systems. The systems biology primarily intends to construct a model, which is most closely related to the biological system under study, and to reveal thereby new regulatory and dynamic properties as well as structure–mechanism relationships of this system. The criterion of adequacy of the mathematical model to relevant biological system is a quality of simulation of all possible experimental data obtained for this biological object.

The models constructed using kinetic modeling approach (4–17) usually take into consideration all currently reported regulatory and dynamic features of studied biological systems. The kinetic model considers biochemical pathways as the whole set of processes catalyzed by intracellular enzymes and is described by a system of non-linear differential equations. Hence, each enzyme is represented by a rate equation, which determines the rate of enzymatic reaction as a function of concentrations of not only substrate and product but also the intermediates involved in considered pathways as inhibitors and/or activators of the enzyme. This description allows one to take into account all currently reported regulatory properties of the studied biological system. Since all parameters of rate equations have unambiguous physical and biological meanings, this makes it possible to model any mutations affecting the dynamic and regulatory features of enzyme, or any therapeutic agonist or antagonist mechanism of action. Moreover, the kinetic model can be used to describe the responses of biochemical system to any modifications including those required for constructing the strains capable of overproducing the target products. The merits of kinetic models mentioned above arise from detailed description of all processes occurring in considered biological system. The high level of detailing gives rise to a large number of parameters, which requires a great body of

experimental data for evaluation of these parameters. The high data capacity of kinetic models may be regarded as a disadvantage in comparison with other models. However, these are the models that look very promising for application to analysis and interpretation of a large amount of multilevel experimental data.

In this chapter, we describe an approach to construction of mathematical models, their study, and application for reconstructing the dynamic and regulatory behavior of biochemical systems using multilevel experimental data.

2. Methods

2.1. Basic Principles of Kinetic Model Construction

The term kinetic model refers to a system of mechanistic ordinary differential equations that determine the temporal state of the corresponding system of biochemical reactions. In these equations, there is mass conservation between the production and consumption of each species:

$$dX/dt = V_{\text{production}} - V_{\text{consumption}}$$

where $V_{\text{production}}$ and $V_{\text{consumption}}$ are the respective rates of production and consumption of species X. X designates any entity (compound, protein, RNA) involved both in a biological pathway and in transcription or translation processes. This model describes dynamics of intracellular processes occurred in the cells of some cellular assemblage. Such an assemblage can represent, for example, a cellular suspension consisting of 10^4–10^8 cells. The concentration of entity X is defined as the total number of molecules of this entity in all cells of the assemblage divided by the total volume of these cells.

The construction of kinetic models for biological systems can be accomplished in several stages.

The first stage of model construction is elucidation of a static model of the system, i.e., identification of all cellular players (proteins, intermediates, enzymes, small molecules, and co-factors) and all non-enzymatic processes occurring in the cellular network. The result of the elucidation is a network (i.e., a directed bond graph) of all interactions connecting all the species. For the network to be correct, each of the entity must participate in at least one reaction or serve as a co-factor. In **Note 1** section we will illustrate all details of static model development by the example of histidine biosynthesis pathway in *Escherichia coli* (18).

Once the appropriate static network has been chosen, **the second stage** of model construction is the generation of rate equations that describe the dependence of each reaction rate on the concentrations of intermediates involved in the studied

pathway. In order to make the models scalable and comparable with various experimental data, we have developed detailed and reduced descriptions of every biochemical process involved in the model.

The detailed description of biochemical reaction (i.e., enzyme catalytic cycle) implies the authentic view of the molecular mechanism of this reaction and takes into account all the possible states of the protein involved, including its possible inactivated states (because of phosphorylation) or dead-end inhibitor complexes. Usually, the detailed description comprises a combined set of ordinary differential flux equations and non-linear algebraic equations (if steady-state or conservation constraint assumptions are made).

A *reduced* description represents the reaction rate as an explicit analytic function of the concentrations of substrates, products, inhibitors, and activators, as well as the total protein concentration and all kinetic constants of the processes. Derivation of rate equation is accomplished in the following stages:

1) Construction of the enzyme catalytic cycle using structural and kinetic data (in most cases, the information on the mechanism of enzyme functioning is available in the literature).
2) Derivation of the rate equation in terms of parameters of the catalytic cycle (rate constants and dissociation/equilibrium constants of elementary stages of the catalytic cycle) on the basis of quasi-steady-state or rapid equilibrium approaches (19).
3) Derivation of the equations that express parameters of the catalytic cycle in terms of kinetic parameters (Michaelis constants, inhibition constants, catalytic constants).
4) Derivation of the rate equation in terms of kinetic parameters of the enzymatic reaction.

In the framework of the KM approach proposed in this chapter, the level of detailing the catalytic cycle and the complexity of the rate equation derived on the basis of this cycle are fully determined by availability of the experimental data on structural and functional organization of the enzyme. Indeed, once the catalytic cycle of the enzyme has been established and experimentally proved, one can use this information to derive the rate equation. In case that the mechanism of enzyme functioning is unknown, we suggest that a minimal catalytic cycle should be developed, which

- satisfies all structural and stoichiometric data available in the literature;
- allows derivation of the rate equation describing available kinetic experimental data; and
- is the simplest of all possible catalytic cycles that satisfy the first two preceding requirements.

To derive a rate equation on the basis of reconstructed catalytic cycle, there are various techniques. These techniques are based on application of different assumptions to describe function of individual processes of catalytic cycle and enable us to take into account diversity of dynamic and regulatory properties of different enzymes. We consider three approaches to derive rate equation: quasi-equilibrium, quasi-steady-state, and combined quasi-equilibrium, quasi-steady-state approaches. Main features of these approaches are exemplified via consideration of various catalytic cycles and derivation of corresponding rate equations (*see* **Note 2** section).

2.2. Basic Principles of Kinetic Model Verification

The third stage of the model development is the parameter evaluation. For evaluation of kinetic parameters, we used in vitro and in vivo experimental data from the following sources:

- literature data on the values of K_m, K_i, K_d, rate constants, etc.;
- electronic databases (only a few databases with specific kinetic content are available at present, in particular, the EMP database (20) and BRENDA (21));
- experimentally measured dependences of the initial rates of enzymatic reaction on the concentrations of substrates, products, inhibitors, and activators;
- time series data obtained from kinetic experiments;
- in vitro data obtained with cell-free extracts; and
- in vivo data that describe the intracellular kinetics of metabolic pathway under study.

2.3. Verification of Kinetic Model Using In Vitro Experimental Data Measured for Purified Enzymes

The kinetic parameters of rate equations were usually estimated as follows. First, we search for all the in vitro experimental data on kinetics of studied enzyme available in the literature. These data are usually presented as dependences of either the initial reaction rate on the substrate/product concentration or as dependences of the substrate/product concentration on time. A special technique was elaborated for quantitative description of all available in vitro experiments. We used an explicit rate equation applicable to individual enzymes for fitting the initial reaction rate dependences on the substrate/product concentration. The reaction rate equation was also used for determination of inhibition parameters. In order to describe the time series experiments carried out with purified enzymes, we constructed minimodels, i.e., the systems of ordinary differential equations, which have the solutions corresponding to the measured time dependences. Rate laws and the concentrations of experimentally measured intermediates were used as variables in the construction of these minimodels. The kinetic parameters of rate equations were evaluated by fitting the minimodel solutions against experimentally measured time series.

2.4. Verification of the Kinetic Model Using In Vitro and In Vivo Experimental Data Measured for Biochemical System

The preceding sections show how one can reconstruct the catalytic cycle of enzyme and derive the rate equation on the basis of this cycle using structural information and the kinetic data obtained for purified enzyme preparation. Then, the parameters of the rate equation can be estimated using the in vitro experimental dependences characterizing the kinetic properties of this enzymatic reaction. However, it may also happen that the in vitro data characterizing the kinetics of purified enzyme will be insufficient to estimate all parameters of the rate equation. For example, only the dependences of initial rate on the concentration of substrate have been measured for many enzymes but the capacity of products to inhibit the enzymatic reaction has not been studied yet. There are two possibilities to estimate the values of these unknown parameters by using

1) in vitro kinetic data obtained for the same enzyme isolated from another (micro)organism;
2) in vitro and in vivo data that characterize the kinetics of entire biochemical pathway or its fragment containing the studied enzyme.

There are two types of data that characterize the kinetics of entire biochemical pathway or its fragment containing the studied enzyme:

a) in vitro data obtained with cell-free extracts;
b) in vivo data that describe the intracellular kinetics of biochemical pathway under study.

The data obtained in experiments with cell-free extracts can be used for verification of the models that describe only metabolic and signaling pathways and do not take into account the dependence of expression of enzymes on the concentrations of intermediates formed in this pathway. The models of this type cannot be used for interpretation of experimental data obtained in in vivo experiments. The data obtained in in vivo experiments can be used for verification of the models that take into account the dependence of expression of enzymes on the concentration of intermediate formed in the pathway under study. These models describe both the metabolism and its interaction with transcription and translation as well as their mutual regulation.

2.5. Software for Kinetic Modeling: DBSolve7

A system of ordinary differential equations describing any part of intracellular processes is numerically integrated using specialized software packages.

There are many stand-alone software packages available for systems biology and kinetic modeling (22). Most are available as a result of the efforts of the SBML community (23). However, only a few contain a full range of tools to allow kinetic model creation, parameter fitting, and analysis. DBSolve is one of these

packages. DBSolve is a software environment for creation, analysis, and visualization of kinetic models of biological processes. A number of versions have been released during more than 10 years of software development (24–27). During this 10-year period, DBSolve has been extensively used in Moscow State University and by GlaxoSmithKline to create hundreds of kinetic models for both research and teaching. The package has built-in algorithms and tools for constructing models and fitting parameters to the experimental data. All the models are considered to be systems of non-linear ordinary differential equations and/or non-linear algebraic differential equations with arbitrary right-hand sides. These features allow modelers to expand the class of possible applications to include chemical, PK/PD, ecological, or other biomedical systems.

DBSolve includes the following methods:

1. Generation of stoichiometric matrix based on the list of the reactions describing the system.
2. Automatic analysis of the stoichiometric matrix.
3. Automatic generation of the systems of ordinary differential equations and conservation laws based on the stoichiometric matrix.
4. Calculation of functional dependences defined explicitly.
5. Numerical solution of non-linear ODE system and visualization of the solution.
6. Calculation of functional dependences defined implicitly as a system of algebraic equations (generally non-linear).
7. Automatic search of optimal values of the parameters of a system based on the experimental data (fitting).
8. Analysis of stability of the dynamic system (bifurcation) and calculation of the control coefficients as defined in metabolic control analysis.

DBSolve is written in C++. The software has an object-oriented structure containing a range of methods and associated data that can be viewed from the main application window. DBSolve has an internal language for building and storing models and an internal compiler for processing the models and running simulations. A derived model can be saved in the internal format (with the SLV extension) and/or exported to other systems biology software as an ASCII text file or as an SBML file. The SLV file (extended SBML) contains all the necessary information about the mathematical model: the stoichiometric matrix, system equations, initial values, the experimental parameter values, information about the biological components together with the reactions/processes, and links to external databases, ontology, and controlled vocabularies.

The full description of the SLV files is available from the website http://www.insysbio.ru. DBSolve supports both import and export to SBML 2.0 format.

2.6. Conclusion

In this chapter, we have described the KM approach in detail. The primary advantage of kinetic modeling over existing approaches is the incorporation of reaction kinetics and the utilization of different types of temporal dependences. It allows to map high-throughput data onto existing knowledge of organ, tissue, cells and cellular interactions, phenotypes, and disease etiology.

More broader the KM approach enables

- Knowledge management and understanding at a high level of resolution, allowing
  - data integration and explanation, even with very large data sets, data variability;
  - quantitative studies of biological processes as whole systems; and
  - the identification of knowledge gaps.
- Hypothesis generation
  - cellular bioengineering to address specific challenges, the prediction of cellular functions;
  - mimicking of different therapeutic, environmental, physiological and genetic conditions;
  - prediction of the consequences of system perturbations;
  - mechanistic understanding of microdosing and combination therapies;
  - drug safety and cope with adverse effects; and
  - chronotherapy and individual dose prediction.
- Hypothesis testing
  - enabling cheaper and faster methods that are complementary to in vitro, ex vivo, and in vivo experiments or animal models;
  - conducting the rational design of pathways, cells, biomarkers, organisms; and
  - theoretical and computational support of synthetic biology studies.

3. Notes

3.1. Note 1

This pathway is depicted in **Fig. 11.1**. List of intermediates of the pathway is shown in **Table 11.1**. Chemical equations of reactions of the pathway are summarized in **Table 11.2**. Taking into account this information we create stoichiometric matrix:

v_1 v_2 v_3 v_4 v_5 v_6 v_7 v_8 v_9 v_{10} v_{11} v_{12} v_{13} v_{14} v_{15} v_{16} v_{17}

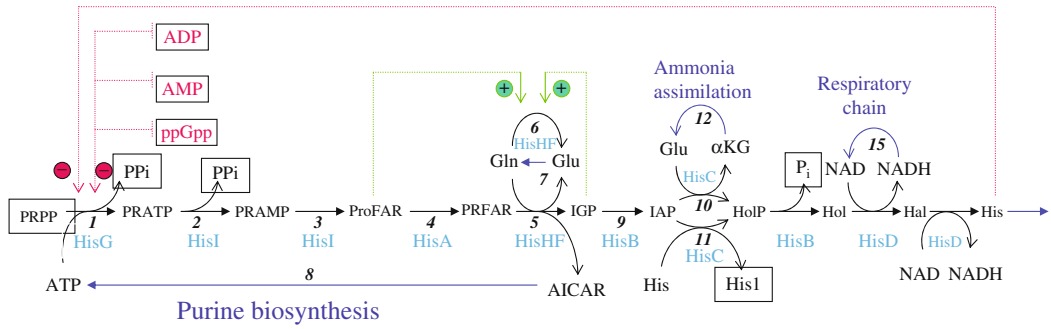

Fig. 11.1. Pathway of histidine biosynthesis.

Table 11.1
Intermediates of histidine biosynthesis pathway in *E. coli*

Intermediate designation	Chemical name of intermediate
PRATP	N1-(5′-phosphoribosyl)-ATP
PRAMP	N1-(5′-phosphoribosyl)-AMP
ProFAR	Pro-phosphoribosyl-formimino-5-aminoimidazole-4-carboxamide ribonucleotide
PRFAR	Phosphoribosyl-formimino-5-aminoimidazole-4-carboxamide ribonucleotide
IGP	Imidazoleglycerol phosphate
AICAR	5-Aminoimidazole-4-carboxamide ribonucleotide
IAP	Imidazoleacetol phosphate
HolP	L-Histidinol phosphate
Hol	L-Histidinol
Hal	L-Histidinal
His	L-Histidine
ATP	Adenosine triphosphate
Gln	Glutamine
αKg	α-Ketoglutarate
Glu	Glutamate
NAD	Nicotineamide adenine dinucleotide phosphate oxidized
NADH	Nicotineamide adenine dinucleotide phosphate reduced

Table 11.2
Reactions of histidine biosynthesis pathway in *E. coli*

Reaction number	Chemical equation	Enzyme	Gene
1	PRPP + ATP = PRATP + PPi	ATP-phosphoribosyltransferase	**1 HisG**
2	PRATP = PPi + PRAMP	Phosphoribosyl-ATP-pyrophosphohydrolase: Phosphoribosyl-AMP cyclohydrolase	**1.1 HisI**
3	PRAMP = ProFAR	Phosphoribosyl-ATP-pyrophosphohydrolase: Phosphoribosyl-AMP cyclohydrolase	*HisI*
4	ProFAR = PRFAR	Phosphoribosyl-formimino-5-amino-1-phosphoribosyl-4-imidazole-carboxamide isomerase	**1.2 HisA**
5	PRFAR + Gln = Glu + IGP + AICAR	IGP synthase	*HisHF*
6	Gln = Glu	IGP synthase	*HisHF*
7	Glu = Gln	Glutamine synthatase	
8	AICAR = ATP	Purine biosynthesis	
9	IGP = IAP	IGP dehydratase	*HisB*
10	IAP + Glu = HolP + αKG	Histidinol phosphate aminotransferase	*HisC*
11	IAP + His = HolP + His1	Histidinol phosphate aminotransferase	*HisC*
12	αKG = Glu	Ammonia assimilation	
13	HolP = Hol + Pi	Histidinol phosphatase	*HisB*
14	Hol + NAD = Hal + NADH	Histidinoldehydrogenase	*HisD*
15	NADH = NAD	Respiratory chain	
16	Hal + NAD = His +NADH	Histidinaldehydrogenase	*HisD*
17	His →	Histidine consumption	

$$\begin{pmatrix}
-1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & -1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 1 & -1 & 0 & 0 & -1 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 1 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0
\end{pmatrix}
\begin{matrix}
\text{ATP} \\ \text{PRATP} \\ \text{PRAMP} \\ \text{ProFAR} \\ \text{PRFAR} \\ \text{Gln} \\ \text{Glu} \\ \text{IGP} \\ \text{AICAR} \\ \text{IAP} \\ \text{His} \\ \text{HolP} \\ \alpha\,\text{KG} \\ \text{Hol} \\ \text{NAD} \\ \text{NADH} \\ \text{Hal}
\end{matrix}$$

Columns of the matrix correspond to reactions of the pathway and rows correspond to metabolites. Construction of stoichiometric matrix of selected biochemical system corresponds to development of its "static" model. Next stage on the way of development of kinetic model of the system consists in that using static model (or, in other words, stoichiometric matrix) we can write out system of differential equations describing dynamics of selected pathway:

$$\frac{d\mathbf{x}}{dt} = \mathbf{N} \cdot \mathbf{v}, \quad \mathbf{x}(\mathbf{0}) = \mathbf{x_0} \qquad [1]$$

Here, $\mathbf{x} = [x_1,\ldots,x_m]^{\mathrm{T}}$ is a vector of intermediate concentrations, $\mathbf{x_0} = [x_{10},\ldots,x_{m0}]^{\mathrm{T}}$ is a vector of initial concentrations of intermediates, $\mathbf{v} = [v_1,\ldots,v_n]^{\mathrm{T}}$ is a vector of reaction rates, and $\mathbf{N}$ is the stoichiometric matrix which has n columns and m rows. In case of pathway of histidine biosynthesis both m and n are equal to 17 and vectors of intermediate concentrations and initial conditions are

$$\mathbf{x} = [\text{ATP}, \text{PRATP}, \text{PRAMP}, \text{ProFAR}, \text{PRFAR}, \text{Gln}, \text{Glu}, \text{IGP}, \text{AICAR}, \text{IAP}, \text{His}, \text{HolP}, \alpha\text{KG}, \text{Hol}, \text{NAD}, \text{NADH}, \text{Hal}]^{\mathrm{T},}$$

$$\mathbf{x}_0 = [\text{ATP}_0,\ \text{PRATP}_0,\ \text{PRAMP}_0,\ \text{ProFAR}_0,\ \text{PRFAR}_0,\ \text{Gln}_0,\ \text{Glu}_0,\ \text{IGP}_0,\ \text{AICAR}_0,\ \text{IAP}_0,\ \text{His}_0,\ \text{HolP}_0,\ \text{KG}_0,\ \text{Hol}_0,\ \text{NAD}_0,\ \text{NADH}_0,\ \text{Hal}_0]^{\mathrm{T}}$$

Before going into methods, peculiarities, and details of rate equation derivation, we will focus on what static model enables us to understand about the system of interest. It turns out that stoichiometric matrix allows us, first, to derive relationships between steady-state fluxes and, second, to find out number of conservation laws, and to write out their expressions. Indeed, solving the system of linear algebraic equations

$$\mathbf{N} \cdot \mathbf{v} = \mathbf{0} \qquad [2]$$

we find that any steady-state reaction rate (steady-state flux), v_i, $i = 1, \ldots, n$, can be expressed as a linear combination of s-independent steady-state rates. Number s, equal to dimension

of kernel of matrix **N**, and coefficients of relationships expressing any steady-state rate in terms of *s*-selected independent rates are fully determined by stoichiometric matrix. As an example we consider relationships between steady-state rates of histidine biosynthesis pathway (*see* **Fig. 11.1**):

$$v_{16} = v_{14} = v_{13} = v_9 = v_8 = v_5 = v_4 = v_3 = v_2 = v_1,$$
$$v_7 = v_1 + v_6,$$
$$v_{10} = v_{17} = v_{12},$$
$$v_{11} = v_1 - v_{12},$$
$$v_{15} = 2 \cdot v_1$$

From these relationships it follows that any steady-state rate can be expressed in terms of the three independent rates v_1, v_6, and v_{12}, i.e., s is equal to 3. Conservation laws are the first, linear integrals of the system of differential equations [1] describing kinetics of the selected biochemical system. As a simplest example of the conservation law valid for the pathway of histidine biosynthesis we can consider the following algebraic expression:

$$\mathrm{NAD} + \mathrm{NADH} = \mathrm{const}_1 \qquad [3]$$

This relationship results from summing up and integration of differential equations describing how concentrations NAD and NADH change with time. The meaning of equation [3] consists in that sum of concentrations of NAD and NADH does not change with time. It is easy to show that the number of conservation laws of kinetic model describing biochemical system consisting of m intermediates connected with n reactions is given by the following formula:

$$(\text{number of conservation laws}) = m - n + s \qquad [4]$$

In case of histidine biosynthesis pathway both m and n are equal to 17 and s is equal to 3. Consequently, in accordance with equation [4], one obtains that the number of conservation laws of kinetic model of histidine biosynthesis pathway shown in **Fig. 11.1** is equal to 3. Relationship [3] is one of the three conservation laws. Two others are given by the following expressions:

$$\begin{gathered}\mathrm{PRATP} + \mathrm{PRAMP} + \mathrm{ProFAR} + \mathrm{PRFAR} + \mathrm{AICAR} + \mathrm{ATP} = \mathrm{const}_2 \\ \mathrm{Glu} + \mathrm{Gln} + \alpha\mathrm{KG} = \mathrm{const}_3\end{gathered} \qquad [5]$$

Since equations [3] and [5] are true for any moment of time, including time equal to 0, then values of parameters, const_i, $i = 1, 2, 3$, are completely determined by initial conditions:

$$\mathrm{const}_1 = \mathrm{NAD}_0 + \mathrm{NADH}_0,$$
$$\mathrm{const}_2 = \mathrm{PRATP}_0 + \mathrm{PRAMP}_0 + \mathrm{ProFAR}_0 + \mathrm{PRFAR}_0 + \mathrm{AICAR}_0 + \mathrm{ATP}_0$$
$$\mathrm{const}_3 = \mathrm{Glu}_0 + \mathrm{Gln}_0 + \alpha\mathrm{KG}_0$$

3.2. Note 2

3.2.1. Quasi-equilibrium Approach

This approach can be applied to derive rate equations for the catalytic cycles with the following characteristic features:

(i) All stages of catalytic cycle can be subdivided into group of fast reactions and group of slow reactions.

(ii) Fast reactions can be considered at quasi-equilibrium in comparison with slow reactions.

(iii) All concentrations of enzyme states can be expressed in terms of parameters of catalytic cycle and substrate/product/effector concentrations on the basis of equilibrium relationships valid for fast reactions.

We exemplify application of quasi-equilibrium approach via derivation of reaction rate of the enzyme functioning in accordance with Random Bi Bi mechanism (Cleland (28)). Catalytic cycle of the enzyme is depicted in **Fig. 11.2**.

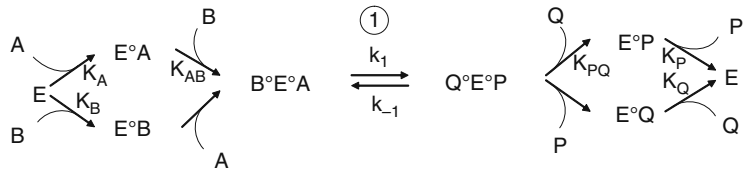

Fig. 11.2. Catalytic cycle of the enzyme with Random Bi Bi mechanism.

To derive the equations which describe the dependence of the reaction rate on parameters of catalytic cycle and concentrations of the substrates, products and effectors, we assume that the rates of all reactions of the substrate binding and dissociation of the products are significantly higher than the rates of catalytic reaction designated as 1 in **Fig. 11.2**. This means that each of these "fast" reactions can be considered as a quasi-equilibrium one (the dissociation constants are given near the corresponding reactions in **Fig. 11.2**); thus, we can write the following relationships:

$$\begin{aligned} K_{\mathrm{A}} &= \frac{E \cdot A}{E \bullet A}, \quad K_{\mathrm{P}} = \frac{E \cdot P}{E \bullet P} \\ K_{\mathrm{B}} &= \frac{E \cdot B}{B \bullet E}, \quad K_{\mathrm{Q}} = \frac{E \cdot Q}{Q \bullet E} \\ K_{\mathrm{AB}} &= \frac{B \cdot E \bullet A}{B \bullet E \bullet A}, \quad K_{\mathrm{PQ}} = \frac{Q \cdot E \bullet P}{Q \bullet E \bullet P} \end{aligned} \qquad [6]$$

For concentrations of the enzyme states, the following conservation law is also fulfilled:

$$E + E \bullet A + B \bullet E + B \bullet E \bullet A + E \bullet P + Q \bullet E + Q \bullet E \bullet P = \mathrm{e}_0 \qquad [7]$$

where e_0 is the total concentration of the enzyme. Solving the system of linear (relative to concentrations of the enzyme states) algebraic equations [6] and [7], we obtain the following expressions for the concentrations of the enzyme states:

$$B \bullet E \bullet A = \frac{A}{K_A}\frac{B}{K_{AB}}\frac{e_0}{\Delta}, \quad Q \bullet E \bullet P = \frac{P}{K_P}\frac{Q}{K_{PQ}}\frac{e_0}{\Delta} \qquad [8]$$
$$\Delta = 1 + \frac{A}{K_A} + \frac{B}{K_B} + \frac{A}{K_A}\frac{B}{K_{AB}} + \frac{P}{K_P} + \frac{Q}{K_Q} + \frac{P}{K_P}\frac{Q}{K_{PQ}}$$

According to the scheme of the catalytic cycle presented in **Fig. 11.2**, the rate equations for the reaction can be written as follows:

$$v = k_1 \cdot B \cdot E \cdot A - k_{-1} \cdot Q \cdot E \cdot P \qquad [9]$$

Substitution of equation [8] into [9] gives the following equation for the reaction rate:

$$v = \frac{e_0}{\Delta} \cdot \frac{k_1}{K_A}\frac{1}{K_{AB}}\left(A \cdot B - P \cdot Q \cdot \frac{k_{-1}}{k_1}\frac{K_A}{K_P}\frac{K_{AB}}{K_{PQ}}\right) \qquad [10]$$

where K_A, K_B, K_P, and K_Q are the dissociation constants of the substrates A, B and products P, Q from free enzyme; K_{AB} and K_{PQ} are the dissociation constants of the substrates B and Q from the enzyme complex with the substrates A and P, respectively; k_1, k_{-1} are the rate constants of catalytic stage of the enzyme cycle. The dissociation and rate constants are presented in **Fig. 11.2** near the corresponding reactions.

3.2.2. Quasi-steady-state Approach

This approach can be applied to derive rate equations for the catalytic cycles with the following characteristic features:

(i) All stages of catalytic cycle cannot be subdivided into group of fast reactions and group of slow reactions.

(ii) All concentrations of enzyme states can be expressed in terms of parameters of catalytic cycle and substrate/product/effector concentrations on the basis of steady-state solution of system of ordinary differential equation describing the catalytic cycle

We exemplify application of quasi-steady-state approach via derivation of reaction rate of the enzyme functioning in accordance with Ordered Uni Bi mechanism (Cleland (28)). Catalytic cycle of the enzyme is depicted in **Fig. 11.3** and the dissociation and rate constants are given near the corresponding stages.

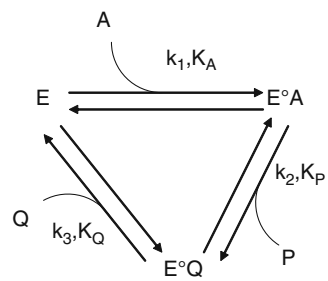

Fig. 11.3. Catalytic cycle of the enzyme with Ordered Uni Bi mechanism.

To derive the equations which describe the dependence of the reaction rate on parameters of catalytic cycle and concentrations of the substrates, products, and effectors, we assume that there are no fast and slow stages in the catalytic cycle but all reaction rates are of the same order of magnitude. This means that dynamics of the catalytic cycle is described by the following system of differential equations:

$$\begin{aligned} &\frac{dE}{dt} = v_1 - v_2 \\ &\frac{dE \bullet A}{dt} = v_2 - v_3 \\ &E + E \bullet A + E \bullet Q = e_0 \end{aligned} \qquad [11]$$

where e_0 is the total concentration of the enzyme. Rate equations of individual stages of the catalytic cycle are expressed in the following manner:

$$\begin{aligned} v_1 &= k_1 \cdot (E \cdot A / K_A - E \cdot A) \\ v_2 &= k_2 \cdot (E \cdot A - P \cdot E \cdot Q / K_P) \\ v_3 &= k_3 \cdot (E \cdot Q - Q \cdot E / K_Q) \end{aligned} \qquad [12]$$

Solving the system of [11] and [12] at steady state, we obtain the following expressions for the concentrations of the enzyme states:

$$\begin{aligned} E &= \frac{e_0}{\Delta} \cdot \left(k_3 \cdot (k_1 + k_2) + k_1 \cdot k_2 \frac{P}{K_P} \right), \\ E &= \frac{e_0}{\Delta} \cdot \left(k_1 \cdot k_3 \frac{A}{K_A} + k_1 \cdot k_2 \frac{A}{K_A} \frac{P}{K_P} + k_2 \cdot k_3 \frac{Q}{K_Q} \frac{P}{K_P} \right) \\ \Delta &= k_3 \cdot (k_1 + k_2) + k_1 \cdot (k_2 + k_3) \frac{A}{K_A} + k_1 \cdot k_2 \frac{P}{K_P} + k_3 \cdot (k_1 + k_2) \frac{Q}{K_Q} \\ &\quad + k_1 \cdot k_2 \frac{A}{K_A} \frac{P}{K_P} + k_2 \cdot k_3 \frac{Q}{K_Q} \frac{P}{K_P} \end{aligned} \qquad [13]$$

Substitution of equation [13] into the first equation of [12] gives the following expression for the reaction rate:

$$v = \frac{e_0}{\Delta} \cdot \frac{k_1 \cdot k_2 \cdot k_3}{K_A} \left(A \cdot B - P \cdot Q \cdot \frac{K_A}{K_P} \frac{1}{K_Q} \right) \qquad [14]$$

where K_A, K_P, and K_Q are the dissociation constants of the substrate A and products P, Q; k_i, $i = 1, 2, 3$, are the rate constants of corresponding stages of catalytic cycle.

3.2.3. Combined Quasi-equilibrium, Quasi-steady-state Approach

This approach can be applied to derive rate equations for the catalytic cycles with the following characteristic features:

(i) All stages of catalytic cycle can be subdivided into group of fast reactions and group of slow reactions.

(ii) Fast reactions can be considered at quasi-equilibrium in comparison with slow reactions.

(iii) Initial catalytic cycle of the enzyme can be reduced to the catalytic cycle including slow processes only.

(iv) All concentrations of enzyme states can be expressed in terms of parameters of catalytic cycle and substrate/product/effector concentrations on the basis of both equilibrium relationships valid for fast reactions and steady-state solution of system of ordinary differential equation describing reduced catalytic cycle.

We exemplify application of combined quasi-equilibrium, quasi-steady-state approach via derivation of reaction rate of the enzyme functioning in accordance with Ping Pong Bi Bi mechanism (Cleland (28)). Catalytic cycle of the enzyme is depicted in **Fig. 11.4** and the dissociation and rate constants are given near the corresponding stages.

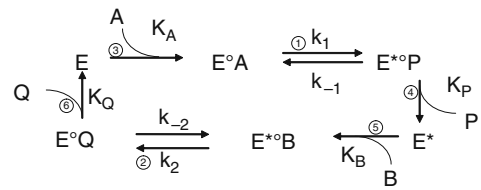

Fig. 11.4. Catalytic cycle of the enzyme with Ping Pong Bi Bi mechanism.

Dynamics of the catalytic cycle is described by the following system of differential equations:

$$\begin{aligned}
\frac{dE}{dt} &= v_6 - v_3 \\
\frac{dE \bullet A}{dt} &= v_3 - v_1 \\
\frac{dQ \bullet E}{dt} &= v_2 - v_6 \\
\frac{dE^*}{dt} &= v_4 - v_5 \\
\frac{dE^* \bullet P}{dt} &= v_1 - v_4 \\
\frac{dB \bullet E^*}{dt} &= v_5 - v_2
\end{aligned} \qquad [15]$$

where rate equations of individual stages of the catalytic cycle are given by the following expressions:

$$\begin{aligned}
v_1 &= k_1 \cdot E \cdot A - k_{-1} \cdot E^* \cdot P \\
v_2 &= k_2 \cdot B \cdot E^* - k_{-2} \cdot Q \cdot E \\
v_3 &= k_3 \cdot w_3 = k_3 \cdot (E \cdot A/K_A - E \cdot A) \\
v_4 &= k_4 \cdot w_4 = k_4 \cdot (E^* \cdot P - E^* \cdot P/K_P) \\
v_5 &= k_5 \cdot w_5 = k_5 \cdot (E^* \cdot B/K_B - B \cdot E^*) \\
v_6 &= k_6 \cdot w_6 = k_3 \cdot (Q \cdot E - E \cdot Q/K_Q)
\end{aligned} \qquad [16]$$

To derive the equations which describe the dependence of the reaction rate on parameters of catalytic cycle and concentrations of the substrates, products, and effectors, we assume that the rates of all

reactions of the substrate binding and dissociation of the products are significantly higher than the rates of catalytic reactions designated as 1 and 2 in **Fig. 11.4**, i.e., $k_i >> k_{\pm j}$, $i = 3, 4, 5, 6$ and $j = 1, 2$. This allows us to subdivide all stages of the catalytic cycle to fast (reactions [3], [4], [5], and [6]) and slow (reactions [1] and [2]).

Let us transform system of differential equations [15] in such a way to have in the resulting system two differential equations which right hand side consists in linear combination of the rates of slow reactions only. In order to do it, we proceed with the following linear transformations of the ODE system:

(a) to add up first three differential equations of the system [15] and resulting differential equation substitutes for the first equation of the system [15];

(b) to add up last three differential equations of the system [15] and resulting differential equation substitutes for the fourth equation of the system [15].

Transformed system of differential equations can be presented in the following way:

$$\begin{gathered}\frac{d(E + Q \bullet E + E \bullet A)}{dt} = v_2 - v_1 \\ \frac{dE \bullet A}{dt} = v_3 - v_1 \\ \frac{dQ \bullet E}{dt} = v_2 - v_6 \\ \frac{d(E^* + E^* \bullet P + B \bullet E^*)}{dt} = v_1 - v_2 \\ \frac{dE^* \bullet P}{dt} = v_1 - v_4 \\ \frac{dB \bullet E^*}{dt} = v_5 - v_2\end{gathered} \qquad [17]$$

where X and Υ stands for the following sums of concentrations of the enzyme states:

$$\begin{gathered}X = E + Q \bullet E + E \bullet A \\ \Upsilon = E^* + E^* \bullet P + B \bullet E^*\end{gathered} \qquad [18]$$

Substituting relationships [16] to system [17] and tending k_i, $i = 3, 4, 5, 6$ to infinity, one obtains

$$\begin{gathered}\frac{dX}{dt} = v_2 - v_1 \\ \frac{d\Upsilon}{dt} = v_1 - v_2\end{gathered} \qquad [19]$$

$$w_3 = 0, w_4 = 0, w_5 = 0, w_6 = 0 \qquad [20]$$

System of differential equations [19] corresponds to reduced catalytic cycle depicted in **Fig. 11.5**. To derive rate equation we should solve the system at steady state. To proceed with it we should express variables of catalytic cycle entering rate equations

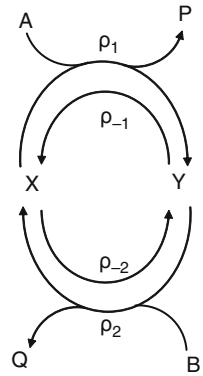

Fig. 11.5. Reduced scheme.

v_1 and v_2 ($E\cdot A$, $E^{*}\cdot P$, $B\cdot E^{*}$, $Q\cdot E$) in terms of new variables X and Υ. Solving system of the algebraic equations [18] and [20], one obtains

$$\begin{aligned} E \bullet A &= \frac{X}{\Delta_1}\cdot\frac{A}{K_A}, \quad Q \bullet E = \frac{X}{\Delta_1}\cdot\frac{Q}{K_Q} \\ E^{*} \bullet P &= \frac{\Upsilon}{\Delta_2}\cdot\frac{P}{K_P}, \quad B \bullet E^{*} = \frac{\Upsilon}{\Delta_2}\cdot\frac{B}{K_B} \\ \Delta_1 &= 1+\frac{A}{K_A}+\frac{Q}{K_Q}, \quad \Delta_2 = 1+\frac{B}{K_B}+\frac{P}{K_P} \end{aligned} \tag{21}$$

Substituting expressions [21] to the rate equations for v_1 and v_2 [16], one arrives at the following rate equations in terms of variables of reduced catalytic cycle X and Υ:

$$\begin{aligned} v_1 &= \rho_1\cdot A\cdot X - \rho_{-1}\cdot P\cdot \Upsilon \\ v_2 &= \rho_2\cdot B\cdot \Upsilon - \rho_{-2}\cdot Q\cdot X \end{aligned} \tag{22}$$

where apparent rate constant can be expressed in terms of parameters of catalytic cycle and substrate/product concentrations in the following manner:

$$\begin{aligned} \rho_1 &= \frac{1}{\Delta_1}\cdot\frac{k_1}{K_A}, \quad \rho_{-1} = \frac{1}{\Delta_2}\cdot\frac{k_{-1}}{K_P} \\ \rho_2 &= \frac{1}{\Delta_2}\cdot\frac{k_2}{K_B}, \quad \rho_{-2} = \frac{1}{\Delta_1}\cdot\frac{k_{-2}}{K_Q} \end{aligned} \tag{23}$$

Solving the system of equations [19], [22], and [23] at steady state, we obtain the following expressions for steady-state concentrations of X and Υ:

$$X = \frac{e_0\cdot(\rho_{-1}\cdot P+\rho_2\cdot B)}{\rho_1\cdot A+\rho_{-1}\cdot P+\rho_2\cdot B+\rho_{-2}\cdot Q}, \quad \Upsilon = \frac{e_0\cdot(\rho_1\cdot A+\rho_{-2}\cdot Q)}{\rho_1\cdot A+\rho_{-1}\cdot P+\rho_2\cdot B+\rho_{-2}\cdot Q} \tag{24}$$

Substitution of equation [24] into first equation of [22] results in the following expression for the reaction rate:

$$v = \frac{e_0\cdot\frac{k_1\cdot k_2}{K_A\cdot K_B}\left(A\cdot B - P\cdot Q\cdot\frac{k_{-1}\cdot k_{-2}}{k_1\cdot k_2}\frac{K_A}{K_P}\frac{K_B}{K_Q}\right)}{\left(k_1\cdot\frac{A}{K_A}+k_{-2}\cdot\frac{Q}{K_Q}\right)\cdot\left(1+\frac{B}{K_B}+\frac{P}{K_P}\right)+\left(k_2\cdot\frac{B}{K_B}+k_{-1}\cdot\frac{P}{K_P}\right)\cdot\left(1+\frac{A}{K_A}+\frac{Q}{K_Q}\right)} \tag{25}$$

where K_A, K_B, K_P, and K_Q are the dissociation constants of the substrates A, B and products P, Q from enzyme states E and E^*; k_i, k_{-i} , $i = 1, 2$ are the rate constants of catalytic stages of the enzyme cycle.

3.2.4. How to Express Parameters of Catalytic Cycle in Terms of Kinetic Parameters

In accordance to our approach described in this chapter, we derive rate equation for the enzyme on the basis of its catalytic cycle applying one of the techniques described above. The resulting rate equation represents fractionally rational function of concentrations of substrates, products, effectors, and parameters of catalytic cycle such as rate and dissociation constants of its individual reactions. However, in the enzyme kinetics the rate equations are usually written using parameters, which characterize kinetic properties of the enzyme as a whole. The Michaelis constants, the turnover number of the enzyme, and the equilibrium constant are used as such kinetic parameters (Cornish-Bowden (19)). In this section we present method allowing us to express parameters of the catalytic cycle in terms of kinetic parameters.

Let equation [26] be the rate equation of the enzyme functioning in which parameters of the catalytic cycle (the rate and dissociation constants of certain stages) are the terms:

$$v = [\mathrm{E}]_{\mathrm{tot}} \cdot f(S_1, \ldots, S_i, \ldots, S_n, P_1, \ldots, P_j, \ldots, P_m, M_1, \ldots, M_h, \ldots, Mq) \quad [26]$$

where $[\mathrm{E}]_{\mathrm{tot}}$ is the total enzyme concentration, and S_i ($i = 1, \ldots, n$), P_j ($j = 1, \ldots, m$), and M_h ($h = 1, \ldots, q$) are concentrations of the substrates, products, and modifiers (inhibitors and activators), respectively. Using biochemical definitions of conventional parameters of enzymatic kinetics (the Michaelis constants of the substrates and products, the equilibrium constants, the turnover number of enzyme in forward reaction in the presence and in the absence of activators and inhibitors), let us find how to express the kinetic parameters via parameters of the catalytic cycle. By definition, the turnover number of the enzyme in the forward reaction is the ratio of the maximal rate of enzyme functioning to the total enzyme concentration at saturating concentrations of all substrates and zero concentrations of all products and modifiers. This means that for calculation of the turnover number of enzyme in the forward reaction, the following expression should be used:

$$k_{\mathrm{cat}}^{\mathrm{f}} = \lim_{\substack{S_i \to \infty, i = 1, \ldots, n \\ P_j = 0, j = 1, \ldots, m \\ M_h = 0, h = 1, \ldots, q}} f(S_1, \ldots, S_n, P_1, \ldots, P_m, M_1, \ldots, M_q) \quad [27]$$

Analogously the maximal number of enzyme cycles at the saturating concentration of modifier (inhibitor or activator) M_r can be calculated as

$$k_{\text{cat}}^{\text{f},M_r} = \lim_{\substack{S_i \to \infty, i = 1, ..., n \\ M_r \to \infty \\ P_j = 0, j = 1, ..., m \\ M_h = 0, h = 1, ...r-1, r+1, ..., q}} f(S_1, ..., S_n, P_1, ..., P_m, M_1, ..., M_q) \quad [28]$$

The equilibrium constant can be found from the following expression:

$$K_{\text{eq}} = \prod_{j=1}^{m} \text{P}_j^{\text{eq}} \Big/ \prod_{i=1}^{n} \text{S}_i^{\text{eq}} \quad [29]$$

where the equilibrium concentrations of the substrates S_i^{eq} (i = 1, ..., n) and products P_j^{eq} (j = 1, ..., m) are solutions of the following equation:

$$f(S_1^{\text{eq}}, \ldots, S_i^{\text{eq}}, \ldots, S_n^{\text{eq}}, P_1^{\text{eq}}, \ldots, P_j^{\text{eq}}, \ldots, P_m^{\text{eq}}, M1, \ldots, M_h, \ldots, M_q) = 0 \quad [30]$$

By definition, the Michaelis constant of the enzyme with some substrate is the concentration of the considered substrate at which the rate of the enzyme functioning is half of its maximal rate under the conditions when the products and modifiers (inhibitors and activators) are absent and concentrations of all other substrates are saturating. In accord with this definition, $K_{\text{m,St}}$, the Michaelis constant of substrate S_t, is a solution of the following equation:
where

$$F_{S_t}(S_t) = \lim_{\substack{S_i \to \infty, i = 1, ..., t-1, t+1, ..., n \\ P_j = 0, j = 1, ..., m \\ M_h = 0, h = 1, ..., q}} f(S_1, ..., S_n, P_1, ..., P_m, M_1, ..., M_q) \quad [31]$$

Analogously, the Michaelis constant of substrate S_t at the saturating concentration of modifier (inhibitor or activator) M_r is

$$F_{S_t}^{M_r}(S_t) = \lim_{\substack{S_i \to \infty, i = 1, ..., t-1, t+1, ..., n \\ M_r \to \infty \\ P_j = 0, j = 1, ..., m \\ M_h = 0, h = 1, ..., r-1, r+1, ..., q}} f(S_1, ..., S_n, P_1, ..., P_m, M_1, ..., M_q) \quad [32]$$

where

$$\frac{k_{\text{cat}}^{\text{f},M_r}}{2} = F_{S_t}^{M_r}\left(K_{\text{m},S_t}^{M_r}\right)$$

and the Michaelis constant of product P_t is calculated as

$$F_{P_t}(P_t) = \lim_{\substack{P_j \to \infty, j = 1, ..., t-1, t+1, ..., m \\ S_i = 0, i = 1, ..., n \\ M_h = 0, h = 1, ..., q}} \left(-f(S_1, ..., S_n, P_1, ..., P_m, M_1, ..., M_q)\right) \quad [33]$$

where

$$\frac{k_{\text{cat}}^{b}}{2} = F_{P_t}\left(K_{\text{m},P_t}\right)$$

In this expression, the turnover number of enzyme in the reverse reaction, k^{b}_{cat}, is calculated as follows:

$$k_{\text{cat}}^{b} = \lim_{\substack{P_j \to \infty, j = 1, ..., m \\ S_i = 0, i = 1, ..., n \\ M_h = 0, h = 1, ..., q}} \left(-f(S_1, ..., S_n, P_1, ..., P_m, M_1, ..., M_q)\right) \quad [34]$$

Applying methods described above we can rewrite rate equations derived in the previous section in terms of kinetic parameters:

3.2.4.1. Random Bi Bi Mechanism

$$v = \frac{e_0 \cdot k_{\text{cat}}^{f}}{\Delta} \cdot \left(A \cdot B - P \cdot Q / K_{\text{eq}}\right) \quad [35]$$

where

$$\Delta = K_A \cdot K_{m,B} + K_{m,B} \cdot A + K_{m,A} \cdot A + A \cdot B + \frac{K_A \cdot K_{m,B}}{K_P} \cdot P + \frac{K_A \cdot K_{m,B}}{K_P} \cdot \frac{K_{m,P}}{K_{m,Q}} \cdot Q + \frac{K_A \cdot K_{m,B}}{K_P \cdot K_{m,Q}} \cdot P \cdot Q$$

3.2.4.2. Ordered Uni Bi Mechanism

$$v = \frac{e_0 \cdot k_{\text{cat}}^{f}}{\Delta} \cdot \left(A - P \cdot Q / K_{\text{eq}}\right)$$

where

$$\Delta = K_{m,A} + A + \frac{K_{m,A}}{\alpha \cdot \delta \cdot K_{m,P}} \cdot P + \frac{K_{m,A}}{\alpha \cdot \delta \cdot K_{m,Q}} \cdot Q + \frac{1 + \frac{1}{\alpha} - \frac{1}{\alpha^2 \cdot \delta}}{\alpha \cdot K_{m,P}} \cdot A \cdot P + \frac{1}{\alpha \cdot K_{eq}} \cdot P \cdot Q \quad [36]$$

3.2.4.3. Random Bi Bi Mechanism

$$v = \frac{e_0 \cdot k_{\text{cat}}^{f}}{\Delta} \cdot \left(A \cdot B - P \cdot Q / K_{\text{eq}}\right) \quad [37]$$

where

$$\Delta = \left(A + \frac{K_{m,A}}{K_{m,Q}} \cdot \phi \cdot Q\right) \cdot \left(K_{m,B} + \frac{K_A - K_{m,A}}{K_A} \cdot B + \frac{K_{m,B}}{K_P} \cdot P\right) + \left(B + \frac{K_{m,B}}{K_{m,P}} \cdot \phi \cdot P\right) \cdot \left(K_{m,A} + \frac{K_{m,A}}{K_A} \cdot A + \frac{K_{m,B}}{K_{m,Q}} \cdot \frac{K_P - K_{m,P}}{K_P} \cdot Q\right)$$

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