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SABIO-RK: Kinetic Data for Reaction Mechanism Steps

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Abstract

SABIO-RK is a curated database containing kinetic information not only for biochemical reactions but also for individual steps of the reaction mechanism manually extracted from literature. Data in SABIO-RK comprises information about reaction participants including enzyme properties, biological locations (organism, tissue etc.), kinetic parameters and rate equations determined for the reaction, and the experimental conditions used for the determination. To understand biochemical reactions and their kinetic behaviour not only reaction details and kinetic properties of the biochemical reactions but also details of the reaction mechanism are essential. To meet these requirements additionally to the kinetic data of the reactions SABIO-RK offers a graphical representation of the mechanism of a selected reaction as a survey and also a detailed listing of all individual reaction steps as separate entries.

Introduction

SABIO-RK (http://sabio.villa-bosch.de/) [1, 2] is a database containing information about biochemical reactions and their kinetic properties based on literature information and data from automatic submissions from lab experiments (publication in progress). The kinetic data

are related to the organism, tissue or cell type and to the environmental conditions in which they were determined. Beside the information about the overall reactions SABIO-RK offers new feature information about individual mechanism steps.

Most of the available pathway and enzyme databases (KEGG [3], BRENDA [4], IUBMB [5], IntEnz [6], Rhea [7], Reactome [8] etc.) contain information about enzymes and biochemical reactions only focussing on the overall biochemical reactions catalysed by enzymes. This does not allow a detailed analysis of the reaction mechanism by searching for elementary steps of the overall reaction. The MACiE database [9] offers stepwise information about the reaction mechanism based on structural information of the proteins and chemical compounds. For each EC sub-subclass where there is a crystal structure and sufficient evidence in the literature to support mechanism information is given in MACiE. The mechanism steps of the reactions include the function of the catalytic residues involved in the reaction and the mechanism by which substrates are transformed into products.

At the moment there is no database available containing kinetic parameters as quantitative data for individual reaction mechanism steps. Since there is no standard data format for the representation of the mechanism in the literature a structured format is needed to store, easily access and export the data.

For a comprehensive analysis of a biochemical reaction and the enzymatic mechanism detailed information about the reaction mechanism and kinetic parameters for the steps are necessary. Linking of the reaction mechanism data to the overall reaction is essential to relate the information to the corresponding general information of the overall reaction available in SABIO-RK and to other external databases.

Reaction Mechanism

The data in SABIO-RK are extracted from literature or automatically submitted by wet lab experimentators. The data include information about biochemical reactions and related pathways, reaction participants, enzyme and protein characteristics such as EC number, cellular location, UniProt [10] accession number, molecular weights of protein complexes and subunits and beyond that the description of the protein complex composition (*e.g.* homohexamer described as (subunit)*6). General information about the organism and tissue are linked to the reactions. Additionally, the source of the data is provided *e.g.* by the literature reference. For the biochemical reactions kinetic details are extracted from the literature which includes kinetic parameters (K_m, V_{max}, k_{cat}, K_i values etc.), the type of the kinetic law (Michaelis-Menten, Ping Pong, bi-bi etc.) and the corresponding kinetic law equation. Moreover, the experimental conditions (pH, temperature and buffer) under which the kinetic parameters were determined are available.

Beside the general description and kinetic characterization of the overall biochemical reactions SABIO-RK also collects information about single mechanism steps representing the individual interactions of the single reaction participants (substrates, products, inhibitors, activators, cofactors etc.) with the catalytic proteins or intermediate states of the protein (Fig. 1). Forward and reverse reactions are handled as separate reactions for the representation of the mechanism steps. For example the biochemical reaction "L-serine + L-homocysteine = cystathionine + H_2O" represents an overall reaction in SABIO-RK for which kinetic parameters are stored. Beyond that kinetic parameter are available in the literature for the individual mechanism steps represented as separate reactions (equations 1 – 8) leading to separate mechanism entries in the database linked to the overall reaction mentioned above.

Equations 1 – 8:

(1) E + A → EA
(2) EA → E + A
(3) EA → EX + Q
(4) EX + B → EXB
(5) EXB → EX + B
(6) EXB → EP
(7) EP → E + P
(8) E + P → EP

E, A, B, P, and Q are the representatives of Enzyme, L-serine, L-homocysteine, cystathionine, and H_2O, respectively. X is the intermediate aminoacrylate formed during the catalytic process. Compared to the MACiE database SABIO-RK displays the mechanism steps in an abstract representation, no structural formulae or structural descriptions of catalytic centres of enzymes nor detailed description or representation of chemical interactions between compounds and proteins are indicated.

The SABIO-RK database contains mechanism information both on a qualitative and quantitative level dependent on the information given in the publication. Qualitative information is the representation of the steps to define the order of interactions without any kinetic parameters. This representation is used to understand the types of interactions of all reaction participants with the enzyme and to define different enzyme complexes and intermediate states of the enzyme. If quantitative data are provided, the corresponding kinetic parameters are stored for each single mechanism step. Kinetic parameters represent mainly rate constants but also include participant concentrations and dissociation constants. Rate constants for the forward and reverse reactions like for example *k+1* and *k-1* are stored in separate entries because they represent different mechanism steps.

One advantage of the representation of individual reaction mechanism steps is the possibility to define protein-ligand interactions such as the binding of reaction participants to the enzyme or to the enzyme-ligand complexes. These definitions of protein-ligand interactions are helpful in the understanding of the mechanism of inhibitor, activator or cofactor interactions.

Each reaction step is related to the overall reaction with its general information (enzyme and protein details, organism, tissue, information source) and is represented equivalently to the overall reaction by its reaction participants (substrates, products, inhibitors, activators etc.), kinetic parameters and corresponding kinetic law information. For each reaction mechanism step individual experimental conditions can be defined independent of the experimental conditions of the overall reaction. For the individual reactions also different kinetic law types and different kinetic law equations compared to the overall reaction can be assigned.

All the mechanism information can be accessed via the overall reaction entries in the SABIO-RK user interface. At the moment web service functions for the mechanism data are not implemented. To search for mechanism entries, the user interface offers a checkbox "Detailed mechanism data (single steps)". Additionally, at least one of the dark blue highlighted parameters (reactant, pathway, enzyme, organism etc.) on the main search page of the user interface must be selected to search for reaction mechanism details.

On the mechanism details page of the SABIO-RK user interface (Fig. 1) the reaction mechanism can be displayed by using several predefined automatic layout algorithms [11]. A code using different colours and shapes for the reaction participants helps to easily understand the graphical representation of the reactions. The colours used to code for substrates, products, activators, and inhibitors are yellow, blue, green, and red, respectively. Chemical compounds (molecules) are displayed as circles and enzymes and enzyme-compound complexes as rectangles. Additionally the graphical representation includes the possibility to select reaction details (triangle) of single graphs representing an individual reaction mechanism step to highlight either the reaction equation of the selected step or the kinetic parameters of the step in a table view. The graphical representation of the mechanism steps was implemented as a Java applet, which provides interactive features to web applications that cannot be provided by HTML alone.

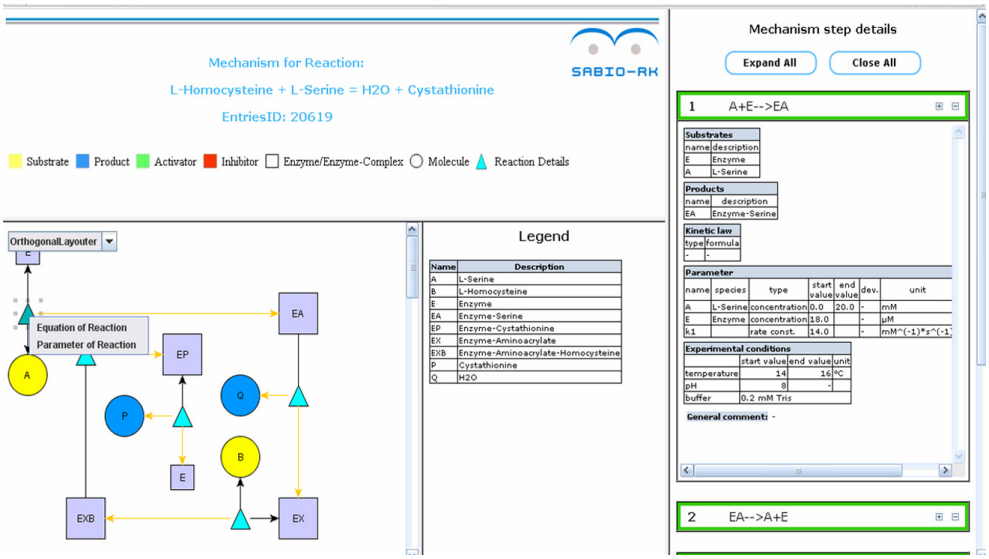

Figure 1. Screenshot of the reaction mechanism details page in the SABIO-RK user interface

Reaction participants of the mechanism steps are explained in more detail in the legend of the mechanism details page. The legend contains a description of the abbreviated step participant names. In the database reaction participants are matched to the reaction participants of the overall reaction. For example a protein complex EAB is related to three different reaction participants of the overall reaction: E, A, and B. They are the representatives of the enzyme, the first substrate (L-serine) and the second substrate (L-homocysteine), respectively, of the reaction mechanism above. This information will be also available as links to the corresponding compound details page in the SABIO-RK user interface in one of the next database releases.

Each single mechanism step is represented in a separate reaction entry containing the detailed information about this step including step equation, step participants details, kinetic parameters and law description, and experimental conditions.

The web-based input interface and the data model of the SABIO-RK database were adapted to the new requirements for the insert and storage of mechanism data. Analogue to the data for main biochemical reactions these mechanism data are manually extracted from literature, are related to overall reaction entries and are manually curated by biological experts. Along the lines of the SABIO-RK data model for overall reactions the mechanism data are represented as similar objects in the database (Fig. 2). Step participants are handled like reactants and modifiers of the main reaction. Mechanism steps are similar to reactions and step parameters to kinetic parameters of overall reactions.

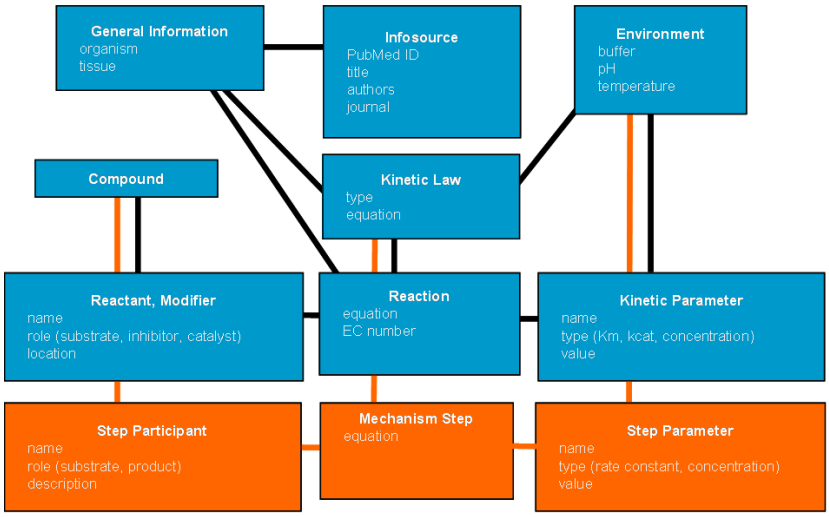

Figure 2. Extended data model (schematic) for representing reaction mechanism

Conclusion and Future perspectives

To meet the requirements for a detailed analysis of biochemical reactions and their kinetic behaviour additionally to the kinetic data of the biochemical reactions SABIO-RK now includes a graphical representation of the mechanism of a selected reaction as a survey and also a detailed listing of all individual reaction steps as separate entries. Each reaction step is related to the overall reaction with its general information (enzyme, organism, information source) and is represented equivalently to the overall reaction by its reaction participants (substrates and products), kinetic parameters like for example rate constants and corresponding kinetic law information. For each reaction mechanism step individual experimental conditions can be defined. Beside the graphical representation of the reaction mechanism using several layout algorithms each mechanism step is represented in a separate reaction entry containing the detailed information about this step including step equation, step participants details, kinetic parameters and law description, and corresponding experimental conditions.

The storage and representation of reaction mechanism steps in SABIO-RK offers the possibility to define protein-ligand interactions like for example the binding of reaction participants to the enzyme or to the enzyme-ligand complexes which is important for the representation of signalling reactions in regulatory pathways. The detailed analysis of protein-ligand interactions helps to understand the mechanism of cofactors, inhibitors, or activators in complex networks.

SABIO-RK provides export functions for the overall reactions and their kinetic properties in SBML format [12] to allow the import of the data into simulation and modelling tools. In the future also mechanism steps will be included in the SBML export to combine information and kinetic data from overall reactions with details of reaction mechanism steps.

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