SulfoSYS (*Sulfolobus* Systems Biology): towards a silicon cell model for the central carbohydrate metabolism of the archaeon *Sulfolobus solfataricus* under temperature variation

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Abstract

SulfoSYS (*Sulfolobus* Systems Biology) focuses on the study of the CCM (central carbohydrate metabolism) of *Sulfolobus solfataricus* and its regulation under temperature variation at the systems level. In Archaea, carbohydrates are metabolized by modifications of the classical pathways known from Bacteria or Eukarya, e.g. the unusual branched ED (Entner–Doudoroff) pathway, which is utilized for glucose degradation in *S. solfataricus*. This archaeal model organism of choice is a thermoacidophilic crenarchaeon that optimally grows at 80°C (60–92°C) and pH 2–4. In general, life at high temperature requires very efficient adaptation to temperature changes, which is most difficult to deal with for organisms, and it is unclear how biological networks can withstand and respond to such changes. This integrative project combines genomic, transcriptomic, proteomic and metabolomic, as well as kinetic and biochemical information. The final goal of SulfoSYS is the construction of a silicon cell model for this part of the living cell that will enable computation of the CCM network. In the present paper, we report on one of the first archaeal systems biology projects.

Systems biology

After an initial period of a substantial diversity of activities that were called and accepted to be systems biology, the discipline has now narrowed down to a defined area of science. The various definitions agree that it is the science that attempts to discover principles governing the emergence of biological function from the interactions of components of living systems [1,2]. The non-linearity and complexity of those interactions invokes mathematics, quantitative experimentation and system-wide approaches. However, these three aspects are derived properties, not defining properties, of systems biology.

For reasons of higher homogeneity and genetic accessibility, systems biology has been particularly strong in micro-organisms. These have included both prokaryotes, such as *Escherichia coli*, and eukaryotes, with *Saccharomyces cerevisiae* as a main object of study. These organisms will have some systems biology principles, such as the summation law for control coefficients [3,4], in common. Others, such as those related to signal transduction, are notably different. For yet others, such as distribution of control [5] and heterogeneity of regulation [6], the situation is still unclear.

Archaea

The third domain of life on earth, i.e. the Archaea, have been much less subject to systems biology studies. Since their discovery, Archaea represent an important comparative lineage to study the evolution and characteristics of central

Table 1 | The SulfoSYS consortium

SulfoSYS partners	Partner universities	Work packages
Project co-ordination:		
Christa Schleper	University of Vienna, Austria	Fermentation and Perturbation,
	University of Bergen, Norway	Transcriptomics
Bettina Siebers	University of Duisburg-Essen, Germany	Genomics, Biochemistry
Project partners:		
Sonja V. Albers, Arnold J.M. Driessen	Max-Planck Institute for Terrestrial Microbiology, Germany	Fermentation and Perturbation, Biochemistry
	University of Groningen, The Netherlands	
Nils-Kåre Birkeland	University of Bergen, Norway	Biochemistry
Hans-Peter Klenk	e.gene Biotechnologie GmbH, Feldafing, Germany	Genomics
Peter Ruoff	University of Stavanger, Norway	Modelling
Dietmar Schomburg	University of Braunschweig, Germany	Metabolomics, Modelling
John van der Oost	Wageningen University, The Netherlands	Fermentation, Transcriptomics
Hans V. Westerhoff	Free University of Amsterdam, The Netherlands	Modelling
	University of Manchester, U.K.	
Phillip C. Wright	University of Sheffield, U.K.	Proteomics

cellular functions in living cells. Archaea represent a mosaic of bacterial and eukaryotic as well as archaeal-specific features. They have gained special interest because mechanisms involved in information processing (e.g. transcription, translation, and replication) represent a simpler version of the eukaryotic equivalents and are different from the bacterial equivalents, and because Archaea harbour several unique metabolic features. The archaeal CCM (central carbohydrate metabolism) is characterized by unusual pathways and enzymes, many of which differ from their bacterial or eukaryotic counterparts (for a review, see [7]), and their regulation as well as energetics is not understood.

Many archaeal species that have been obtained as laboratory cultures to date are adapted to extremely high temperatures. They also experience large and frequent temperature changes in their natural environments, probably more pronounced than mesophilic microbes, and therefore require effective adaptation mechanisms. Temperature changes are the most difficult to deal with for organisms, because of the extreme heat permeability of all known external membranes of living organisms, and the rather substantial temperature dependence of both activity and stability of most proteins. Recently, it was pointed out that biochemical networks should be expected to be exquisitely sensitive to temperature changes unless a temperature-compensation mechanism is in place: even slight differences between the rates of individual reactions in metabolic pathways should cause rapid accumulation or depletion of intermediates with various deleterious effects [8].

To prevent substantial and rapid changes in the concentration of important metabolites with change in temperature, the rates of individual reactions in metabolic pathways must therefore change by precisely the same extent. Organisms could adapt by (i) having identical temperature coefficients of the enzymes, (ii) exhibiting metabolic regulation, (iii) adjusting V_{max} values (e.g. through enzyme phosphorylation), (iv) adjusting translation or protein stability, (v) adjust-
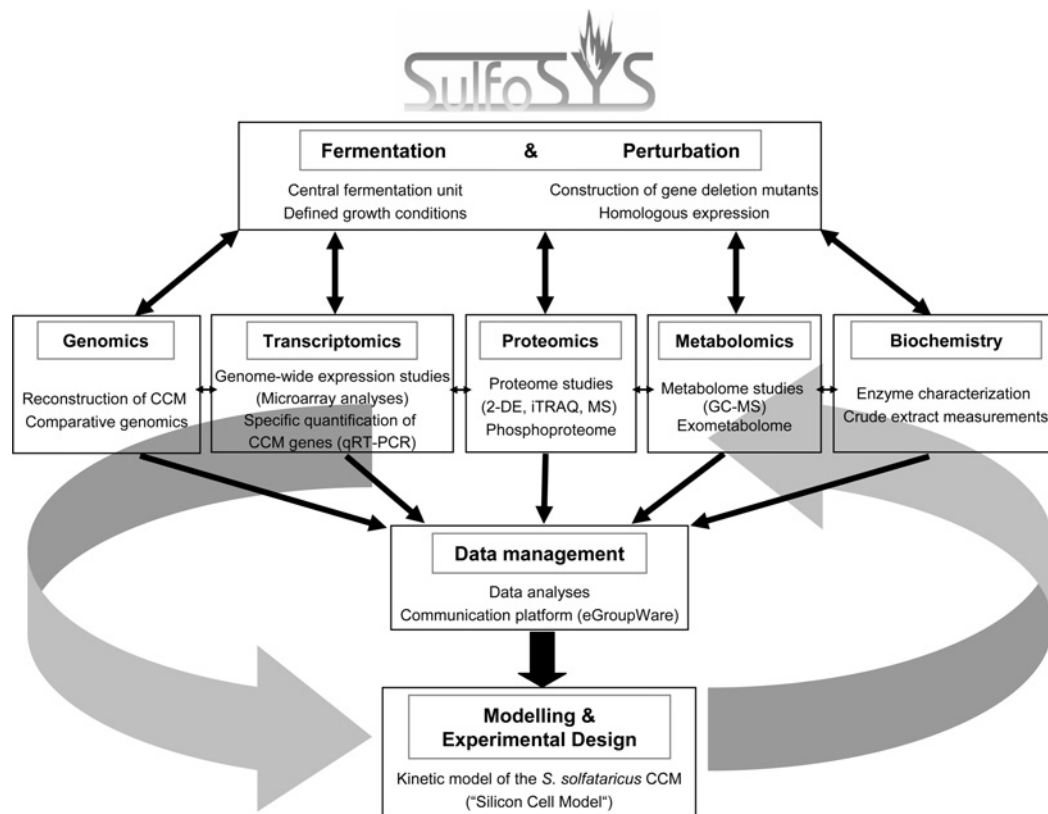
ing transcription or mRNA stability, (vi) re-routing the metabolic flow, (vii) synthesizing compatible solutes, (viii) exporting 'overflow' metabolites, or (ix) going into dormancy. The hypothesis is that several of these mechanisms contribute to different extents, and the aim of this study is to quantify each of these adaptations in a systems biology approach. As the issue should be most acute for (hyper)thermophiles, the study is performed with the crenarchaeon *Sulfolobus solfataricus*. In this integrated systems biology approach, the CCM of *S. solfataricus* and its regulation under temperature variation is analysed by the integration of genomic, transcriptomic, proteomic, metabolomic, kinetic and biochemical information.

Project structure

The SulfoSYS (*Sulfolobus* systems biology) project is one of 11 projects funded within the European trans-national research initiative 'Systems Biology of Microorganisms' (SysMO; http://www.sysmo.net/), supported by six European partner countries (Austria, Germany, Norway, Spain, The Netherlands and the U.K.). In the SulfoSYS project, 11 academic partners from five European countries participate (Table 1). The overall SulfoSYS structure comprises three work packages (Fermentation and Perturbation, Biochemistry, Genomics and Post-Genomics) embedded in the modelling platform (Figure 1). The Fermentation and Perturbation work package offers a central fermentation unit, which provides biological samples for all partners. In addition, *S. solfataricus* knockout as well as expression strains with increased levels of key enzymes are constructed. The Biochemistry work package contributes biochemical and kinetic information of enzymes and analyses their regulatory properties, all in response to temperature change. Finally, the Genomics and Post-Genomics work package performs whole-cell high-throughput analyses (Transcriptomics, Proteomics, Metabolomics and Comparative Genomics), which allow for the analysis of changes at different

Figure 1 | SulfoSYS project structure

The different work packages, their major tasks, the work flow and interactions are indicated. The iterative and hypothesis-driven experimental verification and improvement of the model is indicated by grey arrows. 2-DE, two-dimensional electrophoresis.

hierarchical levels. The experimental groups are embedded in the Analysis, Modelling and Experimental Design Platform, which collects and analyses the respective data and integrates them in the established Blueprint Model. The modelling platform in turn proposes new experiments in order to challenge and by that improve the model (Figure 1).

The aim is that all data, as well as prior knowledge, will be collected into a single mathematical model. Part of this model will be of the silicon cell/JWS type (http://www.siliconcell.net [9]), i.e. a model with rate equations for each enzyme-catalysed reaction and a balance equation for each metabolite, which are then all integrated together. Where this is not yet possible because of a lack of data, more qualitative modelling methods will be used. The model will also be used for experimental design and for systems-biology-driven data management.

The procedure is iterative, and follows the principle that at any time a model can be incomplete, but should reflect all the relevant knowledge about the components of the network (often the kinetic parameters of the enzymes). Thereby the models are almost invariably wrong, or at least incomplete, and this is why they are called Blueprint Models. The models may still be better, however, than the quasi-intuitive prediction on the basis of data that is standard procedure at present.
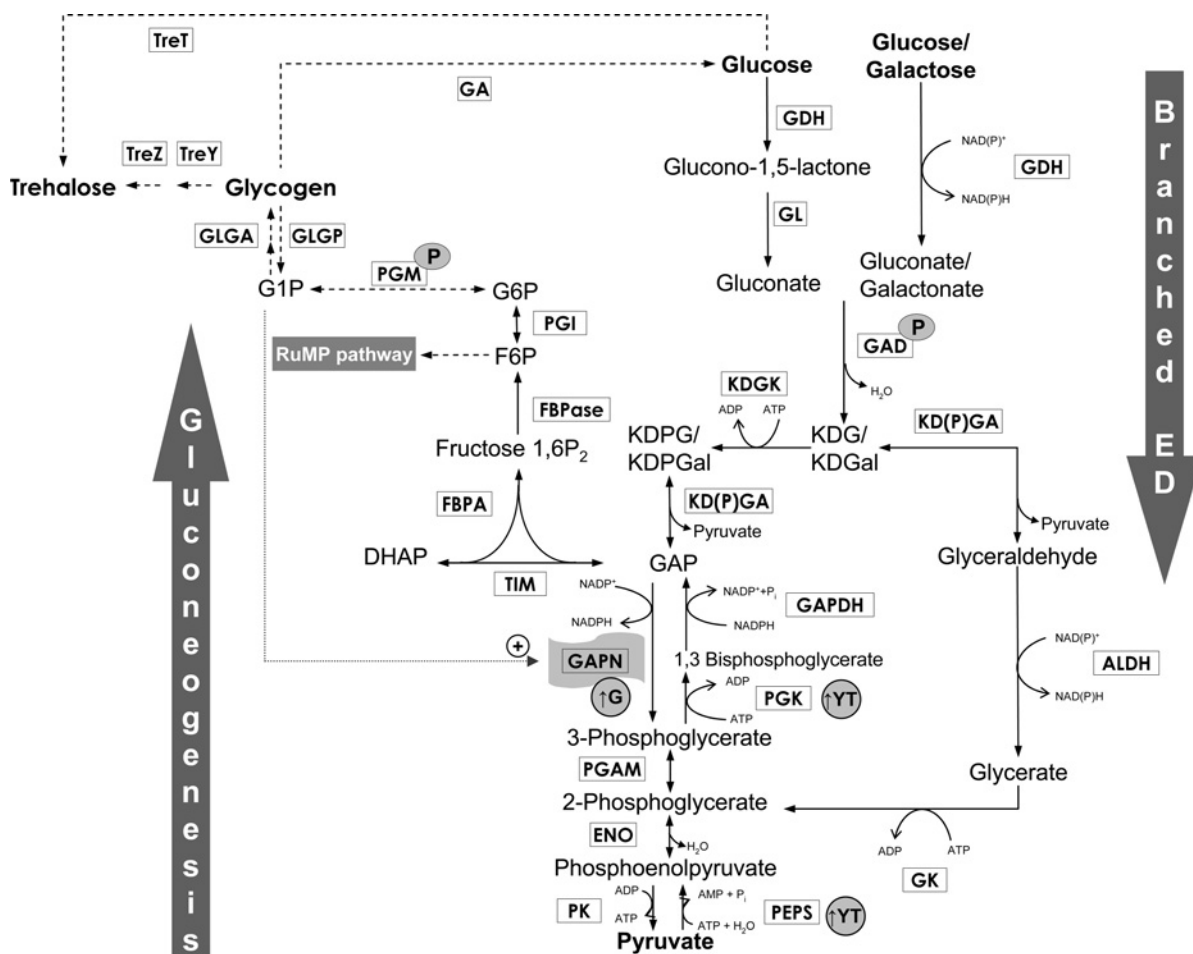
The model system: *Sulfolobus solfataricus* (P2, DSM 1617)

S. solfataricus P2 is a thermoacidophilic crenarchaeon that grows optimally at 80°C (growth range 60–92°C) and pH 2–4 [10]. The organism is a strict aerobe and grows heterotrophically on various carbon sources, amino acids and peptides [11]. No autotrophic or anaerobic growth is observed.

As mentioned above, (hyper)thermophilic archaea are still lacking from the common systems biology model organisms. *S. solfataricus* qualifies for such a role, as (i) its genome has been sequenced and annotated [12], (ii) it can be cultured easily under defined growth conditions [11], (iii) comprehensive biochemical and functional genomics data have been assembled over 25 years (e.g. [13]; reviewed, in [7,14,15], but see [15a]), and (iv) the organism is meanwhile amenable to genetic manipulations. Methods for the construction of directed gene-deletion mutants [16–18] and overexpression strains [19,20] have been established. In addition, proteins of this thermophile surpass proteins from mesophiles in terms of (i) rigidity (favouring crystallization, purification and protein–protein interaction studies), and (ii) the exploitation of thermostable bioproducts in biotechnological applications (extremozymes, white biotechnology). Therefore, and also

Figure 2 | The molecular network: CCM of *S. solfataricus*

The engaged glycolytic reactions of the branched ED pathway and gluconeogenesis via the EMP pathway are shown. Furthermore, the reverse ribulose monophosphate (RuMP) pathway, which is used for pentose generation as well as glycogen and trehalose metabolism, is indicated (broken arrows). The two phosphoproteins of *S. solfataricus* identified are indicated (P). In addition, the results of the transcriptomic/proteomic CCM analysis [14] are given: induction during growth on yeast/tryptone (↑YT) and glucose medium (↑G). The allosteric regulation of the GAPN by glucose 1-phosphate is indicated by a dotted arrow. Enzymes: ALDH, aldehyde dehydrogenase; ENO, enolase; FBPA, fructose-1,6-bisphosphate aldolase; FBPase, fructose-1,6-bisphosphatase; GA, glucan-1,4-α glucosidase; GAD, gluconate dehydratase; GDH, glucose dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAPN, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase; GK, glycerate kinase; GL, gluconolactonase; GLGA, glycogen synthase; GLGP, glycogen phosphorylase; KD(P)GA, 2-keto-3-deoxy-(6-phospho)-gluconate aldolase; KDGK, 2-keto-3-deoxygluconate kinase; PEPS, phosphoenolpyruvate synthetase; PGAM, phosphoglycerate mutase; PGI, phosphoglucose isomerase; PGK, phosphoglycerate kinase; PGM, phosphoglucomutase; PK, pyruvate kinase; TIM, triose phosphate isomerase; TreT, trehalose glycosyltransferring synthase; TreY, malto-oligosyltrehalose synthase; TreZ, malto-oligosyltrehalose trehalohydrolase. Intermediates: DHAP, dihydroxyacetonephosphate; Fructose 1,6P2, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; G1P, glucose 1-phosphate; G6P, glucose 6-phosphate; GAP, glyceraldehyde 3-phosphate; KD(P)G, 2-keto-3-deoxy-(6-phospho)-gluconate; KD(P)Gal, 2-keto-3-deoxy-(6-phospho)-galactonate.

because of its use as a production strain for proteins and its use for the development of drugs and diagnostics, *S. solfataricus* is an emerging biotechnology organism of the domain Archaea.

The molecular network

The network under study is the CCM of *S. solfataricus*, i.e. the catabolic branched ED (Entner–Doudoroff) pathway,

the gluconeogenic EMP (Embden–Meyerhof–Parnas) pathway, the reverse ribulose monophosphate pathway for pentose formation and the tricarboxylic acid cycle, as well as glycogen and trehalose metabolism (Figure 2). The initial focus of the project is on the archaeal-specific branched ED pathway of *S. solfataricus* [21–26]. This modified pathway has been reported first for *S. solfataricus* and *Thermoproteus*

tenax [21]; however, genomics-based studies indicate that this is the common ED modification found in archaea. Biochemical studies in *S. solfataricus* revealed that glucose is catabolized exclusively by the branched ED pathway. The pathway is characterized by a common shunt, which harbours the conversion of glucose via gluconate into KDG (2-keto-3-deoxygluconate), as catalysed by GDH (glucose dehydrogenase) and GAD (gluconate dehydratase). The GAD belongs to the enolase superfamily and shares no similarity to the classical ED enzyme 6-phosphogluconate dehydratase. KDG is the characteristic intermediate of this pathway. It is either cleaved directly by a unique archaeal-type bi-functional KD(P)G [2-keto-3-deoxy-(6-phospho)gluconate] aldolase, forming glyceraldehyde and pyruvate, in the non-phosphorylative branch or is phosphorylated via KDG kinase, forming KDPG, in the semi-phosphorylative branch [21]. KDPG is cleaved to GAP (glyceraldehyde 3-phosphate) and pyruvate by the same bifunctional KD(P)G aldolase, which is active on phosphorylated as well as non-phosphorylated substrates and is therefore a key player in both branches of the pathway. The enzyme is a member of the NAL (N-acetylneuraminate lyase) superfamily and shows no similarity to the classical ED aldolase. In the non-phosphorylative branch, glyceraldehyde is oxidized further, yielding glycerate via glyceraldehyde dehydrogenase or oxidoreductase. Glycerate kinase catalyses the phosphorylation of glycerate, forming 2-phosphoglycerate, which enters the final part of the EMP pathway and is converted into a second molecule of pyruvate via enolase and pyruvate kinase. In the semi-phosphorylative branch, GAP enters the lower shunt of the EMP pathway. As a specific feature of *S. solfataricus* and hyperthermophiles (organisms with optimal growth above 80°C, e.g. *T. tenax*, *Thermococcus kodakaraensis*), GAP is converted directly into 3-phosphoglycerate by the uni-directional GAPN (non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase). The enzyme substitutes for the classical enzyme couple GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and PGK (phosphoglycerate kinase), which were shown to have a main function in the gluconeogenesis of these different (hyper)thermophilic archaea [14,27–29]. This has been supported recently for *S. solfataricus* by a combined transcriptome/proteome analysis of the CCM [13] that revealed an induction of the GAPN in glucose grown cells, in contrast with an up-regulation of PGK as well as PEPS (phosphoenolpyruvate synthetase) in cells grown on yeast/tryptone. Finally, 3-phosphoglycerate is converted into pyruvate via phosphoglycerate mutase, enolase and pyruvate kinase.

Strikingly, the pathway has been shown to be promiscuous for glucose as well as galactose degradation in *S. solfataricus* with the respective enzymes [GDH, GAD, KDG kinase, KD(P)G aldolase] being active on both substrates and their respective derivatives [22,23]. There is no net energy gain of the branched ED pathway, bearing in mind that the GAPN is used in the semi-phosphorylative ED branch.

The physiological role of the two ED branches, as well as their regulation, is still unclear [21,30]. GAPN is the only enzyme of the pathway known so far to be subject to allosteric control (Figure 2). The enzyme is activated by glucose 1-phosphate, an intermediate of glycogen metabolism [27]. Interestingly, regulation by protein phosphorylation/dephosphorylation was reported for the GAD and the PGM (phosphoglucomutase) (Figure 2) of *S. solfataricus* [24,31,32].

In vitro measurements indicate that some of the intermediates (e.g. GAP, 1,3-bisphosphoglycerate) of the semi-phosphorylated ED branch are unstable at the high temperatures that are physiological for this organism, suggesting that metabolic thermoadaptation might play an important role in the utilization of the two ED branches. One example that strongly supports this hypothesis is the almost exclusive presence of GAPN or GAP oxidoreductase in hyperthermophilic archaea. Both enzymes avoid the formation of the thermolabile 1,3-bisphosphoglycerate (half-life of less than 2 min at 60°C, [30]) by the direct oxidation of GAP to 3-phosphoglycerate. The pathway then foregoes the production of ATP via the classical GAPDH–PGK enzyme couple. Therefore this part of the CCM is highly suited to analyse possible temperature-dependent shifts and the resulting re-routing of metabolic flow between pathways in response to these temperature changes.

The EMP pathway is supposed to be active only in the gluconeogenic direction, since no classical or archaeal-type sugar kinase activities (glucokinase, hexokinase or phosphofructokinase) have been detected in *S. solfataricus* and no obvious candidate genes encoding homologues have been identified in the genome. As characteristic enzymes of the anabolic EMP pathway, candidate genes encoding PEPS, PGK, GAPDH and fructose bisphosphatase (archaeal-type, class V) were identified in the genome.

Achievements and outlook

Several enzymes of the branched ED pathway have been characterized previously (e.g. [21–24,26,27]). The ongoing reconstruction of the pathway using the genome sequence information, however, revealed several paralogues for some of the enzymes, raising questions about their function in the network. Therefore one focus of the project is the identification of all players in the network. In addition, and most importantly for a joint systems biology approach, besides providing scientific fundamentals [e.g. SOPs (standard operating procedures), recombinant enzymes, appropriate strains], an efficient communication platform has been established (eGroup-Ware, open source). Furthermore, SOPs have been improved and/or established in the different work packages [e.g. control of genomic strain stability, stock handling, fermentation, metabolite isolation, proteome analysis (iTRAQ)]. The first models for the network under investigation have been developed, and the first experimental pilot projects were performed, in which all partners tested and, if necessary, refined their protocols and generated first-run quantitative data on the same shared biomass samples. The same biomass sample philosophy was of course a vital standardization SOP needed to gain cohesive and meaningful results.

The long-term goal of this highly integrated project is to build a sufficiently precise replica for this part of the living cell, i.e. a 'silicon cell model' that should enable the computation of life at the systems level. This model will be the first detailed kinetic model of a pathway in an archaeon, and should highlight differences as well as similarities between members of all three domains of life. The model should definitely enable us to look at the general properties of metabolic control analysis and at robustness. With additional experimentation determining the changes in enzyme levels with the temperature, we shall also be able to determine to what extent the organism manages its defence against temperature fluctuation and variations through regulated gene expression, through direct metabolic regulation or through signal transduction leading to covalent modification. The results will be the first instance of a comprehensive, 'live' dataset of an archaeon's carbon and energy metabolism. It should also serve as a scaffold for storing and managing other data concerning the functioning of the organism. And it will be 'alive' indeed: its system behaviour can be calculated by anyone through our web interface (see http://jjj.bio.vu.nl/).

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References

1 Alberghina, L. and Westerhoff, H.V. (eds) (2005) Systems Biology: Definitions and Perspectives (Topics in Current Genetics Vol, 13), Springer, Berlin

2 Marcus, F.B. (2008) Bioinformatics and Systems Biology: Collaborative Research and Resources, Springer, Berlin

3 Kacer, H. and Burns, J.A. (1973) The control of flux. Symp. Soc. Exp. Biol. **27**, 65–104

4 Heinrich, R. and Rapoport, T.A. (1974) A linear steady-state treatment of enzymatic chains: general properties, control and effector strength. Eur. J. Biochem. **42**, 89–95

5 Groen, A.K., Vervoorn, C.R., Van der Meer, R. and Tager, J.M. (1983) Control of gluconeogenesis in rat liver cells. I. Kinetics of the individual enzymes and the effect of glucagon. J. Biol. Chem. **258**, 14346–14353

6 Rossell, S., van der Weijden, C.C., Lindenbergh, A., van Tuijl, A., Francke, C., Bakker, B.M. and Westerhoff, H.V. (2006) Unraveling the complexity of flux regulation: a new method demonstrated for nutrient starvation in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **103**, 2166–2171

7 Siebers, B. and Schönheit, P. (2005) Unusual pathways and enzymes of central carbohydrate metabolism in Archaea. Curr. Opin. Microbiol. **8**, 695–705

8 Ruoff, P., Zakhartsev, M. and Westerhoff, H.V. (2007) Temperature compensation through systems biology. FEBS J. **274**, 940–950

9 Olivier, B.G. and Snoep, J.L. (2004) Web-based kinetic modelling using JWS Online. Bioinformatics **20**, 2143–2144

10 Zillig, W., Stetter, K.O., Wunderl, S., Schulz, W., Priess, H. and Scholz, I. (1980) The *Sulfolobus*–"*Caldariella*" group: taxonomy on the basis of the structure of DNA-dependent RNA polymerases. Arch. Microbiol. **125**, 259–269

11 Grogan, D.W. (1998) Phenotypic characterization of the archaebacterial genus *Sulfolobus*: comparison of five wild-type strains. J. Bacteriol. **171**, 6710–6719

12 She, Q., Singh, R.K., Confalonieri, F., Zivanovic, Y., Allard, G., Awayez, M.J., Chan-Weiher, C.C.Y., Groth Clausen, I., Curtis, B.-A., De Moors, A. et al. (2001) The complete genome of the crenarchaeon *Sulfolobus solfataricus* P2. Proc. Natl. Acad. Sci. U.S.A. **98**, 7835–7840

13 Snijders, A.P.L., Walther, J., Peter, S., Kinnman, I., de Vos, M.G.J., van de Werken, H.J.G., Brouns, S.J.J., van der Oost, J. and Wright, P.C. (2006) Reconstruction of central carbon metabolism in *Sulfolobus solfataricus* using a two-dimensional gel electrophoresis map, stable isotope labelling and DNA microarray analysis. Proteomics **6**, 1518–1529

14 van der Oost, J. and Siebers, B. (2007) The glycolytic pathways of Archaea: evolution by tinkering. In Archaea: Evolution, Physiology and Molecular Biology (1st edn) (Garrett, R.A. and Klenk, H.-P., eds), pp. 247–260, Blackwell Publishing, Oxford

15 Verhees, C.H., Kengen, S.W., Tuininga, J.E., Schut, G.J., Adams, M.W.W., De Vos, W.M. and van der Oost, J. (2003) The unique features of glycolytic pathways in Archaea. Biochem. J. **375**, 231–246

15a Erratum (2004) Biochem. J. **377**, 819–822

16 Worthington, P., Hoang, V., Perez-Pomares, F. and Blum, P. (2003) Targeted disruption of the α-amylase gene in the hyperthermophilic archaeon *Sulfolobus solfataricus*. J. Bacteriol. **185**, 482–488

17 Schelert, J., Dixit, V. Hoang, V. Simbahan, J., Drozda, M. and Blum, P. (2004) Occurence and characterization of mercury resistance in the hyperthermophilic archaeon *Sulfolobus solfataricus* by use of gene disruption. J. Bacteriol. **186**, 427–437

18 Albers, S.V. and Driessen, A.J.M. (2007) Conditions for gene disruption by homologous recombination of exogenous DNA into the *Sulfolobus solfataricus* genome. Archaea **2**, 145–149

19 Jonuscheit, M., Martusewitsch, E., Stedman, K.M. and Schleper, C. (2003) A reporter gene system for the hyperthermophilic archaeon *Sulfolobus solfataricus* based on a selectable and integrative shuttle vector. Mol. Microbiol. **48**, 1241–1252

20 Albers, S.V., Jonuscheit, M., Dinkelaker, S., Urich, T., Kletzin, A., Tampe, R. Driessen, A.J.M. and Schleper, C. (2006) Production of recombinant and tagged proteins in the hyperthermophilic Archaeon *Sulfolobus solfataricus*. Appl. Environ. Microbiol. **72**, 102–111

21 Ahmed, H., Ettema, T.J., Tjaden, B., Geerling, A.C., van der Oost, J. and Siebers, B. (2005) The semi-phosphorylative Entner–Doudoroff pathway in hyperthermophilic archaea: a re-evaluation. Biochem. J. **390**, 529–540

22 Lamble, H.J., Heyer, N.I., Bull, S.D., Hough, D.W and Danson, M. (2003) Metabolic pathway promiscuity in the archaeon *Sulfolobus solfataricus* revealed by studies on glucose dehydrogenase and 2-keto-3-deoxygluconate aldolase. J. Biol. Chem. **278**, 34066–34072

23 Lamble, H.J., Theodossis, A., Milburn, C.C., Taylor, G.L., Bull, S.D., Hough, D.W and Danson, M. (2005) Promiscuity in the part-phosphorylative Entner–Doudoroff pathway of the archaeon *Sulfolobus solfataricus*. FEBS Lett. **579**, 6865–6869

24 Kim, S. and Lee, S.B. (2005) Identification and characterization of *Sulfolobus solfataricus* D-gluconate dehydratase: a key enzyme in the non-phosphorylated Entner–Doudoroff pathway. Biochem. J. **387**, 271–280

25 Kim, S. and Lee, S.B. (2006) Catalytic promiscuity in dihydroxy-acid dehydratase from the thermoacidophilic archaeon *Sulfolobus solfataricus*. J. Biochem. **139**, 591–596

26 Kim, S. and Lee, S.B. (2006) Characterization of *Sulfolobus solfataricus* 2-keto-3-deoxy-D-gluconate kinase in the modified Entner–Doudoroff pathway. Biosci. Biotechnol. Biochem. **70**, 1308–1316

27 Ettema, T.J.G., Ahmed, H., Geerling, A.C.M., van der Oost, J. and Siebers, B. (2008) The non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN) of *Sulfolobus solfataricus*: a key enzyme of the semi-phosphorylative branch of the Entner–Doudoroff pathway. Extremophiles **12**, 75–88

28   Zaparty, M., Zaigler, A., Stamme, C., Soppa, J. and Hensel R., Siebers B.
      (2008) DNA microarray analysis of the central carbohydrate
      metabolism: glycolytic/gluconeogenic carbon switch in the
      hyperthermophilic Crenarchaeum *Thermoproteus tenax*. J. Bacteriol.
      190, 2231–2238
29   Zaparty, M., Tjaden, B., Hensel, R. and Siebers, B. (2008) The central
      carbohydrate metabolism of the hyperthermophilic crenarchaeote
      Thermoproteus tenax: pathways and insights into their regulation.
      Arch. Microbiol. **190**, 231–245
30   Ahmed, H., Tjaden, B., Hensel, R. and Siebers, B. (2004)
      Embden–Meyerhof–Parnas and Entner–Doudoroff pathways in
      Thermoproteus tenax: metabolic parallelism or specific adaptation?
      Biochem. Soc. Trans. **32**, 2–4
31   Solow, B., Bichoff, K.M., Zylka, M.J. and Kennelly, P.J. (1998) Archaeal
      phosphoproteins. Identification of a hexosephosphate mutase and the
      α-subunit of succinyl-CoA synthetase in the extreme
      acidothermophile *Sulfolobus solfataricus*. Protein Sci. **7**, 105–111
32   Ray, W.K., Keith, S.M., DeSantis, A.M., Hunt, J.P., Larson, T.J., Helm, R.F.
      and Kennelly, P.J. (2005) A phosphohexomutase from the Archaeon
      Sulfolobus solfataricus is covalently modified by phosphorylation on
      serine. J. Bacteriol. **187**, 4270–4275