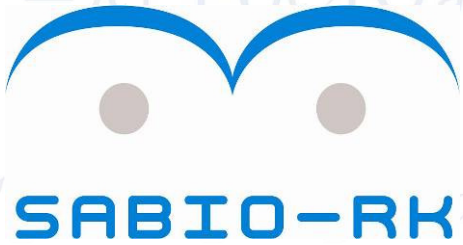
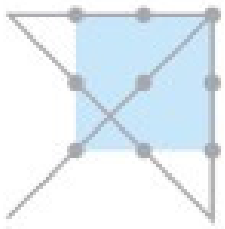


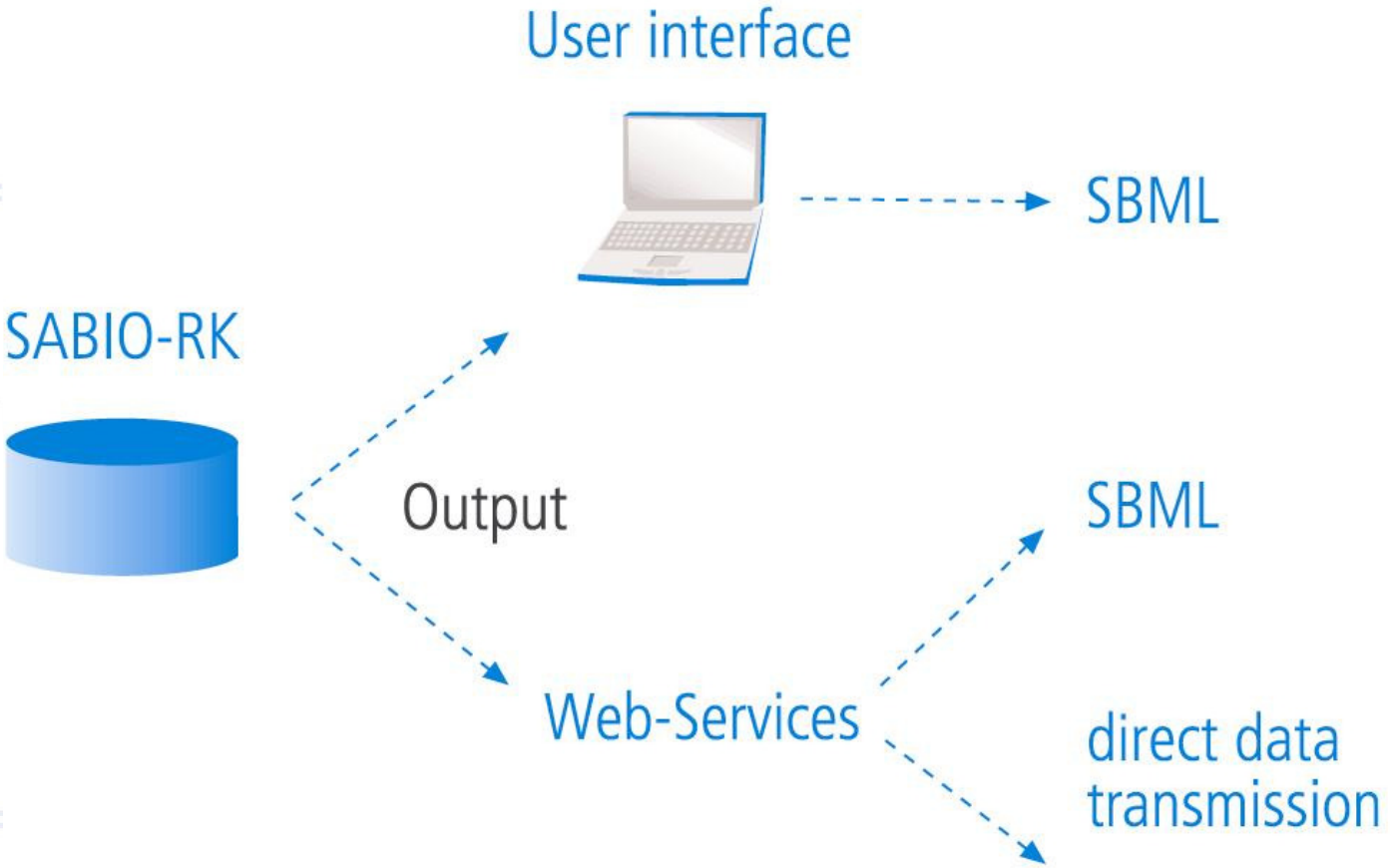


EML Research

SABIO-RK
User Interface

Ulrike Wittig

Data Access in SABIO-RK



SABIO-RK User Interface

- Search for general reaction information, kinetic laws, kinetic parameters, experimental conditions etc.
- Colour-coded representation of results

– *Kinetic data available matching search criteria*

– *Kinetic data available but not matching search criteria (other organism, tissues etc.)*


– *No kinetic data available*

- Links to external databases

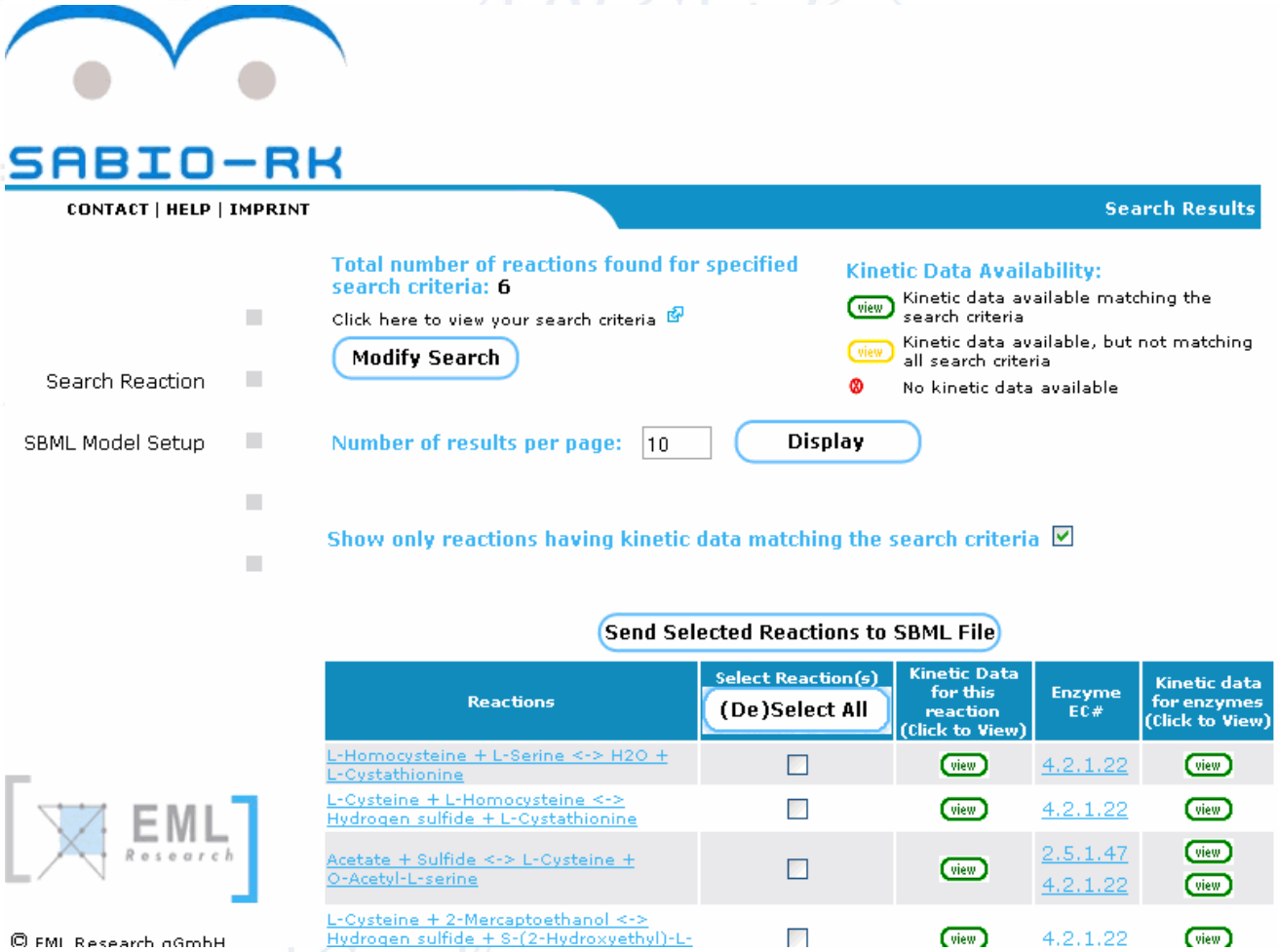
Queries

- Search for reactions by defining substrates /products (compound names)
- Search for reactions by defining enzymes (EC number or name)
- Search for reactions by defining proteins (UniProt identifier)
- Search for publications (author name or PubMed identifier)
- Search for reactions for a given pathway, organism, tissue, cellular location (name)
- Search for reactions containing defined kinetic parameters or laws (select from list)
- Search for reactions under defined experimental conditions (specify values)

→ ***Definition of queries by the combination of all search criteria***

http://sabio.villa-bosch.de/SABIORK

http://sabio.villa-bosch.de/SABIORK



http://sabio.villa-bosch.de/SABIORK

Reaction Details

Stochiometric Equation	L-Homocysteine + L-Serine <-> H2O + L-Cystathionine
Substrates	L-Homocysteine L-Serine
Products	H2O L-Cystathionine

Enzymes known to catalyse this reaction (curated information)

EC Number	in Organism	SWP/UnitProt Link	PubMedID
4.2.1.22	Homo sapiens	SWP/UnitPro	9675031
4.2.1.22	Homo sapiens	SWP/UnitPro	681363
4.2.1.22	Homo sapiens	SWP/UnitPro	15520012
4.2.1.22	Homo sapiens	SWP/UnitPro	15581573
4.2.1.22	Aeropyrum pernix	SWP/UnitPro	12644499
4.2.1.22	Rattus norvegicus	SWP/UnitPro	5456996

Pathways	Glycine, Serine and Threonine metabolism Methionine metabolism

Compound Details

Back | List of Reactions

Common Name	L-Homocysteine
Synonyms	L-2-Amino-4-mercaptobutyric acid
SABIO-Compound-ID	1950
External Links	
CAS-ID	6027-13-0
KEGG-ID	C00155
PUBCHEM-ID	3455
ChEBI-ID	17588
HepatoSys-ID	C00155 (Only accessible for HepatoSys project members)

Enzyme Details

Enzyme (recommended) name	Cystathionine beta-synthase
Alternative names	L-serine hydro-lyase (adding homocysteine) Serine sulfhydrase beta-thionase Methylcysteine synthase Cysteine synthase Serine sulfhydrylase
EC Classification	4.2.1.22
Classification	Lyases Carbon-oxygen lyases Hydro-lyases
Catalyses reactions	L-Serine + Sulfide <-> L-Cysteine + H2O L-Homocysteine + L-Serine <-> H2O + Cystathionine L-Homocysteine + L-Serine <-> H2O + L-Cystathionine Selenohomocysteine + L-Serine <-> Selenocystathionine + H2O L-Cysteine + L-Homocysteine <-> Hydrogen sulfide + L-Cystathionine Acetate + Sulfide <-> L-Cysteine + O-Acetyl-L-serine L-Cysteine + 2-Mercaptoethanol <-> Hydrogen sulfide + S-(2-Hydroxyethyl)-L-cysteine
External links	Expasy KEGG IntEnz (EBI) IUBMB Reactome HepatoSys (Only accessible for HepatoSys project members)

http://sabio.villa-bosch.de/SABIORK

Kinetic
L-Homocysteine +

Expa

Entry Nr. 3101

Organism:	**Homo sapiens**
Tissue:	**unknown**
EC Class: 4.2.1.22	wildtype

Entry Nr. 3102

Organism:	**Homo sapiens**
Tissue:	**unknown**
EC Class: 4.2.1.22	mutant delta411-551

Entry Nr. 3103

Organism:	**Homo sapiens**
Tissue:	**unknown**
EC Class: 4.2.1.22	wildtype

Entry Nr. 5535

Organism:	**Homo sapiens**
Tissue:	**unknown**
EC Class: 4.2.1.22	wildtype

Entry Nr. 8224

Tissue:	**liver**
EC Class: 4.2.1.22	wildtype
Recombinant	Expressed in Escherichia coli

Substrates

name	location	comment
L-Homocysteine	-	-
L-Serine	-	-

Products

name	location	comment
H2O	-	-
L-Cystathionine	-	-

Modifiers

name	location	effect	comment	protein complex
Cystathionine beta-synthase(Enzyme)	-	Modifier-Catalyst	-	(P35520)*4;

Enzyme (protein data)

	UniProt-ID	name	mol. weight (kDa)	deviation (kDa)
subunit	-	-	-	-
complex	-	-	252.0	-

Kinetic Law

type	formula
Michaelis-Menten	E*kcat*S/(Km+S)

Parameters

name	species	type	start value	end value	deviation	unit	comment
E	Enzyme	concentration	-	-	-	-	-
S	L-Serine	concentration	0.005	6.4	-	mM	-
A	L-Homocysteine	concentration	5	-	-	mM	-
Km	L-Serine	Km	3	-	-	mM	-
kcat_Km	L-Serine	kcat/Km	2	-	-	mM^(-1)*s^(-1)	-
kcat	-	kcat	6	-	-	s^(-1)	-

Experimental conditions

	start value	end value	unit
pH	8.6	-	-
temperature	37	-	°C
buffer: 10 mM Tris/HCl			

General comment: -

PUBMEDID: 9675031

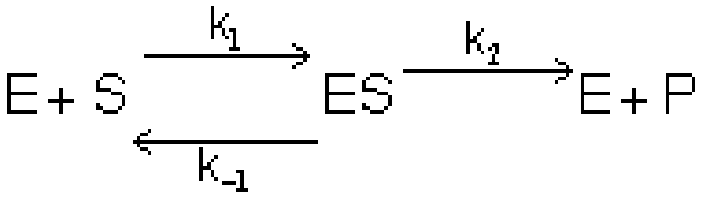
Compound Classification

Search for general compounds/ compound classes

Example: Amino acid

→ search result gives all reactions with any amino acid as substrate or product

Reaction Mechanism



- For simulation experiments detailed information about the reaction mechanism and kinetic parameters for the steps are necessary
- No quantitative data for mechanism steps available in structured format
- Relation of the reaction mechanism data to the overall reaction

Sources for Reaction Mechanism

Literature

- no standard representation
- manual information extraction

isolated the carboxylated enzyme, which was capable of transferring its carboxyl to acetyl-CoA. The active carboxyl in the carboxylated enzyme was shown to be bound to the 1′-*N*-atom of biotin linked to the ε-amino group of a lysine residue in the enzyme. These results support the following reaction mechanism involving two partial reactions.

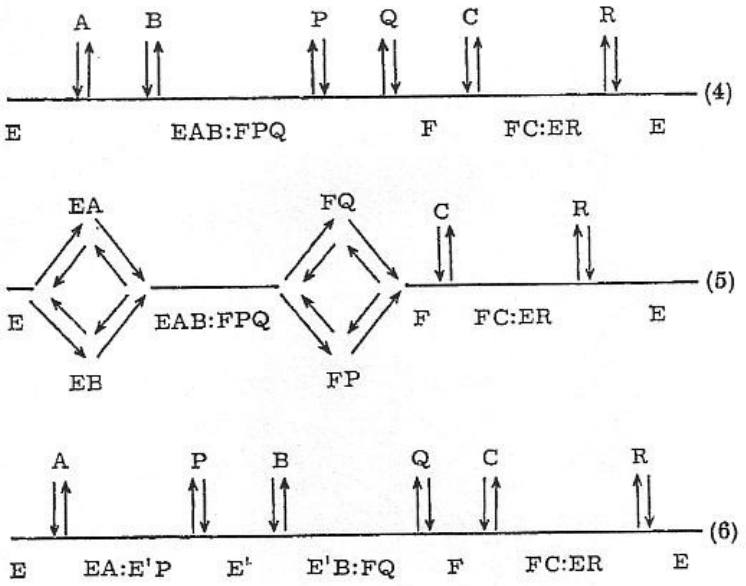
$$\text{ATP} + \text{HCO}_3^- + \text{E-biotin} \overset{Mg^{2+}}{\rightleftharpoons} \text{E-biotin}\sim\text{CO}_2 + \text{ADP} + \text{P}_i \quad (1)$$

$$\text{E-biotin}\sim\text{CO}_2 + \text{acetyl-CoA} \rightleftharpoons \text{E-biotin} + \text{malonyl-CoA} \quad (2)$$

Overall:

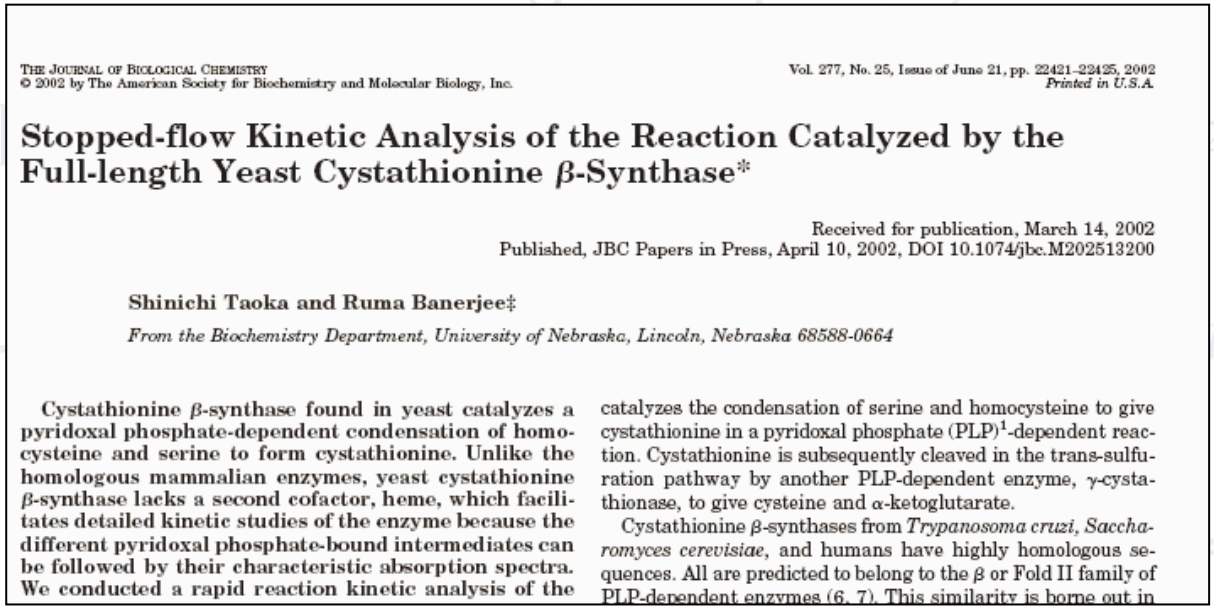
$$\text{ATP} + \text{HCO}_3^- + \text{acetyl-CoA} \overset{Mg^{2+}}{\rightleftharpoons} \text{ADP} + \text{P}_i + \text{malonyl-CoA} \quad (3)$$

Enzymes. Acetyl-CoA carboxylase or acetyl-CoA : CO_2 ligase (ADP) (EC 6.4.1.2); pyruvate kinase or ATP : pyruvate phosphotransferase (EC 2.7.1.40); lactate dehydrogenase or L-lactate : NAD oxidoreductase (EC 1.1.1.27); hexokinase or ATP : D-hexose 6-phosphotransferase (EC 2.7.1.1); glucose-6-phosphate dehydrogenase or D-glucose-6-phosphate : NADP oxidoreductase (EC 1.1.1.49); citrate synthase or citrate oxaloacetate-lyase (CoA-acetylating) (EC 4.1.3.7); malate dehydrogenase or L-malate : NAD oxidoreductase (EC 1.1.1.37).



A, B, P and Q represent substrates and products of the first partial reaction [Eqn. (1)], and C and R acetyl-CoA and malonyl-CoA, respectively. E and F represent two forms of the enzyme, *i.e.* enzyme-

Mechanism Paper



THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 277, No. 25, Issue of June 21, pp. 22421–22425, 2002
Printed in U.S.A.

Stopped-flow Kinetic Analysis of the Reaction Catalyzed by the Full-length Yeast Cystathionine β-Synthase*

Received for publication, March 14, 2002
Published, JBC Papers in Press, April 10, 2002, DOI 10.1074/jbc.M202513200

Shinichi Taoka and Ruma Banerjee‡

From the Biochemistry Department, University of Nebraska, Lincoln, Nebraska 68588-0664

Cystathionine β-synthase found in yeast catalyzes a pyridoxal phosphate-dependent condensation of homocysteine and serine to form cystathionine. Unlike the homologous mammalian enzymes, yeast cystathionine β-synthase lacks a second cofactor, heme, which facilitates detailed kinetic studies of the enzyme because the different pyridoxal phosphate-bound intermediates can be followed by their characteristic absorption spectra. We conducted a rapid reaction kinetic analysis of the

catalyzes the condensation of serine and homocysteine to give cystathionine in a pyridoxal phosphate (PLP)[1]-dependent reaction. Cystathionine is subsequently cleaved in the trans-sulfuration pathway by another PLP-dependent enzyme, γ-cystathionase, to give cysteine and α-ketoglutarate.

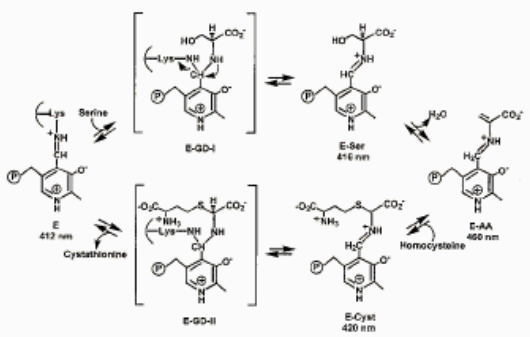
Cystathionine β-synthases from *Trypanosoma cruzi*, *Saccharomyces cerevisiae*, and humans have highly homologous sequences. All are predicted to belong to the β or Fold II family of PLP-dependent enzymes (6, 7). This similarity is borne out in

Cystathionine beta-synthase (EC 4.2.1.22)

L-Serine + L-Homocysteine = Cystathionine + H2O

Overall Reaction

L-Serine + L-Homocysteine = Cystathionine + H2O



SCHEME I. **Intermediates in the reaction catalyzed by cystathionine β-synthase and their absorption maxima as determined in this study.** The two gem-diamine intermediates (*GD-I* and *GD-II*), shown in *square brackets*, were not observed in the pre-steady-state kinetic analysis of the full-length yeast enzyme. *AA* and *Cyst* denote aminoacrylate and cystathionine, respectively.

A. Forward Reaction

$$E \underset{21\ s^{-1}}{\overset{14\ mM^{-1}\ s^{-1}}{\rightleftharpoons}} E{\cdot}Ser \overset{15\ s^{-1}}{\rightleftharpoons} E{\cdot}AA$$

$$24\ s^{-1} \uparrow\downarrow 35\ mM^{-1}\ s^{-1}$$

$$E \underset{(1.5\ mM^{-1}\ s^{-1})}{\overset{(2.3\ s^{-1})}{\rightleftharpoons}} E{\cdot}Cyst \overset{180\ s^{-1}}{\rightleftharpoons} E{\cdot}AA{\cdot}Hcy$$

B. Reverse Reaction

$$E \underset{2.3\ s^{-1}}{\overset{1.5\ mM^{-1}\ s^{-1}}{\rightleftharpoons}} E{\cdot}Cyst \overset{fast}{\rightleftharpoons} E{\cdot}AA{\cdot}Hcy$$

SCHEME II. **Minimal kinetic scheme for reaction catalyzed by yeast cystathionine β-synthase in the forward and reverse directions.** The values shown in *parentheses* in *A* are taken from *B*, where the reaction was followed in the reverse direction.

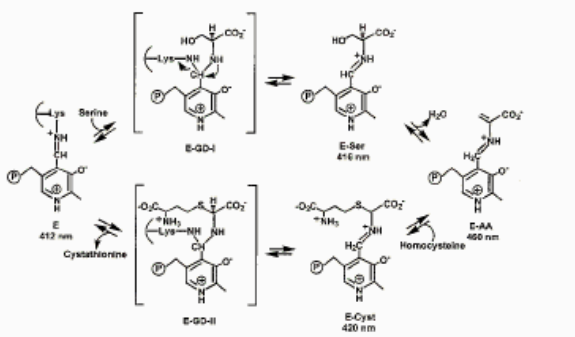
→ Not useful for the storage in the SABIO-RK database

→ Useful for the storage in the SABIO-RK database

Overall Reaction

L-Serine + L-Homocysteine = Cystathionine + H2O

A **B** **P** **Q**



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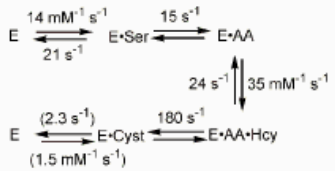
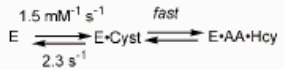
A. Forward Reaction



B. Reverse Reaction



SCHEME II. **Minimal kinetic scheme for reaction catalyzed by yeast cystathionine β-synthase in the forward and reverse directions.** The values shown in *parentheses* in *A* are taken from *B*, where the reaction was followed in the reverse direction.

Mechanism Steps

E + A → EA
EA → E + A
EA → EX + Q
EX + B → EXB
EXB → EX + B
EXB → EP
EP → E + P
E + P → EP

Overall Reaction

L-Serine + L-Homocysteine = Cystathionine + H2O

A **B** **P** **Q**

Mechanism Steps

E + A → EA
EA → E + A
EA → EX + Q
EX + B → EXB
EXB → EX + B
EXB → EP
EP → E + P
E + P → EP

Insertion using the SABIO-RK Input Interface

User Interface – Overall Reaction

L-Serine + L-Homocysteine = Cystathionine + H2O

User Interface – Overall Reaction

L-Serine + L-Homocysteine = Cystathionine + H2O

Entry Nr. 20619 [⊞][⊟] Select Show Mechanism

Organism:	**Saccharomyces cerevisiae**
Tissue:	**unknown**
EC Class: 4.2.1.22	wildtype

Substrates

name	location	comment
L-Serine	-	-
L-Homocysteine	-	-

Products

name	location	comment
H2O	-	-
Cystathionine	-	-

Modifiers

name	location	effect	comment	protein complex
Cystathionine beta-synthase(Enzyme)	-	Modifier-Catalyst	-	P32582;

Enzyme (protein data)

	UniProt-ID	name	mol. weight (kDa)	deviation (kDa)
subunit	-	-	-	-
complex	-	-	56.0	-

Kinetic Law

type	formula
-	-

Parameters

name	species	type	start value	end value	deviation	unit	comment
Kd	L-Serine	Kd	1.5	-	0.3	mM	-
E	Enzyme	concentration	18	-	-	µM	-
A	L-Serine	concentration	0	20	-	mM	-
kcat	-	kcat	1.7	-	-	s^(-1)	-

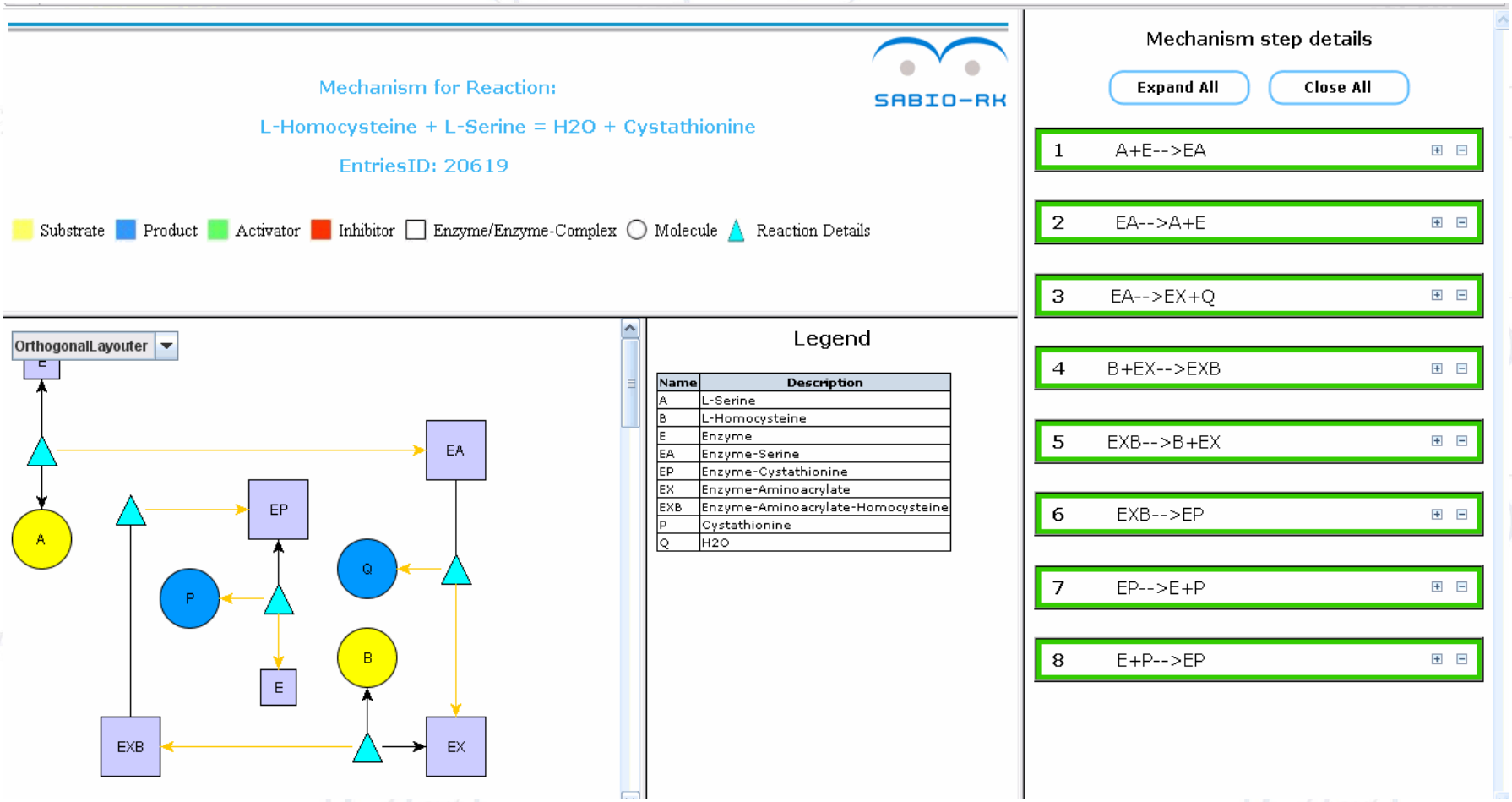
Experimental conditions

	start value	end value	unit
pH	8	-	-
temperature	14	16	°C
buffer: 0.2 mM Tris			

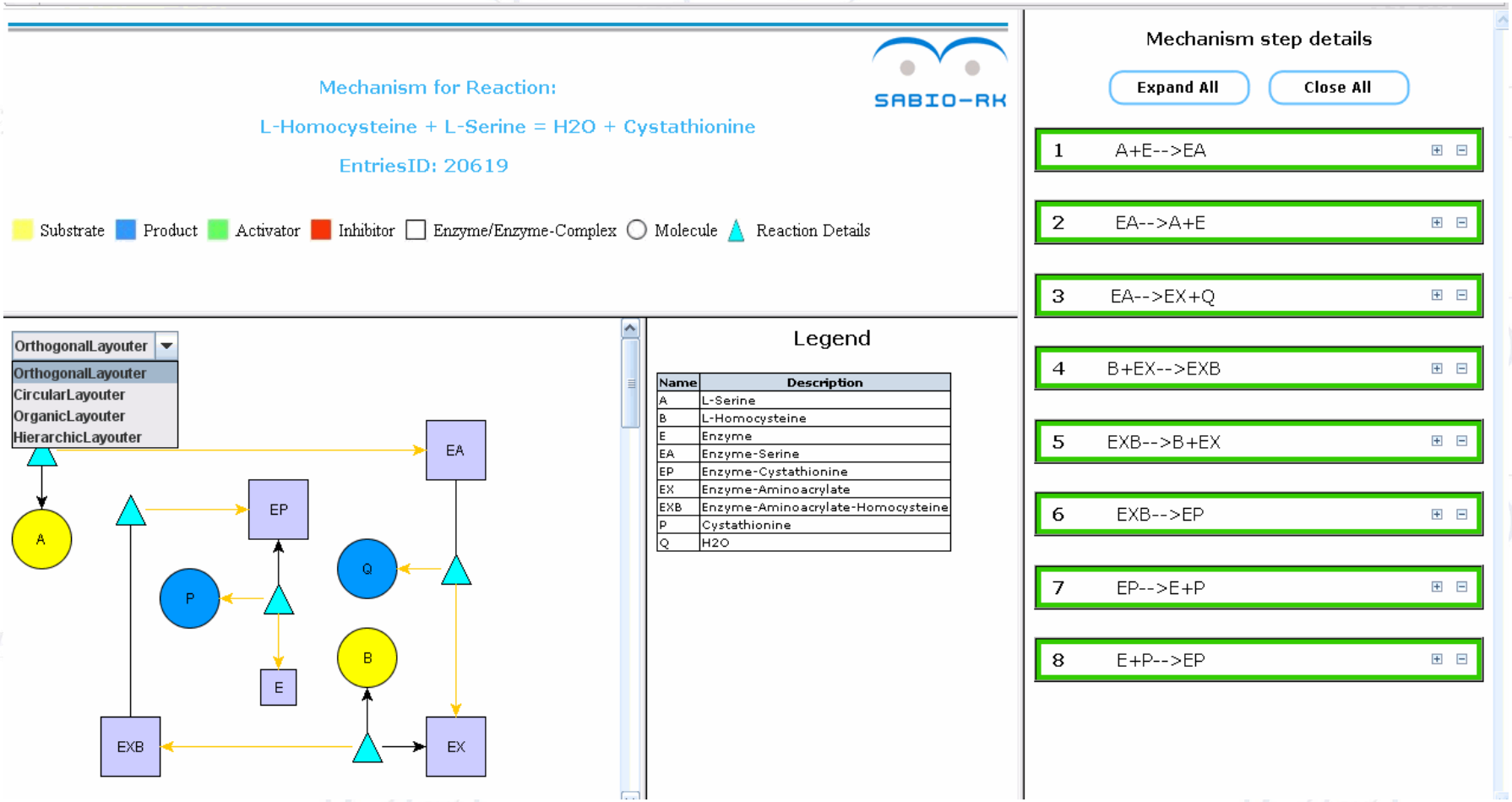
General comment: -

PUBMEDID: 11948191

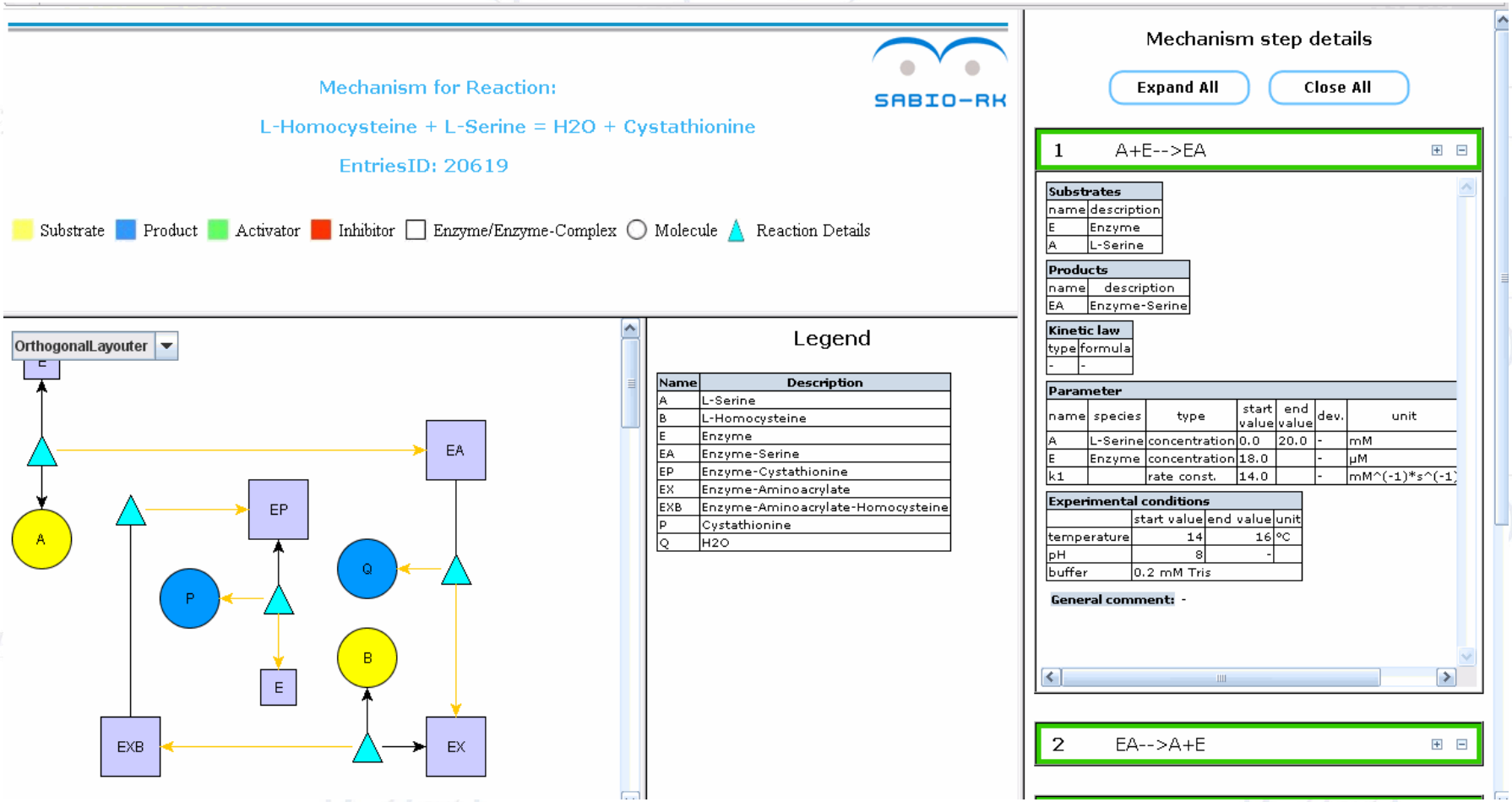
User Interface – Mechanism



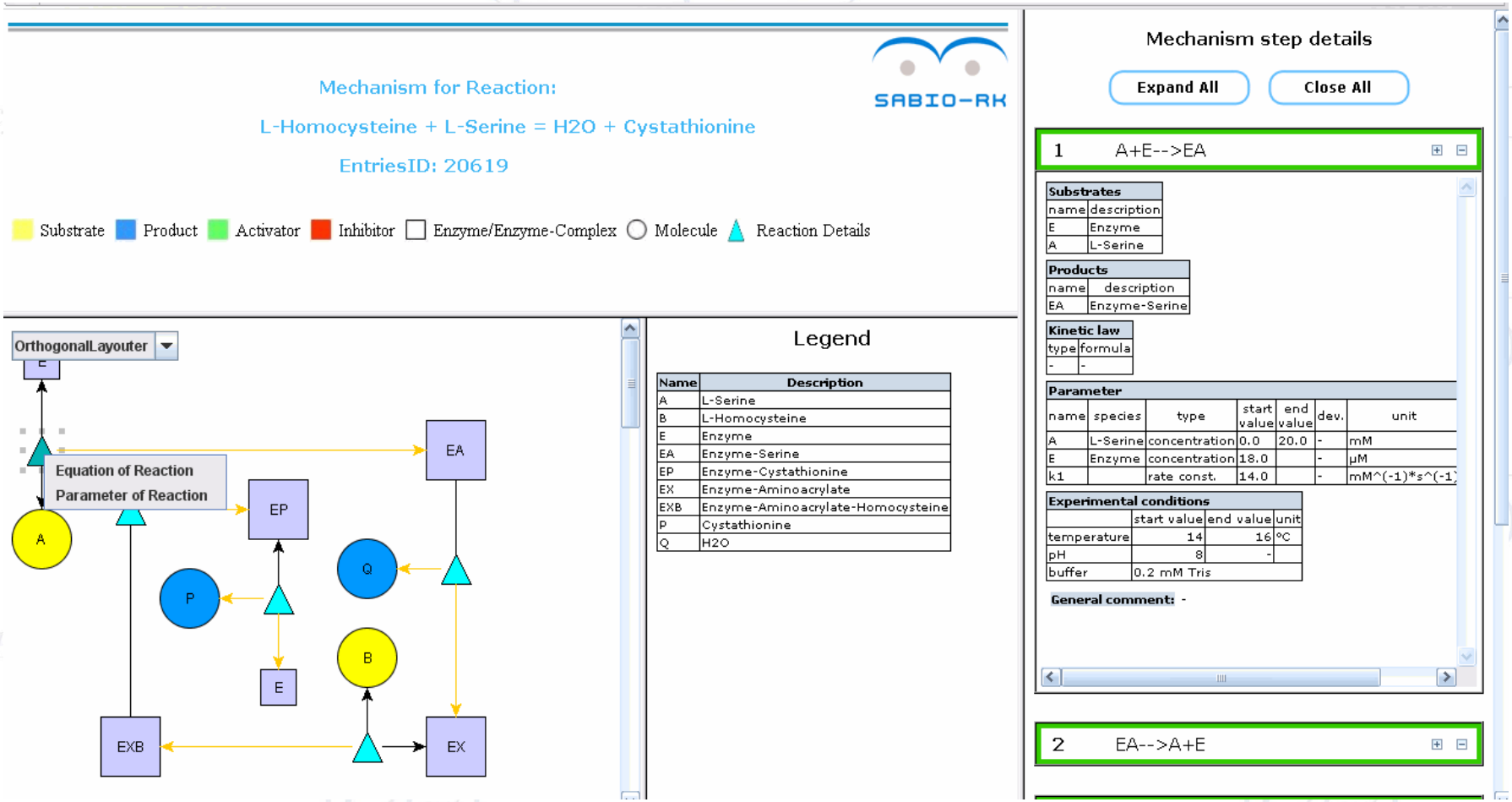
User Interface – Mechanism



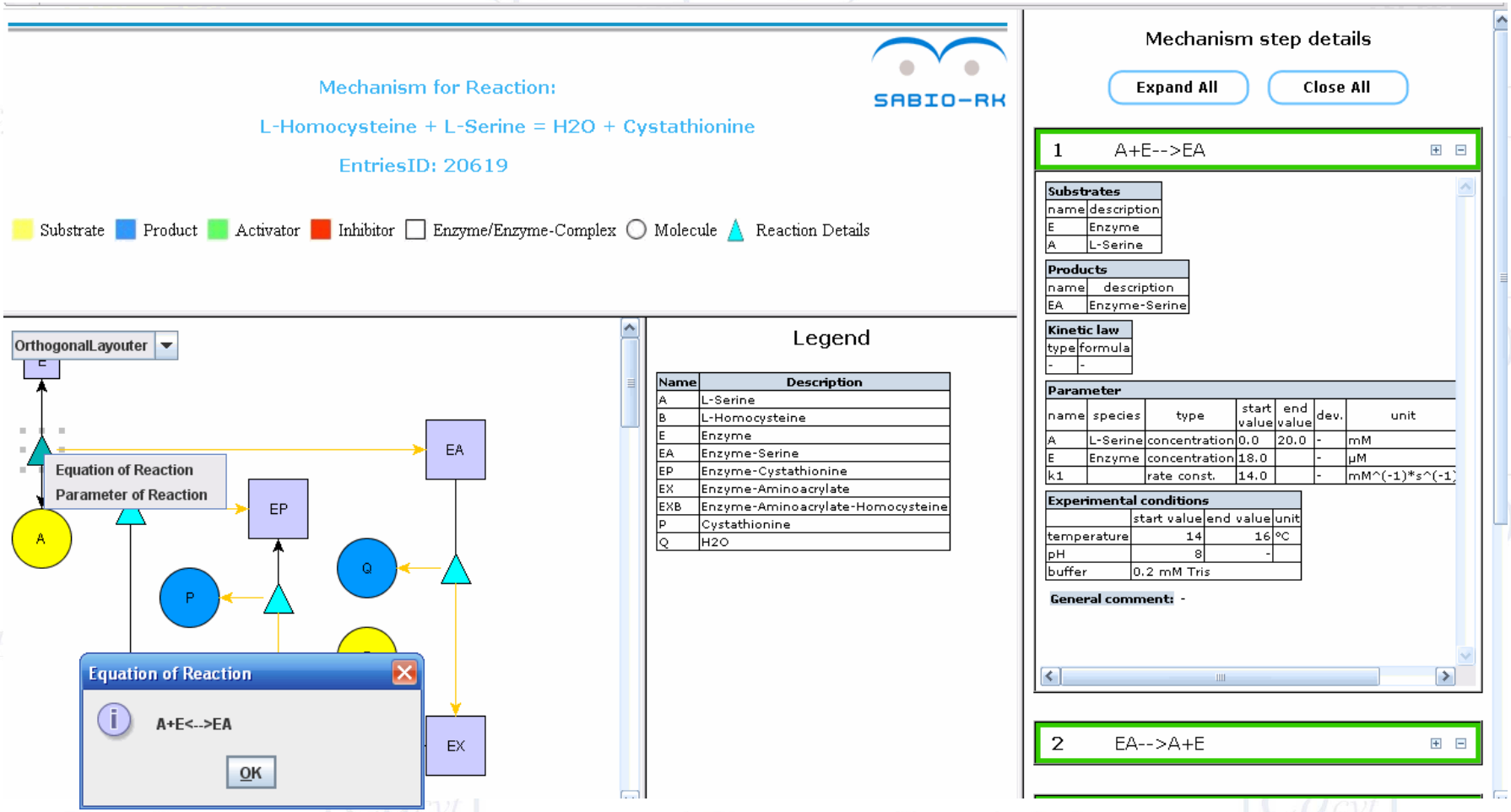
User Interface – Mechanism



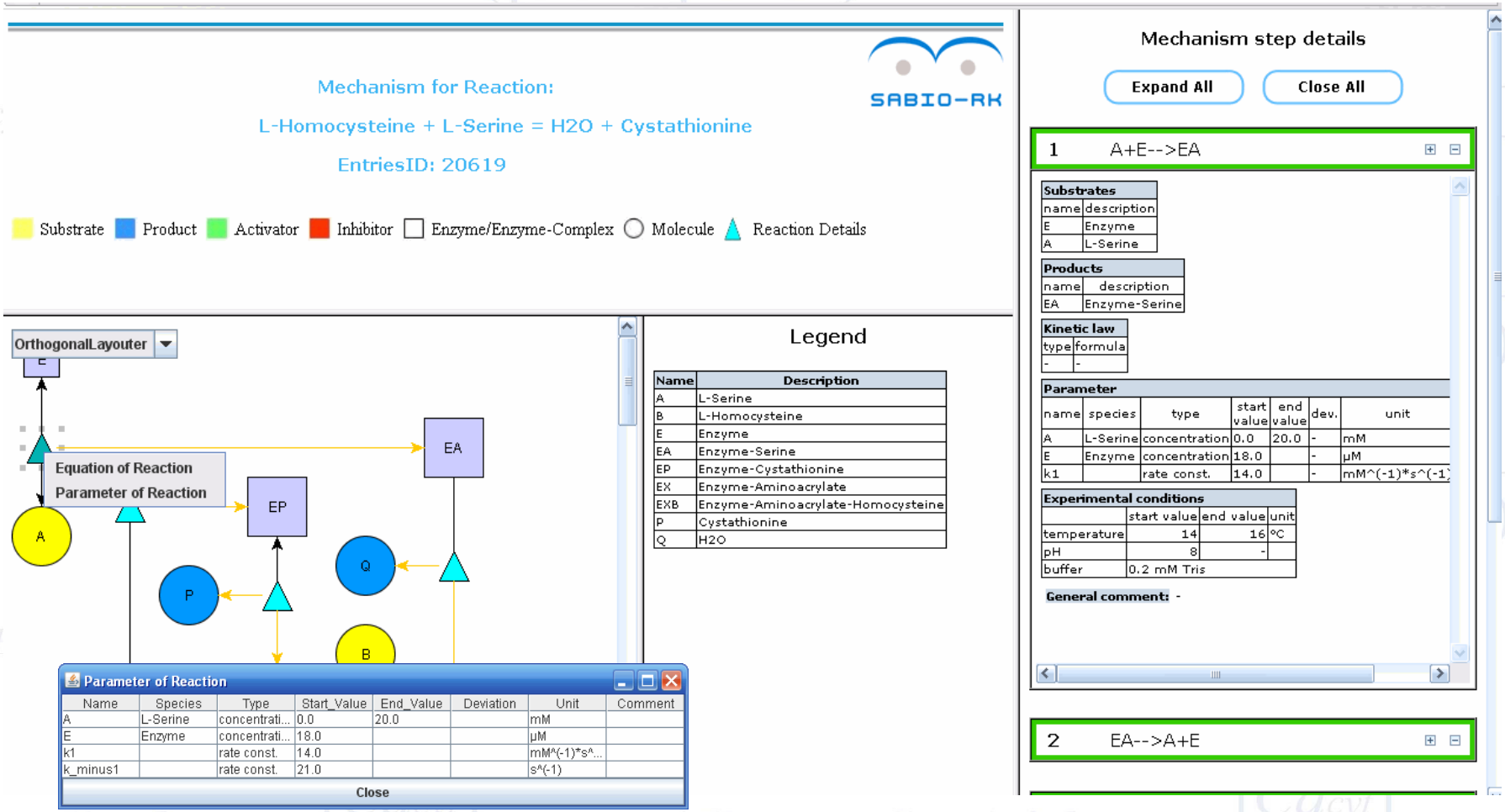
User Interface – Mechanism



User Interface – Mechanism



User Interface – Mechanism



Future Search Functions

- expansion of compound classification
- classification of organisms, tissues/ cell types, cell locations
- search for mechanism
- search for modifiers: inhibitor/ activator/ cofactor

- less reaction oriented search

  Examples:

  → search for all enzymes in a tissue/ organism etc.

  → search for all organisms containing specific enzyme