BEILSTEIN SYMPOSIUM

From Molecular Mechanisms to High-performance Systems

Beilstein Enzymology Symposium 2023

September 12–14, 2023
Hotel Jagdschloss Niederwald
Rüdesheim (Rhein), Germany

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 webinars and supporter of open data initiatives. We believe that free access to scientific research results, giving everyone in the world an equal chance to participate in the exchange of experimental findings and data, is the best way to advance science.

Open Science is a new paradigm to scientific research. It is based on cooperation and creates 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, which is an essential contribution to the foundation's mission to advance the chemical and related sciences. All journal articles, conference proceedings, webinars 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 diamond 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. In addition, as our journals are Plan S compliant, they meet all technical specifications and policy requirements as outlined by cOAlition S. This means publishing with our journals will ensure compliance with all major funding agency publishing mandates including NIH, NSF, UKRI, ERC, DFG, RCUK, European Commission and Wellcome Trust.

Both journals publish thematic issues on subjects of high contemporary interest; these are often edited by guest editors, further expanding our network and outreach.

Launched in April 2019, a further addition to our publishing platform is the *Beilstein Archives*. This is the preprint server for the Beilstein journals. During the manuscript submission process, authors have the option to request that the manuscript is posted as a preprint, which is the version of the manuscript before peer review. Our preprints are posted on average within two days, allowing authors to rapidly disseminate their research results and ensuring that they claim priority for their work. Currently, over 25% of our authors select the preprint option.

The Beilstein-Institut runs two data standards projects: STRENDA which is concerned with the reporting of enzymology data and MIRAGE which is working on guidelines for 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 a unique event, 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. The talks also provide a framework and catalyse discussions which often go on into the night and have led to subsequent cooperation projects. The resulting exchange between researchers, at all stages of their careers, is the underlying goal of the meeting and gives the Beilstein Symposium their unique character.

Upcoming events in this year are:

4th EnzymeML Workshop

September 26–27, 2023, Rüdesheim, Germany

Scientific Program:
Jürgen Pleiss and Carsten Kettner

https://www.beilstein-institut.de/en/projects/strenda/meetings/4th-enzymeml-workshop/

Beilstein Nanotechnology Symposium 2023
Nanocrystal Surfaces and Defects

October, 17–19, 2023, Rüdesheim, Germany

Scientific Program:
Shelley A. Claridge and
Liberato Manna

https://www.beilstein-institut.de/en/symposia/nanocrystals/

Beilstein Organic Chemistry Symposium 2023
π-Conjugated Molecules and Materials

November, 7–9, 2023, Limburg, Germany

Scientific Program:
Chunyan Chi and
Aurelio Mateo-Alonso

https://www.beilstein-institut.de/en/symposia/pisystems/

You will find regularly updated information about our symposia at www.beilstein-symposia.org.

Book of Abstracts

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Overview

The beginning of this conference series date back to 2003, initialised by a nebulous idea. This idea became concrete through the lectures and discussions at the Beilstein Bozen Symposium 2002 - Molecular Informatics: Confronting Complexity. In preparation for this symposium, the organisers, Martin Hicks and Carsten Kettner, stated in the overview [1] that "the flood of information generated as a result of research in genomics and proteomics is often completely overwhelming", which makes it inherently difficult to use this information for analysis, confirmation, interpretation, and even to understand the experimental results and to distinguish between real findings and assumptions. As much of the experimental data is reused for model development, e.g., in systems and structural biology and in drug discovery and targeting, there is a requirement to ensure accuracy and contextual quality of this data. Contextual data is data that describes the experimental data with unambiguous attributes and is now called metadata.

An international panel of molecular informatics researchers presented results of their analysis and understanding of the storage, processing and distribution of information encoded by molecules and molecular interactions, including protein structure, pattern recognition, drug discovery, design and delivery, and software tools for analysis and prediction. A unifying theme throughout the workshop was the goal of gaining insight into the behaviour of biological and molecular systems through computer simulations.

From the energetic discussions that followed the presentations, it quickly became clear that the software tools presented could only run successfully with very well defined data sets. It also became clear that the limitations in data quality were preventing researchers and modellers from generating knowledge that went significantly beyond hypotheses. Even subsequent data-driven research was considered ineffective, if not impossible.

It is a small step from structural proteomics to enzymology (and biocatalysis). Enzymologists monitor the activities of enzymes and obtain metrics such as reaction rates, substrate specificities and enzyme efficiencies. The outcome of the discussions with researchers during the symposium and with other biochemists, particularly enzymologists, after the symposium reinforced Carsten's personal experience of the limitations encountered while characterising the kinetics of membrane-bound ion transporters: it was almost impossible to comprehensively interpret published kinetic data to corroborate one's own findings, as incomplete descriptions of the methodology precluded matching the experimental data.

Encouraged, in particular by Athel Cornish-Bowden (BIP-CNRS, Marseille), Rolf Apweiler (EBI, Cambridge) and Friedrich Lottspeich (MPI for Biochemistry, Martinsried), Carsten proposed the organisation of a workshop to the directors of the Beilstein-Institut. The aim of this workshop was to confirm the prevailing opinion that standardisation of experiments and methods for the comprehensive characterisation of enzymes is necessary and that initial suggestions should be presented to the general scientific community. This workshop had he unwieldy name "ESCEC – Experimental Standard Conditions of Enzyme Characterizations" and took place at the Hotel Jagdschloss Niederwald Hotel in October 2003. The goal was surpassed by the end of a round table discussion where several participants, including Rolf Apweiler, Athel Cornish-Bowden, Jan-Henrik Hofmeyr, Thomas Leyh, Dietmar Schomburg, and Keith Tipton, spontaneously agreed to work out a proposal for standards. This working group constituted itself as the STRENDA Commission in February 2004 [2].

In parallel, Carsten laid the groundwork for the STRENDA Commission when he analysed the data deposited in BRENDA relating both the experimental conditions and the experimental results of the key enzymes of the glycolysis of four well-studied model organisms [3]. The result was sobering: although these three enzymes had been well investigated with respect to their structures and sequences, the kinetic data was incomplete and showed wide ranges for some parameters. Although the experimental studies had been carried out by seemingly comparable methods, crucial differences stood out where the methods were comprehensively described (which was rarely the case). Similarly, to the lack of functional data making it nearly impossible to numerically analyse metabolic pathways, cells, tissues or entire organs [4], those observations clarified the dilemma of enzymology: "the purpose of enzymology is to bring about advances in the understanding of enzymes through enzyme characterisation, but in doing so it generates large quantities of data, the value of which is limited by the lack of standard experimental procedures."[3]. The standardisation of procedures crucial as experiments are carried out in different laboratories under different conditions. In his epilogue on the Beilstein Bozen Symposium 2002, Gisbert Schneider (today ETH Zurich) described this finding in plain language: "The choice of methods and objects strongly depends on the scientific background and individual skills of a researcher."[5].

Despite its importance, the STRENDA Commission quickly recognised that standardisation of experimental procedures was a problem that could not be satisfactorily solved in a short timeframe.

The Commission therefore approached the challenge from the opposite direction: it developed guidelines for reporting enzymology data, namely the STRENDA Guidelines which are now recommended by more than 55 biochemistry journals [6,7] and which form the basis of STRENDA DB, a web-based database whose data submission form automatically checks the manuscript data for compliance with the STRENDA Guidelines before or during the publication process [8]. However, this is a story that will be presented later during this conference.

Since 2003, this conference series has been held every two years. In 2011, it was renamed the Beilstein Enzymology Symposium. With this renewed focus, and with the help of the co-organisers, the Beilstein Enzymology Symposia embrace structural, computational and biological disciplines, and bring together established and early career researchers to discuss the diverse roles of enzymes in biology, and to explore the limitations and challenges of holistic studies that attempt to integrate microscopic views of protein function into complex biological behaviour.

With this symposium, the organisers will not only celebrate the 20th anniversary of this series along with all participants, but will also span the arc from the history of enzymology and its impact on the past, present and the future of the study of enzyme mechanisms, through current insights into enzyme mechanisms and regulation, as well as directed/engineered and natural evolution of enzyme functions, to the computational prediction of structures and functions, and the application of this combined knowledge to the investigation of reaction cascades and their use in biotechnology.

All participants are encouraged to discuss their latest results, approaches and methodologies in experimental, theoretical and computational enzymology.

Enjoy the Symposium!

Edda Klipp, Joelle Pelletier, Jürgen Pleiss and Carsten Kettner

References

[1] https://www.beilstein-institut.de/en/symposia/archive/bozen/bozen-2002/

[2] https://www.beilstein-institut.de/en/projects/strenda/

[3] Kettner, C., and Hicks, M.G. (2005) The Dilemma of Modern Functional Enzymology. *Current Enzyme Inhibition* **1**(2):3-10. DOI:10.2174/1573408054022234.

[4] Stelling, J., Klamt, S., Bettenbrock, K. *et al.* Metabolic network structure determines key aspects of functionality and regulation. *Nature* **420**, 190–193 (2002). DOI:10.1038/nature01166.

[5] Schneider, G. (2003) Epilogue – Complexity Challenges Research in Molecular Informatics. In: Proceedings of the Beilstein Bozen Symposium – Molecular Informatics: Confronting Complexity. Eds. M. Hicks & C. Kettner. Logos Verlag Berlin. https://www.beilstein-institut.de/download/750/schneider_3.pdf.

[6] Apweiler, R., Cornish-Bowden, A., Hofmeyr, J.-H.S., Kettner, C., Leyh, T.S., Schomburg, D. and Tipton, K.T. (2005) The importance of uniformity in reporting protein-function data. *Trends Biochem. Sci.* **30**:11-12. DOI:10.1016/j.tibs.2004.11.002.

[7] https://www.beilstein-institut.de/en/projects/strenda/guidelines/, and Tipton, K.F., Armstrong, R.N., Bakker, B.M., Bairoch, A., Cornish-Bowden, A., Halling, P.J., Hofmeyr, J.-H.S., Leyh, T.S., Kettner, C., Raushel, F.M., Rohwer, J., Schomburg, D., Steinbeck, C. (2014) Standards for Reporting Enzyme Data: The STRENDA Consortium: What it aims to do and why it should be helpful. *J. PiSc.* **1:131-137.** DOI:10.1016/j.pisc.2014.02.012**.**

[8] 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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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 773 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. 353 EUR per person.**

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

Conference Venue

Both, the conference and lunches and dinners will take place at the conference hotel, i.e.:

Hotel Jagdschloss Niederwald
Niederwald 1
65385 Rüdesheim
Germany

T +49 (0)6722-7106-0
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www.niederwald.de
jagdschloss@niederwald.de

The hotel offers wireless internet access free of charge. The hotel accepts MasterCard, Visa and EC-Cash (Maestro-Cards).

The Symposium

The symposium will be held from September 12 to 14, 2023, with the 11th and the 15th 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 12th, and

end in the late afternoon (ca. 6:30 pm) on Thursday, the 14th.

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 40 min slot includes 30 min talk + 10 min for questions). We will have an LCD projector connected to a Windows PC available.

Presentations of Posters

Poster Exhibition: Tuesday, 12 and Wednesday, 13 September, during the coffee breaks

Location of the posters

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

Poster mounting

Please mount your poster on Monday, 11th from 6:00 pm or on Tuesday, 12th at the 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 after the afternoon coffee break on Thursday, 14th** otherwise it will be taken down on time and disposed by the organizers. The organizers cannot take any responsibility for this material.

Poster material

The size of your poster board is 120 x 90 cm (height x width) and will be marked with your poster number. Hanging material for the poster boards will be provided on-site.

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 have delivered your final presentation to the organizers in time.

We will have an LCD projector (XGA) 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, September 11

19.30 Welcome reception

20.00 Dinner

Tuesday, September 12

09:00	Opening and Introductory Remarks	Carsten Kettner
	Session Chair: Jürgen Pleiss	
09:20	Enzyme Kinetics: Still a Valuable Tool for Shedding Light on Regulation, Mechanism, and Even Structure	María Luz Cárdenas Athel Cornish-Bowden
10:10	Enzymology Under New-to-nature Conditions	John M. Woodley
10:50	Poster Lightning Talks #1 Posters #1, 3–5	
11:15	*Coffee Break and Poster Session*	
11:45	Thermodynamic Considerations on Enzyme Kinetics in Biochemical Reaction Networks	Edda Klipp
12:25	Elucidation of the Biosynthetic Pathways for the Construction of the Capsular Polysaccharides in the Human Pathogen *Campylobacter jejuni*	Frank M. Raushel
13:05	*Lunch*	
	Session Chair: John Richard	
14:20	A Tail of Tails – the Remarkable SULT2B1b	Thomas S. Leyh
15:00	Coenzyme A Metabolism in Inherited Disorders of Fatty-acid Metabolism	Barbara M. Bakker
15:40	Poster Lightning Talks #2 Posters #6–11	
16:10	*Conference Photo, Coffee Break and Poster Session*	
16:40	When Nature Stumbles on a Good Thing: From Promiscuous Binding to Essential Enzyme Activity	Joelle Pelletier
17:20	(Re)designing Enzymes as Therapeutics and Biosensors	Karen N. Allen

18:00	Evolution of Energy Landscapes and its Exploitation for Enzyme and Drug Design	Dorothee Kern
18:40	*Close*	
19:30	*Dinner*	

Wednesday, September 13

09:00	*Opening*	
	Session Chair: Santiago Schnell	
09:05	Stepping into the Universe of Proteoformes	Hartmut Schlüter
09:45	Reinventing Pathways: Convergent Evolution of Specialized Metabolites in Flowering Plants	Tobias Köllner
10:25	Poster Lightning Talk #3 Poster #2	Hans V. Westerhoff
10:40	*Coffee Break*	
11:10	Predicting the Effects of Mutations in Proteins by Data-driven Methods	Stanislav Mazurenko
11:50	The EnzymeML Toolbox: Digitalization of Biocatalysis by Scalable Workflows and FAIR Data	Jürgen Pleiss
12:30	STRENDA: What, Why, How and Where to?	Johann M. Rohwer
13:10	*Lunch*	
14:00 – 18:00	*Excursion*	
19:30	*Dinner*	

Thursday, September 14

09:00	*Opening*	
	Session Chair: Edda Klipp	
09:05	Approaches to the Design of of Protein Folds and Functions	Birte Höcker
09:45	Can We Computationally Design Efficient Enzymes	Sílvia Osuna
10:25	Software Lightning Talk PRankMe - A New Algorithm to Predict Metabolic Alterations at Genome scale	Ines Heiland
10:45	*Coffee Break*	
11:15	Design Needs Evolution: Theory and Practice for Fine-tuned Bioengineering	Victor de Lorenzo
11:55	Use of Mechanistic Understanding to Improve Enzyme Performance	Pimchai Chaiyen
12:35	Enzymes for Sustainable Biomanufacturing – Expanding the Molecular Repertoire and Getting there Faster	Ee Lui Ang
13:15	*Lunch*	
	Session Chair: Joelle Pelletier	
14:30	Features and Adaption Strategies of Cold-active Enzymes	Marina Lotti
15:10	Improvement of α-amino Ester Hydrolase Stability via Computational Protein Design	Andreas S. Bommarius
15:50	*Coffee Break*	
16:20	Tuning Multi-enzyme Catalyzed Processes – Modular Set-up, Activity Regulation, and Integration with Microbial and Chemical Transformations	Dörte Rother
17:00	Biocatalysis and Beyond: Developing (Photo)enzymatic Strategies for more Sustainable Synthesis	Sandy Schmidt
17:40	Photocatalytic Enzymes by Design and Evolution	Adrian Bunzel
18:20	*Closing Remarks*	Carsten Kettner

List of Posters

The poster presentation includes a short (5 min) oral presentation on Tuesday, September 12, and the poster sessions during the coffee breaks on Tuesday and Wednesday. The posters will be displayed throughout the entire symposium from Tuesday, September 12, to Thursday, September 14.

#1	Cyanobacterial Indole Alkaloid Biosynthesis: Exploring the Role of Rieske Oxygenases	Niels de Kok
#2	What is Life Like *in vivo*? Standards for Enzymology	Hans V. Westerhoff
#3	Triosephosphate Isomerase: The Crippling Effect of the P168A/I172A Substitution at the Heart of an Enzyme Active Site	John Richard
#4	Structure-function Relationships in NDP-sugar Active SDR Enzymes: Fingerprints for Functional Annotation and Enzyme Engineering	Koen Beerens
#5	Structure-driven Drug Discovery of Human NADPH Oxidases Specific Inhibitors	Sara Marchese
#6	Needs for and Challenges in the Analysis of Proteoformes	Bente Siebels
#7	Computational Design of Enantioselective Photoenzymes Using Deep Learning	Florence Hardy
#8	OpenTECR – the Open Database on Thermodynamics of Enzyme-catalyzed Reactions	Robert Giessmann
#9	Immobilizing Mutant Xylosidase by Cross-linked Enzymatic Aggregates and onto Chitosan Support through Entrapment of Covalent Bonding	Jeff Wilkesman
#10	Sequence-dynamics-function Relationships in Protein Tyrosine Phosphatases	Michael Robinson
#11	Prediction of Protein Interaction Sites in the mTOR Signalling Network	Yin-Chen Hsieh

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Abstracts

Tuesday

09:20

Enzyme Kinetics: Still a Valuable Tool for Shedding Light on Regulation, Mechanism, and Even Structure

María Luz Cárdenas & Athel Cornish-Bowden

CNRS-BIP
Department de Bioenergétique et Ingénierie des Protéines
Marseille, France

The Beilstein Enzymology Symposia have their roots in a conversation at a Beilstein Symposium at Schloß Korb, near Bolzano, in 2002, between Carsten Kettner and Athel Cornish-Bowden, who had written the IUBMB recommendations on enzyme kinetics and had participated in discussions of enzyme classification. For many years the Henri-Michaelis-Menten equation and simple applications such as competitive inhibition have formed a central role in the teaching of biochemistry. However, with the rise of molecular biology they have started to be regarded as old-fashioned, and relegated to a secondary position.

Are all the equations really necessary for educating students?

Yes: deviations from expected behaviour in properly done experiments need to be analysed and understood, as they often lead to important discoveries, such as cooperativity and allosteric interactions. They can even lead to a better understanding of enzyme structure.

We shall briefly consider several cases where a knowledge and understanding of kinetics has been essential for advancing biochemistry, starting with a discussion of modelling aspartate metabolism in plants, which sheds light on the functioning of feedback inhibition and cooperative effects *in vivo*, and on the distribution of control between the different enzymes (including isoenzymes) in metabolic pathways. A second example concerns the relationships between types of inhibition, inhibition constants and the concentration for half-inhibition, still very widely used in pharmacology and medical studies.

Next, we show how enzyme kinetic studies revealed the unexpected order of product release in hexokinase D ("glucokinase").

Finally, we discuss the mechanism of inhibition of fructose 1,6-bisphosphatase by excess of its substrate, fructose 1,6-bisphosphate, which induces the appearance of a second class of binding site with lower affinity and catalytic activity.

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Tuesday
10:10

Enzymology Under New-to-nature Conditions

John M. Woodley

Technical University Denmark
Department of Chemical and Biochemical Engineering
Kgs. Lyngby, Denmark

Conventionally, enzymology is the discipline dealing with the study of enzymes, largely under conditions found in Nature. Over many years' laboratory-based tests have also been developed to make easy and rapid measurement of the various thermodynamic, kinetic and stability features of a given enzyme. More recently, enzymes have also found a very valuable role as industrial catalysts, in particular in the pharmaceutical sector. Combined with protein engineering, not only are new reactions being explored and but also reactions under entirely new conditions. Such new-to-nature conditions can be found, for example, in industrial reactors [1].

Here, conditions may include high concentrations of substrate and product (to ease downstream processing), substrate concentration gradients (caused by mixing challenges at larger scales), as well as the presence of gas-liquid interface (when carrying out oxidation reactions with molecular oxygen). Such conditions are often essential in order to fulfil the economic requirements of commercial operation at scale [2]. In my lab at DTU, we have started to focus on the study of enzymes in such new-to nature conditions [3]. In this presentation, I will discuss examples of such conditions and the development of novel lab-scale apparatus to test the effect of such conditions on enzymes.

References

[1] Erdem, E. and Woodley, J.M. 2022. *Chem Catalysis* **2**, 2499.

[2] Meissner, M.P. and Woodley, J.M. 2022. *Nature Catal.* **5**, 2.

[3] Wang, J., Erdem, E. and Woodley, J.M. 2023. *Org. Proc. Res. Dev.* **27**, 1111.

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Tuesday

11:45

Thermodynamic Considerations on Enzyme Kinetics in Biochemical Reaction Networks

Edda Klipp

Humboldt University Berlin
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Life depends on the input of energy, either directly provided by sunlight or in form of high-energy matter. The rules and conditions for the conversion of chemical or electromagnetic energy into living structure and all the processes related with life are governed by the laws of thermodynamics. Hence, to understand the potential and the limitations of cell growth and metabolism, it is unavoidable to take these laws into account. During the last years, systems biology has developed many mathematical models aiming to describe steady states and dynamic behavior of cellular processes in qualitative and quantitative terms. The validity of the model predictions depends strongly on whether the model formulation is in agreement with the laws of physics, chemistry, and, specifically, thermodynamics.

Here, we review basic principles of thermodynamics for equilibrium and non-equilibrium processes as well as for closed and open systems as far as they concern processes of life and development. We illustrate the application of thermodynamic laws for some practical cases that are currently intensively studied in systems and computational biology. Specifically, we will discuss the concept of entropy production and energy dissipation for isolated and open systems and its interpretation for the feasibility of biological processes, especially metabolism.

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Tuesday

12:25

Elucidation of the Biosynthetic Pathways for the Construction of the Capsular Polysaccharides in the Human Pathogen *Campylobacter jejuni*

Frank M. Raushel

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Department of Chemistry
College Station, TX, United States of America

Campylobacter jejuni is the leading cause of food poisoning in the United States and Europe. The exterior cell surface of *C. jejuni* is coated with a capsular polysaccharide (CPS) that is essential for the maintenance and integrity of the bacterial cell wall and evasion of the host immune response. The identity and sequences of the monosaccharide components of the CPS are quite variable and dependent on the specific strain of *C. jejuni*. At least 23 of the most common strains/serotypes of *C. jejuni* utilize a heptose moiety within their capsular polysaccharides, including 6-deoxy-heptoses and 3,6-dideoxy-heptose modifications. From the associated gene clusters for GDP-heptose biosynthesis we have identified 20 different enzymes that are utilized to produce at least 12 GDP-heptose variants.

The precursor heptose in each serotype has been shown to be GDP-D-*glycero*-α-D-*manno*-heptose (GMH). For biosynthesis of GDP-6-deoxy-heptose sugars, oxidation of GMH at C4 enables subsequent epimerization reactions at C3, C4, and C5 that can be coupled to the dehydration/reduction at C5/C6 and eventual reduction of C4 to make 6 of the 8 possible diastereomers at C3, C4, and C5.

For the biosynthesis of GDP-6-hydroxy-heptoses it was shown that the enzyme responsible for the critical oxidation of C4 within GMH to GDP-D-*glycero*-4-keto-α-D-*lyxo*-heptose requires the presence of α-ketoglutarate to recycle the tightly bound NADH nucleotide in the active site, which does not dissociate from the enzyme during catalysis. Oxidation of C4 enables the epimerization of C3 and/or C5 by different epimerases and substrate-specific C4-reductases enables the synthesis of 4 of the 8 possible diastereomers of GDP-heptose.

For biosynthesis of 3,6-dideoxy-heptoses a PLP-dependent 3-dehydratase catalyzes the removal of the hydroxyl group at C3 from GDP-6-deoxy-4-keto-α-D-*lyxo*-heptose.

Subsequent epimerization at C5 and stereospecific reduction at C4 enables the biosynthesis of GDP-3,6-dideoxy-β-L-*ribo*-heptose and GDP-3,6-β-L-*xylo*-heptose. Mixing different combinations of the purified enzymes has enabled the chemo-enzymatic synthesis of 8 additional GDP-activated heptoses that have not previously been identified in nature

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Tuesday

14:20

A Tail of Tails – The Remarkable SULT2B1b

Ian Cook[1], Mary Cacace[2], Alex Deiters[2], and Thomas S. Leyh[1]

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Bronx, NY, United States of America

[2]University of Pittsburgh
Department of Chemistry
Pittsburgh, PA, United States of America

SULT2B1b is distinct among human cytosolic sulfotransferases by virtue of its catalytic selectivity and its atypically long N- and C-termini. The enzyme exhibits a pronounced catalytic preference for cholesterol (CH) and related oxysterols — signaling compounds that, along with their sulfonates, are intricately engaged in regulating and coordinating metabolic processes often across multiple cell types.

The SULT2B1b N-terminus is 18-residues longer than any other SULT. We've shown recently that the terminus structures into a highly selective cholesterol-metabolite sensor that inhibits turnover by "fastening shut" the active-site cap upon binding CH precursors. We determined the sensor-pocket structure and use it as a template to create isoform specific, allosteric inhibitors to test hypotheses related to the role of 2B1b in the molecular etiology of Alzheimer's disease (AD) — the most common cause of age-related dementia worldwide. *Ex-vivo* studies will demonstrate that 2B1b inhibition slows astrocyte Ab plaque production 10-fold and stimulates its phagocytic consumption 12-fold; further, inhibition reduces neuronal tau fibril formation 25-fold; and finally, inhibition reduces the sterile immune response, which contributes to rapid decline in end-stage AD, 9-fold. These findings reveal that 2B1b simultaneously regulates four major AD processes and recommend it as a promising, novel therapeutic target.

The 37-residue C-terminal domain of SULT2B1b is highly proline enriched (~34%), its structure is not known and structural domain homologues are not available. Domain phosphorylation, at S340, results in 2B1b transport to the nucleus, wherein its functions are unknown.

All-atom molecular dynamics studies indicate that phosphorylation causes the C-tail to transition from a disordered state to a stable structure “tied” together by inner and outer, charged coordination spheres that condense around the phosphate. When placed in the context of the 2B1b dimer, the apposed C-termini form a negatively charged groove (50L x 19W x 20H Å) suggestive of a scaffolding function. SULT2B1b has emerged as a promising anti-cancer target. Consistent with the scaffolding hypothesis, *ex-vivo* studies using MF7-cells (an immortalized estrogen dependent human breast cancer cell line) will demonstrate that the 2B1b pro-cancer functions are dependent on its nuclear localization and independent of its catalytic functions.

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Tuesday

15:00

Coenzyme A Metabolism in Inherited Disorders of Fatty-acid Metabolism

Ligia Akemi Kiyuna[1], Christoff Odendaal[1], Madhulika Singh[2], Anne-Claire M.F. Martines[1], Marianne van der Zwaag[3], Miriam Langelaar-Makkinje[1], Albert Gerding[1], Ody Sibon[3], Amy Harms[2], Barbara M. Bakker[1]

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Coenzymes play a pivotal role as 'hub molecules' that connect distant metabolic pathways and thereby support a healthy, balanced metabolic network. Coenzymes are synthesized in the body from vitamins and act as carriers of for instance Gibbs energy (ATP), redox equivalents (NAD(P)H, $FADH_2$), or fatty acids (CoA). There is an increasing awareness of the potential of coenzyme precursors to normalize the rewiring of metabolism an a plethora of diseases. Specifically, CoA is essential for lipid metabolism and thereby important for diseases in which lipid metabolism is implicated. The latter range from rare and severe inherited metabolic diseases to age-related multifactorial diseases, such as diabetes and cancer.

To understand the properties of complex metabolic networks, computational models are a powerful tool. We developed computational models of fatty-acid metabolism and the role of CoA therein in rat and humans. We predicted that the topology of the pathway confers an intrinsic risk of accumulation of fatty-acid-CoA esters and depletion of free CoA. In addition, CoA availability also depends on the regulation of CoA biosynthesis, CoA degradation and hydrolysis of CoA esters.

We studied the dynamics of CoA metabolism experimentally in the context of an inherited fatty-acid oxidation disorder (medium-chain acyl-CoA dehydrogenase (MCADD) deficiency), using stable isotope labelling, targeted metabolomics, proteomics and transcriptomics in MCAD deficient liver cell lines, mice, and patient-derived organoids. Under conditions of energetic stress, the CoA availability was reduced and the balance between CoA generating and utilizing pathways was drastically altered. Computational modelling of fatty-acid metabolism in individual patients, using their personal proteomics profile, suggests that coenzyme availability is an important determinant of disease severity, which may ultimately be used in personalized diagnosis and treatment.

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Tuesday

16:40

When Nature Stumbles on a Good Thing: From Promiscuous Binding to Essential Enzyme Activity

Joelle Pelletier

Université de Montréal
Department of Chemistry
Montréal, Québec, Canada

We investigate the prevalence and evolutionary origin of an emerging antibiotic resistance enzyme and track its modern context in multi-drug resistance contexts carried in pathogenic microbes. DfrB enzymes were first identified in the 1970's for providing resistance to the antimicrobial trimethoprim due to their dihydrofolate reductase activity. Intriguingly, DfrB enzymes have no evolutionary homology to any characterized protein, such that their evolutionary origin is unknown. Their active site is formed upon homotetramerization of an SH3 fold, indicative of an atypical path to evolution of enzyme function.

Through kinetic and biophysical characterization as well as inhibitor discovery, we demonstrate that structurally diverse, putative proteins sharing with DfrB only its fold, recreate the DfrB active site environment and provide high trimethoprim resistance. Our results contribute important insights into the evolutionary path that finds the fold of DfrB enzymes included in the modern resistome.

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Tuesday

17:20

(Re)designing Enzymes as Therapeutics and Biosensors

Karen N. Allen

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Department of Chemistry
Boston, MA, United States of America

Pseudomonas putida nicotine oxidoreductase (NicA2) oxidizes *S*-nicotine to *N*-methyl-myosmine (followed by non-enzymatic hydrolysis to form pseudooxynicotine). Taking advantage of its unique evolutionary adaptation, we aim to refine the inherent catalytic function and structural features of NicA2 to develop a tools for nicotine biosensor development and biotherapeutic for nicotine addiction and nicotine poisoning. The X-ray crystal structure of the NicA2/S-nicotine complex refined to 2.6 Å resolution reveals a substrate-binding domain with a unique composition of the aromatic cage (W427 and N462) flanking the flavin isoalloxazine ring. NicA2 is specific for *S*-nicotine with a K_m of 44 nM, but with a very slow catalytic rate (k_{cat} of 6.64×10^{-3} s^{-1}), yielding an "apparently efficient" enzyme with $k_{cat}/K_m = 1.5 \times 10^5$ $M^{-1}s^{-1}$. To address the low k_{cat}, the apparent tight binding and slow rate of NicA2 was further explored using stopped-flow and single-turnover kinetics. These studies reveal the rate-limiting reaction step to be in the half-reaction with oxygen and together with structural information, yield insight into the mechanism and specificity of NicA2. Unexpectedly in the flavin-dependent superfamily, a cytochrome, CycN acts as a co-substrate. The use of an internal verses external electron donor to reduce flavin may affect evolvability and hence expansion in monooxygenases.

Although enzymes like NicA2 are well suited for applications such as biosensing in terms of their stability other enzymes are less tractable. We have developed a machine-learning method to identify amino-acid substitutions that contribute to thermal stability based on comparison of the sequences of homologues derived from psychrophilic, mesophilic and thermophilic bacteria. The method, called Machine-learning Enhanced Target Thermostabilization (MEnTaT), compares protein sequences based on the structural and physicochemical properties of the amino-acids.

The method was used to identify stabilizing substitutions in three well-studied systems and to successfully predict stabilizing substitutions in a polyamine oxidase from the flavin amine oxidase superfamily. The method can be used to provide insight into fundamental aspects of protein structure and evolution.

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Tuesday

18:00

Evolution of Energy Landscapes and its Exploitation for Enzyme and Drug Design

Dorothee Kern

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Waltham, MA, United States of America

Why can we not design efficient enzymes or highly selective drugs to date? While one can solve high resolution structures of ground states experimentally, and even now predict them with Alphafold; for biological function proteins need to traverse the entire energy landscape from the lowest energy state over the transition states into higher energy states.

Therefore, I will first share a novel approach to visualize the structures of transition-state ensembles (TSEs), that has been stymied due to their fleeting nature despite their crucial role in dictating the speed of biological processes. We determined the transition-state ensemble in the enzyme adenylate kinase by a synergistic approach between experimental high-pressure NMR relaxation during catalysis and molecular dynamics simulations [1].

Second, a novel general method to determine high resolution structures of high-energy states that are often the biologically reactive species will be described [2]. With the ultimate goal to apply this new knowledge about energy landscapes in enzyme catalysis for designing better biocatalysts, in “forward evolution” experiments, we discovered how directed evolution reshapes energy landscapes in enzymes to boost catalysis by nine orders of magnitude relative to the best computationally designed biocatalysts. The underlying molecular mechanisms for directed evolution, despite its success, had been illusive, and the general principles discovered here (dynamic properties) open the door for large improvements in rational enzyme design [3].

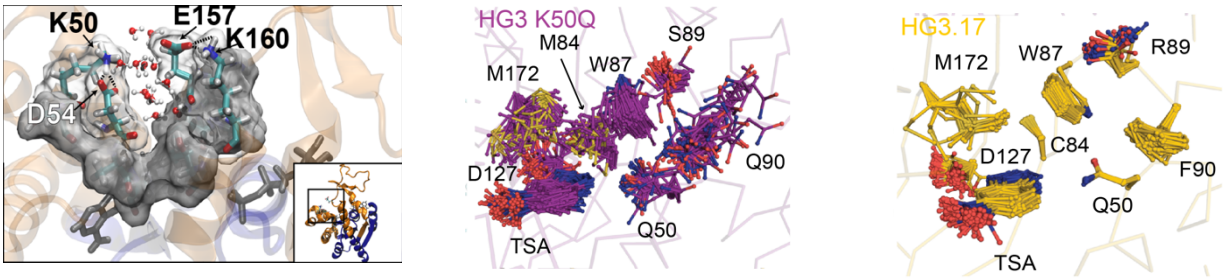

Fig.1: Transition state ensembles (TSE) for adenylate kinase rate limiting conformational change combining high pressure NMR dynamics with MD simulations (left) and TSE for designed enzyme (middle) and after directed evolution (right) for Kemp eliminase using x-ray crystallography ensemble refinement of transition state analogue (TSA) bound enzymes.

To gain insight into one of the most fundamental evolutionary events, the development of circadian rhythms, we find and characterize the most ancient, primitive biological clock [4]. Finally, visions (and success) for putting protein dynamics at the heart of drug design are discussed.

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Wednesday
09:05

Stepping into the Universe of Proteoforms

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Section Mass Spectrometric Proteomics
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An urgent need for decreasing the ambiguity of structure–function and biological process relationships of genes and their proteins.
(How the Beilstein Workshops on Experimental Standard Conditions of Enzyme Characterizations catalyzed the development of the proteoform concept since 2003)

Currently, we have the problem that for specific genes and their corresponding proteins often dozens of functions and biological processes are listed in knowledgebases, especially if they are very well known and scientifically investigated since decades. E.g. for the protein angiotensinogen (gene: AGT) more than 90 functions and biological processes are listed in UniProt. Thus, there is the risk that interpretation of transcriptomics and proteomics data is getting arbitrary, moving science into science fiction. Getting out of this dilemma is possible with the concept of proteoforms also termed protein species (synonym), first described in depth by Jungblut [1] and Schlüter [2].

A proteoform is defined as smallest unit of the proteome. From a specific gene usually many proteoforms are generated. It is estimated that more than a billion proteoforms are appearing during the lifetime of a human [3]. Proteoforms coded by the same gene can be very similar, differing in a few atoms only, but also very different comprising alterations in molecular weight of more than 90%. The specific biologic process of a proteoform and its underlying function and activity is determined by the composition of its atoms, structure and binding partners. The new Human Proteoform Atlas (HPrfA, built by Neil Kelleher and his team [4]) connected with the knowledgebase UniProt and repositories like PRIDE, is aiming to collect experimentally derived data describing exactly the identity of proteoforms and to assign them to their functions and biological processes.

The concept of the proteoform file card (first draft presented in [5]) is a central tool for this assignment. In addition, tools like the five-level classification system for identification of proteoforms [6] will increase the accuracy of knowledge about them. Moving proteomics to proteoformics in the future will help to understand more precisely what is going on in organisms on the molecular level system wide.

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09:45

Reinventing Pathways: Convergent Evolution of Specialized Metabolites in Flowering Plants

Matilde Florean, Katrin Luck, Benke Hong, Yoko Nakamura, Sara E. O'Connor, and Tobias G. Köllner

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Department for Natural Product Biosynthesis
Jena, Germany

To cope with their sessile nature, plants synthesize a number of specialized metabolites that serve as defenses against a multitude of biotic and abiotic threats. Benzoxazinoids (BXDs) form a class of indole-derived plant defense compounds with broad antimicrobial and antifeedant properties. Unlike many other specialized plant metabolites, which tend to be lineage-specific, BXDs occur sporadically in a number of distantly related plant orders, including Poales, Ranunculales, Lamiales, and Gentianales, raising the question of whether BXD biosynthesis arose independently in different lineages. Decades of research in the grasses have led to the complete elucidation of the BXD metabolism in this monocot family; however, the biosynthesis of BXDs in eudicots is still unknown.

We used a metabolomic and transcriptomic-guided approach to identify BXD enzymes in three eudicot species belonging to different plant families. Interestingly, the elucidated pathways all utilize a dual-function flavin-containing monooxygenase in place of two distinct cytochrome P450s, as is the case in the grasses. In addition, we identified several evolutionarily unrelated cytochrome P450s, a UDP-glucosyltransferase, an oxoglutarate-dependent dioxygenase, and a methyltransferase involved in BXD formation in these species. Transient expression of the identified enzymes in the plant *Nicotiana benthamiana* allowed us to fully reconstruct the respective BXDs pathways.

Our results represent the first discovery of BXD pathways in eudicots. Moreover, the heterogeneous pool of identified enzymes represents a remarkable example of metabolic plasticity, in which the ability to produce BXDs according to a similar chemical logic, but using a different set of metabolic enzymes, has evolved independently in distantly related plant families.

Wednesday

10:25

Extended Lightning Talk

What is Life? *In vivo* Standards vor Enzymology

Hans V. Westerhoff[1,2,3,4]

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[3]University of Manchester, School of Biological Sciences Manchester, United Kingdom

[4]Wallenberg Research Centre at Stellenbosch University Stellenbosch Institute for Advanced Studies Stellenbosch, South Africa

The essence of genomics is that the DNA sequence of the whole has been determined. The same holds for transcriptomics, proteomics and metabolomics. But to understand the whole one must not only study the whole but also the components and the ways those components interact to function as a whole. 'Enzymomics' as the integral of proteomics and metabolomics constitutes the most successful example to date. Thanks to the principle of mass balance and the extensive homology between genes encoding enzymes with the same function in different organisms, Genome wide metabolic maps have been made and with Flux Balance Analysis the catalysis by complete pathways has been explained on the basis of the enzymologies of the enzymes mapped into the pathway. Optimal flux patterns in living cells can be understood as Elementary Flux Modes through pathways and for these it is becoming possible to integrate the kinetic properties known from the enzymology of the participating enzymes into 'watchmaker models' of pathway function. In this way the intense understanding of enzymes achieved by enzymology can be applied to biological function in health and to malfunction in disease. The catalytic and regulatory properties of the individual enzymes depend strongly on temperature, pH and pMg however, as do the equilibrium constants. The integration of the enzymological information into pathway functioning is thereby impossible unless the enzyme properties have (also) been determined all under the same experimental conditions, which should be representative of the situation *in vivo*.

However, and for good reasons, the parameters of enzyme catalyzed reactions have not usually been determined under the same condition for all enzymes: rather, conditions were optimized for every enzyme and were thereby often specific for that enzyme, and, as biochemistry had its roots in physical chemistry, standard conditions of the latter prevailed, at least in reporting, leading to biological absurdities such as reporting for pH=pMg=0.

This presentation will report on our attempt to improve on this situation. From the cacophony of databases on energies of formation of chemical species, we constructed a single new database showing also the so-called 'metabolic energies' of approximately 1000 metabolites, at intracellular standard conditions. It is proposed that the latter be defined as T=310 K (37° C), p=1 Bar (atmosphere), pH=7, pMg=3, pCa=3, Ionic strength=0.15M and osmotic strength =0.5 M, with an additional proposal for how these standard conditions should be achieved by mixtures of salts like Kglutamate and Na_2H_2EDTA. From these metabolic energies the effective equilibrium constants can be calculated for metabolic interconversions. Through the Haldane relationship this provides one dependency between the kinetic constants of the enzyme catalyzed reaction. We here propose that in enzymological experiments other kinetic and regulatory properties are (also) determined under the same standard *in vivo* conditions.

Not only this proposal but also the choice of the best in vivo standard conditions (why is pCa not chosen as 8, for instance?) will be the subject of discussion in and around this presentation.

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Wednesday

11:10

Predicting the Effects of Mutations in Proteins by Data-driven Methods

Stanislav Mazurenko

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Department of Experimental Biology & RECETOX
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Protein engineering is a fast-developing field with multiple well-established subdomains and advanced computational tools, even available for non-experts. Nonetheless, predicting mutational effects on protein properties, such as folding, stability, solubility, function, or dynamics, remains a significant challenge due to the complex nature of the phenomenon and intricate networks of interactions between protein residues. The last decade has seen an increased interest in using data-driven methods based on machine learning to tackle the challenge, primarily due to the growing data availability and inspiring progress of such methods in other disciplines. In my talk, I will briefly discuss the recent trends in applying machine learning to protein engineering. I will also share our experience with manual curation of mutational stability (FireProt^{DB}) and solubility (SoluProtMut^{DB}) data, as well as training ML-based predictors for various protein engineering tasks. Finally, I will touch upon promising directions for future research and technology.

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11:50

The EnzymeML Toolbox: Digitalization of Biocatalysis by Scalable Workflows and FAIR Data

Jürgen Pleiss

University of Stuttgart
Institute of Biochemistry and Technical Biochemistry
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The design of biocatalytic reaction systems is highly complex due to the dependency of the estimated kinetic parameters on the enzyme, the reaction conditions, and the modelling method. Consequently, reproducing enzymatic experiments and reusing enzymatic data are challenging.

To enable storage, retrieval, and exchange of enzymatic data such as the reaction conditions, the measured time courses of substrate and product, the selected kinetic model, and the estimated kinetic parameters, the XML–based markup language EnzymeML has been developed [1]. EnzymeML is based on SBML and the community Standards for Reporting Enzyme Data (STRENDA). The EnzymeML toolbox supports biocatalysis research by making enzymatic data findable, accessible, interoperable, and reusable (FAIR).

An EnzymeML document contains information about reaction conditions and the measured time course of substrate or product concentrations [2]. It is generated from an EnzymeML spreadsheet or by the webtool BioCatHub [3]. Kinetic modelling is performed by uploading EnzymeML documents to the modelling platforms COPASI or PySCeS. The EnzymeML document containing the experimental and modelling results is then uploaded to a Dataverse installation or to the reaction kinetics database SABIO-RK. The workflow of a project is encoded as Jupyter Notebook, which can be re-used, modified, or extended.

EnzymeML serves as a seamless communication channel between experimental platforms, electronic lab notebooks, tools for modelling of enzyme kinetics, publication platforms, and enzymatic reaction databases. The feasibility and usefulness of the EnzymeML toolbox was demonstrated in six scenarios, where data and metadata of different enzymatic reactions are collected, analysed, and uploaded to public data repositories for future re-use [4].

EnzymeML documents are interoperable and reusable by other groups. Because an EnzymeML document is machine-readable, it can be used in an automated workflow for storage, visualization, data analysis, and re-analysis of previously published data, without limitations of the size of each dataset or the number of experiments.

EnzymeML is open, transparent, and invites the community to contribute. Tools, documentation, and examples are available at *https://enzymeml.org/*.

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Wednesday

12:30

STRENDA: What, Why, How and Where to?

Johann Rohwer

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STRENDA stands for “STandards for Reporting ENzymology Data”. The STRENDA project has been constituted in 2004 under the auspices of the Beilstein Insitut, which has supported it ever since. The project has three main aims:

- to establish publication standards for enzyme function, activity and kinetics data;
- to formulate standard assay conditions; and
- to develop STRENDA DB, an electronic validation system and database for storage of enzyme function data.

In parallel with the launching of the STRENDA project, the STRENDA Commission was formed. It consists of an international panel of scientists who bring in diverse areas of expertise such as biochemistry, enzyme nomenclature, bioinformatics, systems biology, modelling, mechanistic enzymology and theoretical biology.

As part of the process of scientific discovery, it is essential that researchers can compare, evaluate, interpret and reproduce experimental results published in the literature and databases. The STRENDA project identified significant gaps in this regard, specifically concerning enzyme function data. As part of its initial work, the Commission thus set about formulating a set of guidelines to define a list of minimum information for describing assay conditions as well as enzyme activity results. These STRENDA Guildelines aim to improve the quality of enzyme function data published in the literature and were developed through extensive consultation with the biochemistry community. The Guidelines are currently recommended by more than 50 international biochemistry journals, which include them in their Instructions for Authors.

In addition, STRENDA supports authors by providing STRENDA DB – a web-based database whose data submission form automatically checks the manuscript data for compliance with the STRENDA Guidelines prior to or during the publication process. Once all the checks have passed, these are summarised automatically in a fact sheet that can be submitted with the manuscript, e.g. as supplementary information. Each dataset is also assigned a DOI for persistent reference.

This presentation will provide an historical overview of STRENDA and highlight possible future directions such as automated capturing of data and metadata, tools for processing and data deposition, as well as assisting with experimental design to conform to the STRENDA guidelines.

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Thursday

09:05

Approaches to the Design of Protein Folds and Functions

Birte Höcker

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Department of Biochemistry
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In protein design we aim to build new proteins with novel functions. Methods range from generating and screening of mutant libraries via repurposing of active sites or binding pockets all the way to *de novo* design. In many of these approaches protein structures form the basis for the designs and structures are solved to validate the hypotheses.

I will discuss advantages and difficulties of the different approaches and show some highlights from our recent work that ranges from biosensors to the *de novo* design of TIM-barrels. As structure prediction and design are greatly influenced by the rapidly developing AI-based methods, I will also touch on this topic, in particular the use of natural language processing.

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Thursday

09:45

Can We Computationally Design Efficient Enzymes?

Sílvia Osuna

Universitat de Girona
Institut de Química Computacional i Catàlisi and
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Girona, Spain

Life could not be sustained without the presence of enzymes, which are responsible for accelerating the chemical reactions in a biologically compatible timescale. Enzymes present additional advantageous features such as high specificity and selectivity, plus they operate under very mild biological conditions. Inspired by these extraordinary characteristics, many scientists wondered about the possibility of designing new enzymes for industrially-relevant targets.

Enzymes exist as an ensemble of conformational states, whose populations can be shifted by substrate binding, allosteric interactions, but also by introducing mutations to their sequence. Tuning the populations of the enzyme conformational states through mutation enables evolution towards novel activity [1]. A common feature observed in many laboratory-evolved enzymes, is the introduction of remote mutations from the catalytic center, which often have a profound effect in the enzyme catalytic activity [2]. As it happens in allosterically regulated enzymes, distal mutations regulate the enzyme activity by stabilizing pre-existing catalytically important conformational states.

In this talk, the rational enzyme design approaches our group has developed based on inter-residue correlations from microsecond time-scale Molecular Dynamics (MD) simulations and enhanced sampling techniques will be presented [3,4]. Our work along the years in many different enzyme systems evidences that the current challenge of distal active site prediction for enhanced function in computational enzyme design can be ultimately addressed [3].

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Thursday

10:25

Extended Lightning Talk

PrankMe – A New Algorithm to Predict Metabolic Alterations at Genome Scale

Suraj Sharma[1], Roland Sauter[2], Marc Niere[1], Kathrin Thedieck[3], Marcel Kwiatkowski[3], Mathias Ziegler[1], and Ines Heiland[2,4]

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Metabolic alterations are a hallmark of disease progression caused by changes in the expression of the corresponding enzymes. Although analysis of gene expression data has become a standard approach, predicting large scale metabolic alterations is still challenging. We here present a novel mathematical framework that is based on the centrality of nodes in a directed graph. Through integration of differential expression data from either transcriptomics or proteomics we can predict metabolic alterations for a large set of metabolites but avoid artificial biomass or energy constraints required for other genome-scale modelling approaches. We demonstrate the efficacy of our approach using multi-omics data from modified cell lines and patient data from different diseases.

We furthermore extended our approach to trace the experimentally confirmed metabolic alterations back to the expression changes enabling network based multi-omics integration.

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Thursday

11:15

Design Meets Evolution: Theory and Practice for Fine-tuned Bioengineering

Victor de Lorenzo

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The prevailing view of biological evolution is not unlike bricolage/pastiche/tinkering—in sharp contrast with rational engineering. Yet, different paths often lead to solutions that coincide or converge whether they emerge from naturally-occurring evolution or rationally designed. Such a conjunction—often presented as a mere anecdote— in fact reveals the ability of biological systems to physically explore solution spaces and gravitate towards information-rich attractors, which can be found through different routes. This scenario evokes one of heterotic computing, a non-conventional type of data processing in which the solution to a problem is not delivered through numerical calculations but through its embodiment in a material object. Once left to undergo a physical process the object manages a large number of parameters for reaching a multi objective optimum.

The course of information is thus a physical flow and the outcome is a physical currency. The consequences of this notion for bioengineering are remarkable, as it enables solutions to multi-objective optimization challenges not yet amenable to all-rational approaches. The ensuing technical question is how to bring about hyper-diversification not only of genomic sequences but also environmental and context-dependent parameters for securing the desired performance of a given synthetic device.

This issue will be illustrated with a number of practical cases where naturally-occurring or artificially enhanced variability was key to find ideal outcomes to otherwise intractable design hitches of interest for industrial and environmental biotechnology.

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Use of Mechanistic Understanding to Improve Enzyme Performance

Pimchai Chaiyen

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Our group interests are in the broad areas of enzyme catalysis, enzyme engineering, systems biocatalysis, metabolic engineering and synthetic biology. In this talk, I will highlight our recent and current work which leads to applications in biocatalysis, detection technology and circular economy. We used mechanistic understanding and rational-engineering to improve performance of a flavin-dependent halogenase and also reroute reactions of selected flavin-dependent monooxygenases to perform non-native reactions. We also developed of an enzymatic cascade and engineering of a flavin-dependent monooxygenase, HadA, which catalyzes the dehalogenation and denitration of the toxicants, nitro- and halogenated phenols, to benzoquinone. The HadA reaction was applied in one-pot reactions towards the de novo synthesis of D-luciferin.

Currently, this technology allows us to develop a new method for synthesizing various D-luciferin analogues. As nitro- and halogenated phenols are key indicators of human overexposure to pesticides commonly used worldwide and indicators of pesticide contamination, the technology provides a sensitive and convenient tool for biomedical and environmental detection at ppb sensitivity in biological samples without the requirement for any pre-treatment. The last system is a showcase of our work in metabolic engineering where we have developed cofactor and energy boosting systems to support whole cell biocatalysts for production of valuable compounds.

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12:35

Enzymes for Sustainable Biomanufacturing – Expanding the Molecular Repertoire and Getting there Faster

Wan Lin Yeo[1], Dillon W.P. Tay[2], Jhoann M.T. Miyajima[3], Shreyas Supekar[3], Tong Mei Teh[1], Jin Xu[2], Yee Ling Tan[1], Jie Yang See[2], Hao Fan[2,3,4,5], Sebastian Maurer-Stroh[3,7,8], Yee Hwee Lim[2,4], Ee Lui Ang[1,4]

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Alcohol oxidation is a key chemical transformation in synthetic routes for specialty chemicals including active pharmaceutical ingredients (APIs). Carbonyl (C=O) functional groups introduced through alcohol oxidation are convenient reactive handles amenable toward further modification. However, chemical alcohol oxidation typically involves elevated temperatures, stoichiometric oxidants, and toxic by-products. In contrast, biocatalytic alcohol oxidation possesses advantages such as high selectivity, mild reaction conditions, benign by-products, and sustainable operation in water instead of organic solvents.

In this work, we combined the power of prediction models with a systematic and comprehensive saturation mutagenesis survey of the key domains of the galactose oxidase enzyme to create an industrially relevant enzyme panel for secondary alcohol oxidation.

We first significantly improve its activity against a challenging bulky secondary alcohol substrate. Subsequent engineering further expanded the galactose oxidase's substrate scope to include new substrates not accepted by the parent, improved its stability, and increased its solubility, thus improving the overall properties of the enzyme for biomanufacturing.

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Thursday

14:30

Features and Adaptation Strategies of Cold-active Enzymes

Marina Lotti, Marco Mangiagalli, Marco Orlando, Alessandro Marchetti

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To thrive in cold environments, psychrophilic organisms produce enzymes that are active at low temperatures. In many cases, cold-active enzymes are sensitive to heat and can undergo inactivation/denaturation even at mild temperatures. However, we have observed some notable exceptions, suggesting that the world of "cold" biocatalysts is more multifaced than previously thought. For example, a cold active beta-glycosidase from *Marinomonas* sp., in addition to being active at 4° C, shows highest activity at 55° C and is thermostable. In recent years we have studied a range of cold active enzymes from polar marine microorganisms, including glycosidases and esterases, finding evidence for different heat dependencies and structural adaptation strategies.

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15:10

Improvement of α-amino Ester Hydrolase Stability via Computational Protein Design

Colton E. Lagerman, Emily A. Joe, Martha A. Grover, Ronald W. Rousseau, **Andreas S. Bommarius**

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Amino ester hydrolases (AEHs) are capable of rapid synthesis of cephalexin but suffer from rapid deactivation at low temperatures. Previous efforts to engineer AEH have generated several improved variants but have been limited in scope in part due to limitations in activity assay throughput for β-lactam synthesis reactions. Rational design of 'whole variants' was explored to rapidly improve AEH thermostability by mutating between 3 – 15% of residues. Most variants were found to be inactive due to a mutated calcium binding site, the function of which has not previously been described. Four active variants, all with improved melting temperatures, were characterized in terms of synthesis and hydrolysis activity, unfolding by differential scanning fluorimetry, and deactivation at 25° C. Two variants were found to have improved total turnover numbers relative to the initial AEH variant; however, a clear tradeoff exists between improved stability and overall activity of each variant with an approximate linear tradeoff between the catalytic rate constant and deactivation rate constant for each variant studied.

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16:20

Tuning Multi-enzyme Catalysed Processes – Modular Set-up, Activity Regulation, and Integration with Microbial and Chemical Transformations

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There is an urgent need for the development of greener syntheses procedures if we want to maintain an environment worth living in and keep a high standard in material comfort (or reach a higher one in developing countries). The establishment of more biocatalytic steps in chemical syntheses is one possible solution, as enzymes and whole cells offer sustainable advantages, such as biodegradability, intoxicity, high selectivity, and many more. As a myriad of enzymatic reactions exist for almost any product, their potential is immense. Great scientific achievements and new techniques have enabled the design of economically and ecologically feasible one-step and multi-step enzyme catalysed reactions. This presentation will highlight the advantages of multi-enzyme catalysed processes especially with respect to atom- and step efficiency, selectivity and modularity.

However, with new opportunities, also new challenges arise. Two new possibilities, including our efforts to circumvent the associated disadvantages, will be subsequently presented: (A) avoiding cross-reactivity in (self-) regulated one-enzyme cascades and (B) the potential of using renewable resources as starting materials when microbial cell factories, enzymes and chemical catalysts are combined effectively.

The more enzyme steps are combined, the higher the risk of undesired cross-reactivity. Separation in space or on time can solve this issue. In the ‘LightCas’-project we investigate the possibilities to avoid cross- reactivity in one-pot systems by separation of reaction steps in time.

With the help of sequential enzyme addition and on-demand light-induced enzyme inactivation [1], a tight control of each biocatalytic step in a one-pot cascade is possible. This results in high product purity. Due to online analytics and automation, our ultimate goal is in close reach: setting up a one-pot multi-step light-controlled enzyme reactor yielding tetrahydroisoquinolines [2] from cheap substrates with high selectivity and concentration in a technically self-controlled manner.

The combination of suitable chemical and biological catalysts with their intrinsic advantages holds the potential to develop processes that are truly superior to current production methods in terms of efficiency and sustainability. In such hybrid processes, e.g. microorganisms can use substrates from human waste streams from agriculture and the food industry to provide simple chemical building blocks. Then, enzymes are used to diversify these compounds *in vitro*, enabling the construction of product platforms of valuable fine chemicals, complex (chiral) building blocks and active pharmaceutical ingredients.

In addition, chemical transformations can complement and diversify this product portfolio. This approach will be shown on the examples of (i) a hybrid process for the bio-based production of chiral amino alcohols from xylose and glucose [3,4] (Figure 1) and (ii) the adaptive use of renewable raw materials and the integration of CO_2 in the chemo-enzymatic production of chiral diols and dioxolanes [5]. Challenges, like the identification of suitable reaction conditions for each individual catalysts and the need of integrating downstream processing [6] to make these hybrid processes truly advantageous over other strategies will be discussed.

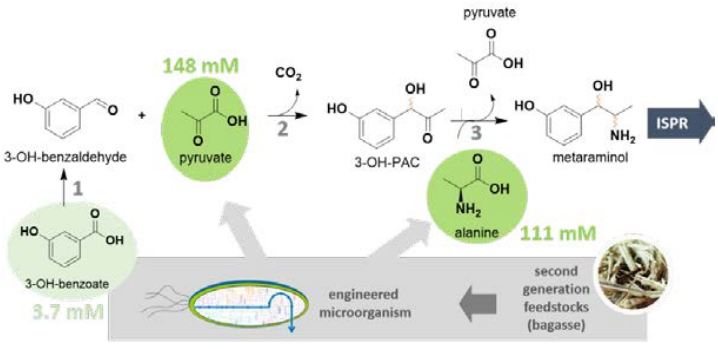

Figure 1. Enzymatic cascade towards metaraminol starting from renewables. The cascade comprises of a carboxylic acid reduction (1), a carboligation (2) and a transamination (3) step. The aliphatic precursors pyruvate and L-alanine can be produced microbially from second generation feedstocks (D-glucose, D-xylose) in suitable amounts. *In situ* product removal (ISPR) is used to shift the reaction equilibrium of the transamination step.

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17:00

Biocatalysis and Beyond: Developing (Photo)enzymatic Strategies for more Sustainable Synthesis

Sandy Schmidt

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Enzyme catalysis and photocatalysis are two research areas that have become of great interest in organic synthesis. This is mainly because both represent attractive strategies for making chemical synthesis more efficient and sustainable. It is therefore not surprising that bio- and photocatalytic approaches are now often combined to exploit the exquisite selectivity of enzymes and the unique chemical transformations accessible to photocatalysis [1-3].

In recent work, we have investigated the application of photobiocatalytic strategies using different photosensitizers and sacrificial electron donors to drive Rieske oxygenase (RO)-catalyzed hydroxylations *in vivo* [4], demonstrating that light-induced electron transfer leads to similar catalytic activities as in whole-cell reactions supplemented with glucose. We are currently exploring the possibility of using this light-induced electron transfer via photosensitizers as a simple way of overcoming the need for the natural redox partners of ROs. This is important because the identification of the physiological redox partner(s) of a given RO is often hampered by the fact that host genomes typically contain numerous candidate genes encoding redox partner proteins, most of which are not located near the RO gene(s). Thus, a light-driven approach to screen for RO activity without the physiological redox partner(s) would provide an effective surrogate electron supply system for functional characterization and/or biocatalytic application of ROs.

In another example, we have shown that the combination of bio- and photocatalysis provides a highly valuable approach to building molecular complexity from simple, cheap and widely available starting materials [5].

By combining photocatalytic C-C bond formation with enzymatic asymmetric reduction, we have demonstrated the direct conversion of simple aldehydes and acrylates or unsaturated carboxylic acids to chiral -lactones. The photochemoenzymatic synthesis of aliphatic and aromatic -lactones was thereby achieved with up to >99% *ee* and 99% yield.

References

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Thursday
17.40

Photocatalytic Enzymes by Desgin and Evolution

Eleftheria K. Stratidaki[1], Philipp Elbers[1], Adrian J. Mulholland[2], J. L. Ross Anderson[3], and H. Adrian Bunzel[1,2]

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Basel, Switzerland

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[3]University of Bristol, School of Biochemistry
Bristol, United Kingdom

The global energy crisis challenges us to develop more efficient strategies for sustainable energy production. Given the excellent efficiency of natural photo-biocatalysts, biohybrid photovoltaic devices present an attractive solution for solar energy conversion. However, their limited stability and complexity prevent their inclusion in photovoltaic devices. Here, we combined computational design and directed evolution to overcome these limitations and create tailor-made photoenzymes (Fig. 1).

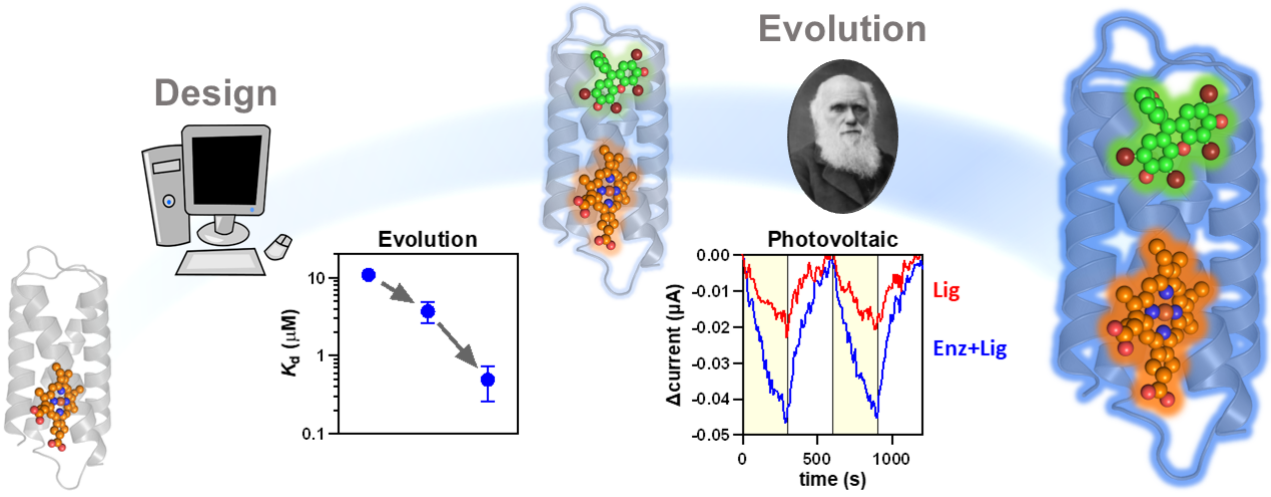

Figure 1: Computational design and directed evolution affords in new-to-nature photoenzymes.

Photo-biocatalysts were designed by introducing photosensitizer binding sites into heme-containing helical bundle proteins. The designed binding sites were specific for the target photosensitizer and could be readily transplanted into other helical bundles.

The designs were highly evolvable and reached nanomolar ligand affinity through mutagenesis and screening. The most efficient photoenzyme generated 2.6 times higher photocurrents than the photosensitizer alone, primarily driven by increased photostability.

Our work provides a robust methodological framework for creating efficient photoactive enzymes through design and evolution. Evolvability is a unique advantage of our protein-based approach over abiological photovoltaic and will be critical to developing efficient biohybrid systems. In addition to solar energy production, the versatility of our approach will enable the sustainable targeting of numerous critical transformations, including nitrogen fixation, CO_2 reduction, hydrogen production, and water splitting.

Reference

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Poster Abstracts

Tuesday

Poster
#1

Cyanobacterial Indole Alkaloid Biosynthesis: Exploring the Role of Rieske Oxygenases

Niels de Kok, Michael E. Runda,. Jannik Witte, Sandy Schmidt

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Department of Chemical and Pharmaceutical Biology, Groningen
The Netherlands

Global healthcare is facing several challenges in the near future, for instance antimicrobial resistance is on the rise, and a growing and aging global population causing an increase in age-related diseases such as cancer. As such, there is an urgent need for the development of novel pharmaceuticals to fight these challenges. Cyanobacteria are known to produce hapalindole-type indole-alkaloids, a diverse group of molecules with promising biological activities, including antitumor, antibacterial, antifungal and insecticidal properties [1]. The late-stage diversification of these indole-alkaloids, where essential modifications and functionalizations of the complex carbon scaffold are introduced, has been suggested to be catalyzed by Rieske oxygenases (Ros) [2,3]. ROs are non-heme iron-dependent multi-component enzymes that liberate single electrons from biological reducing agents, such as NAD(P)H, to activate molecular oxygen and catalyze oxidations in a highly regio- and stereoselective manner [4]. The exact role of the ROs in the late-stage diversification of indole alkaloids is unclear. Therefore, we aim to elucidate their role in indole alkaloid biosynthesis; and in anticipation of their unique reaction chemistries[5], further explore their catalytic capabilities for the synthesis of pharmaceutical building blocks.

We have identified and purified several putative ROs involved in the indole alkaloid biosynthetic pathway of *Hapalosiphon welwitschii*. Moreover, we have identified various indole alkaloids in hydrophobic extracts of *H. welwitschii* as a source of reference compounds and native substrates. Subsequently, the reactivity of identified ROs against native substrates and several substrate analogs is investigated to elucidate the role of ROs in the late-stage diversification of indole alkaloids. The outcome of this work paves the way towards exploring the application of Rieske oxygenases as biocatalysts for more sustainable drug production.

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Poster #2

(Extended Lightning Talk)

What is Life? *In vivo* Standards vor Enzymology

Hans V. Westerhoff[1,2,3,4]

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[2]University of Amsterdam, Swammerdam Institute for Life Sciences Amsterdam, The Netherlands

[3]University of Manchester, School of Biological Sciences Manchester, United Kingdom

[4]Wallenberg Research Centre at Stellenbosch University Stellenbosch Institute for Advanced Studies Stellenbosch, South Africa

The essence of genomics is that the DNA sequence of the whole has been determined. The same holds for transcriptomics, proteomics and metabolomics. But to understand the whole one must not only study the whole but also the components and the ways those components interact to function as a whole. 'Enzymomics' as the integral of proteomics and metabolomics constitutes the most successful example to date. Thanks to the principle of mass balance and the extensive homology between genes encoding enzymes with the same function in different organisms, Genome wide metabolic maps have been made and with Flux Balance Analysis the catalysis by complete pathways has been explained on the basis of the enzymologies of the enzymes mapped into the pathway. Optimal flux patterns in living cells can be understood as Elementary Flux Modes through pathways and for these it is becoming possible to integrate the kinetic properties known from the enzymology of the participating enzymes into 'watchmaker models' of pathway function. In this way the intense understanding of enzymes achieved by enzymology can be applied to biological function in health and to malfunction in disease.

The catalytic and regulatory properties of the individual enzymes depend strongly on temperature, pH and pMg however, as do the equilibrium constants. The integration of the enzymological information into pathway functioning is thereby impossible unless the enzyme properties have (also) been determined all under the same experimental conditions, which should be representative of the situation *in vivo*. However, and for good reasons, the parameters of enzyme catalyzed reactions have not usually been determined under the same condition for all enzymes: rather, conditions were optimized for every enzyme and were thereby often specific for that enzyme, and, as biochemistry had its roots in physical chemistry, standard conditions of the latter prevailed, at least in reporting, leading to biological absurdities such as reporting for pH=pMg=0.

This presentation will report on our attempt to improve on this situation. From the cacophony of databases on energies of formation of chemical species, we constructed a single new database showing also the so-called 'metabolic energies' of approximately 1000 metabolites, at intracellular standard conditions. It is proposed that the latter be defined as T=310 K (37° C), p=1 Bar (atmosphere), pH=7, pMg=3, pCa=3, Ionic strength=0.15M and osmotic strength =0.5 M, with an additional proposal for how these standard conditions should be achieved by mixtures of salts like Kglutamate and Na_2H_2EDTA. From these metabolic energies the effective equilibrium constants can be calculated for metabolic interconversions. Through the Haldane relationship this provides one dependency between the kinetic constants of the enzyme catalyzed reaction. We here propose that in enzymological experiments other kinetic and regulatory properties are (also) determined under the same standard *in vivo* conditions.

Not only this proposal but also the choice of the best in vivo standard conditions (why is pCa not chosen as 8, for instance?) will be the subject of discussion in and around this presentation.

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Poster
#3

Triosephosphate Isomerase: The Crippling Effect of the P168A/I172A Substitution at the Heart of an Enzyme Active Site

Rania Hegazy and John P. Richard

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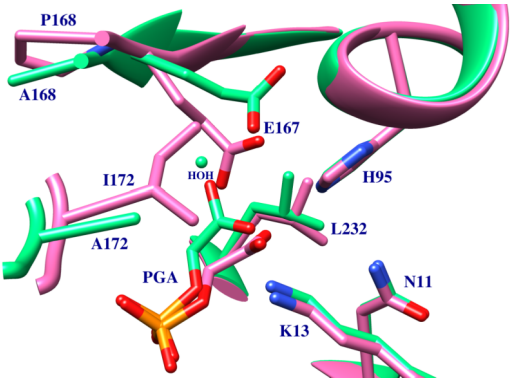

The P168 and I172 side chains sit at the heart of the active site of triosephosphate isomerase (TIM) and play important roles in catalysis of the isomerization reaction (Figure). The phosphodianion of substrate gylceraldehyde 3-phosphate (GAP) activates TIM for catalysis by driving a protein conformational change that creates a steric interaction with the P168 side chain that is relieved by movement of P168 that carries the basic E167 side chain into a clamp that consists of the hydrophobic I172 and L232 side chains. The P168A/I172A substitution at TIM from *Trypanosoma brucei brucei* (*Tbb*TIM) causes a large 120000-fold decrease in k_{cat} for isomerization of GAP that eliminates most of the difference in the catalytic activity of TIM compared to the small amine base quinuclidinone for deprotonation of catalyst-bound GAP. The I172A substitution causes a > 2-unit decrease in the pK_a > 10 for the E167 carboxylic acid in a complex to the intermediate analog PGA: the P168A substitution at the I172A variant has no further effect on this pK_a. The P168A/I172A substitution causes a 5-fold decrease in K_m for isomerization of GAP from a 0.9 kcal/mol stabilization of the substrate Michaelis complexes. The results show that the P168 and I172 side chains play a dual role in destabilizing the ground state Michaelis complex to GAP and in stabilizing the transition state for substrate isomerization. This shows that these side chain play an important role in an induced fit reaction mechanism for enzyme activation by the phosphodianion driven protein conformational change [Richard, J. P. (2022) Enabling Role of Ligand-Driven Conformational Changes in Enzyme Evolution. *Biochemistry* **61**:1533-1542].

Tuesday

Poster #4

Structure-function Relationships in NDP-sugar Active SDR Enzymes: Fingerprints for Functional Annotation and Enzyme Engineering

Matthieu Da Costa[1], Ophelia Gevaert[1], Stevie Van Overtveld[1], Carlos Alvarez-Quispe[1], Joanna Lange[2], Henk-Jan Joosten[2], Tom Desmet[1], Koen Beerens[1]

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Short-chain Dehydrogenase/Reductase enzymes that are active on nucleotide sugars (abbreviated as NS-SDR) are of paramount importance in the biosynthesis of rare sugars and glycosides. Some family members have already been extensively characterized due to their direct implication in metabolic disorders or in the biosynthesis of virulence factors. In this study, we combine the knowledge gathered from studies that typically focused only on one NS-SDR activity with an in-depth analysis and overview of all of the different NS-SDR families (169,076 enzyme sequences). Through this structure-based multiple sequence alignment of NS-SDRs retrieved from public databases, we could identify clear patterns in conservation and correlation of crucial residues. Supported by this analysis, we suggest updating and extending the UDP-galactose 4-epimerase “hexagonal box model” to an “heptagonal box model” for all NS-SDR enzymes [1].

This specificity model consists of seven conserved regions surrounding the NDP-sugar substrate that serve as fingerprint for each specificity. The specificity fingerprints highlighted in this study will be beneficial for functional annotation of the large group of NS-SDR enzymes, selection of novel specificities and form a guide for future enzyme engineering efforts focused on the biosynthesis of rare and specialty carbohydrates.

As such, we have already discovered one new specificity (i.e., promiscuous GDP-sugar 4-epimerases [2]) and have successfully created improved variants via enzyme engineering based on this *in silico* analysis and novel specificity model.

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Poster
#5

Structure-driven Discovery of Human NADPH oxidases Specific Inhibitors

Sara Marchese[1], Marta Massari[1], Joana Reis[2], Christoph Gorgulla[2], Lorenzo Basile[1], Yuriy Chaban[4], Sergio Valente[3], Antonello Mai[3], Haribabu Arthanari[2], Andrea Mattevi[1]

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[4]Diamond Light Source, Didcot, United Kingdom

The investigation of NADPH oxidases (NOXs), the only systems that produce reactive oxygen species (ROS) as main product and not as by-product, affords great insight into the fine-tuned mechanisms of regulation to maintain the ROS physiological/pathological balance.

Our approach to generate effective inhibitors against NOXs included *in silico*, *in vitro* and *in cellulo* techniques. We screened millions of potential inhibitors *in silico* through the *VirtualFlow* platform targeting the solved by us three-dimensional structure of the active site of *Cylindrospermum stagnale* NOX5 (*cs*NOX5). By means of a robust workflow for medium-throughput *in vitro* and *in cellulo* screening on the isolated human NOXs of the selected positive hits we achieved promising results especially with one lead: M41, significantly effective on *h*NOX2. To deeply investigate the effect of this inhibitor in cancer cell models we utilized PRISM technology.

We have identified, as a result, M41-responsive cell lines mostly in lymphocyte sub-lineages where NOX2 is overexpressed. Moreover, with the high-resolution crystal structures of the potent hit M41 bound to the active site of *cs*NOX5 we revealed its binding mode.

In this regard, the understanding of the mechanism of activation of NOXs is of great interest as it provides the foundation for an effective and isoform- selective generation of NOX inhibitors. However, the progress in the field has been long delayed by the lack of structural data because of proteins instability. Here, we describe the first Cryo-EM initial model of the activated NOX2 in the presence of M41, discovered to be a NOX2-stabilizer and consequently used as a tool for structural purposes. This is a crucial achievement and as such it provides reliable insights for NOX research in the structural field, as well as for a structure-driven investigation of ligands with a potential pharmacological relevance.

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Poster #6

Needs for and Challenges in the Analysis of Proteoforms

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Jungblut *et al.* 2008 and Schlüter *et al.* 2009 defined and described in detail the meaning of protein species and stated that from a single gene many different protein species are derived, which can differ in the quality and quantity of functions and can participate in very different, even opposed biological processes. In 2013 Smith *et al.* introduced the term proteoform, a synonym of protein species.

If in the future we want to understand, what is really going on in cells or tissues, it is essential that we not only quantify proteins, as we are doing with e.g., Western blots or bottom-up proteomics, but quantify specific individual proteoforms. Only the latter are directly associated with the quality and quantity of biological functions. Aebersold *et al.* claimed that the human genome is comprising more than a billion proteoforms.

Are we ready to tackle this large diversity? The challenge starts on the level of sampling and sample prepara4on: During conven4onal homogenization of tissues original proteoforms are converted into new artificial proteoforms, e.g., by proteases, released from the compartments. Chemical and enzyma4c reac4ons occurring *in vitro* significantly alter the quantities of proteoforms during conventional mechanical procedures, while sampling of tissues with the infrared-laser technology significantly reduces the diverse reactions (Kwiatkowski *et al.* 2016). The next level in the analysis of proteoforms usually comprises their enrichment or separation. On this level, closely related proteoforms, differing in few atoms only (e.g., containing methionine or oxidated methionine), are often not well separated. Current chromatographic materials provide a low resolution for the separation of proteoforms in contrast to capillary electrophoresis.

However, the latter technique is not suited for the prepara4ve separation of proteoforms. Mass spectrometry of intact proteoforms (top-down proteomics) is currently the technique with the highest resolution and selectivity for the identification and quantification of proteoforms. Promising are ion-mobility mass analyzer integrated in mass spectrometers, which can separate molecules with the same molecular mass but different shape. In conclusion, proteoform analysis is in its infancy and further progress is needed. This is also true for bioinforma4c tools, enabling the interpretation of mass spectrometric data in form of identification and quantification of proteoforms as well as databases and repositories.

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Tuesday

Poster #7

Computational Design of Enantioselective Photoenzymes Using Deep Learning

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Early efforts towards computational enzyme design relied on retrofitting natural proteins to embed quantum mechanically calculated transition states. These methods met with partial success, delivering modestly active biocatalysts that required extensive evolutionary optimisation to increase their efficiency towards a handful of model reactions [1]. Although impressive, this approach is computationally expensive, reliant on natural protein scaffolds, and generally does not provide the level of accuracy required to create efficient catalysts. With the recent emergence of powerful deep-learning algorithms for accurate protein design and structure prediction [2-4], there is a fresh opportunity to revisit the enzyme design challenge.

Here, we present the computational design of an enantioselective photoenzyme using the latest deep learning (DL) tools. We selected an intramolecular [2+2]-cycloaddition of a quinolone derivative to serve as the target reaction for design. The mechanistic blueprint for which was provided by our recently reported photoenzyme, developed through targeted introduction of the noncanonical amino acid 4-benzoylphenylalanine (BpA) into a β-propellor scaffold (EnT1.0), followed by iterative rounds of directed evolution to optimise efficiency and specificity (EnT1.3) [5].

Remarkably, from a panel of 75 designed photoenzyme sequences, we were able to identify 56 that achieve higher conversions than EnT1.0, and four higher than EnT1.3 (in clarified cell lysate), unprecedented success rates for computational enzyme design. The strategy was directed towards producing a single regio- and stereo-isomer of the product and indeed, designs were identified with high selectivity (up to 14:1 r.r. and up to 83% *e.e.*).

These results highlight how the latest DL-derived tools for protein design and structure determination hold great promise for building enzymes with new function.

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Poster #8

OpenTECR – the Open Database on Thermodynamics of Enzyme-catalyzed Reactions

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openTECR (Open database on Thermodynamics of Enzyme-Catalyzed Reactions) is a database and a community of ~30 researchers.

We create a reliable, free, machine-actionable data collection of apparent equilibrium constants of enzyme-catalyzed reactions, with a clear change process to integrate new data and correct errors.

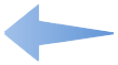
There exist diverse data collections for thermodynamic parameters (e.g. Gibbs energies of formation, apparent equilibrium constants, ...) of enzyme-catalyzed reactions in the field, but they are either only-human-readable PDF files, an online database which is only human-usable, or in the form of a free-floating csv file. Further, there are actually different versions of the same data set used by different software packages. We unify those data collections.

We cross-reference and utilize other databases, e.g. Rhea, to exploit division of labor. All sources of data, i.e. mostly the primary literature publications, are referenced and made available to researchers. Everyone can propose to integrate new experimental data. One can alert us of potential errors in the database and propose corrections.

We use GitHub for storing data in json files, with a strict versioning scheme (no information is ever lost). This heap of files is compiled into formats for use in different down-stream applications, e.g. eQuilibrator and COBRA. You can find the data and code online, starting from our main page: https://github.com/opentecr We use an open Google Groups mailinglist to communicate our requests and negotiate implementation; you can find it here: https://w3id.org/opentecr

Conceptually, our project provides a blueprint on how to set-up databases with no cost for infrastructure / developer capacity, given a specific set of technological skills and freely available resources.

Our effort for the openTECR database serves computational and experimental scientists in the fields of metabolic engineering, genome-scale metabolic modelling, biocatalysis and related fields by providing curated information.

 Back to Program

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Poster
#9

Immobilizing Mutant β-xylosidase by Cross-linked Enzymatic Aggregates and onto Chitosan Support Through Entrapment or Covalent Bonding

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The attractiveness behind xylan degrading enzymes are, besides their degradation properties, their potential applications in paper manufacturing, waste treatment, and fuel production, where lignocellulosic biomass is converted into ethanol and xylitol. β-xylosidase, an enzyme catalyzing the cleavage of xylobiose by attacking the non-reducing ends of short xylo-oligosaccharides and releasing xylose, plays a key catalytic role in xylan degradation, as it removes the end-product that inhibits endoxylanases, which in turn hydrolyzes xylan. A mutant β-xylosidase XynB2^{Y509E} from *Geobacillus stearothermophillus* was shown to possess, beyond its natural xylosidase activity, a newly discovered exo-xylanase activity. This enzyme was immobilized through three different techniques: cross-linked enzyme aggregates (CLEAs) and onto chitosan spheres through either entrapment or covalent bonding. XynB2^{Y509E}-CLEAs retained 92.3% of the original β-xylosidase activity at pH 6.5 and 65 °C.

Interestingly, the xylanase activity caused by the mutation prevailed after the immobilization, and yet was lost when immobilized by entrapment. A key factor in preserving the dual enzyme activity during the covalent bond immobilization were keeping pH 8 during the glutaraldehyde treatment, and the subsequent protein binding. The XynB2^{Y509E} activity after immobilization by covalent bonding was 23% higher compared to the entrapment immobilization (i.e., 122.3 vs. 99.4 U·g^{-1}, respectively). Both chitosan immobilized-XynB2^{Y509E} showed thermal and pH stability, along with an improved storage capacity, retaining 40% and 88% of their activity after a 60-day- storage at 4 °C. Reusability tests showed that after ten cycles, XynB2^{Y509E} immobilized by covalent binding retained 92% of its activity. With this type of eco-friendly immobilization technique, a long-term stability of both biocatalyst forms was enhanced. Our findings contribute to the growing list of successful immobilizations using activated chitosan to immobilize enzymes and offer valuable insights into the optimization of supports and/or enzymes to enhance biocatalyst performance.

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Poster #10

Sequence-dynamics-function Relationships in Protein Tyrosine Phosphatases

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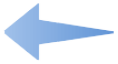
Protein tyrosine phosphatases play a major role in a variety of cellular signaling pathways, and are of significant potential interest as pharmaceutical targets. Unlike many enzymes, their activity is controlled by the motions of a conserved loop region, the WPD loop. This loop contains a catalytically active residue, and loop dynamics control the positioning of this residue in the active site. In this work, chimeric PTPs were created in which parts of the WPD loop sequence of PTP1B were grafted onto an YopH scaffold. In addition to the catalytically active closed state, and the inactive open state, these chimeric PTPs showed a wide-open state which was previously known only from structures with small-molecule inhibitors bound. Computational investigation of these chimeras was performed, showing minimal differences in the energetic components of the catalysis.

However, significant differences were observed in the dynamical behavior of these chimeras, particularly in the relatively stability of the wide-open conformation of the WPD loop, leading to a significant population shift towards a wide-open conformation. This differences in stability were then connected to individual mutations through the use of interaction network analysis.

This work demonstrates the connection between the loop dynamics observed and the chemical changes associated with specific mutations, and provides insight into a method in which protein engineering is performed through modification of an internal protein interaction network.

 Back to Program

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Poster #11

Prediction of Protein Interaction Sites in the mTOR Signaling Network

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Structure identifiability is one of the major issues limiting the predictive power of signal transduction modeling approaches. The reason is that the underlying protein interaction networks are very complex and governed by the competition for interaction sites. To identify protein interaction sites and their regulation via potential binding competition of downstream targets and identifying binding partners experimentally is tedious and time consuming. The power of computational approaches to predict these interactions has so far been limited. Recently, AlphaFold2, an algorithm originally developed to predict protein structures of single proteins, has been released and shown to have a very high accuracy to predict protein structures not only for single proteins but also for protein complexes.

We therefore use this approach for *de novo* prediction of protein complexes and their interaction sites. For the prediction of interaction sites, we explore AlphaFold2 capabilities to predict stably-bound and transiently-bound complexes. We complement these analyses with phylogenetic approaches looking at the degree of conservation and coevolution of amino acids in the interaction site. Generally, comparison with partial experimental structures available indicates good agreement and we thus used this method to explore and predict protein interactions in the mTOR network.

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