

# E-cyanobacterium.org: Tutorial

## Introduction

Cyanobacterium is one of the simplest organisms that developed oxygenic photosynthesis and helped to establish conditions suitable for life as we know it nowadays. It exists mostly in unicellular or filamentous organization without nuclei or an internal membrane system and provides excellent minimal photosynthetic system for research. Even though compared to much more complex photosynthetic organisms like algae and plants, knowledge about processes and functions involved in cyanobacteria metabolic and genetic regulation is still far beyond our complete understanding [1, 2].

There are many standard biological techniques available for Synthetic Biology applications in *Escherichia coli* that can be also adopted for cyanobacteria research. To facilitate such effort we developed web-based platform for public sharing, annotation, analysis, and visualization of dynamical models and wet-lab experiments.

As a case study used within this tutorial we have chosen biological processes of extreme evolutionary importance for almost any living organism on Earth – circadian rhythm. For simple photosynthetic organisms such as cyanobacteria this process plays a significant role in regulation of many biological processes, e.g., antagonistic processes of photosynthesis and nitrogen fixation. The molecular mechanism of the circadian oscillator in cyanobacteria was discovered in late '90s and consists of three components (kinases *KaiA*, *KaiB*, *KaiC*) creating a post-translational oscillator (together with ATP) [3].

The platform (E-cyanobacterium.org) harbors repositories for cyanobacteria related models and experiments. Model repository allows online analyses and simulation of mathematical models while experiments repository enables to store and share experimental data.

## 1. Homepage and navigation

The home page contains description of platform's purpose and explanation of its three major parts – Biochemical space, Model repository, and Experiments repository (Fig. 1.1). Login and registration panel can be found in the top right corner (Fig. 1.2).

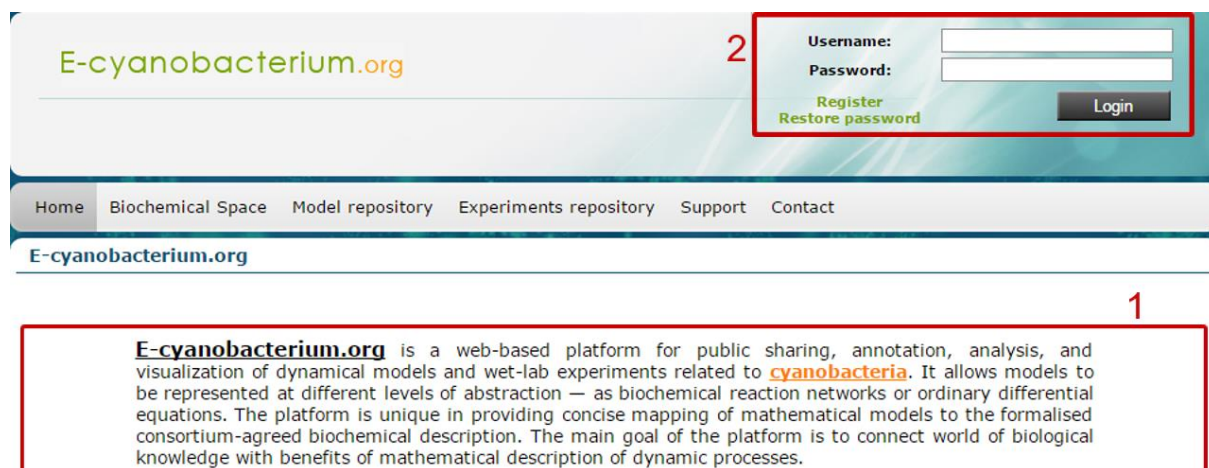


Fig. 1 – homepage of e-cyanobacterium.org

There are three main navigation elements:

- Main menu (Fig. 2.1): main navigation to corresponding pages,
- Navigation panel (Fig. 2.2): navigation in Biochemical space or in Model repository,
- Information panel (Fig. 2.3): divides information about selected item into tabs.

You can view help tooltip by clicking on question mark in blue circle in the top right corner (Fig. 2.4).

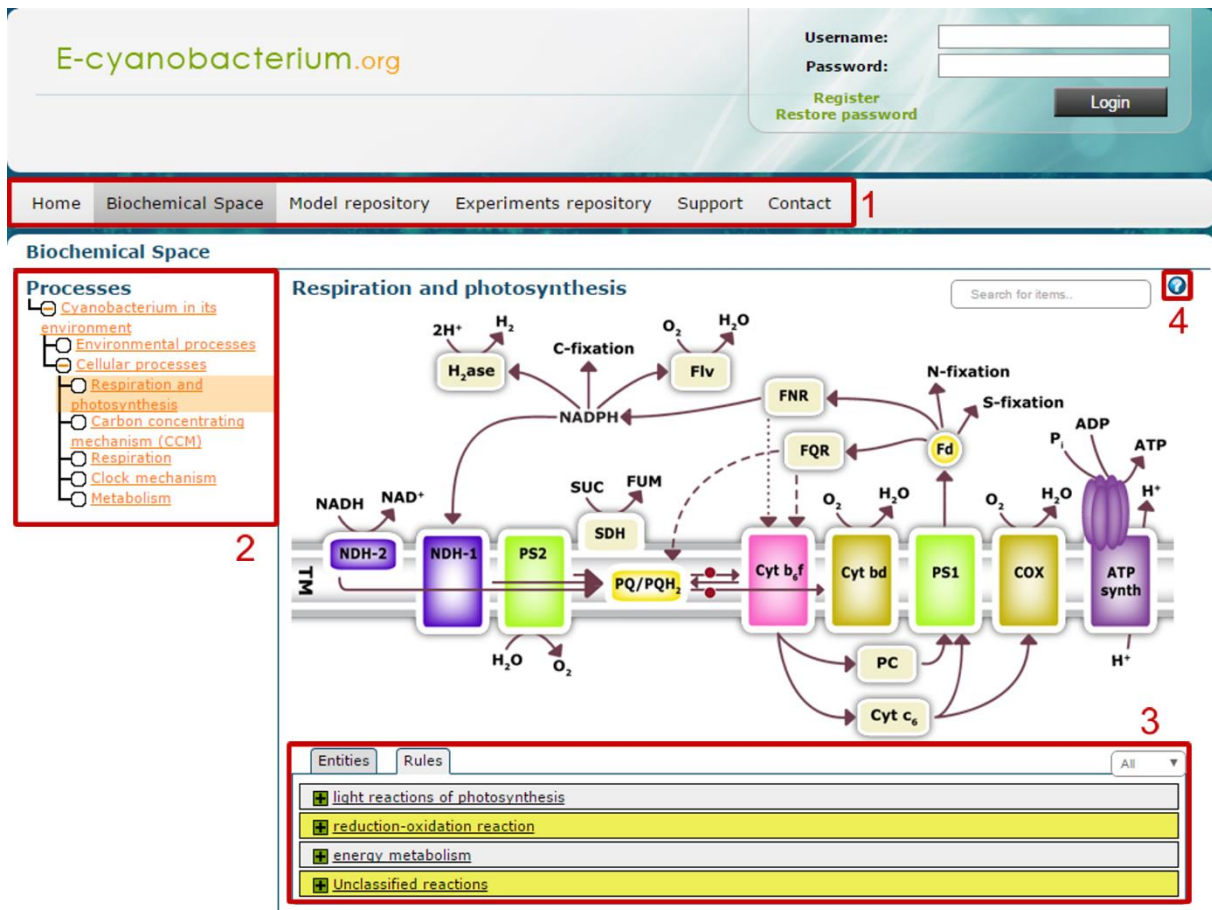
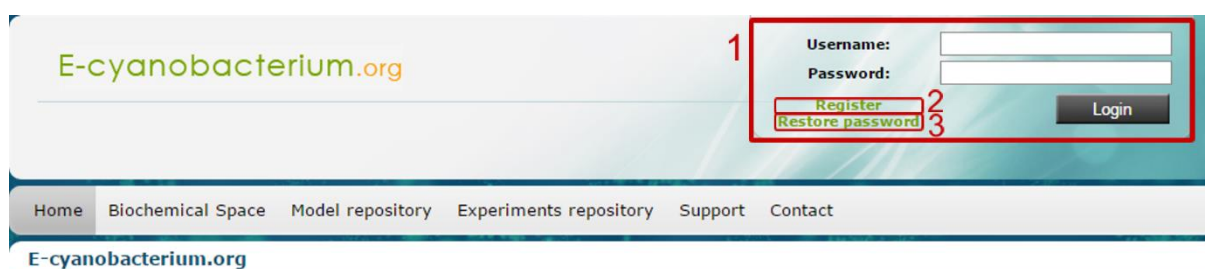


Fig. 2 – main navigation elements

## 2. Registration

Registration is necessary for access to full features of the website. It allows a registered user to customize parameters in models, import and edit own experiments and contact support team if necessary. The login panel can be found in the top right corner of the header (Fig. 3.1). Below is Registration link (Fig. 3.2) which redirects you to the registration page. Username, password, and email address fields are compulsory, other fields are optional. Immediately after registration the user can login and use website as a registered user. In case the password is forgotten, there is possibility to restore the password (Fig. 3.3).



**E-cyanobacterium.org** is a web-based platform for public sharing, annotation, analysis, and visualization of dynamical models and wet-lab experiments related to **cyanobacteria**. It allows models to be represented at different levels of abstraction — as biochemical reaction networks or ordinary differential equations. The platform is unique in providing concise mapping of mathematical models to the formalised consortium-agreed biochemical description. The main goal of the platform is to connect world of biological knowledge with benefits of mathematical description of dynamic processes.

Fig. 3 – home page of e-cyanobacterium.org, login and registration panel

After registration, e-cyanobacterium.org platform is fully operational.

### 3. Biochemical Space

Section Biochemical space [4] is located in the Main menu (Fig. 4.1). Navigation is possible either through Navigation panel (left column) named Processes or via interactive schemes.

We demonstrate the usage on the example of the cyanobacteria circadian clock mechanism. As it has been mentioned before, there are two options how to do it. First one is through the selection tree structure on the left – Cyanobacterium in its environment → Cellular processes → Clock metabolism (Fig. 4.2). After clicking on the link, the picture displays simplified scheme of the circadian clock in cyanobacteria. Another way to get there is by clicking on the particular element in the illustration (Fig. 4.3, Fig. 5.1). Each entity is supplied with a description when moving the cursor over it.

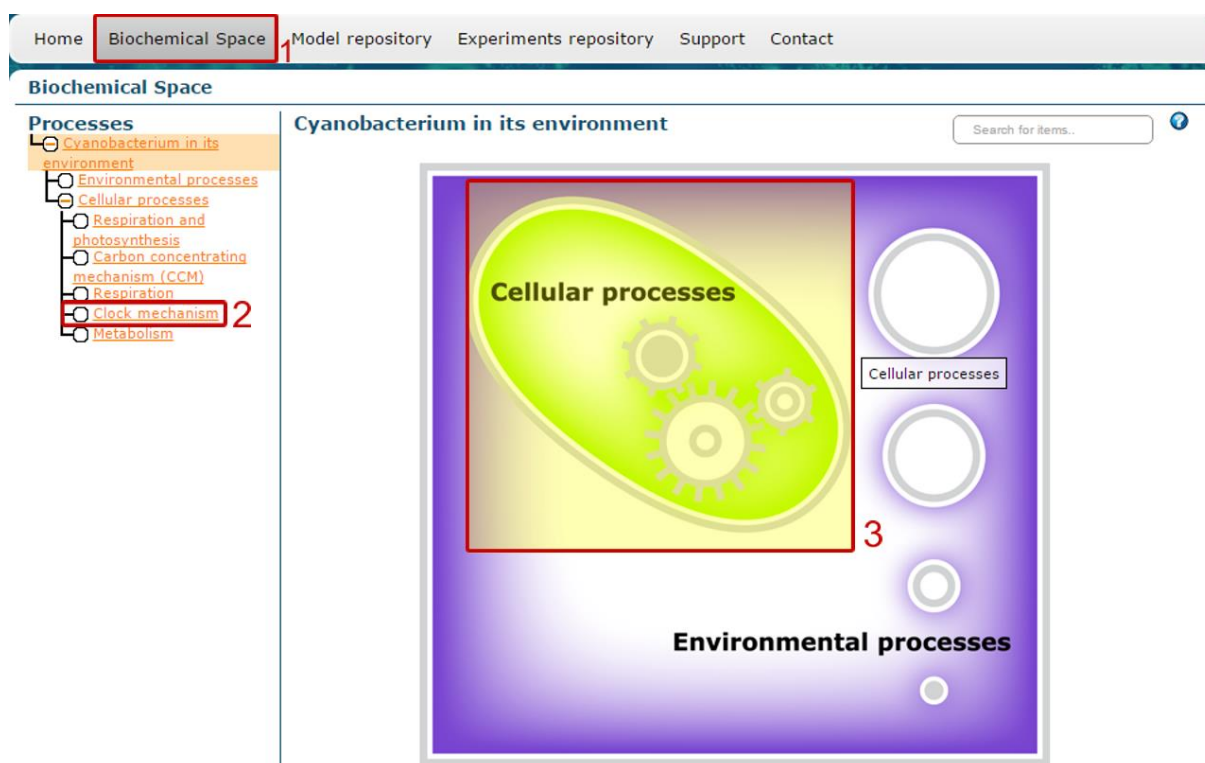


Fig. 4 – navigation through Biochemical space, main page

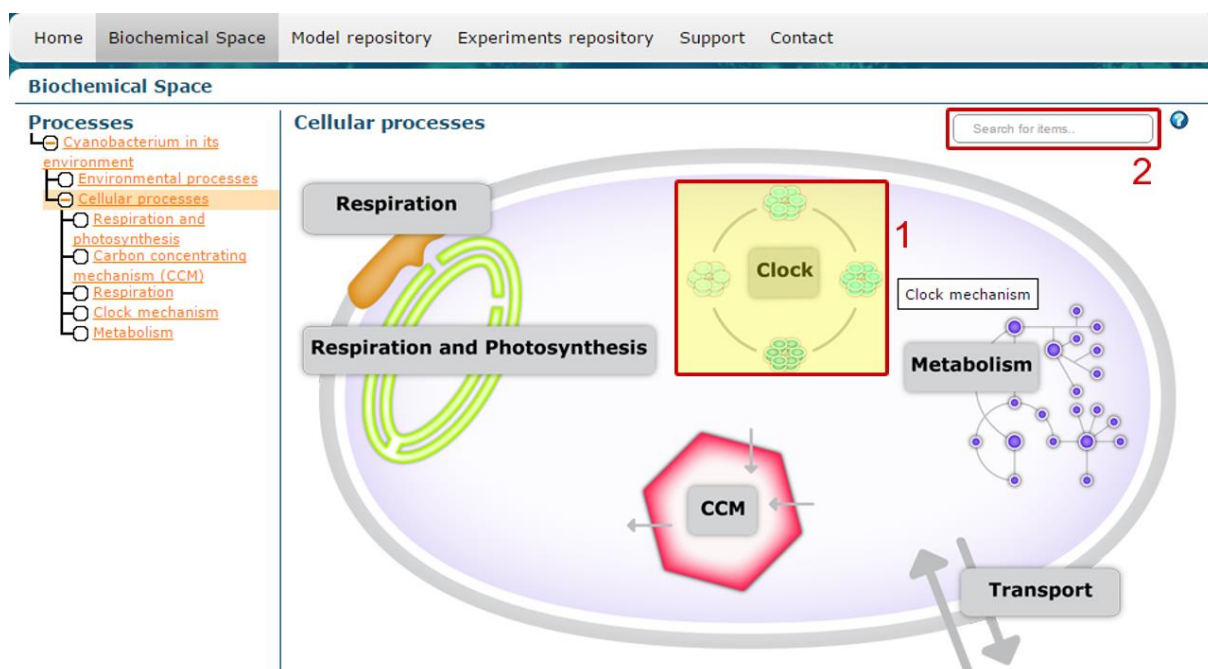


Fig. 5 – navigation through Biochemical space, cellular processes; search field

There is a search field in the top right corner above the scheme (Fig. 5.2). When a term is inserted, the corresponding element is highlighted. It is helpful especially in complex schemes. By clicking on an entity, the user will be redirected to detailed description of the entity.

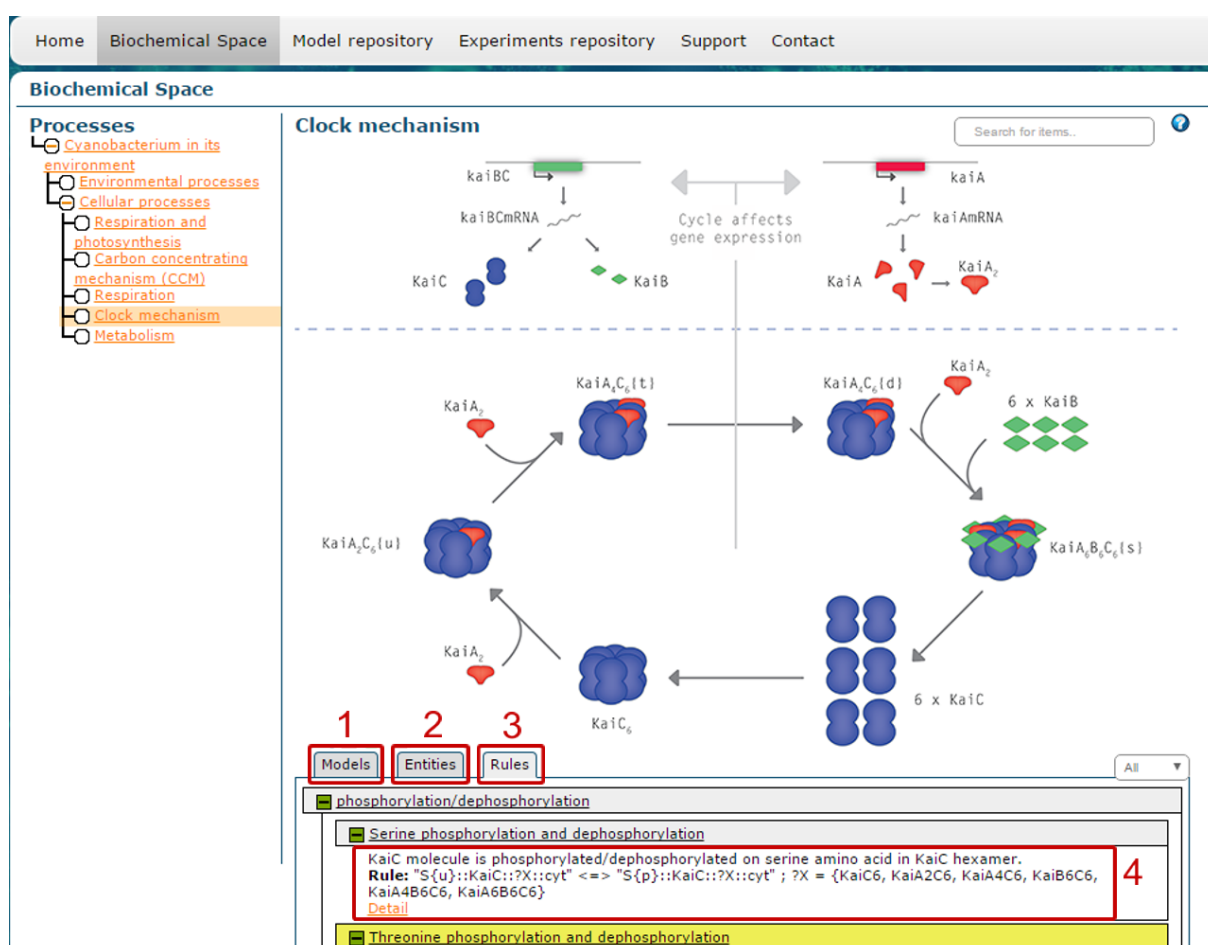


Fig. 6 – Biochemical space, circadian clock of cyanobacteria; rules tab expanded

There are three tabs beneath the picture – Models, Entities and Rules (Fig. 6). The Models tab (Fig. 6.1) contains information about models related to process selected in the Navigation panel. It is possible to get into the particular model by expanding the corresponding row and clicking on the Detail button. In the Entities tab (Fig. 6.2), there are listed entities occurring in the selected process. There is detailed annotation when expanded. The Rules tab (Fig. 6.3) contains categories of rules as displayed in Fig. 6. When expanded, there is a list of rules. We can display short description and rule (written in Biochemical Space Language) by clicking on the exact rule (Fig. 6.4).

## 4. Models

Except the method described above, another way to get to models is by clicking on the Model repository tab in the Main menu (Fig. 7.1).

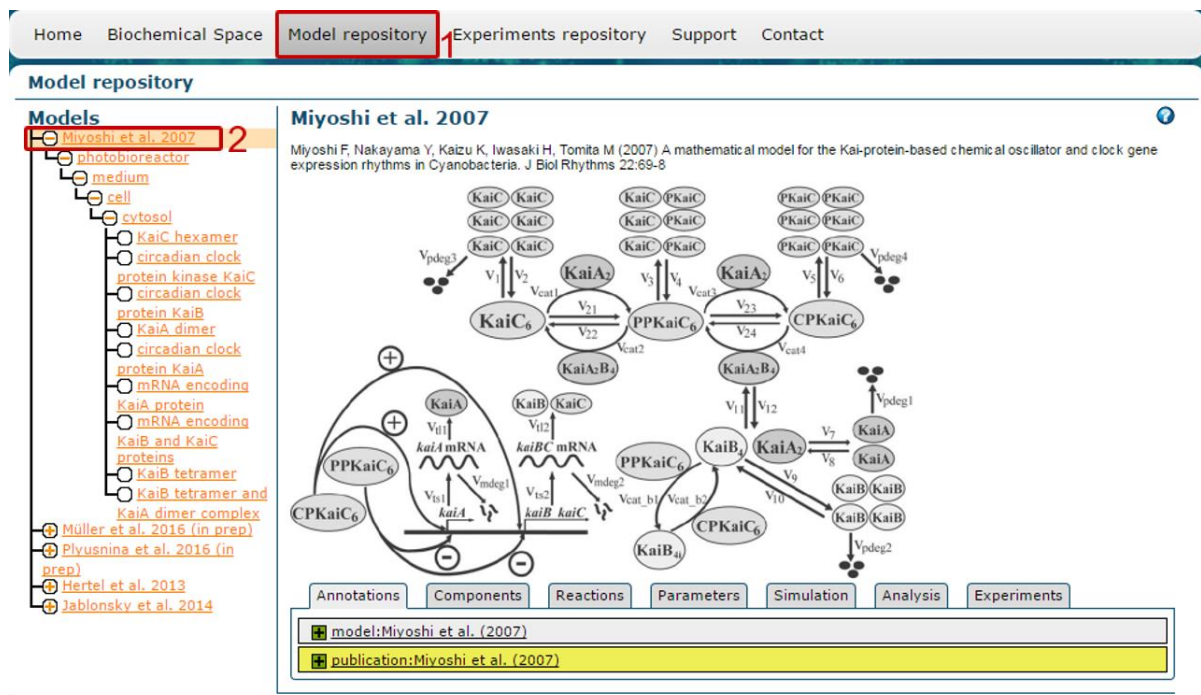


Fig. 7 – Model repository, Model Miyoshi et al. 2007

Suppose we would like to see model Miyoshi et al. 2007 [5]. We can go to the page of this model by clicking on *Miyoshi et al. 2007* in the navigation on the left (Fig. 7.2). Corresponding model unfolds by clicking on the plus sign in the Navigation panel and we can explore entities of selected model.

There are seven tabs beneath the picture in the Information panel (Fig. 7):

- The Annotation tab which contains annotation for the model and link to the respective publication.
- The Components tab where are listed all entities of the selected model. By clicking on the Detail link, details of the entity in Biochemical Space will be displayed.
- The Reactions tab contains all mathematical properties of reactions of the selected model. By clicking on the Detail link, details of the appropriate rule in Biochemical Space will be displayed.
- The Parameters tab includes Constant and Assigned quantities.
- The Simulation tab allows a registered user to change parameters of the simulation (Fig. 8.1), save them as a dataset (Fig. 8.2) and use their own datasets or datasets

created by someone else. To run simulation and show results in the chart you need to click on Simulate button (Fig. 8.3). There are several options what to do with the chart, all of them are described in the help (Fig. 9.1). It is also possible to download the simulation results in several formats (Fig. 9.2). In our example we chose the graphset of protein complexes KaiB4 and light adapted dataset that represents cyanobacteria in environment with diurnal rhythms of light (Fig. 9.3). Simulation tab also contains possibility to export model to SBML [6] (Fig. 8.4). Platform offers several simulation engines<sup>1</sup> which can be chosen exclusively by model administrator

- The Analysis tab is dedicated to people who are interested in models. It contains static analysis for finding out the properties of the model, specifically mass conservation, flux modes and incidence matrix. It is possible to export the results of all of these analyses to a file in SBRML [7] format.
- The Experiment tab shows experiments associated with the model. By clicking on the Experiment detail, the link to the corresponding experiment is shown.

Annotations Components Reactions Parameters Simulation Analysis Experiments

**Initial conditions**

Name	Value	Dataset value
cytosol	1	<input type="text"/>
KaiC6	139.22	<input type="text"/>
PPKaiC6	779.158	<input type="text"/>
CPKaiC6	1229.563	<input type="text"/>
KaiC	932.446	<input type="text" value="1000"/>
PKaiC	110.829	<input type="text"/>
KaiB4i	3251.369	<input type="text"/>
KaiB4	0.173	<input type="text"/>
KaiB	1130	<input type="text"/>
KaiA2	166.559	<input type="text"/>
KaiA	9.998	<input type="text"/>
kaiA_mRNA	2.856	<input type="text"/>
kaiBC_mRNA	2.865	<input type="text"/>
KaiA2B4	51.022	<input type="text"/>

**Parameters**

**Options**

Dataset

Load dataset

Default  
dark adapted (read only)  
light adapted (read only)

Dataset Name

Fig. 8 – model repository, simulation, datasets and export to SBML

<sup>1</sup> For example Octave <https://www.gnu.org/software/octave/> or COPASI [8] <http://copasi.org/>

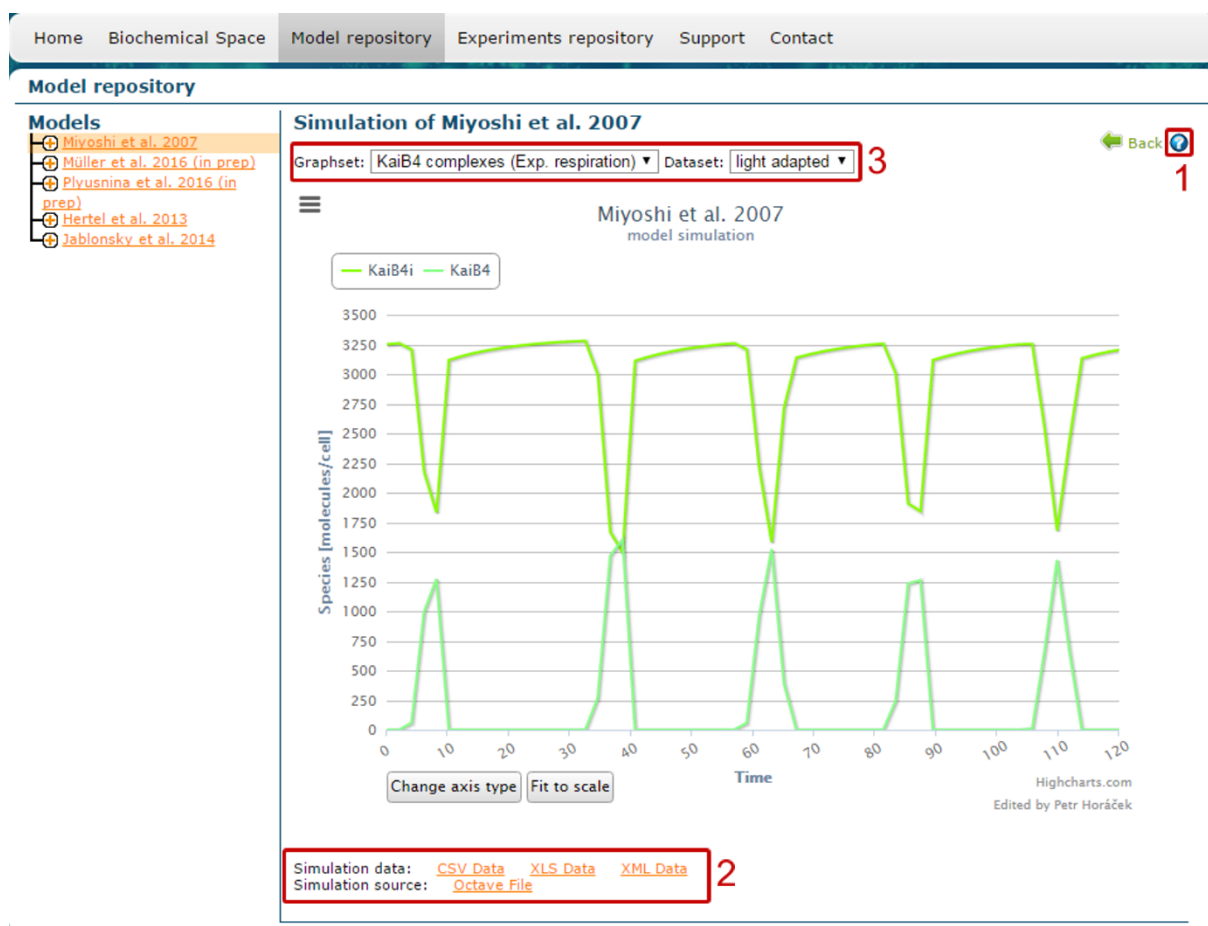


Fig. 9 – model repository, chart of simulation

## 5. Experiments

There are two options to enter Experiments repository. First is the one mentioned before – Experiments tab in Model repository refers to all experiments related to the selected model. The second way is by clicking on Experiments repository in Main menu (Fig. 10.1).

The screenshot shows the 'Experiments repository' page. The top navigation bar has 'Experiments repository' highlighted with a red box labeled '1'. Below the navigation bar, there is a search and filter section. A red box labeled '2' highlights the 'Filters: Add', 'Order: Add', 'Show fields: Add', and 'Found: 7 experiments' area. Below this, there is a table with the following columns: Name, Series, Started, and Inserted. The table contains several rows of experiment data. A red box labeled '3' highlights the 'Light/Dark 16:8h Entrainment' row. A red box labeled '4' highlights the 'Light/Dark 16:8h Entrainment: Data dO2' row. The table data is as follows:

Name	Series	Started	Inserted
<a href="#">Light Characterization</a>	Synechocystis 6803 GT-L Turbidostat, Temperature 32oC	2014-01-04 14:20:00	2016-02-09 08:30:01
<a href="#">Light/Dark 16:8h Entrainment: Data dO2</a>	Cyanothece Metabolic Oscillations		2015-11-28 16:49:10
<a href="#">Light/Dark 16:8h Entrainment: Data dCO2</a>	Cyanothece Metabolic Oscillations		2015-11-28 16:34:05
<a href="#">Light/Dark 16:8h Entrainment</a>	Cyanothece Metabolic Oscillations	2008-07-12 02:15:00	2015-11-28 15:38:14
<a href="#">Growth Curve 35oC, 440/25 R/B</a>	Synechocystis 6803 GT-L Batch, Temperature 35oC	2014-02-03 17:46:47	2015-10-21 13:35:31
<a href="#">Growth Curve 35oC, 220/25 R/B - continuation</a>	Synechocystis 6803 GT-L Batch, Temperature 35oC	2014-02-04 07:27:14	2015-10-16 11:24:36
<a href="#">Growth Curve 35oC, 220/25 R/B</a>	Synechocystis 6803 GT-L Batch, Temperature 35oC	2014-02-03 17:46:28	2015-10-16 11:13:04

Fig. 10 – experiments repository, default view

This page allows the user to adjust the view, filter and order experiments and also select a desired one (Fig. 10.2). Choosing *Light/Dark 16:8h Entrainment* (Fig. 10.3) we proceed to a page with experiment details.

Information in tabs Series detail and Experiment detail are self-explanatory.

Some experiments are measured on several devices simultaneously. Therefore we need to associate these measurements. For that reason there is a possibility of having parent-child relation between experiments as shown on Fig. 10.4 and Fig. 11. *Data dCO2* and *Data dO2*, measured by devices of different types, are child experiments of *Light/Dark 16:8h Entrainment* measured by *Photobioreactor*.

Home Biochemical Space Model repository **Experiments repository** Support Contact

**Light/Dark 16:8h Entrainment - Experiment detail**

Series detail **Experiment detail** Variables Data Annotations

**Name:** Light/Dark 16:8h Entrainment

**Description:** Exp #720

**Started:** 2008-07-12 02:15:00

**Device Type:** Photobioreactor-035

**Device Name:** PBR A

**Device ID:** 002

**Experiment gassing:** 10 000 ppm CO<sub>2</sub>, 300 ml/min

**Medium:** ASP2 -N

**Organism:** Cyanothece ATCC 51142

**Stirring:** 180 rpm

**Name:** Data dCO<sub>2</sub>

**Description:** Dissolved carbon dioxide was measured externally by METTLER TOLEDO SevenMulti

**Device Type:** METTLER TOLEDO SevenMulti

**Device Name:** Multimeter NH

**Device ID:** 000

**Name:** Data dO<sub>2</sub>

**Description:** Dissolved oxygen was measured externally by PSI OxyCorder

**Device Type:** PSI OxyCorder 401

**Device Name:** OxyCorder NH

**Device ID:** 000

Fig. 11 – experiments repository, experiment detail

In tab Variables all variables of selected experiment are shown. Data tab contains two possible options – data export and show chart. The data can be exported in different file formats (Fig. 12.2) and as a whole, parent or child experiments (Fig. 12.1). Clicking on Show chart all variables are plotted in interactive chart. There are several possibilities what to do with the chart, all of them are described in the help (Fig. 12.3). Selecting desired variables and fitting to scale are the essential ones – in the Fig. 12 we have hidden all parameters except *O<sub>2</sub> resp* and fitted the view to scale.

Tab Annotations contains all annotations related to selected experiment.

As long as it is possible to view data for both models and experiments we can compare these outputs. Comparing the chart of simulation of model Miyoshi et al. 2007 mentioned earlier to experimental data we can monitor relation between components of model and data measured in experiments. The last four periods of values of *O<sub>2</sub> resp* from experiments (Fig. 12) and *KaiBi* variable in model Miyoshi et al. 2007 (Fig. 13) really show some qualitative similarity.

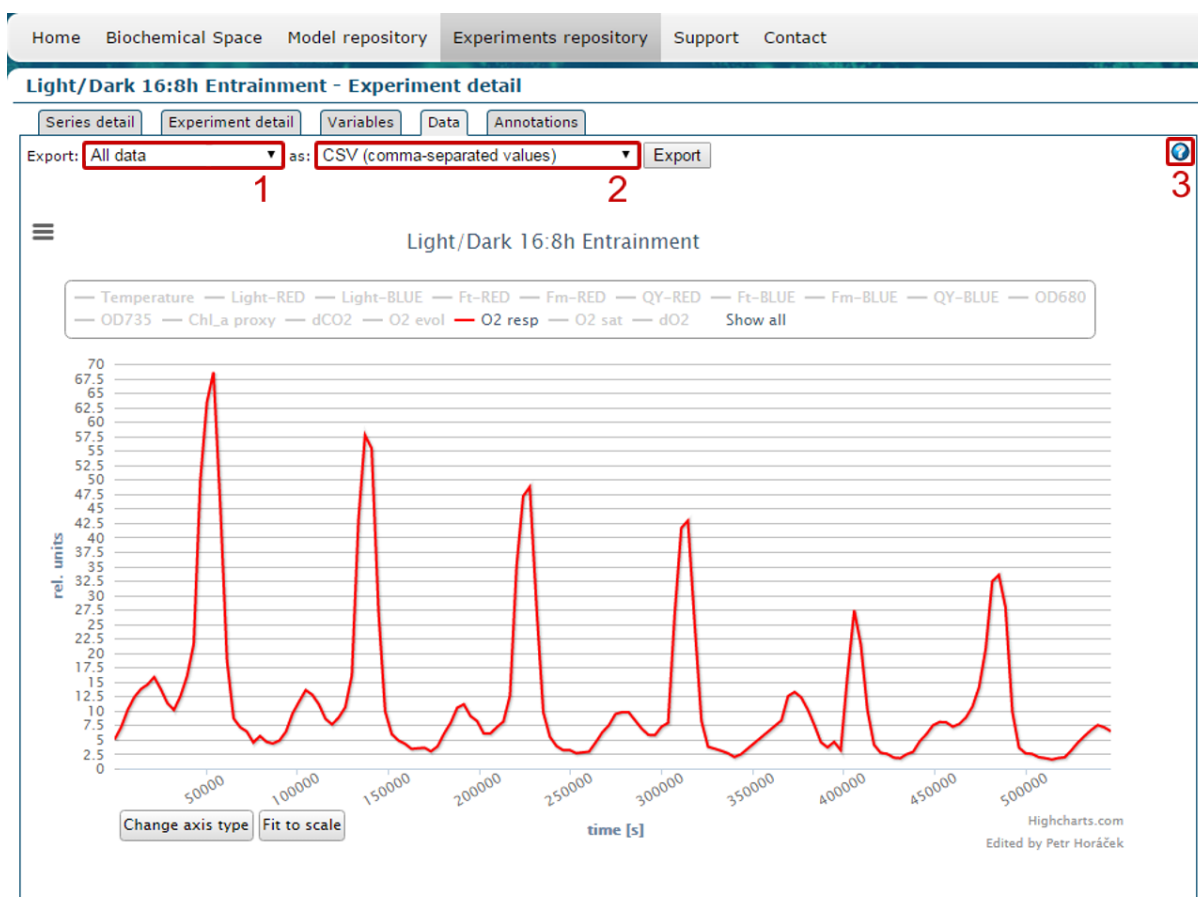


Fig. 12 – experiments repository, chart of measured data

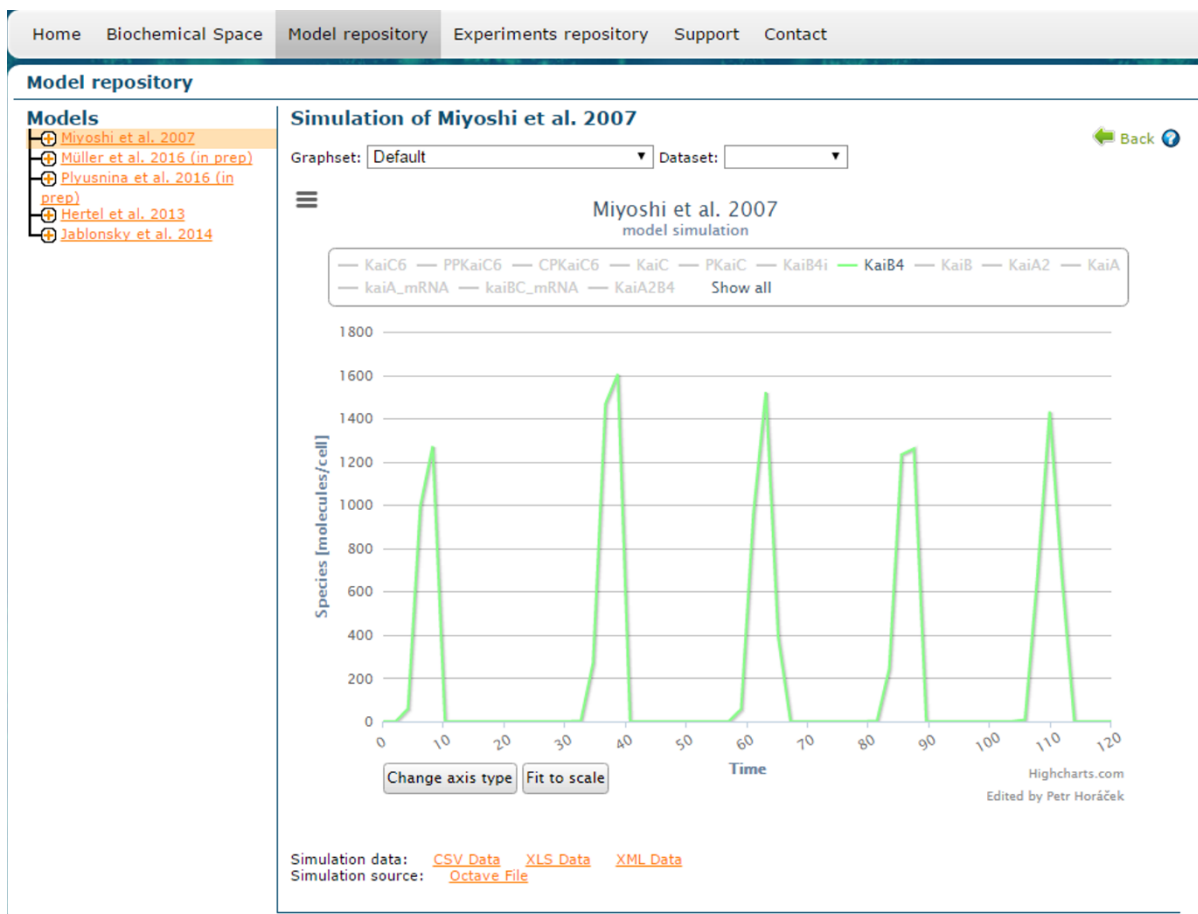


Fig. 13 – model Miyoshi et al. 2007, chart of KaiB4 complexes

In case of additional question see FAQ in Support tab or use contact form which can be found in Contact tab, both in Main menu.

*e-cyanobacterium.org team*

## References

- [1] Cardona, T., Murray, J. W., & Rutherford, A. W. Origin and evolution of water oxidation before the last common ancestor of the cyanobacteria. *Molecular Biology and Evolution*, 2015, 32(5), 1310–1328. <http://doi.org/10.1093/molbev/msv024>;
- [2] Zhou, J., Zhu, T., Cai, Z., & Li, Y. From cyanochemicals to cyanofactories: a review and perspective. *Microbial Cell Factories*, 2016, 15(1), 2. <http://doi.org/10.1186/s12934-015-0405-3>
- [3] Axmann, I. M., Hertel, S., Wiegard, A., Dörrich, A. K., Wilde, A. Diversity of KaiC-based timing systems in marine Cyanobacteria. *Marine Genomics*, 2014, 14, 3–16. <http://doi.org/10.1016/j.margen.2013.12.006>
- [4] Troják, M., Děd, T., Šafránek, D., Klement, M., Červený J., Brim L. Biochemical Space: A Language for Formal Description and Annotation of Complex Biological Processes. In 13th Conference on Computational Methods in Systems Biology, 2015. <http://dx.doi.org/10.1016/j.entcs.2014.06.013>
- [5] Miyoshi, F., et al.: A mathematical model for the kai-proteinbased chemical oscillator and clock gene expression rhythms in cyanobacteria. *Journal of Biological Rhythms*, 2007, 22(1), 69–80. <http://dx.doi.org/10.1177/0748730406295749>
- [6] Hucka, M., et al.: The systems biology markup language (sbml): a medium for representation and exchange of biochemical network models. *Bioinformatics*, 2003, 19(4), 524–531. <http://doi.org/10.1093/bioinformatics/btg015>
- [7] DADA, J. O., et al. SBRML: a markup language for associating systems biology data with models. *Bioinformatics*, 2010, 26.7: 932-938. <http://dx.doi.org/10.1093/bioinformatics/btq069>
- [8] Hoops, D., et al.: Copasi – a complex pathway simulator. *Bioinformatics*, 2006, 22(24), 3067–2074 <http://doi.org/10.1093/bioinformatics/btl485>