DOE/ER/15349

Bayly

June 2003

TO:	U.S. Department of Energy Office of Science
RE:	**Special Report**
PROJECT TITLE:	An integrative approach to energy, carbon, and redox metabolism in the cyanobacterium *Synechocystis* sp. PCC 6803
PRINCIPAL INVESTIGATOR:	Ross Overbeek (630)-567-7677 ross@.....
RECIPIENT ORGANIZATION:	Integrated Genomics, Inc. 2201 West Campbell Park Drive Chicago, Illinois 60612
DOE AWARD NUMBER:	DE-FG02-02ER15349
PROJECT PERIOD:	from 09/30/2002 through 09/29/2004
BUDGET PERIOD COVERED BY THIS REPORT:	from 09/30/2002 through 09/29/2003

DOE Patent Clearance Granted

MPDvorscak Apr 6, 2004

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Office of Intellectual Property Law

DOE Chicago Operations Office

Signature of Principal Investigator

DISCLAIMER

Special Report

The accompanying Progress Report covers the period from 9/30/2002 to 9/29/2003 for the above-referenced grant. Please note that this Report, originally scheduled as a 9-month progress report, establishes that Integrated Genomics, Inc. ("IG") has successfully completed the entire initial-year goals three months early, by July 2003. The early completion of the first-year requirements was facilitated by management changes at IG.

Specifically, in March 2003, ownership changes at IG resulted in replacement of the company's top-level management. Concurrent with those changes, Ross Overbeek (the named PI) resigned from the company. In light of Dr. Overbeek's resignation the company has decided not to continue work on *Synechocystis* 6803.

In order to meet grant obligations to DOE, Dr. Overbeek and IG intensified efforts to complete the first-year goals for delivery by July 2003. Accordingly, the percentage of work-time by all grant participants in May and June of 2003 has been significantly increased: Dr. Gerdes devoted 100% of her time to grant related tasks; while Dr. Ivanova, V. Joukov and T. Walunas each devoted from 60% to 80% of their time to the grant. An additional software designer, Yuri Grechkin, was also added to the project team. These efforts have allowed us to accomplish our first-year goals--i.e. qualitative metabolic reconstruction and compilation of a formally-encoded reaction network of *Synechocystis* 6803. See accompanying Progress Report for the details

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_____
Signature of Principal Investigator

_____
Svetlana Gerdes (co-PI)

NOTE:
This project is a part of a collaborative research effort involving three other groups lead by:
Willem F.J. Vermaas (Arizona State University) coordinating PI
Julian Whitelegge and Kym Faull (University of California, Los Angeles)
Robert W. Roberson (Arizona State University)

Progress Report

Integrated Genomics, Inc. (IG) has begun work on this GTL Project in October 2002. The main objectives for the first year were:

I. To produce a detailed metabolic reconstruction of *Synechocystis* sp. PCC 6803 especially in the interrelated arrears of photosynthesis, respiration, and central carbon metabolism to support a more complete understanding and modeling of this organism. Provide a web-based access to the intermediate results to our collaborators for joint curation and refinement, and for projection and interpretation of experimental data. Ultimately deliver these results to the whole scientific community via specialized web-based resource and publications.

II. To provide detailed bioinformatic analysis of selected functional systems related to carbon and energy generation and utilization, and of the corresponding pathways, functional roles and individual genes in order to support wet lab experimental projects of our collaborators on this grant.

The completion of these tasks initially planned for a year (from 9/30/2002 to 9/29/2003) was achieved ahead of schedule - by July 2003. See accompanying Special Report for explanation.

Specific aim I.

I.1. Qualitative metabolic reconstruction of Synechocystis sp. PCC 6803. A draft metabolic reconstruction of *Synechocystis* sp. PCC 6803 has been automatically generated in ERGO™ based on comparative analysis of several hundred microbial genomes. The draft reconstruction was further subjected to detailed manual curation by a team of bioinformaticians, focusing on the functional systems related to carbon generation and utilization.

Briefly, this work included: i) refinement of automatic functional annotations, ii) detailed bioinformatics analysis of "unknown" ORFs using unique ERGO software tools, as well as publicly available programs in order to adequately predict their functions, iii) building a collection of metabolic and non-metabolic pathways expected to be present in *Synechocystis* sp. PCC 6803 based on analysis its genome sequence, iv) manual connection of ORFs with the corresponding pathways (see (3) for detailed description of metabolic reconstruction technology utilized at IG).

The following results have been produced to date:

- In the course of this work functional roles were suggested for a much higher number of *Synechocystis* 6803 ORFs (64%, or 2101 out of 3264 total ORFs, see Table 1), than it was previously available for the cyanobacterial community through Cyanobase (http://www.kazusa.or.jp/cyanobase/Synechocystis/), its main genomics resource, where only 51% (1673 out of 3264) ORFs have specific functional annotations;
- Dozens of new pathways were added to ERGO collection (see Table 1), including composition of cyanobacterial respiratory and photosynthetic membrane complexes, inorganic carbon concentration and fixation mechanisms, several pigment and cofactor biosynthetic pathways (phycobilins, carotenoids, chlorophyll, tocopherols, etc.);

- A complete draft has been obtained for the following functional systems: central carbon metabolism, CO_2 fixation, respiratory and photosynthetic electron transport, biosynthesis of photosynthetic pigments, amino acid biosynthesis, major vitamins and cofactor biosynthesis, and membrane transport;
- The up-to-date status of the reconstruction is shown in Table 1;

The results of the *Synechocystis* 6803 genome analysis performed by Integrated Genomics are available to the whole scientific community for browsing, analysis and display through ERGOLight platform. This resource is freely available to the research community at light (http://www.ergo-light.com/ERGO/) and currently includes 7 genomes: *Bacillus cereus* ATCC 14579, *Bacillus anthracis* A2012 (public TIGR genome), *Bacillus thuringiensis israelensis* ATCC 35646, *Brucella melitensis* 16M, *Fusobacterium nucleatum* ATCC 25586, and *Fusobacterium nucleatum vincentii* ATCC 49256. ERGO™ Light will be one of the major conduits at IG for the distribution of data obtained from publicly funded projects.

Features of ERGO™ Light include:

- Access to IG metabolic and non-metabolic pathway database;
- A user-friendly graphical interface
- Manually curated annotations
- Chromosomal clustering data
- Detailed information about all genes
- Connectivity to other public databases, including KEGG, NCBI, SwissProt.

The qualitative metabolic reconstruction in its current state has been made publicly available at: `http://www.ergo-light.com/RN/CY_Tree.html` (user ID: PCC6803, password: DOE_GTL). This specialized resource is providing a hierarchical presentation of metabolic systems and pathways hyperlinked to ERGO Light at the level of pathways and individual genes (for additional information and examples, see Appendix A)

Further improvement and updates of the reconstruction will be performed during the second year of the project. We expect the input from our collaborators as well as from the broader scientific community to aid in further improvement of the quality of ORF calling, functional annotations, and reaction network in *Synechocystis* 6803 reconstruction. For instance, as a part of the current project an effort to improve predictions of ORF starting codons in *Synechocystis* 6803 has been initiated by Ross Overbeek in collaboration with Dr. Lee Ann McCue (Harvard University, Cambridge, MA).

Table 1. The current status of the *Synechocystis* sp. PCC 6803 metabolic reconstruction

Category	Counts:	% of Total:
ORFs total	**3,264**	**100.00%**
ORFs with assigned function	2,101	64.37
ORFs without assigned function	1,163	35.63
ORFs without function or similarity	228	6.95

ORFs without function, with similarity	935	28.65
ORFs in asserted pathways	1,634	50.06
ORFs not in asserted pathways	1,630	49.94
ORFs with assigned function but no pathway	468	14.34
ORFs in the functional overview	1,606	49.20
Functions assigned	**1,428**	**100.00%**
Functions assigned, hypothetical	19	1.33
Functions assigned, connected to asserted pathways	1,049	73.46
Functions assigned, not connected to asserted pathways	379	26.54
Functions missing from asserted pathways	121	
Functions with no sequence	12	
Pathways asserted total	**1,037**	**100.00**

I.2. Converting metabolic reconstruction into a mathematical model of Synechocystis sp. PCC 6803 *metabolism.* A qualitative metabolic reconstruction above is a first mandatory step towards mathematical modeling of cellular metabolism. Next step is development of formally encoded reaction network suitable for modeling. However, the following problems are common for most available network encodings (both at IG and in any public integrations), which preclude a possibility of building useful quantitative models:

1. redundancy and imprecision of compound names (synonyms and typos);
2. inconsistent formula representation (such as salt versus free acid etc);
3. lack of convention in treating generic compound names (such as "alcohol");
4. non-balanced reactions (lost reactants, wrong stoichiometry, etc);
5. undefined direction/reversibility;
6. abundance of irrelevant reactions, that are not supported by reconstruction;
7. lost relevant reactions that are supported experimentally and/or by reconstruction;
8. inconsistency in enzyme names, functions, and E.C. numbers;
9. inconsistency in encoding of complex reactions (polymerization, degradation, etc).

The following results have been produced to date:

- Integrated Genomics, Inc. has developed software tools to automatically convert the qualitative metabolic reconstruction into a formal computer-readable version of *Synechocystis* sp. PCC 6803 reaction network.

This software also supports curation of compounds, reactions and functional roles necessary to resolve all the problems mentioned above. Appendix B illustrates the web-based user interface designed and implemented at IG (`www.ergo-light.com/RN` , user ID: PCC6803, password: DOE_GTL) to support curation of reaction network as collaborative effort by all participating labs;

- System-by-system curation effort was started. It includes:
  a. manual curation of compounds, adding, reconciling and refining:
     i. names/synonyms;
     ii. atomic formulas;

iii. structure representations ("smiles")

b. manual curation of reactions:
   i. mass-balance;
   ii. reversibility/direction;
   iii. assignment with functional roles (E.C. numbers);
   iv. hierarchical placement (pathways, systems)

c. manual curation of functional roles and genes:
   i. names and E.C. numbers;
   ii. connection to genes (reconcile annotations);
   iii. revealing missing genes (and produce conjectures)

- Curation of the following subsystems has been largely completed: central carbon metabolism (CCM), amino acids biosynthesis (AAB), and nucleotide and cofactor biosynthesis. Statistics illustrating the current progress in this effort are summarized in Table 2.
- Reactions are connected to pathways and individual genes in ERGO Light.

Table 2. The current status of reactions/compounds curation

	Carbohydrate metabolism	**Amino-acid metabolism**	**Coenzyme metabolism**	**Nucleotide metabolism**
Total number of functional roles (distinct E.C. numbers)	102	105	58	34
Total number of reactions:	144	113	54	52
mass-balanced	95%	99%	78%	96%
Total number of compounds:	91	63	59	20
fully refined	90%	94%	81%	100%

Refinement of other functional systems relevant for the scope of the funded project will be continued during its second year. The produced reaction network will constitute an invaluable novel resource for our collaborators and for the broader scientific community needed to support mathematic modeling of cellular sub-networks, search for missing components, interpretation of experimental results, and deeper understanding of physiology of cyanobacteria, as well as other organisms such as plants.

Specific aim II.

The current version of the *Synechocystis* sp PCC 6803 metabolic reconstruction produced during the first year of this project already allows insight into *Synechocystis* 6803 physiology and genetics, that has proved very useful for experimental research in this organism and other cyanobacteria.

The following focused research projects were conducted at Integrated Genomics during the reported period to support wet lab experimental projects of our collaborators on this grant:

1. *Detailed reconstruction of carotenoid biosynthesis in Synechocystis* 6803 was performed. This pathway has several "missing genes" (functions not connected to any sequenced gene), which are being pursued by Dr. Vermaas's lab. We suggested a candidate ORF for a "missing" lycopene cyclase (beta- and gamma-carotene synthase) - hypothetical gene RCY45362 (*slr0941*). This prediction is currently being pursued experimentally in the lab of Dr. Vermaas.

2. *Prediction of photoheterotrophic growth capability for Thermosynechococcus elongates.* The dominant mode of growth of cyanobacteria is photoautotrophy, in spite of the fact that they possess a respiratory activity and many enzymes of sugar catabolism. A thermophilic cyanobacterium capable of photoheterotrophic growth is needed for the purpose of crystal structure analysis of membrane protein complexes. Availably of the complete genome sequence makes *T. elongates* a strong candidate for this role. To facilitate experimental attempts by Dr. Vermaas's group to grow this strain under conditions other than photoautotrophic ones, we performed detailed comparative analysis of central carbon metabolism reconstructed from the complete genome sequences of several cyanobacteria with known growth capabilities. Several consistent differences were detected between obligate and facultative photoautotrophs in organization of glycolysis and tricarboxylic acid (TCA) cycle (manuscript in preparation). This analysis has shown that central metabolism of *Thermosynechococcus elongates* is close to that of facultative photoautotrophs. We predict that introduction into its genome of a gene for (S)-malate dehydrogenase (for instance, *Synechocystis* 6803 *sll0891*) may confer photoheterotrophic growth capacity. This prediction is currently being pursued experimentally in the lab of Dr. Vermaas.

3. *Predictions regarding fixed-carbon compounds taken up by Thermosynechococcus elongates.* The detailed comparative analysis of membrane transporters present in the genome of *T. elongates* resulted in prediction of several compounds as potential substrates for its photoheterotrophic growth. One of them, N-acetyl-D-glucosamine has been proven very useful in propagation of this organism.

5. *Reconstruction of NAD metabolism in Synechocystis 6803 and several other cyanobacterial species.* We performed *in silico* reconstruction of NAD metabolism based on the presence or absence of orthologs of all known genes involved in NAD biosynthesis, salvage, recycling, and transport in genomic sequences of cyanobacteria available to date. The resultant model was further refined and verified based on *in vitro* characterization of several key enzymes of NAD biosynthesis (NaMN adenylyltransferase, nicotinamide phosphoribosyltransferase, and NAD synthetase), and on genetic analysis of the *nadD*⁻ and *nadM*⁻ mutants in *Synechocystis* 6803 (manuscript in preparation: (2)).

6. We have initiated a systematic analysis of regulatory sites in the genome of *Synechocystis* 6803 based on literature data and on prediction of novel regulatory elements and the corresponding regulons, using methodology described in (1, 4). An example of this effort is

analysis of coregulated cobalamin (vitamin B_{12}) metabolism genes in the genomes of *Synechocystis* 6803 and several other cyanobacteria (manuscripts in preparation: 5, 6) The results of this on-going effort will be highly relevant for metabolic modeling, as well as for generating functional predictions for hypothetical cyanobacterial ORFs.

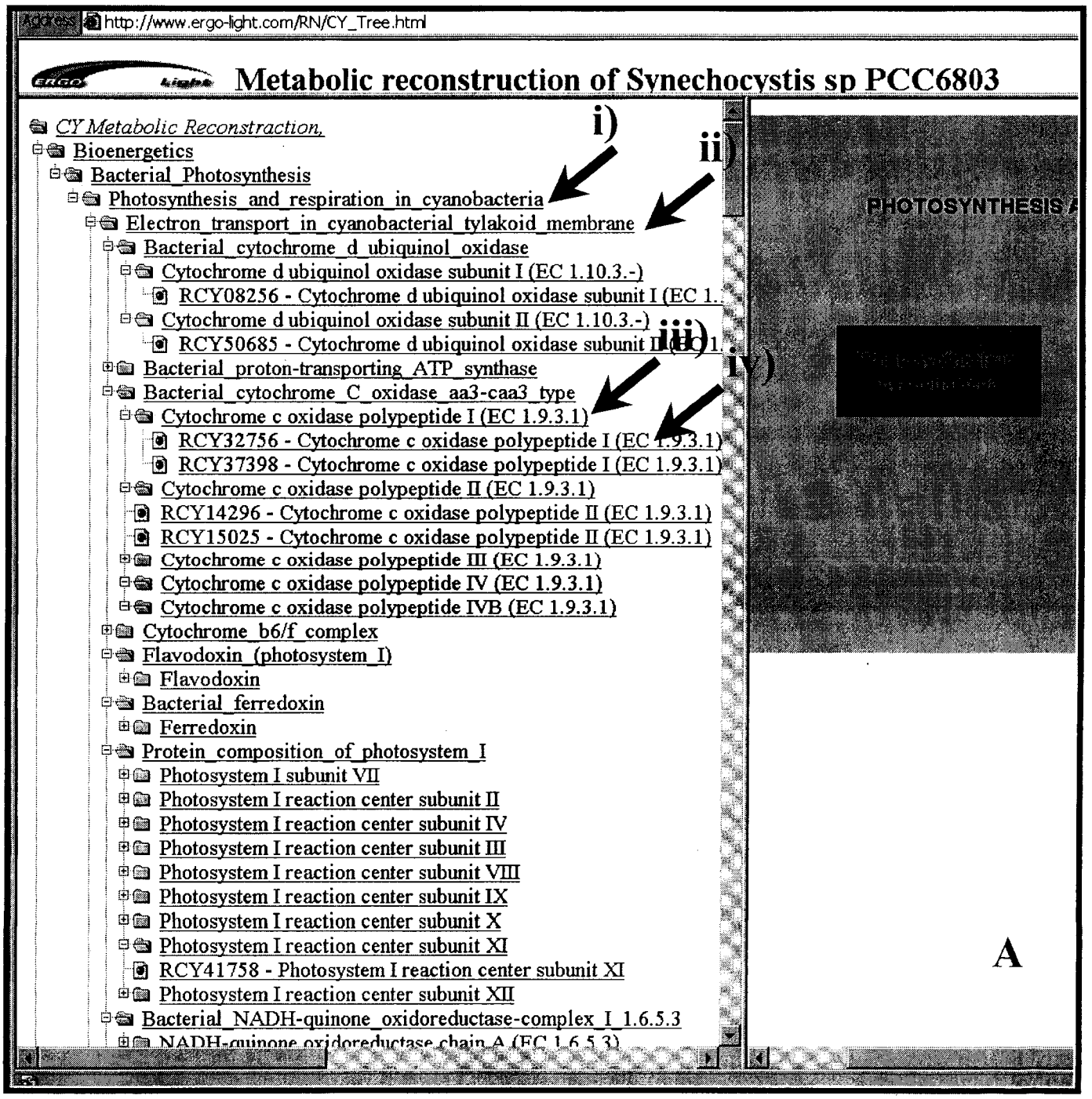
References:

1. **Gelfand, M. S., P. S. Novichkov, E. S. Novichkova, and A. A. Mironov.** 2000. Comparative analysis of regulatory patterns in bacterial genomes. Brief Bioinform **1**:357-71.
2. **Kurnasov, O., S. Gerdes, K. Shatalin, B. Polanuyer, and A. Osterman.** 2003. Genomics of NAD biosynthesis and recycling in Cyanobacteria. manuscript in preparation.
3. **Overbeek, R., N. Larsen, T. Walunas, M. D'Souza, G. Pusch, E. J. Selkov, K. Liolios, V. Joukov, D. Kaznadzey, I. Anderson, A. Bhattacharyya, H. Burd, W. Gardner, P. Hanke, V. Kapatral, N. Mikhailova, O. Vasieva, A. Osterman, V. Vonstein, ., M. Fonstein, N. Ivanova, and N. Kyrpides.** 2003. The ERGO$^{(TM)}$ genome analysis and discovery system. Nucleic Acids Res. **31**:164-171.
4. **Rodionov, D. A., A. A. Mironov, and M. S. Gelfand.** 2002. Conservation of the biotin regulon and the BirA regulatory signal in eubacteria and archaea. Genome Res **12**:1507-16.
5. Rodionov, D. A., A. G. Vitreschak, A. A. Mironov, and M. S. Gelfand. 2003. Comparative genomics of the vitamin B12 metabolism and regulation in prokaryotes. (manuscript in preparation).
6. **Vitreschak, A. G., D. A. Rodionov, A. A. Mironov, and M. S. Gelfand.** 2003. Conserved RNA structural element regulates vitamin B12 metabolism in bacteria. (manuscript in preparation).

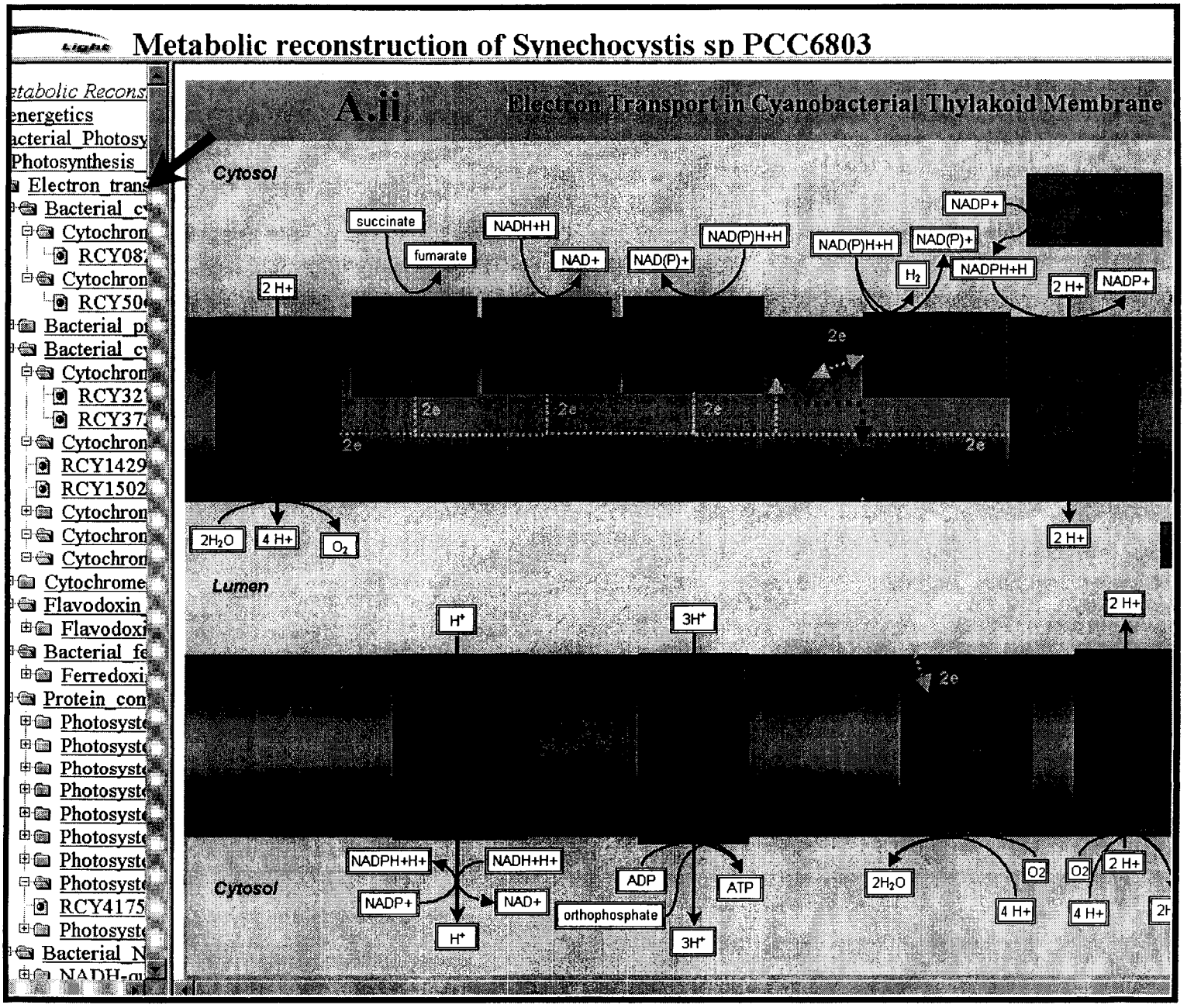
Appendix A

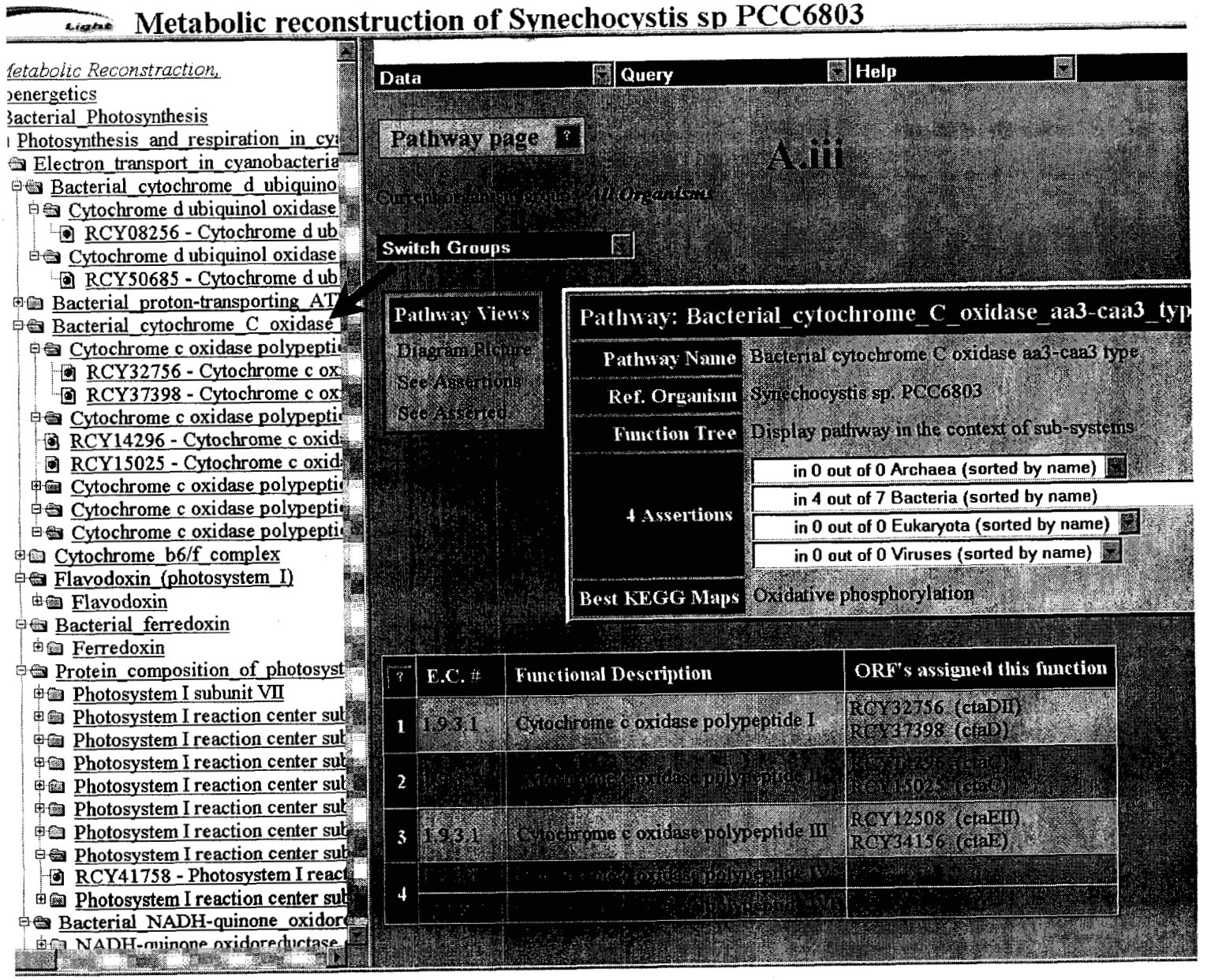
Qualitative metabolic reconstruction of *Synechocystis* sp. PCC 6803 posted at `http://www.ergo-light.com/RN/CY Tree.html` (user ID: PCC6803, password: DOE_GTL). Examples with interface screenshots.

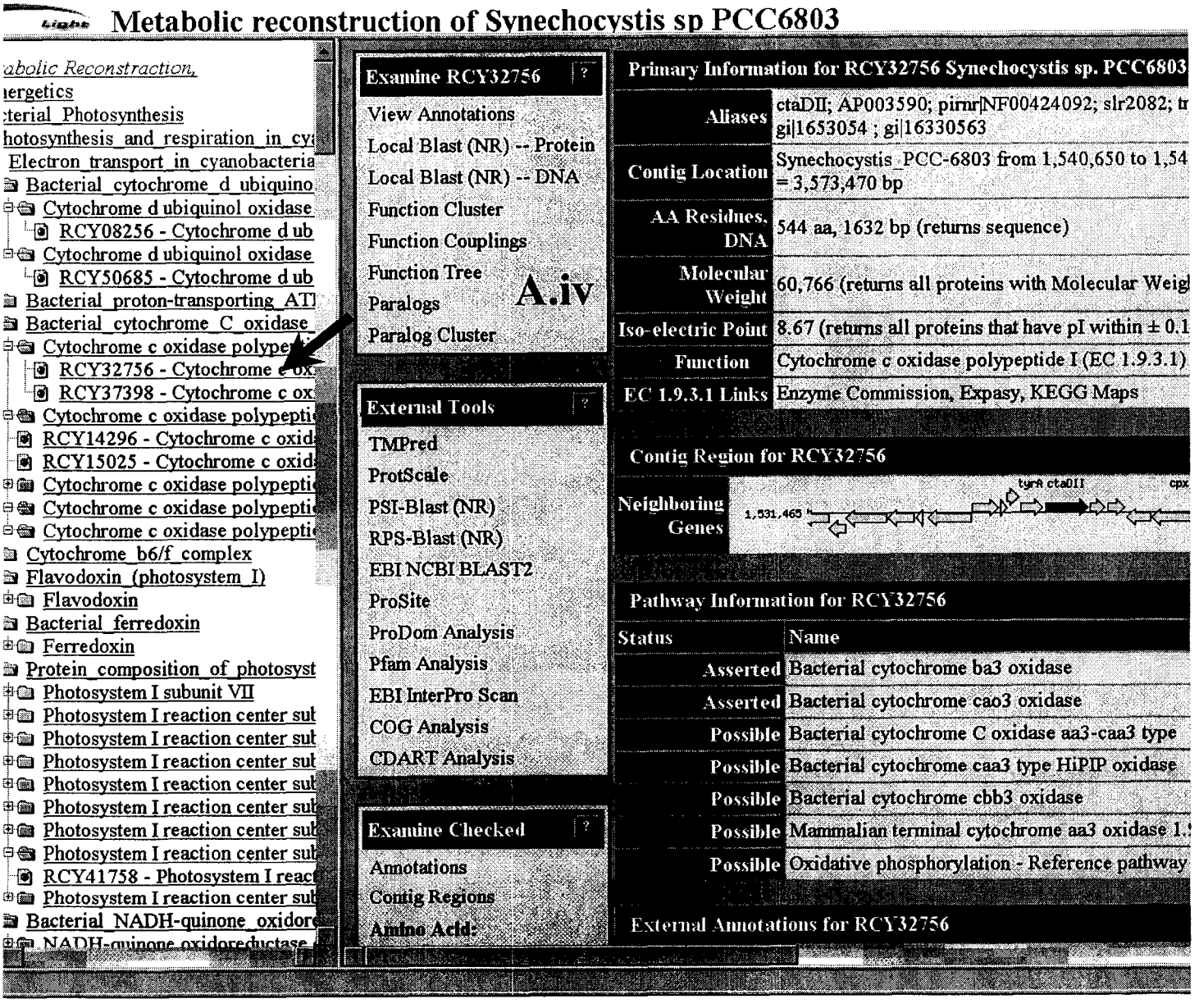
A: Hierarchical functional overview of metabolism of *Synechocystis* sp. PCC 6803 reconstructed from its genome sequence. Arrows point to links which allow the user to access: i) large functional blocks; ii) graphic panels illustrating functional subsystems; iii) individual pathway pages; iv). individual protein pages for *Synechocystis* 6803 ORFs. The last two links connect the user directly with the corresponding pages in ERGO light.



Examples of the tree expansion to the pages listed above are provided in the corresponding panel A.ii, A.iii, A.iv




Metabolic reconstruction of Synechocystis sp PCC6803
Metabolic Reconstraction,
Energetics
Bacterial_Photosynthesis
Photosynthesis_and_respiration_in_cy
Electron_transport_in_cyanobacteria
Bacterial_cytochrome_d_ubiquino
Cytochrome d ubiquinol oxidase
RCY08256 - Cytochrome d ub
Cytochrome d ubiquinol oxidase
RCY50685 - Cytochrome d ub
Bacterial_proton-transporting_AT
Bacterial_cytochrome_C_oxidase
Cytochrome c oxidase polypepti
RCY32756 - Cytochrome c ox
RCY37398 - Cytochrome c ox
Cytochrome c oxidase polypepti
RCY14296 - Cytochrome c oxid
RCY15025 - Cytochrome c oxid
Cytochrome c oxidase polypepti
Cytochrome c oxidase polypepti
Cytochrome c oxidase polypepti
Cytochrome_b6/f_complex
Flavodoxin_(photosystem_I)
Flavodoxin
Bacterial_ferredoxin
Ferredoxin
Protein_composition_of_photosyst
Photosystem I subunit VII
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
RCY41758 - Photosystem I reac
Photosystem I reaction center sul
Bacterial_NADH-quinone_oxidor
NADH-quinone oxidoreductase
Data
Query
Help
Pathway page
A.iii
Switch Groups
Pathway Views
Diagram Picture
See Assertions
See Asserted
Pathway: Bacterial_cytochrome_C_oxidase_aa3-caa3_typ
Pathway Name
Bacterial cytochrome C oxidase aa3-caa3 type
Ref. Organism
Synechocystis sp. PCC6803
Function Tree
Display pathway in the context of sub-systems
4 Assertions
in 0 out of 0 Archaea (sorted by name)
in 4 out of 7 Bacteria (sorted by name)
in 0 out of 0 Eukaryota (sorted by name)
in 0 out of 0 Viruses (sorted by name)
Best KEGG Maps
Oxidative phosphorylation
? E.C. # Functional Description ORF's assigned this function
1 1.9.3.1 Cytochrome c oxidase polypeptide I RCY32756 (ctaDII) RCY37398 (ctaD)
2 RCY14296 (ctaCI) RCY15025 (ctaC)
3 1.9.3.1 Cytochrome c oxidase polypeptide III RCY12508 (ctaEII) RCY34156 (ctaE)
4


Metabolic reconstruction of Synechocystis sp PCC6803
abolic Reconstraction,
ıergetics
:terial_Photosynthesis
hotosynthesis_and_respiration_in_cy
Electron_transport_in_cyanobacteria
Bacterial_cytochrome_d_ubiquino
Cytochrome d ubiquinol oxidase
RCY08256 - Cytochrome d ub
Cytochrome d ubiquinol oxidase
RCY50685 - Cytochrome d ub
Bacterial_proton-transporting_ATI
Bacterial_cytochrome_C_oxidase
Cytochrome c oxidase polype
RCY32756 - Cytochrome c ox
RCY37398 - Cytochrome c ox
Cytochrome c oxidase polypepti
RCY14296 - Cytochrome c oxid
RCY15025 - Cytochrome c oxid
Cytochrome c oxidase polypepti
Cytochrome c oxidase polypepti
Cytochrome c oxidase polypepti
Cytochrome_b6/f_complex
Flavodoxin_(photosystem_I)
Flavodoxin
Bacterial_ferredoxin
Ferredoxin
Protein_composition_of_photosyst
Photosystem I subunit VII
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
Photosystem I reaction center sul
RCY41758 - Photosystem I react
Photosystem I reaction center sul
Bacterial_NADH-quinone_oxidore
NADH-quinone oxidoreductase
Examine RCY32756
View Annotations
Local Blast (NR) -- Protein
Local Blast (NR) -- DNA
Function Cluster
Function Couplings
Function Tree
Paralogs
Paralog Cluster
A.iv
External Tools
TMPred
ProtScale
PSI-Blast (NR)
RPS-Blast (NR)
EBI NCBI BLAST2
ProSite
ProDom Analysis
Pfam Analysis
EBI InterPro Scan
COG Analysis
CDART Analysis
Examine Checked
Annotations
Contig Regions
Amino Acid:
Primary Information for RCY32756 Synechocystis sp. PCC6803
Aliases
ctaDII; AP003590; pirnr|NF00424092; slr2082; tr gi|1653054 ; gi|16330563
Contig Location
Synechocystis_PCC-6803 from 1,540,650 to 1,54 = 3,573,470 bp
AA Residues, DNA
544 aa, 1632 bp (returns sequence)
Molecular Weight
60,766 (returns all proteins with Molecular Weigl
Iso-electric Point
8.67 (returns all proteins that have pI within ± 0.1
Function
Cytochrome c oxidase polypeptide I (EC 1.9.3.1)
EC 1.9.3.1 Links
Enzyme Commission, Expasy, KEGG Maps
Contig Region for RCY32756
Neighboring Genes
1,531,465
tyrA
ctaDII
cpx
Pathway Information for RCY32756
Status
Name
Asserted
Bacterial cytochrome ba3 oxidase
Asserted
Bacterial cytochrome cao3 oxidase
Possible
Bacterial cytochrome C oxidase aa3-caa3 type
Possible
Bacterial cytochrome caa3 type HiPIP oxidase
Possible
Bacterial cytochrome cbb3 oxidase
Possible
Mammalian terminal cytochrome aa3 oxidase 1.
Possible
Oxidative phosphorylation - Reference pathway
External Annotations for RCY32756

Appendix B

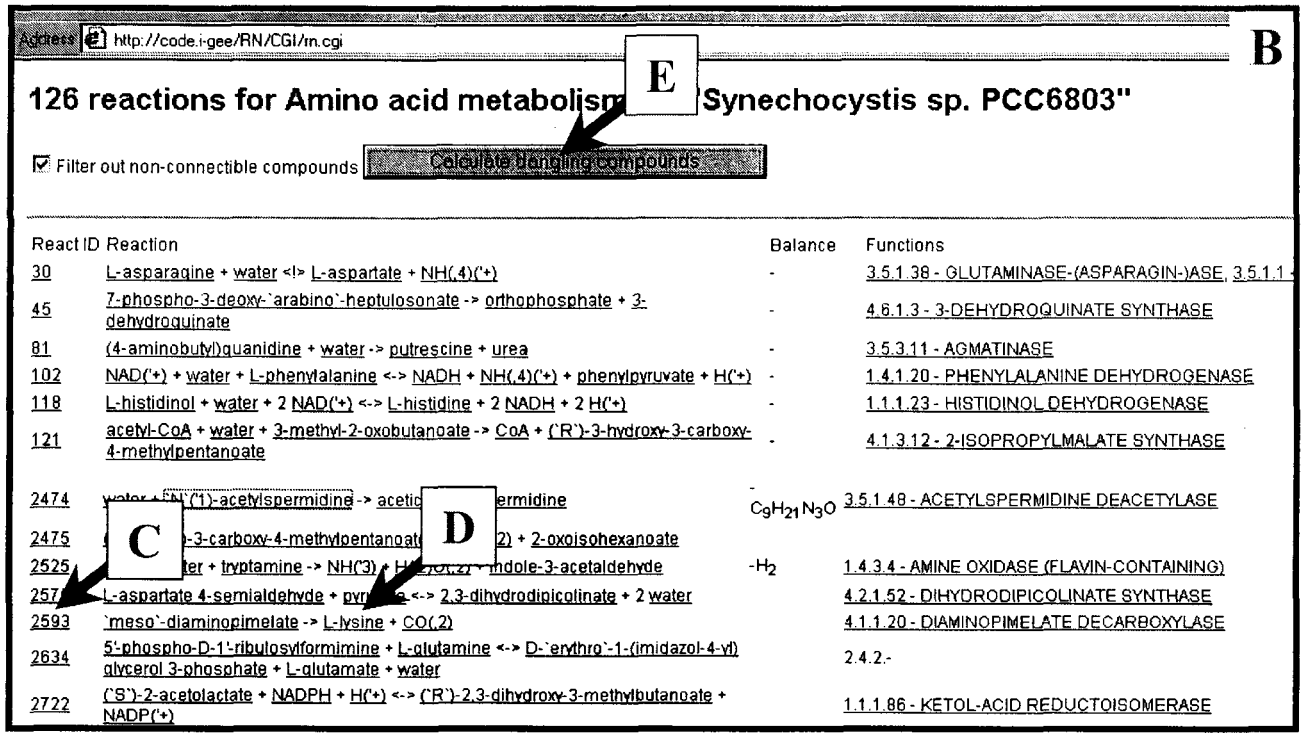
Software for collaborative curation of reactions, compounds, and enzymes within the *Synechocystis* sp. PCC 6803 metabolic reaction network posted at http://www.ergo-light.com/RN (user ID: PCC6803, password: DOE_GTL). Examples with interface screenshots.

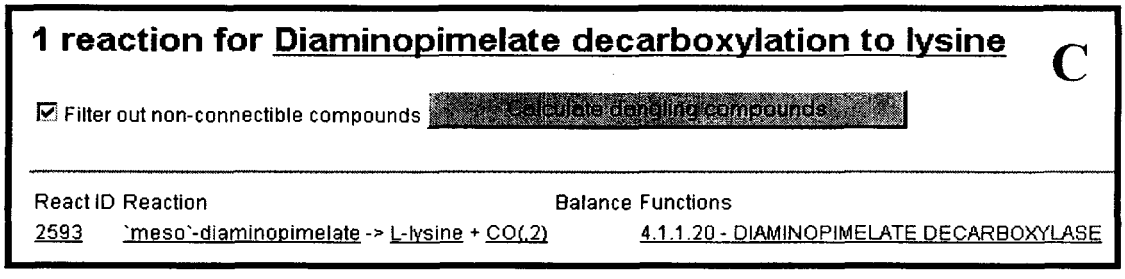
A: The hierarchically organized list of reactions related to amino acid biosynthesis in *Synechocystis* 6803, automatically generated from metabolic reconstruction in ERGO. Arrows point to links which allow the user to retrieve: i) all reactions; ii) reactions for a given node (pathway). **B:** List of reactions relevant for the functional node "amino acid metabolism" retrieved from the report. Arrows point to links, which allow the user to retrieve a summary/curation window for: i) an individual reaction; ii) an individual compound. **C:** The summary/curation window for the reaction # 2593, retrieved from the previous screen (B). **D:** The summary/curation window for the compound L-lysin, retrieved from the previous screen (B). Arrow indicates a link to smile representation of the compound (shown as inset). The list of "dangling" compounds for the node "amino acid metabolism". All shown windows allow manual editing.

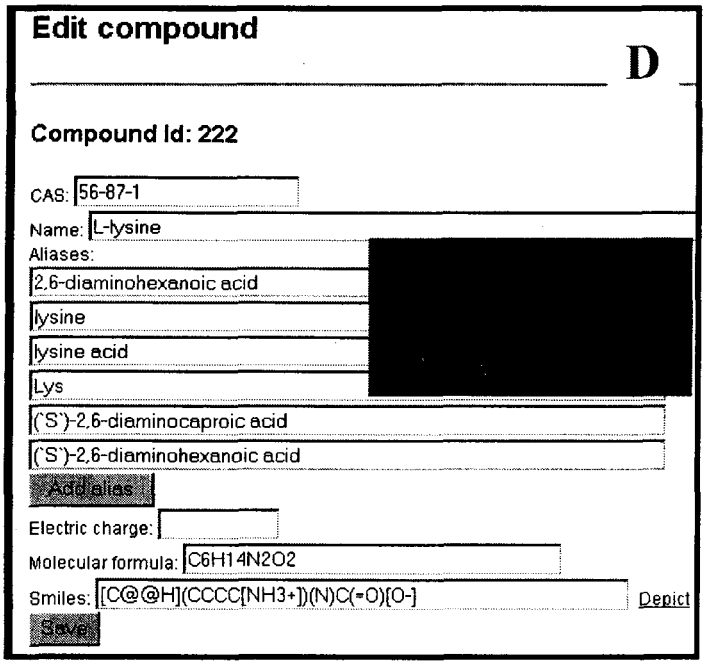
Reactions for Amino acid metabolism for "Synechocystis sp. PCC6803"

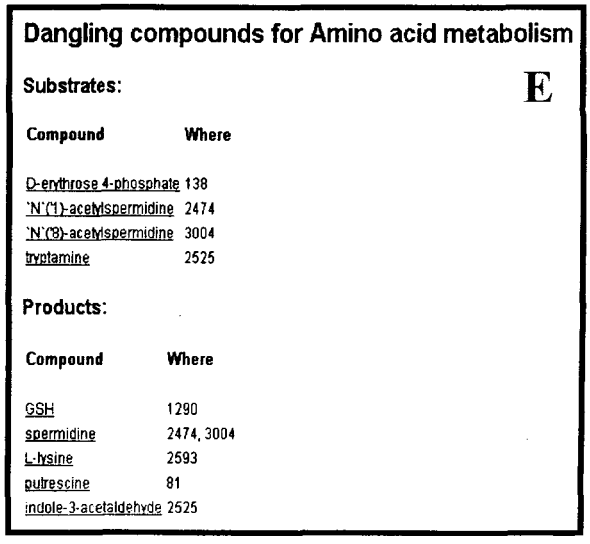
A

- Peptide metabolism
  - glutathione biosynthesis
- Polyamine metabolism
  - Polyamine degradation
    - Acetylspermidine deacetylation
    - Gamma-aminobutyraldehyde dehydrogenase reaction
  - Polyamine biosynthesis
    - Norspermidine biosynthesis via carboxynorspermidine
    - L-arginine decarboxylation to agmatine
    - Putrescine biosynthesis from agmatine via agmatinase
- Amino acid biosynthesis
  - L-asparagine biosynthesis
    - Asparagine biosynthesis via Asn synthase glutamine-hydrolysing
  - L-methionine biosynthesis
  - L-homocysteine biosynthesis
  - isoleucine biosynthesis from threonine
  - threonine biosynthesis from homoserine
  - L-aspartate 4-semialdehyde--homoserine anabolism (NADPH, NADH) (cytosol)
  - L-lysine biosynthesis
    - Diaminopimelate decarboxylation to lysine
    - Diaminopimelate epimerase
    - Tetrahydrodipicolinate biosynthesis
  - aspartate--aspartate 4-semialdehyde, orthophosphate anabolism
  - L-glutamate, oxaloacetate--2-oxoglutarate, L-aspartate catabolism (cytosol)
  - aspartate decarboxylation via EC 4.1.1.11
  - L-ornithine, carbamoyl phosphate--L-arginine, fumarate anabolism (ATP) (cytosol)
  - L-proline biosynthesis from L-1-pyrroline 5-carboxylate


Address http://code.i-gee/RN/CGI/rn.cgi
B
126 reactions for Amino acid metabolism Synechocystis sp. PCC6803"
E
Filter out non-connectible compounds
Calculate dangling compounds
React ID Reaction
Balance
Functions
30 L-asparagine + water <I> L-aspartate + NH(,4)(+) - 3.5.1.38 - GLUTAMINASE-(ASPARAGIN-)ASE, 3.5.1.1
45 7-phospho-3-deoxy-`arabino`-heptulosonate -> orthophosphate + 3-dehydroquinate - 4.6.1.3 - 3-DEHYDROQUINATE SYNTHASE
81 (4-aminobutyl)guanidine + water -> putrescine + urea - 3.5.3.11 - AGMATINASE
102 NAD(+) + water + L-phenylalanine <-> NADH + NH(,4)(+) + phenylpyruvate + H(+) - 1.4.1.20 - PHENYLALANINE DEHYDROGENASE
118 L-histidinol + water + 2 NAD(+) <-> L-histidine + 2 NADH + 2 H(+) - 1.1.1.23 - HISTIDINOL DEHYDROGENASE
121 acetyl-CoA + water + 3-methyl-2-oxobutanoate -> CoA + (`R`)-3-hydroxy-3-carboxy-4-methylpentanoate - 4.1.3.12 - 2-ISOPROPYLMALATE SYNTHASE
2474 `N`(1)-acetylspermidine -> acetic ermidine - $C_9H_{21}N_3O$ 3.5.1.48 - ACETYLSPERMIDINE DEACETYLASE
2475 -3-carboxy-4-methylpentanoat 2) + 2-oxoisohexanoate
2525 ter + tryptamine -> NH(3) + H ndole-3-acetaldehyde -H_2 1.4.3.4 - AMINE OXIDASE (FLAVIN-CONTAINING)
257 L-aspartate 4-semialdehyde + pyr <-> 2,3-dihydrodipicolinate + 2 water 4.2.1.52 - DIHYDRODIPICOLINATE SYNTHASE
2593 `meso`-diaminopimelate -> L-lysine + CO(,2) 4.1.1.20 - DIAMINOPIMELATE DECARBOXYLASE
2634 5'-phospho-D-1'-ribulosylformimine + L-glutamine <-> D-`erythro`-1-(imidazol-4-yl) glycerol 3-phosphate + L-glutamate + water 2.4.2.-
2722 (`S`)-2-acetolactate + NADPH + H(+) <-> (`R`)-2,3-dihydroxy-3-methylbutanoate + NADP(+) 1.1.1.86 - KETOL-ACID REDUCTOISOMERASE
C
D


1 reaction for Diaminopimelate decarboxylation to lysine
C
Filter out non-connectible compounds
Calculate dangling compounds
React ID Reaction
Balance Functions
2593 `meso`-diaminopimelate -> L-lysine + CO(,2) 4.1.1.20 - DIAMINOPIMELATE DECARBOXYLASE


Edit compound
D
Compound Id: 222
CAS: 56-87-1
Name: L-lysine
Aliases:
2,6-diaminohexanoic acid
lysine
lysine acid
Lys
(`S`)-2,6-diaminocaproic acid
(`S`)-2,6-diaminohexanoic acid
Add alias
Electric charge:
Molecular formula: C6H14N2O2
Smiles: [C@@H](CCCC[NH3+])(N)C(=O)[O-]
Depict
Save


Dangling compounds for Amino acid metabolism
Substrates:
E
Compound Where
D-erythrose 4-phosphate 138
`N`(1)-acetylspermidine 2474
`N`(8)-acetylspermidine 3004
tryptamine 2525
Products:
Compound Where
GSH 1290
spermidine 2474, 3004
L-lysine 2593
putrescine 81
indole-3-acetaldehyde 2525