BEILSTEIN SYMPOSIUM

Molecular Functions, Catalysis and Reguation

Beilstein Enzymology Symposium 2019

10 – 12 September 2019
Hotel Jagdschloss Niederwald
Rüdesheim, Germany

The Beilstein-Institut and Open Science

The non-profit Beilstein-Institut is one of the most respected organizations in the communication and dissemination of high-quality information in chemistry. Since 1951, when the foundation was established by the Max Planck Society, we have been fulfilling our mission to support the scientific community by providing high-quality information that is essential for research.

Our role has evolved over the years: from the production of the Beilstein Handbook and Database, to being one of the first open access journal publishers in chemistry, to host of interdisciplinary symposia and supporter of open data initiatives. We believe that free access to scientific research results, giving everyone in the world an equal chance to read and reuse experimental findings and data, is the best way to advance science.

Open Science is a new approach to scientific research. It is based on cooperation and uses new ways to disseminate information and broaden knowledge through digital technologies and new collaborative tools. It aims to make the primary outputs of publicly funded research results – publications (open access) and the research data (open data) – publicly accessible in digital format with no or minimal restriction.

The Beilstein-Institut supports open science and makes the results of its projects freely available to the scientific community as open access publications. This is an essential contribution to the foundation's mission to advance the chemical and related sciences. All journal articles, conference proceedings and videos are open access to allow the worldwide, unhindered sharing and exchange of ideas. This allows scientists, students, educators and the public the opportunity to inform themselves of the latest developments in research and to build on these ideas to further advance scientific knowledge.

Our two platinum open access journals, the *Beilstein Journal of Organic Chemistry* and the *Beilstein Journal of Nanotechnology*, which we fully fund, have no fees for authors or readers. Both journals are produced and managed by the Beilstein Editorial Office team, who work together with a global scientific network of experts that are responsible for the peer review. In 2015, the Beilstein Journals were awarded the DOAJ Seal which recognizes the exceptionally high level of publishing standards and best practices adhering to these journals.

An essential prerequisite for open science data is reporting guidelines and technical standards that provide the framework for the exchange of data from one laboratory to another without technical and textual barriers.

The Beilstein-Institut runs two data standards projects: STRENDA which is concerned with the reporting of enzymology data and MIRAGE with the reporting of glycomics experimental results. Both of which are now widely accepted and acknowledged by the scientific community.

The direct interaction and the exchange of thoughts and ideas between scientists are supported by a program of regularly hosted symposia. These international meetings are organized by the Beilstein-Institut and cover a variety of topics ranging from organic chemistry and biochemistry to nanotechnology and open science as well as interdisciplinary meetings on contemporary topics.

The Beilstein-Institut has been hosting symposia since 1988. Each meeting is always an interesting event with an open result: the Beilstein-Institut provides the framework and the lively and intense exchange of thoughts and ideas of the participants turn it into a memorable and lasting experience. The number of participants is usually limited to around 50 and the program is designed specifically to allow sufficient time for discussions. In some ways the talks can be seen as providing a catalyst for these discussions which often go on into the night and have led to subsequent cooperation projects. The resulting exchange between researchers is the underlying goal of the meeting and gives the Beilstein Symposium their unique character.

Regularly updated information about our symposia is available at www.beilstein-symposia.org.

Upcoming symposia in 2019/2020:

Beilstein Open Science Symposium 2019 *The What, How and Why of Open Science* 15 – 17 October, 2019, Rüdesheim, Germany	Scientific Program: Martin Hicks and Carsten Kettner www.open-science.beilstein-symposia.org
Beilstein Nanotechnology Symposium 2019 *MXene at the Frontier of the 2D Materials World* 15 – 17 October, 2019, Mainz, Germany	Scientific Program: Yuri Gogotsi, Xinliang Feng, Johanna Rosén https://www.beilstein-institut.de/en/symposia/nano-2d-materials

Beilstein Nanotechnology Symposium 2019
New Directions for Nanoporous Materials

12 – 14 November 2019, Rüdesheim, Germany

Scientific Program:
Sir Fraser Stoddard, Cafer T. Yavuz

https://www.beilstein-institut.de/en/symposia/nano-porous

Beilstein Organic Chemistry Symposium 2020
Stereoselective Alkene Functionalizations

21 – 23 April, 2020, Rüdesheim, Germany

Scientific Program:
Thomas Wirth

https://www.beilstein-institut.de/en/symposia/org-chem-stereo-alkene-functions

Beilstein Bozen Symposium 2020
Models of Convenience

16 – 18 June, 2020, Rüdesheim, Germany

Scientific Program:
Lee Cronin, Tim Clark, Martin G. Hicks and Carsten Kettner

http://www.bozen.beilstein-symposia.org

Beilstein Organic Chemistry Symposium 2020
Earth-abundant 3d Metal Catalysis

22 – 24 July, 2020, Mainz, Germany

Scientific Program:
Lutz Ackermann

https://www.beilstein-institut.de/en/symposia/org-chem-3d-metal-catalysis

Book of Abstracts

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Overview

In enzymology, catalytic reactions usually are investigated *in vitro* under well-defined conditions which may mimic physiological conditions. This experimental design allows the characterization of the kinetic capabilities of enzymes and to draw conclusions on both the mechanisms of the conversion of substrates into products and the effect of co-enzymes, essential metal ions and modifiers on the structure of the enzyme and thus on the overall catalytic reaction. In addition to biochemical methods, optical methods have emerged to study protein-structure function. In particular, cryogenic electron microscopy (cryo-EM) has led to new insights of the structures of large protein complexes at near-atomic resolution whose 3D models reveal how these molecules function in the cell.

However, the single-enzyme kinetics obtained under *in vitro* conditions may be misleading if this data is transferred into *in vivo* conditions due to the high viscosity in the cell. The cellular environment is densely packed with macromolecules such as proteins, RNA, DNA and metabolites which form 'quinary' interactions mediated by repulsing and attracting electrostatic forces. In particular, for metabolic pathways in which multi-step reactions are catalyzed this requires either a close spatial arrangement of the enzymes involved (and which has been proposed a substrate channelling for e.g. glycolysis) or lowered turnover rate through the pathway due to increased diffusion times for the individual substrates. Thus, the spatial and temporal arrangement of enzymes has to be regulated to achieve the necessary pathway efficiency, especially for those being located in the cytoplasm. In addition, pathway kinetics is highly controlled by modifying effectors e.g. the diverse types of inhibition and activation of individual enzymes.

This Symposium takes molecular functions, catalysis and regulation in perspective and addresses the insights in structure-function relationships using cryo-EM and computational biology, the role of cofactors in metabolism and regulation, and systems-wide analysis of metabolic pathways.

The Beilstein Enzymology Symposia embrace structural, computational and biological disciplines, and bring researchers (established and younger workers) together to discuss the many and diverse roles of enzymes in biology, and to explore the limits and challenges of holistic studies that attempt to integrate microscopic views of protein function into complex biological behaviour.

Under the guidance of the STRENDA Commission (www.beilstein-strenda.org), this conference series also provides a platform to present the results of this working group's efforts, to discuss about the requirements for setting up standards in biochemistry and to address the needs making research data findable, accessible, interoperable and reusable. The mission of STRENDA is to establish guidelines for the reliable and accurate reporting of protein function data, and to maintain a database (STRENDA DB, www.beilstein-strenda-db.org) which stores this data after its validation on completeness and compliance with the STRENDA Guidelines.

We are looking toward committed discussions about the latest results, approaches and methodologies presented in experimental, theoretic and bioinformatics enzymology.

Enjoy the Symposium!

Scientific Committee

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Registration

All participants must be registered to have access to the conference area.

Participants can ask the organizers for a confirmation of the payment of the conference registration fee. Insurance of participants against accidents, sickness, cancellation, theft, property damage or loss is not covered. Participants are advised to take out adequate personal insurance (see also „Liability and Insurance“).

Participants are responsible for settling their hotel bills directly with the hotel on departure. The total price for participants staying at the Hotel Jagdschloss Niederwald is 659 EUR and includes both accommodation for four nights and the conference package that covers lunches, dinners and coffee breaks as well as admits access to the conference room.

> **Participants not staying at the Hotel Jagdschloss Niederwald are requested to register with the hotel for booking and paying the conference package, i.e. 291 EUR per person.**

Extras, such as drinks, telephone calls etc. are not included in the price.

The Symposium

The symposium will be held from 10 to 12 September, 2019, with the 9th and the 13th for travelling.

The setting and the limited number of participants (max. 50 persons) provide a very convivial atmosphere for the ready exchange of thoughts and ideas.

The scientific program will take place over three days and will

start at 9.00 am on Tuesday, the 10th, and

end in the late afternoon (ca. 5.30 pm) on Thursday, the 12th.

If you wish to extend your stay, please contact the hotel directly.

For the length of the individual talks, please refer to the program. Speakers should allow sufficient time for discussion at the end of their talks (e.g. a 35 min slot includes 25 min talk + 10 min for questions). We will have an LCD projector connected to a Windows PC available.

Presentations of Posters

Poster Exhibition:
Tuesday, 10th and Wednesday, 11th September during the coffee breaks

Location of the posters

The Poster exhibition will be placed in the coffee room which is close to the conference room. Your poster board will be marked with your poster number which is the same in the abstract book.

Poster mounting

Please mount your poster on the 9th September from 4 pm or on the 10th latest by 8.30 am. Your poster will be on display throughout the Symposium. You are asked to remove all poster materials from the board at the end of the meeting otherwise it will be taken down on time and disposed by the organizers. The organizers cannot take any responsibility for this material.

Presentations

The oral poster presentations will take place as indicated in the scientific program. The presentations should not exceed 5 min. You will have 1 min in addition for questions.

Please make sure that you are using the power point template sent out and that you have delivered your final presentation to the organizers in time.

We will have an LCD projector connected to a Windows PC available.

Liability and Insurance

The Beilstein-Institut will not be liable for any accident, theft or damage to property, nor for any delays or modification in the program due to unforeseen circumstances.

Participants and accompanying persons are advised to arrange personal travel and health insurance.

Scientific Program

Monday, 9th September

19.00 Welcome reception

19.30 Dinner

Tuesday, 10th September

09.00	Opening and Introductory Remarks	Carsten Kettner
	Session Chair: Hans V. Westerhoff	
09.20	New Horizons in Enzymology from Cryo-EM and X-ray Free-electron Laser (XFEL)	Ming-Daw Tsai
09.55	Single Molecule Enzymology and Beyond	Xiaoliang Sunney Xie
10.30	Poster Flash Presentation #1	U. Wittig, N. Agarwal, N. Panti, P. Putthapong M.F. Pinto
11.00	*Coffee Break and Poster Session*	
11.30	Common Mechanisms by Skeletal Muscle Actomyosin and Bacterial Flagellar Motor Revealed by Electron Cryomicroscopy and Optical Nanophtometry	Keiichi Namba
12.05	Structures of Flexible Membrane Proteins are Best Solved Cold	Alexander Hahn
12.40	*Lunch*	
	Session Chair: Barbara M. Bakker	
14.00	Analysis of Allosteric Interactions in a Multi-enzyme Complex by Ancestral Sequence Reconstruction	Reinhard Sterner
14.35	Coenzyme A from Extracellular Sources and the Impact thereof in Health and Disease	Ody C.M. Sibon
15.10	*Tea Break and Conference Photo*	
15.40	Emerging Concepts in Pseudoenzyme Evolution and Cell Signalling	Patrick Eyers
16.15	STYX: a Pseudophosphatase that Regulates MAPK Signalling and SCF Ubiquitin Ligases via Spatial Anchoring	Hesso Farhan

16.50	Silencing Noise: Individualized Biochemistry and its Limitations	Hans V. Westerhoff
17.25	*Close*	
19.30	*Dinner*	

Wednesday, 11th September

	Session Chair: Polly M. Fordyce	
09.00	EMBL-EBI Bioinformatic Infrastructure Provision: Protein Function, Network Biology, Modelling and Beyond	Rolf Apweiler
09.35	Modelling the Minimal Cell: Integration of Experiments, Theory and Simulations	Zan Luthey-Schulten
10.10	Quantum Chemistry as a Tool in Biocatalysis	Fahmi Himo
10.45	*Coffee Break and Poster Session*	
11.15	Half-site Enzymes as Conduits for the Transfer of Chemical Potential	Thomas S. Leyh
11.50	The Role of Active Site Loops in Controlling Catalysis by the Aromatic Amino Acid Hydroxylases	Paul F. Fitzpatrick
12.25	Enzymology of Lignin Degradation	Frank M. Raushel
13.00	*Lunch*	
14.15	*Excursion*	
19.30	*Dinner*	

Thursday, 12th September

	Session Chair: Ody C.M. Sibon	
09.00	Are Peroxygenases the New P450s? Scope and Current Challenges of Peroxygenases for Selective Osyfunctionalisation Chemistry	Frank Hollmann
09.35	The NAD Metabolome – Enzymology and Subcellular Compartmentation	Mathias Ziegler
10.10	Identifying Evolutionary and Kinetic Drivers of NAD-dependent Signalling	Ines Heiland
10.45	*Coffee Break*	
11.15	From Enzymes to Products: Automating Synthetic Biology Routes to Chemical Targets	Neil Swainston
11.50	HT-MEK: a New Microfluidic Platform for Quantitative, High-throughput Enzymology	Polly M. Fordyce
12.25	*Lunch*	
	Session Chair: Paul F. Fitzpatrick	
13.40	The Conserved Myosin 1D Controls Multiscale Chirality in *Drosophila*	Stéphane Noselli
14.15	Computational Modelling of Cerebral Amino Acid and Neurotransmitter Metabolism in Phenylketonuria	Barbara M. Bakker
14.50	*Tea Break*	
15.20	Data Integrated Simulation of Enzymes	Jürgen Pleiss
15.55	The Uncertainty of the Michaelis Constant, K_M, in Experimental Reproducible Enzyme Kinetic Public Data	Santiago Schnell
16.30	STRENDA DB – Monitoring the Completeness of Experimental Enzyme Kinetics Data	Johann M. Rohwer
17.15	Closing Remarks	Carsten Kettner
19.30	*Dinner*	

List of Posters

The poster presentation includes a short (5 min) oral presentation on Tuesday, 10th September, and the poster sessions during the coffee breaks on Tuesday and Wednesday. The posters will be displayed throughout the entire symposium from Tuesday, 10th September, to Thursday, 12th September.

Tuesday, 10th September

#1	SABIO-RK: extraction of enzyme function data from STRENDA DB	Ulrike Wittig
#2	Structural basis of cooperativity in Aspergillus niger NADP-glutamate dehydrogenase	Nupur Agarwal
#3	Domain structure and function of α-1,3-Glucanase from *Streptomyces thermodiastaticus* HF3-3	Niphawan Panti
#4	Efficient synthesis of l-theanine in recombinant Escherichia coli containing Glutamyltranspeptidase gene from Pseudomonas nitroreducens (PnGGT)	Phumsombat Putthapong
#5	interferENZY: a web-based tool for enzymatic assay validation and standardized kinetic analysis	Maria Filipa Pinto

Abstracts

Tuesday

09.20

New Horizons in Enzymology from Cryo-EM and X-ray Free-electron Laser (XFEL)

Ming-Daw Tsai

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Institute of Biological Chemistry
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Structural determination of enzymes has been an integral part of mechanistic enzymology. However, the two common approaches, X-ray crystallography and nuclear magnetic resonance (NMR), can only be applied under specific conditions, whereas mechanistic analysis often covers a broad range of conditions. These limitations are being overcome by two emerging structural biology techniques: Cryo-EM can facilitate structural determination at multiple solution conditions with various intermediate complexes, while XFEL allows determination of multiple intermediate structures at ultrashort time intervals.

In this lecture, I will illustrate applications of high-resolution cryo-EM to the mechanism of ketol-acid reductoisomerase (KARI), and the emerging XFEL to the photoreduction mechanism of a DNA photolyase.

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09.55

Single Molecule Enzymology and Beyond

Xiaoliang Sunney Xie

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Department of Biomedical Pioneering Innovation Center
Beijing, China

Our real time observation of single enzyme turnovers reported in 1998 (Lu et. al, *Science*, 1998) was carried out under the nonequilibrium steady state condition (NSSC). We show under this condition, time traces of enzymatic cycles of a single enzyme molecule are not only stochastic, but also exhibit a resonance at the frequency of one over the enzymatic cycle time.

Interestingly, NSSC is also the condition of a living cell, in which DNA exists as a single molecule. Consequently, gene expression time traces exhibit oscillatory behaviors, which have been extensively reported in recent literature.

I will give an overview about the NSSC dynamics in the context of single-molecule enzymology and live cell gene expression.

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Tuesday

Poster #1

SABIO-RK: Extraction of Enzyme Function Data from STRENDA DB

Ulrike Wittig, Maja Rey, Andreas Weidemann, and Wolfgang Müller

Heidelberg Institute for Theoretical Studies (HITS) gGmbH
Heidelberg, Germany

SABIO-RK (http://sabiork.h-its.org) is a web-accessible, manually curated database for biochemical reactions and their kinetic properties with the focus on both supporting the computational modelling to create models of biochemical reaction networks and allowing experimentalists to gain further knowledge about enzymatic activities and reaction properties. SABIO-RK contains annotations to controlled vocabularies and ontologies and is highly interlinked with different other databases and integrated in the data workflow of several modelling and simulation tools. A flexible way of exporting database search results using web services or in a table-like format is provided. Both the export and the import of data are possible via standard data exchange formats.

Data in SABIO-RK are mainly manually extracted from literature but could also be uploaded directly from laboratories or other resources via SBML. Beside that SABIO-RK supports the STRENDA initiative which defines guidelines for reporting enzyme function data in publications to increase the reusability of data for databases and modelling tools as well as to improve the reproducibility of results for experimentalists. During the paper submission process STRENDA recommends authors to submit enzyme function data to STRENDA DB which automatically checks the manuscript data on compliance with the STRENDA guidelines and supports the reviewing process. After final publication SABIO-RK extracts the kinetic parameters including experimental conditions from STRENDA DB. This whole procedure allows a structured and standardized data storage during the publication process, improves the data extraction and curation process for other databases, and gives more responsibility for final data quality to the authors.

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Poster #2

Structural Basis of Cooperativity in *Aspergillus niger* NADP-glutamate dehydrogenase

Nupur Agarwal and Narayan S. Punekar

Indian Institute of Technology Bombay
Department of Biosciences and Bioengineering
Mumbai, India

Glutamate dehydrogenase (GDH) occurs at the crossroads of carbon and nitrogen metabolism and catalyzes the inter-conversion of 2-oxoglutarate and L-glutamate. NADP-GDH from *Aspergillus niger* (AnGDH) shows a sigmoidal pattern of saturation with 2-oxoglutarate (n_H = 2.9) and a biphasic ammonium saturation only when 2-oxoglutarate is sub-saturating. Despite sharing 88% identity the corresponding enzyme from *A. terreus* (AtGDH) displays distinct Michaelis-Menten kinetics. The structural origins of homotropic interactions in AnGDH were probed by creating six AnGDH-AtGDH protein chimeras. Both the 2-oxoglutarate sigmoidicity and ammonium biphasicity could be ascribed to the C-terminal region (named the D-segment; residues 315-460) of AnGDH (Agarwal *et al*, *Arch. Biochem. Biophys.*, 669:50-60, 2019). The D-segment lies at the monomer-monomer interface in the AnGDH trimer within the native hexamer. Among the fifteen residues different between AnGDH and AtGDH in the D-segment, residues D410, R413 and D414 define a network of possible salt bridges (PDB ID 5XVX). And S401 occurs in hydrogen bonding distance to E402 (PDB ID 5XVI).

Both these potential interactions may not exist in AtGDH structure. Site-directed mutations of these residues could define the nature of allosteric network in AnGDH. A close comparison of the AnGDH-AtGDH pair provides a unique opportunity to understand the structural basis of cooperativity in enzymes.

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Poster #3

Domain Structure and Function of α-1,3-Glucanase from *Streptomyces thermodiastaticus* HF3-3

Niphawan Panti[1], Vipavee Cherdvorapong[1], Wasana Suyotha[2], Kazuyoshi Takagi[3], Shigekazu Yano[4], Yosuke Toyotake[1] and Mamoru Wakayama[1]

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[2] Prince of Songkla University, Department of Industrial Biotechnology, Hat Yai, Thailand
[3] Ritsumeikan University, Department of Applied Chemistry, Shiga, Japan
[4] Yamagata University, Department of Biochemical Engineering, Yamagata, Japan

α-1,3-glucan (mutan) is a water-insoluble, linear -1,3-linked homopolymer of glucose, which is the main component of extracellular polysaccharide. It has been synthesized from sucrose by *Steptococcus mutans* via glycosyltransferases (GTFs) that causes dental plague in human. It has also been found in fungi as a component of cell walls, carbon sources and virulent factors of pathogenic fungi. Considering from this background, we are interested in studying α-1,3-glucanase (mutanase) that can hydrolyze α-1,3-glycosidic bonds of α-1,3-glucans. In the previous study the amino acid sequence of GH 87 was determined, a α-1,3-glucanase from *Streptomyces thermodiastaticus* HF3-3 (Agl-ST2) which was categorized as a new group of α-1,3-glucanase, multi-domains enzyme including N-terminal binding domain, carbohydrate binding module family 35 (CBM35), C-terminal catalytic domain and discoidin domain (DS), respectively. The comparison of Agl-ST2 with the related enzymes revealed high similarity to mycodextranase (85%), whereas it had low homology with α-1,3-glucanase. But the properties indicated that Agl-ST2 belongs to α-1,3-glucanase. Since Agl-ST1 is generated from Agl-ST2 by truncation of DS region, Agl-ST1 has the same multi-domain as Agl-ST2 but without DS. In this study to understand domain structure and function of Agl-ST(1&2), we determined each domain structure and function of the Agl-ST in the α-1,3-glucan binding and hydrolysis by constructing several domain deletions and site-directed mutation in catalytic domain.

The results of Agl-ST with site-directed mutation at the critical amino residues indicated low activities of hydrolysis α-1,3-glucan, while the enzymes lacking CBM35, the catalytic domain and DS shows lower binding activities than the wild type.

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Poster #4

Efficient Synthesis of L-theanine in Recombinant *Escherichia coli* containing Glutamyltranspeptidase gene from *Pseudomonas nitroreducens* (PnGGT)

Phumsombat Putthapong[1], Chiharu Sano[1], Takao Hibi[2], Takafumi Itoh[2], Junji Hayashi[1], Yosuke Toyotake[1], Mamoru Wakayama[1]

[1] Ritsumeikan University, Department of Biotechnology, Shiga, Japan
[2] Fukui Prefectural University, Department of Bioscience, Fukui, Japan

L-Theanine (γ-glutamylethylamide) is a non-protein amino acid present in tea. It contributes to the umami taste and the unique flavor of a green tea infusion. It was reported to have many beneficial physiological effects, especially anti-stress and improvement of concentration for learning ability. It can be obtained by chemical synthesis or extraction from green tea, but both processes involve time consuming, cost-ineffective and complicated operational process. Hence, biotechnological production of L-theanine becomes interesting by using microbial enzymes such as glutamyltranspeptidase (GGT). GGT (EC 2.3.2.2), a heterodimeric enzyme found in various sources from bacteria to mammal catalyzes the transfer of the γ-glutamyl moiety of γ-glutamyl compound to γ-glutamyl acceptors such as amino acids and peptides. Bacterial GGTs have been reported in *Escherichia coli* (EcGGT), *Bacillus licheniformis* (BlGGT), and *Pseudomonas nitroreducens* (PnGGT). Our previous study on site directed mutagenesis of PnGGT suggested that Trp525 was a key amino acid residue in determining the preference of acceptor substrate in the reaction. Moreover, *E. coli* producing W525D mutants of PnGGT (*E. coli* W525D mutant) showed low hydrolysis activity with high transfer activity, causing the increase of L-theanine production from ethylamine and L-glutamine. Improving the efficiency of L-theanine production using *E. coli* W525D mutant was also achieved by whole cell calcium-alginate immobilization with determination of optimal pH, temperature, and molar substrate ratio. Under the suitable condition, pH 11, 50 °C, and substrate molar ratio of 1:10, L-glutamine: ethylamine, *E. coli* W525D mutant exhibited the highest theanine production compared to *P. nitroreducens*, *E. coli* wild type, and other recombinant mutant strains.

Tuesday

Poster #5

InterferENZY: a Web-based Tool for Enzymatic Assay Validation and Standardized Kinetic Analysis

Maria Filipa Pinto[1,2,3,4], Antonio Baici[5], Pedro José Barbosa Pereira[3,4], Sandra Macedo-Ribeiro[3,4], Annalisa Pastore[6], Fernando Rocha[2] and Pedro M. Martins[1,3,4]

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Enzymatic assays are widely employed to characterize enzyme function and activity. Even slight effects, e.g., resulting from the presence of activity modulating compounds or the occurrence of conformational changes can be identified from the analysis of the kinetic data measured in such assays. However, experimental interferences like instrumental drift, enzyme inactivation and unsuspected enzyme inhibition may dramatically affect the accuracy and reproducibility of the kinetic results. The webserver interferENZY is presented here as a new tool for automatic detection of hidden assay interferences, and unbiased estimation of apparent kinetic parameters using a standardized protocol for validated assays. The implementation of interferENZY is based on the recently proposed linearization method, in which the reaction coordinates are represented in a modified linear scale [1]. Users interested in validating their continuous/end-point assays will be able to run the interferENZY algorithm from a publicly available platform. The input data is given in molar units of product concentration build-up (or, alternatively, substrate concentration depletion) over time for at least 5 different values of initial substrate concentration.

The output reports and graphs warrant a tight quality control of enzymatic assays and reproducible data analysis. As such, this methodology should contribute to the advance of both fundamental and applied research in enzymology.

References

[1] Pinto, M.F., et al., *A simple linearization method unveils hidden enzymatic assay interferences.* Biophysical Chemistry, 2019. **252**: p. 106193.

Acknowledgements

PhD fellowship; SFRH/BD/109324/2015 (MFP), FCT – Fundação para a Ciência e a Tecnologia. Funding; Doctoral Program in Biomedical Sciences (ICBAS-UP).

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11.30

Common Mechanisms by Skeletal Muscle Actomyosin and Bacterial Flagellar Motor Revealed by Electron Cryomicroscopy and Optical Nanophotometry

Keiichi Namba

RIKEN SPring-8 Center and Center for Biosystems Dynamics Research
Graduate School of Frontier Biosciences
Osaka University, Japan

Visualization of the structures and dynamics of macromolecular assemblies and cellular architectures is essential for understanding mechanisms of biological functions because they are all determined by their structures and dynamics at atomic and molecular levels. Electron cryomicroscopy (cryoEM) and image analysis has become a powerful tool due to recent technical developments in microscope optics and cryostage control, image detector and the method of sample preparation. In addition, single particle cryoEM image analysis now allows structural analysis to reach near-atomic resolution within a few weeks or months. We looked at the structures of actin filament and its rigid complex with myosin head of skeletal muscle and obtained insights into how the strong binding of myosin to actin filament causes rapid ADP and P_i release from myosin to drive the sliding movement at a much faster speed than the cytoplasmic actomyosin by more than one order of magnitude. Furthermore, we observed how the myosin head dissociates from actin filament upon ATP binding, and how the fast and long-distance sliding of muscle actomyosin (with > 60 nm per ATP molecules hydrolysed) is achieved possibly by their biased Brownian motion. We also developed a nanophotometry method to measure rapid step motions and dwell times of the bacterial flagellar motor that rotates as fast as 300 Hz at sub-nm and which we monitored near µs resolution to observe repeated association and dissociation of the rotor and stator proteins coupled with proton binding within the proton channel of the stator complex and its release to the cytoplasm. The data suggest that the step motion of the flagellar motor is also driven by thermal energy of a biased Brownian motion in a similar manner to skeletal muscle actomyosin. I will discuss common features and mechanisms of force generation by these two, apparently distinct macromolecular motor complexes.

Tuesday

12.05

Structures of Flexible Membrane Proteins are Best Solved Cold

Alexander Hahn

Max Planck Institute of Biophysics
Structural Biology
Frankfurt am Main, Germany

Single particle cryoEM is a powerful method for structure determination of protein complexes in near-native environments without the need to grow protein crystals. The development of direct electron detectors, sophisticated alignment algorithms and increasingly powerful computing infrastructures enabled us to obtain high resolution structures of small membrane protein and to visualize amino acid side chains, metal cofactors and coordinated water molecules. Two recent examples of such complexes are the (i) ATP synthase from chloroplasts and the (ii) cytochrome *bd* oxidase from *E. coli*.

The ATP synthase uses the electrochemical proton gradient across the thylakoid membrane to drive the synthesis of ATP by rotary catalysis. The central stalk is captured in different rotational positions which show the dynamic ATP synthase at work.

The cytochrome *bd*-type quinol oxidases catalyze the reduction of molecular oxygen to water in the respiratory chain of many human-pathogenic bacteria. They are structurally unrelated to the mitochondrial cytochrome *c* oxidases and therefore a prime target for the development of antimicrobial drugs. Our structure shows a unique heme cofactor organization, a ubiquinone-8 cofactor, distinct water-filled proton channels and oxygen conducting pathways.

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Tuesday

14.00

Analysis of Allosteric Interactions in a Multi-enzyme Complex by Ancestral Sequence Reconstruction

Michael Schupfner, Kristina Straub, Rainer Merkl and Reinhard Sterner

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Tryptophan synthase (TS) is a heterotetrameric αββα complex. It is characterized by the channeling of the reaction intermediate indole and the mutual activation of the α-subunit TrpA and the β-subunit TrpB via a complex allosteric network. We have analyzed this allosteric network by means of ancestral sequence reconstruction (ASR), which is an *in silico* method to resurrect extinct ancestors of modern proteins [1]. In a first step, the sequences of TrpA and TrpB from the last bacterial common ancestor (LBCA) were computed by means of ASR. The corresponding LBCA TrpA and TrpB proteins were then produced in *Escherichia coli*, purified, and characterized. The results showed that LBCA TS is reminiscent of modern TS by forming a αββα complex with indole channeling taking place [2].

However, LBCA TrpA decreases the activity of LBCA TrpB by a factor of 5 whereas, for example, the modern ncTrpA from *Neptuniibacter caesarensis* increases the activity of ncTrpB by a factor of 1000. In order to identify those amino acid residues that are responsible for this large difference, all six evolutionary TrpA and TrpB intermediates that stepwise link LBCA TS with *N. caesariensis* TS were produced and characterized. Remarkably, the switching from TrpB-inhibition to TrpB-activation by TrpA occurred between two successive TS intermediates.

The comparison of these intermediates and the mutual exchange of residues by iterative rounds of site-directed mutagenesis allowed us to identify four (out of 420) residues from TrpB that are necessary and sufficient for its allosteric activation by TrpA. Our results demonstrate that ancestral sequence reconstruction can efficiently identify residues essential for allosteric communication and contribute to our understanding of large multi-enzyme complexes [3].

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Coenzyme A from Extracellular Sources and the Impact Thereof in Health and Disease

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Coenzyme A is a cofactor required for over 100 metabolic reactions. The general consensus is that cells obtain coenzyme A via a *de novo* biosynthesis pathway starting with the uptake of vitamin B5. Recently the work of the Sibon laboratory has demonstrated that alternative routes exist for cells and organisms to replenish coenzyme A levels under circumstances of an impaired coenzyme A *de novo* biosynthesis route. These alternative routes are starting points for treatments of a group of cardiac and neurodegenerative inherited diseases cause by mutations in genes coding for enzymes required for the *de novo* coenzyme A biosynthesis pathway. Evidence, obtained in cell-, *Drosophila melanogaster*- and mouse- models for coenzyme A-linked diseases will be discussed. This work demonstrates the presence of alternative routes for cells and organisms to obtain sufficient levels of coenzyme A via the use of membrane passing capacities of coenzyme A precursor molecules.

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Emerging Concepts in Pseudoenyzme Evolution and Cell Signaling

Patrick Eyers

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Pseudoenzymes are proteins shown by sequence homology to belong to enzyme families but which are proven, or predicted, to lack enzyme activity due to mutations in otherwise-conserved catalytic amino acids. The best-studied pseudoenzymes are pseudokinases, although examples from other families are emerging at a rapid rate as experimental approaches "catch-up" with an avalanche of informatics data. Our recent kingdom-wide analysis reveals that many enzyme families contain ‘inactive’ pseudoenzyme members, suggesting that they regulate fundamental cellular processes in prokaryotes, archaea and eukaryotes. Some 5-10% of genes in enzyme families appear to encode these 'inactive' pseudoenzymes, with significant expansions and contractions likely to be associated with specific signaling niches.

Pseudoenzymes can allosterically activate canonical enzymes, act as scaffolds to control assembly of signalling complexes and their localization, serve as molecular switches or regulate signalling networks via substrate or enzyme sequestration. Such molecular-level analysis of pseudoenzymes is rapidly advancing knowledge of how they perform their own non-catalytic functions, and permitting the discovery of surprising, and previously unappreciated, functions of their intensively-studied active enzyme counterparts.

In this talk, I will highlight a selection of new and recent findings relevant to our newly discovered appreciation of pseudoenzyme-based biology.

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STYX: a Pseudophosphatase that Regulates MAPK Signalling and SCF Ubiquitin Ligases via Spatial Anchoring

Hesso Farhan

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Department of Molecular Medicine
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The pseudophosphatase STYX is a catalytically dead protein tyrosine phosphatase, which we use as a model to dissect possible modes of action of pseudoenzymes. In my talk, I will discuss general biologic roles of pseudoenzymes. I will show how we used mathematical modelling and experimentation to identify STYX as a spatial anchor for the mitogen-activated protein kinases ERK1/2, affecting their nucleo-cytoplasmic shuttling. I will also show how STYX serves as an example that a pseudophosphatase has evolved to regulate proteins beyond kinases.

Using a proteomics approach, we identified STYX as a regulator of F-box proteins and thereby is capable of modulating the activity of a variety of SCF-family of ubiquitin ligases. Finally, I will discuss the implications of this regulation for the tumorigenesis of breast cancer.

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16.50

Silencing Noise: Individualized Biochemistry and its Limitations

Hans V. Westerhoff[1,2], Thierry (D.A.G.) Mondeel[1], Stefania Astrologo[3]

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As biochemistry is a natural science, it has to accept that its objects are subject to noise. Position of enzymes in the cell, their catalytic activities, as well as the concentrations of their metabolites must be subject to fluctuations. Experiments could be irreproducible for this very reason. For simple systems, fluctuations are limited in magnitude however and thereby irrelevant whenever molecule numbers exceed 100. And experiments in systems with such numbers were thereby expected to be reproducible.

Yet, experiments in the life sciences are largely irreproducible, to the extent that the trustworthiness of science has been questioned. Some of this irreproducibility is due to limited accuracy of reporting of the experimental or computational procedures. This may seem to be a triviality, but we shall argue that it is not: Due to the very complexity of living cells, it is virtually impossible to be sufficiently accurate in much of the life sciences. *In vivo* biochemistry is thereby irreproducible, as is its application: medicine.

In addition there may be coupled uncertainties in *in vivo* biochemistry: It is hard to determine precisely the molecule number of a metabolite and to be precise about the cell type under investigation, at the same time. Likewise one should expect it to be difficult to know the effectiveness of a medicinal drug if one is certain about the particular person it is applied to.

We shall discuss the Einsteinian way out of this predicament, which has to do with the precise genomics-based definition of individuals or cells, i.e. with individualized biochemistry. Such individualized biochemistry is becoming possible, now through single-cell deep sequencing, and soon through single cell proteomics and metabolomics.

Like in particle physics, there seems to be a limitation to reducing the uncertainties, however. Biology may be home to noise that is much stronger than its thermal equivalent: The hierarchical organization of cell biochemistry is capable of leading to transcription, translation, signaling and metabolic bursting. This may be responsible for the substantial differences between individual cells that are genetically identical. A consequence is that single cell experiments become highly irreproducible. An implication may be that much of the functional importance of intracellular biochemistry might stem from the noise rather than the (mean) signal. This may well be so for microbial biotechnology, developmental biology, oncogenesis and drug resistance.

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09.00

EMBL-EBI Bioinformatics Infrastructure Provision: Protein Function, Network Biology, Modelling, and Beyond

Rolf Apweiler

EMBL Outstation – European Bioinformatics Institute
Hinxton, United Kingdom

At EMBL-EBI we maintain the world's most comprehensive range of freely available molecular data resources. Developed in collaboration with our colleagues worldwide, our databases and tools help scientists share data efficiently, perform complex queries and analyse the results in different ways. Our work supports millions of researchers, who are wet-lab and computational biologists working in all areas of the life sciences, from biomedicine to biodiversity and agri-food research.

In my talk I will give an overview of the EMBL-EBI bioinformatics infrastructure provision and will emphasise current infrastructure and research developments of special importance for biochemists, enzymologists and systems biologists.

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Modelling the Minimal Cell: Integration of Experiments, Theory, and Simulations

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JCVI-syn3A, a robust minimal cell with a 543 kbp genome and 493 genes, provides a versatile platform to study the principles of life (Breuer et al. *eLife* 2019). Using the vast amount of experimental information available on its precursor, *Mycoplasma mycoides capri*, we assembled a near-complete essential metabolic network with 98% of enzymatic reactions supported by annotation or experiment. This coherent model of the minimal metabolism along with maps of all its protein-coding genes and proteomics data point toward specific open questions regarding the minimal genome, which still contains many genes of generic or completely unclear function.

The identification of 30 essential genes with unclear function will motivate the search for new biological mechanisms beyond metabolism. Finally, stochastic dynamics of the combined genetic information processes of DNA replication, transcription, translation and ribosome biogenesis clarifies quantitatively demands on the metabolic network over the two-hour cell cycle.

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Quantum Chemistry as a Tool in Biocatalysis

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Using density functional theory methods, it is today possible to treat quite large systems quite accurately, a development that has made it possible to model enzyme active sites in a more realistic way than ever. Indeed, many mechanistic problems have been addressed and solved for a wide spectrum of enzymatic systems using the quantum chemical approach.

In the last few years we have applied this methodology to study enzymes of biocatalytic interest, in particular enzymes utilized in asymmetric biocatalysis. To be able to reproduce various kinds of selectivities, and in the long run also to predict them, high accuracy in relative transition state energies is required.

This talk will give a brief account of the quantum chemical cluster approach used to study enzyme active sites and reaction mechanisms. Advancements of the methodology will be discussed and recent examples from the field of asymmetric biocatalysis will be presented.

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Half-site Enzymes as Conduits for the Transfer of Chemical Potential

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Nature imbues certain proteins with the ability to couple the energetics of cellular processes through conformational change – for example, the ATP-hydrolysis dependent supercoiling of DNA, the coupling of chemical potential to osmotic gradients, the trafficking of metabolic "cargo" along cytoskeletal microtubules. Consistent with the quintessential mechanistic hallmark of conformational coupling, half-site reactive enzymes use allostery to establish an obligate interdependence of the reactions they catalyze. Given this perspective, we hypothesized that half-site enzymes couple the chemical potentials of the reactions they catalyze. Three well established half-site coupling systems were used to test this hypothesis – the human cytosolic sulfotransferases 1E1, human glutathione S-transferases 4A-4 (GST4a1), and equine alcohol dehydrogenase E. Each of these systems is dimeric, promiscuous and a member of an expansive, highly conserved family of isoforms with remarkably broad metabolic penetrance. Our studies reveal that these systems do indeed transfer chemical potential between the reactions they catalyze, and that, in accordance with the *Haldane Relationship*, the catalytic efficiencies of the reactions change upon coupling. Thus, half-site enzymes distribute chemical potential among the metabolites on which they operate such that unfavorable reactions are accelerated and driven further than their intrinsic chemical potentials allow at the expense of more favorable reactions. These findings significantly expand the known repertoire of cellular energy-coupling systems and offer a metabolic and evolutionary *raison d'être* for half-site mechanisms.

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The Role of Active Site Loops in Controlling Catalysis by the Aromatic Acid Hydroxylases

Paul F. Fitzpatrick

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Crystals structures of the aromatic amino acid hydroxylases phenylalanine hydroxylase, tyrosine hydroxylase, and tryptophan hydroxylases have shown that two surface loops close over the active site when both the amino acid and tetrahydropterin substrates have bound.

The contributions of these loops to substrate specificity and formation of the Fe(IV)O intermediate will be discussed.

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Enzymology of Lignin Degradation

Frank M. Raushel

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College Station, TX, United States of America

Sphingobium sp. SYK-6 is a Gram-negative soil bacterium that contributes to the overall degradation of lignin. Lignin provides structural support and protection to plants as a complex aromatic heteropolymer. The lignin degradation pathway of guaiacyl moieties leads to the intermediate, protocatechuate (PCA), which is further degraded via the 4,5-cleavage pathway where PCA is ultimately metabolized to pyruvate and oxaloacetate.

LigI catalyzes the reversible hydrolysis of 2-pyrone-4,6-dicarboxylate (PDC) to 4-oxalomesaconate (OMA) and is a member of the amidohydrolase superfamily. The pH dependence on the kinetic constants suggests that a single active site residue must be deprotonated for the hydrolysis of PDC. The crystal structures of wild-type LigI and the D248A mutant in the presence of the reaction product were determined to a resolution of 1.9 Å. The carbonyl group of the lactone substrate is activated by electrostatic interactions with H180, H31, and H33. The hydrolytic water molecule is activated by a proton transfer to Asp248. LigU was shown to catalyze the isomerization of (4*E*)-OMA to (3*Z*)-2-keto-4-carboxy-3-hexenedioate (KCH), where the double bond migrates from C4/C5 to C3/C4 via a 1,3-allylic isomerization. The three-dimensional structure of wild-type LigU was determined by X-ray crystallography and the structure of the K66M mutant enzyme was determined in the presence of the substrate OMA. NMR spectroscopy was used to demonstrate that LigU catalyzes the exchange of the pro*S* hydrogen at C5 of KCH with solvent during the isomerization reaction.

Mutation of K66 eliminated the isotope exchange at C5 and mutation of C100 abolished the exchange at C3. The positioning of these two residues in the active site of LigU is consistent with a reaction mechanism that is initiated by the abstraction of the pro*S* hydrogen at C3 of OMA by the thiolate anion of C100 and the donation of a proton at C5 of the proposed enolate anion intermediate by the side chain of K66 to form the product KCH.

The 1,3-proton transfer is suprafacial. LigJ catalyzes the reversible hydration of KCH to 4-carboxy-4-hydroxy-2-oxoadipate (CHA). The 3D structure of wild-type LigJ was determined in the presence and absence of the product CHA. The protein folds as a distorted $(\beta/\alpha)_8$-barrel and a single zinc is bound in the active site at the *C*-terminal end of the central β-barrel. CHA is ligated to the zinc via the displacement of a single water from the metal center in LigJ. The product-bound structure reveals that the enzyme catalyzes the hydration of KCH with formation of a chiral center at C4 with *S*-stereochemistry.

Based on the structure of LigJ in the presence of KCH and CHA, it is proposed that the side chain carboxylate of E284 functions as a general base in the abstraction of a water molecule for nucleophilic attack at C4 of the substrate. The reaction is facilitated by the delocalization of the negative charge to the metal center via the carbonyl group at C2 of the substrate. The overall reaction occurs by the *syn*-addition of water to the double bond between C4 and C3 of the substrate KCH.

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Are Peroxygenases the new P450s?

Scope and Current Challenges of Peroxygenases for Selective Oxyfunctionalization Chemistry

Frank Hollmann

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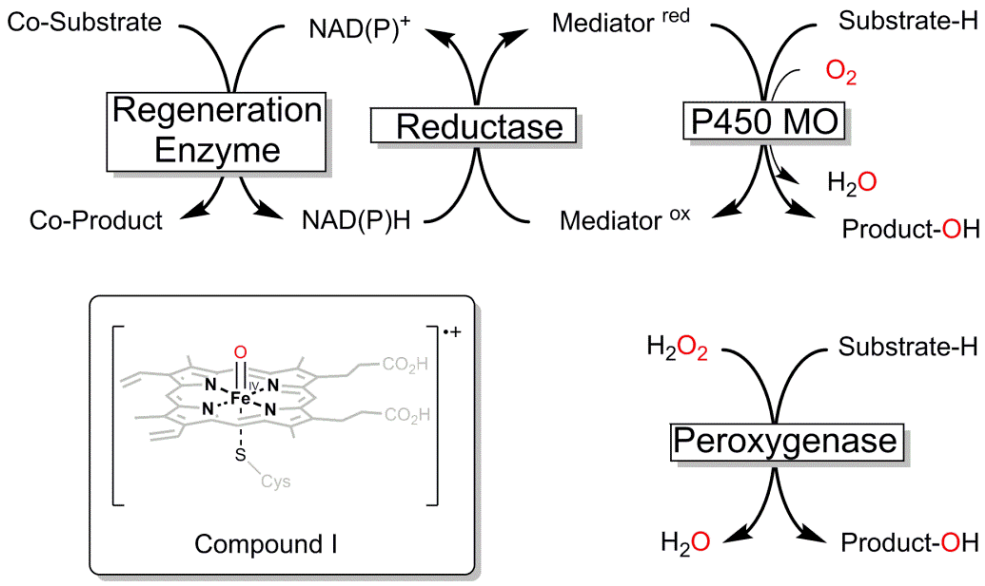
Delft, The Netherlands

Peroxygenases are experiencing a renewed interest as catalysts for selective oxyfunctionalisation chemistry. Peroxygenases are promising alternatives to the well-known P450 monooxygenases due to the significantly simpler regeneration scheme.



Comparison of P450-monooxygenases and peroxygenases with respect to the regeneration of the catalytically Compound I.

New peroxygenases enable selective hydroxylation of non-activated C-H bonds in alkanes and aromatics, epoxidation and heteroatom oxygenation.

Yet, to assess the full scope of this exciting enzyme class a range of challenges need to be met: (1) more enzyme (variants) with tailored properties need to be identified/evolved; (2) new *in situ* H_2O_2-generation systems to minimise oxidative enzyme inactivation need to be established; (3) peroxygenase-reactions in non-aqueous media need to be established.

These issues together with some promising solutions will be discussed.

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The NAD Metabolome – Enzymology and Subcellular Compartmentation

Mathias Ziegler

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NAD is a vital molecule in all organisms. It plays a major role in all cells as substrate for signal transduction and as cofactor in metabolic redox reactions, processes that undergo critical changes in aging and a variety of diseases. NAD^+-dependent signalling pathways include poly- and mono-ADP-ribosylation, protein deacetylation by sirtuins and generation of messengers involved in Ca^{2+} signalling. They regulate fundamental events such as transcription, DNA repair, cell cycle progression and apoptosis and also contribute to the control of metabolism.

Since these signalling reactions include degradation of NAD, perturbations of NAD supply can have severe consequences. The commonly known precursors of NAD biosynthesis are nicotinic acid and nicotinamide (known as vitamin B3). However, their riboside derivatives now appear to be of similar importance. To understand the pathways and molecular mechanisms of NAD biosynthesis, we have developed tools to detect and modulate subcellular NAD pools and to determine turnover of cellular NAD. Using these tools, we have established the pathways of NAD biosynthesis in human cells. Our results indicate that lowering of NAD in individual subcellular compartments is largely compensated suggesting a dynamic interaction of seemingly independent pools. Nevertheless, there are compartment-specific differences in the extent and mechanisms of counteracting NAD losses. Since in aging NAD levels are decreased, therapeutic approaches should be aimed at re-establishing the appropriate balance between NAD consumption and synthesis.

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Identifying Evolutionary and Kinetic Drivers of NAD-dependent Signalling

Mathias Bockwoldt[1], Dorothée Houry[2], Marc Niere[3], Toni I. Gossmann[4], Mathias Ziegler[3], and Ines Heiland[1]

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NAD provides an important link between metabolism and signal transduction and has emerged as central communication hub between bioenergetics and all major cellular events. How the balance between NAD supply and NAD-consuming signalling, e.g. by sirtuins and PARPs, is maintained and regulated has remained unknown. Using phylogenetic analyses, mathematical modelling and experimental verification, we show that diversification of NAD-dependent signalling in deuterostomes required the transition of NAD biosynthesis to exclusive usage of nicotinamide phosphoribosyltransferase (NamPT). Moreover, occurrence of nicotinamide N-methyltransferase (NNMT) was essential to enable higher NAD turnover. It diverts nicotinamide (Nam) from recycling into NAD and thereby prevents feedback inhibition by accumulating Nam, which is produced in the signalling reactions. Concomitantly, NamPT underwent structural adaptations to attain an unusually high affinity towards Nam to match signalling with respective NAD biosynthesis rates. Our results have implications for therapeutic strategies of NAD supplementation and the use of NNMT or NamPT inhibitors in cancer treatment.

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From Enzymes to Products: Automating Synthetic Biology Routes to Chemical Targets

Neil Swainston[1], Pablo Carbonell[2], Andrew Currin[2], Marc Dunstan[2], Katherine Hollywood[2], Adrian Jervis[2], Christopher Robinson[2], Cunyu Yan[2], Paul Mulherin[2], Sandra Taylor[2], Ros Le Feuvre[2], Rainer Breitling[2], Douglas Kell[1], Eriko Takano[2], Nick Turner[2], Nigel Scrutton[2]

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The microbial production of chemical targets provides a promising biosustainable manufacturing solution that has led to the successful production of a growing catalogue of natural products and high-value chemicals. Such approaches are dependent upon the selection and assembly of both naturally-occurring and engineered enzymes into either existing or entirely novel metabolic pathways.

Synthetic biology methods are becoming increasingly prevalent in this field, and are typified by the implementation of an iterative Design-Build-Test-Learn (DBLT) cycle. Such DBLT cycles involve the design of synthetic DNA encoding the selected collection of enzymes into one or more plasmid variants. These plasmids are then expressed in selected hosts and tested for a desired phenotype, in this case, target chemical production.

This approach is applicable to the production of an enormous range of chemical targets, and this talk will focus on the development of a generic pipeline for microbial chemical production that is agnostic to the target chemical class [1]. The pipeline covers the whole DBLT cycle, and includes novel software for pathway design [2], enzyme selection [3], DNA design [4], automated DNA assembly [5], and next-generation sequencing [6], along with mass spectrometry approaches for target quantification.

There will also be a consideration of machine learning approaches to Learn from these accumulated datasets, allowing for the generation of hypotheses to be tested in subsequent iterations [7].

Taken together, this work allows for the rapid development and optimisation of synthetic enzymatic pathways, which when used in tandem with related approaches such as enzyme directed evolution [8] and host engineering, lead towards the development of efficient and sustainable routes to the production of high-value chemicals.

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HT-MEK: a New Microfluidic Platform for Quantitative, High-throughput Enzymology

Polly M. Fordyce

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How enzymes achieve their enormous rate enhancements and exquisite specificities remains elusive, as evidenced by our limited ability to engineer new enzymes. A fundamental reason for this lack of understanding is the large number of interconnected residues and long-range interactions that cannot be revealed via traditional, low-throughput quantitative assays. While high-throughput screens and selections can identify enzymes with desired changes, the improvements obtained are typically modest and the qualitative or limited quantitative data obtainable by these approaches is insufficient to reveal functional interconnections between remote and active site residues.

We have developed a new technology, HT-MEK (High-throughput Microfluidic Enzyme Kinetics), that allows measurement of multiple quantitative biochemical parameters for 1500 rationally chosen enzyme variants in a single experiment. Using this platform, we carried out deep functional analysis of multiple substitutions at each position within a single enzyme, the Alkaline Phosphatase superfamily member PafA. For each substitution, we measured multiple biochemical parameters, including Michaelis-Menten constants for cognate and non-cognate substrates and inhibition constants for multiple inhibitors and transition state analogs. We observe distinct physically contiguous 'functional units' throughout the enzyme that tune specific aspects of function, yielding blueprints for future rational design efforts.

HT-MEK is applicable to enzyme function and stability, provides data that rivals or exceeds the precision typically obtained from standard assays, and reduces the time, effort, and cost to quantitatively profile the effects of individual substitutions on enzyme function by orders of magnitude, providing a technology to access previously inaccessible questions across multiple systems and disciplines.

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The Conserved Myosin 1D Controls Multiscale Chirality in *Drosophila*

Stéphane Noselli

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Breaking Left-Right (LR) symmetry in Bilateria embryos is a major event in body plan organization. LR asymmetry plays a fundamental role in the morphogenesis and function of brain and visceral organs. In human, aberrant LR asymmetry results in severe anatomical defects leading to embryonic lethality, spontaneous abortion and congenital disorders.

Our laboratory pioneered the study of LR asymmetry in *Drosophila*, providing a simple and genetically amenable model to study the molecular basis of laterality. We identified the conserved *myosin1D* (*myo1D*) gene as a major Dextral determinant and showed recently that *myo1D* has a conserved role in both Xenopus and zebrafish. These results indicate that vertebrates and invertebrates share common molecular mechanisms involving actin and associated factors to control LR polarity. Interestingly, genetic screening allowed the identification of specific actin regulators acting in concert with *myo1D* to control laterality. Our recent work further showed that *myo1D* is sufficient to trigger *de novo* LR asymmetry, being able to induce directional twisting at all biological scales (from F-actin turning *in vitro*, to cell, organ, whole-body as well as behavioral chirality).

Myo1D thus represents a unique, conserved chiral factor whose determinant activity depends on the motor domain of the protein and a chiral interaction with F-actin.

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Computational Modelling of Cerebral Amino Acid and Neurotransmitter Metabolism in Phenylketonuria

Agnieszka B. Wegrzyn, Danique van Vliet, Francjan J. van Spronsen, and Barbara M. Bakker

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Phenylketonuria (PKU) is caused by a deficiency of the hepatic phenylalanine hydroxylase enzyme, which normally converts phenylalanine into tyrosine. Despite a phenylalanine-deprived diet, many adult PKU patients display disorders of executive functions. These are hypothesized to be caused by an imbalance in cerebral monoaminergic neurotransmitters. The relationship between plasma amino acids and brain biochemistry in PKU as well as in other diseases remains, however, largely speculative.

To better understand the pathophysiology of PKU in particular, and the relationship between plasma and brain amino acids and monoaminergic neurotransmitter biochemistry in general, we constructed a computational model. The model comprises the transport of large neutral amino acids (LNAA) across the blood-brain barrier as well as cerebral amino acid and monoamine metabolism. It accurately describes the uptake of amino acids into the brain, as validated by direct measurements of brain amino acid concentrations in PKU mice on various diets. Furthermore, model simulations indicate that the observed decrease in neurotransmitter levels are caused predominantly by the inhibition of tyrosine and tryptophan hydroxylases by phenylalanine, and cannot be rescued by addition of tyrosine and tryptophan alone. In agreement with the experimental data, diets that successfully reduce the brain phenylalanine levels, such as LNAA with or without threonine, lead to an increase in the brain neurotransmitter levels displaying a potential to alleviate PKU symptoms.

In conclusion, we present for the first time a complete model of the LNAA transport through the blood-brain barrier and subsequent brain neurotransmitter metabolism. The model leads to a better understanding of the pathophysiological mechanisms and the impact of individual amino acids in the diet on the underlying brain dysfunction in PKU. Furthermore, it can be readily applied for studies of other neurological disorders, in which the relation between diet, brain amino acids, and neurotransmitters is important.

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Data-integrated Simulation of Enzymes

Jürgen Pleiss

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Institute of Biochemistry and Technical Biochemistry
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Enzymes are promising catalysts for a wide range of biocatalytic processes. Because protein engineers still lack a deep understanding of biocatalytic systems, finding the optimal biocatalyst is a tedious process, and trial-and-error strategies are widely used. Combining data mining and molecular modeling is a promising strategy to improve the efficiency of enzyme development processes, because genomics and metagenomics projects provide us with a rapidly increasing stream of novel enzyme candidates [1], and increasingly available computing resources allow us to simulate realistic enzyme-substrate-solvent systems [2].

Therefore, we merge two modelling approaches: the mining of sequence data by systematically analyzing enzyme family databases [3] and the mechanistic modelling of activity and selectivity by combining molecular simulations of enzyme-substrate interactions [4] with thermodynamic modeling of substrate-solvent mixtures [5] and kinetic modeling of biocatalytic reactions [6]. This approach bridges between microscopic and macroscopic scales and seeks to derive kinetic parameters from molecular models.

As a merger of data mining and mechanistic modelling, data-based simulation of enzymes is applied to identify novel enzyme candidates in sequence databases, to guide protein engineering, and to optimize reaction conditions.

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The Uncertainty of the Michaelis Constant, K_M, in Experimental Reproducible Enzyme Kinetics Public Data

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The maximum accuracy of a physical-chemistry measurand depends on the quality of the measurement protocol and experimental data. In enzyme kinetics, the Michaelis constant, K_M, is a critical measurand used to characterize the catalytic efficiency of a substrate for an enzyme. In measurement science, reproducibility occurs when a measurand, like K_M, can be obtained with stated precision using "the same experimental conditions" by a different team, a different measuring system, and in a different location on multiple trials. However, there is no general agreement about the stated precision of K_M in the enzyme kinetics literature. Estimating the experimental uncertainty of K_M will define an upper limit in the predictive performance possible for enzyme kinetic assays.

In this work, our goal is to make a first estimate of the experimental uncertainty of K_M in the enzyme kinetic literature. To achieve this, we performed an analysis of K_M values for 142 enzymes measured under "same assays conditions" from experiments recorded and curated in the SABIO-RK database. The "same essays conditions" are defined as enzyme kinetic experiments carried out with an enzyme and substrate pair for the same organism under identical pH, temperature and buffer.

Our analysis reveals that 88% of the enzymes have positively skewed mean-to-median ratio for their K_M values reported in the literature. A statistical test for normality shows that only 25% of the enzymes studied under the same assay conditions have normally distributed K_M values, and 53% of the enzymes have K_M values with coefficient of variations much greater than one - i.e. the standard deviation is much greater than the mean. As a matter of fact, 88% of the K_M values deviate more than 5% of their mean, and removal of outliers had no impact on K_M estimates.

The range of reported K_M is high as 70% of the K_M values vary by at least by one order of magnitude, with a maximal reported variation of six orders of magnitude (10^6). This variation is independent from the number of reproducible experiments.

Our analysis suggests that experimental uncertainty of independent measurements is large and could be the result of numerous factors. Incomplete collection or reporting of experimental assay conditions or measurement protocols may conceal necessary information for experimental reproducibility. Additionally, systematic errors in the enzyme kinetic assays may directly affect the distribution of K_M values, or in some cases experimentalist measurements might be approaching the natural upper limits to the predictive performance of these assays. A major reproducibility study will help us to evaluate the capabilities of independent groups to measure the K_M and determine the causes of variability presently observed in the literature.

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16.30

STRENDA DB – Monitoring the Completeness of Experimental Enzyme Kinetics Data

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Technical and methodological advances in almost all natural scientific disciplines led to enormous amounts of experimental and theoretical data available in the written literature and databases. An essential requirement for scientific progress is unrestricted access to research results in a form that is directly usable by researchers. However, there are many deficiencies in the way that data are currently reported, resulting often in incomplete, irreproducible and even unusable data sets that are not suitable for subsequent research and knowledge generation. In the daily work, this causes difficulties when data move between researchers whose data are supplied by laboratories that use different methods, and can, in the worst cases, lead to misinterpretation of laboratory findings. In addition, the quality of data in databases is highly dependent on the reliability and depth of literature reports, which in turn only provides high information quality if the experimental context in which the data was generated is comprehensively reported and if metadata, i.e. unambiguous identifiers (e.g. for proteins, genes, assays, chemical compounds etc.) are determined. Reality, however, shows that many of these descriptors often are inadvertently or deliberately incomplete which may become a severe obstacle for the exchange of both quantitative data and models as nobody understands the meaning of this data beside their initial generators.

Thus, more than a decade ago, the STRENDA Commission (www.beilstein-strenda.org), has established standards for data reporting in enzymology research. The aim of these STRENDA Guidelines is to improve the quality of data published in the scientific literature and to enable researchers to compare, evaluate, interpret and reproduce experimental research results published in the literature and databases [1].

STRENDA DB [2] is a storage and search platform supported by the Beilstein-Institut that incorporates the STRENDA Guidelines in a user-friendly, web-based system. If you are an author who is preparing a manuscript containing functional enzymology data, STRENDA DB provides you the means to ensure that your data sets are complete and valid before you submit them as part of a publication to a journal. Data entered in the STRENDA DB submission form are automatically checked for compliance with the STRENDA Guidelines; users receive warnings informing them when necessary information is missing.

Here, STRENDA and STRENDA DB will be presented followed by a discussion with the audience about the pitfalls, challenges and requirements for the complete reporting of enzymology data.

References

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[2] Swainston, N., Baici, A., Bakker, B.M., Cornish-Bowden, A., Fitzpatrick, P.F., Halling, P., Leyh, T.S., O'Donovan, C., Raushel, F.M., Reschel, U., Rohwer, J.M., Schnell, S., Schomburg, D., Tipton, K.F., Tsai, M.-D., Westerhoff, H.V., Wittig, U., Wohlgemuth, R. and Kettner, C. (2018) STRENDA DB: enabling the validation and sharing of enzyme kinetics data. *The FEBS J.* **285**(12)**:**2193-2204. DOI:10.1111/febs.14427.

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