23rd PSDI 2015
Protein Structure Determination in Industry

8. – 10. November 2015

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Program

Day 1: Sunday, November 8, 2015		
15:00–19:00	Registration	
18:20–18:30	Opening Remarks Proteros	
18:30–19:30	Robert Huber, Max-Planck-Institut für Biochemie, Martinsried, Germany	*Protease control in health and disease, my experience with its translation into practice*
19:30–23:00	Welcome Reception – food, drinks and networking	

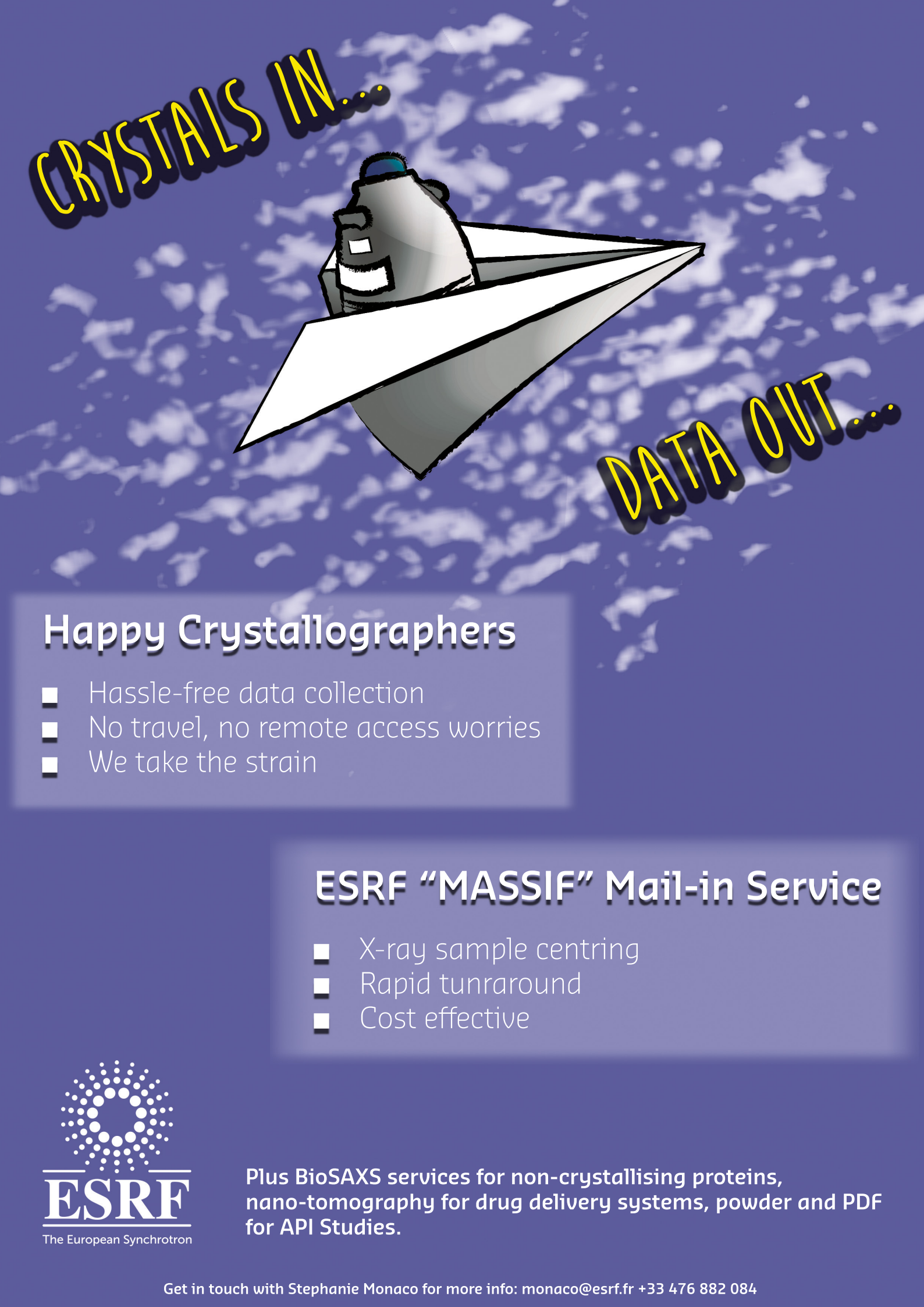
Day 2: Monday, November 9, 2015		
8:30–10:30	Session 1: New structures / Hot targets	
Chair	Herbert Nar, Boehringer Ingelheim	
8:30–8:50	Michael Hennig, Roche, Basel, Switzerland	*Extension of the structure based drug discovery to membrane proteins, update on the Roche activities and recent examples*
8:50–9:10	Armin Ruf, Roche, Basel, Switzerland	*Crystal structures of the human doublecortin C-terminal and N-terminal domains in complex with specific antibodies*
9:10–9:30	Hans Brandstetter, Universität Salzburg, Austria	*Orthogonal activities in legumain and their specific inhibition*
9:30–9:50	Aengus Mac Sweeney, Actelion Pharmaceuticals, Allschwil, Switzerland	*Discovery and optmization of inhibitors of the complement system*
9:50–10:10	Thomas Gossas, Beactica, Uppsala, Sweden	*Discovery of Allosteric Modulators of the α7 Nicotinic Acetylcholine Receptor*
10:10–10:30	Patrik Johansson, AstraZeneca, Mölndal, Sweden	*Inhibition of the membrane-embedded Microsomal prostaglandin synthase-1*
10:30–11:00	Morning break	
11:00–12:50	Session 2: Computational Chemistry, Biostructures & Biophysics	
Chair	Torsten Hoffmann, Proteros	
11:00–11:30	Hans-Joachim Böhm	*Future impact of biostructural and biophysical approaches on drug discovery*
11:30–11:50	Frech Matthias, Merck Serono, Darmstadt, Germany	*Hsp 90 as a model system to explain the kinetics of the interaction of small molecules*
11:50–12:10	Gisela Schnapp, Boehringer Ingelheim, Biberach, Germany	*Analysis of binding kinetics and thermodynamics of DPP-4 inhibitors and their relationship to structure*
12:10–12:30	Lars Neumann, Proteros Biostructures GmbH, Martinsried, Germany	*Case study on CDK8/CycC: Structure guided fragment evolution towards long residence time*
12:30–12:50	Simon Holton,Bayer Pharma AG, Berlin, Germany	*Biophysical approaches for hit finding and evaluation at Bayer: Discovery and characterization of the SMYD2 chemical probe BAY-598“*
12:50–13:50	Lunch	

13:50–15:30	Session 3: Technology 1 / Crystallisation / Crystal treatment / Data Collection / Synchrotron / Automation	
Chair	Martina Schäfer, Bayer	
13:50–14:10	Henry N. Chapman, DESY, Hamburg, Germany	*Structure determination using X-Ray FELs*
14:10–14:30	Stefan Raunser, Max Planck Institute of Molecular Physiology, Dortmund, Germany	*Strengths of Single Particle Cryo-EM*
14:30–14:50	Patrick Shaw Stewart, Douglas Instruments, East Garston, UK	*Understanding random crystal screening with microseeding – how new strategies can improve productivity*
14:50–15:10	Isabel Moraes, Membrane Protein Lab Diamond, Oxford, UK	*Structure Determination of Membrane Proteins The Present and the Future*
15:10–15:30	Frank von Delft, Diamond, Oxford, UK	*Fragment screening in crystals comes of age: 500 soaks in a week at Diamond I04-1*
15:30–16:00	Break	
16:00–17:40	Session 4: Exhibitors	
Chair	Stephan Krapp, Proteros	
16:00–16:10	Paul Thaw, TTP Labtech, Royston, UK	*Flying through optimization screening with dragonfly*
16:10–16:20	Jochen Mueller-Dieckmann, Formulatrix, Inc., Bedford MA, USA	*Advancements in Automated Imaging: Trace Fluorescence Labeling and SONICC*
16:20–16:30	Joe Ferrara , Rigaku, Houston TX, USA	*Reassessing Rigaku Oxford Diffraction's HomeLab Rotating Anode X-ray Sources*
16:30–16:40	Gregory Warren, OpenEye, Santa Fe, NM, USA	*Review of AFITT for automatically fitting ligand to density, refitting protein side chains and optimizing ligand conformations in BUSTER and PHENIX*
16:40–16:50	Hillary McNeill, Douglas Instruments, East Garston, UK	*The Oryx Range by Douglas Instruments: Special Tools for Crystallization*
16:50–17:00	Torsten Hoffmann, Proteros Biostructures GmbH, Martinsried, Germany	*Lead Discovery at the Cutting Edge of Epigenetic Targets*
17:00–17:10	Edward Pryor, Anatrace, Maumee, OH, USA	*Discussion of new tools and techniques for membrane protein purification and crystallization*
17:10–17:20	Andreas Förster, DECTRIS Ldt., Baden, Switzerland	*Technological advances in rational drug design*
17:20–17:30	Artem Evdokimov, HarkerBIO, Buffalo, NY, USA	*If the dog won't hunt: Advanced mutagenesis strategies for crystallization of recalcitrant targets.*
17:30–17:40	Vernon Smith, Bruker AXS GmbH, Karlsruhe, Germany	*Latest Developments in Home-Lab Macromolecular Crystallography*

Conference Dinner	
18.30	Departure to Conference Dinner
19:15–22:45	Dinner at Herzogliches Bräustüberl Tegernsee
22:00	Bus 1 departure back to the hotel
22:30	Bus 2 departure back to the hotel
22:50	Bus 3 departure back to the hotel

Day 3: Tuesday, November 10, 2015		
8:30–10:30	Session 5: Use of structures in Medicinal Chemistry, case and success stories	
Chair	Hans-Joachim Böhm	
8:30–8:50	Sandra Cowan-Jacob, Novartis, Basel, Switzerland	*Discovery of kinase inhibitors binding outside the ATP site*
8:50–9:10	Alex Milbradt, AstraZeneca, Macclesfield, UK	*Structure and Biophysics: Fragment-based discovery of the first known inhibitors of PHGDH*
9:10–9:30	Martina Schäfer,Bayer Pharma AG , Berlin, Germany	*Discovery of BAY 85-8501, a Novel and Highly Potent Induced-Fit Binder of Human Neutrophil Elastase for Pulmonary Diseases*
9:30–9:50	Steven Sheriff, Bristol-Myers Squibb, NJ, USA	*Structure of the kinase domain of TGFβR2*
09:50–10:10	Djordje Musil, Merck Serono, Darmstadt, Germany	*Discovery and optimization of small-molecule inhibitors of transforming growth factor β receptor type I (TβRI)*
10:10–10:30	Bernhard Loll, moloX GmbH, Berlin, Germany	*Production of macrocyclic diterpenes in bacterial hosts*
10:30–11:20	Morning break	
11:20–13:00	Session 6: Technology 2 / Software	
Chair	Michael Mrosek, Proteros	
11:20–11:40	Gerard Bricogne, Global phasing Ltd., Cambridge, UK	*Optimising and driving synchrotron experiments as a third party*
11:40–12:00	Randy J Read, Cambridge Institute for Medical Research, Cambridge, UK	*Recent developments in Phaser*
12:00–12:20	Paul Emsley, University Cambridge, Cambridge, UK	*Using Coot tools for protein-ligand complex model validation*
12:20–12:40	Joshua Salafsky, Biodesy, San Francisco, CA, USA	*Second-harmonic generation (SHG) as a sensitive, real-time probe of protein structure*
12:40–13:00	Stephanie Monaco, ESRF, Grenoble, France	*New tools and techniques at the service of drug discovery at ESRF*
13:00–14:00	Lunch	

14:00–16:20	Session 7: Biopharmaceuticals (structures for design and validation)	
Chair	Charlie Eigenbrot, Genentech	
14:00–14:20	Guy Georges, Roche, Penzberg, Germany	*Why do we need biologics structures, especially antibody-antigen complex structures? Structure-guided antibody engineering and humanization*
14:20–14:40	Alexey Rak, Sanofi, Paris, France	*Biologics structure based rational design, validation and characterization*
14:40–15:00	Tom Ceska, UCB, Oxford, UK	*Antibodies and NCEs Opportunities at the Interface*
15:00–15:20	Alexey Teplyakov, Johnson & Johnson, Spring House, PA, USA	*Structure-based antibody engineering*
15:20–15:40	Herbert Nar, Boehringer Ingelheim, Biberach, Germany	*Specific Antidotes For Dabigatran: Structure-Guided Affinity Optimisation and Functional Characterisation*
15:40–16:00	David Hargreaves, AstraZeneca, Cambridge, UK	*Novel Mcl-1 Antibody Assisted Crystal System: supporting a DNA encoded library hit finding strategy targeting Protein-Protein Interactions*
16:00–16:20	Matthew Bottomley, GSK Vaccines , Siena, Italy	*Structural Biology in Vaccine Research*
16:20	Closing Remarks	
16:20	Coffe Break	



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Abstracts

Extension of the structure based drug discovery to membrane proteins, update on the Roche activities and recent examples

Michael Hennig

F. Hoffmann – La Roche Ltd., Pharmaceutical Research&Early Development
Roche Innovation Center, 4070 Basel, Switzerland

Today, structure based drug discovery is well implemented in the the drug discovery engine of many pharmaceutical companies. Whereas soluble proteins are managed well within the project timelines and portfolio flexibility, transmembrane proteins still represent a significant challenge. At Roche, we implemented a membrane protein platform with an internal portfolio of targets as well as collaborations. Two examples are presented illustrating the progress made to apply biophysical methods for compound screening, hit identification, confirmation and structural analyis of ligand complexes to inspire chemistry. The outlook includes a discussion on the expected impact of serial crystallography and the XFEL to enhance structure determination for pharmaceutical industry.

Crystal structures of the human doublecortin C-terminal and N-terminal domains in complex with specific antibodies

Armin Ruf[1)], Jörg Benz[1)], Dominique Burger[1)], Brigitte D'Arcy[1)], Maja Debulpaep[2),3)], Paola Di Lello[4,(*)], David Fry[4)], Walter Huber[1)], Thomas Kremer[1)], Toon Laeremans[2),3)], Hugues Matile[1)], Alfred Ross[1)], Markus G. Rudolph[1)], Arne Rufer[1)], Ashwani Sharma[5)], Michel O. Steinmetz[5)], Jan Steyeart[2),3)], Guillaume Schoch[1)], Martine Stihle[1)], Ralf Thoma[1)]

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[2)]*Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium*

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[5)]*Paul Scherrer Institute, OFLC/111, 5232 Villigen, Switzerland*

[(*)]*Present address: Department of Structural Biology, Genentech, 1 DNA way, South San Francisco, CA 94080*

Doublecortin is a microtubule-associated protein which is essential for human brain development. Missense mutations in the doublecortin gene cause defective cortical neuronal migration leading to the brain formation disorders X-linked lissencephaly and subcortical band heterotopia. Anti-doublecortin-antibodies are important research tools in neuroscience and widely used to immunohistochemically detect newborn neurons in brain sections. To create new understanding of the molecular processes in neurogenesis by better characterizing the doublecortin interaction with tubulin we raised novel DCX-domain specific antibodies against doublecortin and solved new crystal structures illustrating the conformational flexibility of the DCX domains.

Orthogonal activities in legumain and their specific inhibition

Elfriede Dall[1)], Florian Zauner[1)], Zhentao Zhang[2)], Julia Fegg[1)], Peter Briza[1)], Keqiang Ye[2)], Hans Brandstetter[1)]

[1)]*Department of Molecular Biology, University of Salzburg, Austria*

[2)]*Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA*

Legumain or Asparagine Endopeptidase (AEP) is usually associated with its cysteine endopeptidase activity in lysosomes where it contributes to antigen processing for class II MHC presentation. However, newly recognized functions disperse previously assumed boundaries with respect to their cellular compartmentalisation and enzymatic activities. Legumain is also found extracellularly and even translocates to the cytosol and the nucleus, with seemingly incompatible pH and redox potential. These different milieus translate into changes of legumain's molecular properties, including its (auto-)activation, conformational stability and enzymatic functions.

We show that legumain can develop a carboxypeptidase activity which remains stable at neutral pH, contrasting its endopeptidase activity. Moreover, legumain features a peptide ligase activity, which dominates at near neutral pH. These non-classical legumain activities become particularly relevant in pathological settings, such as cancer or Alzheimer's disease (AD), where the proper association of legumain activities with the corresponding cellular compartments is breached. Legumain is upregulated and activated in normally aged brain and human AD brain, and plays a critical role in mediating the pathophysiology of AD. We report the crystal structure of an orally bioactive and brain permeable legumain inhibitor that blocks the cleavage of tau and APP, and alleviates the cognitive deficits in mouse models of AD.

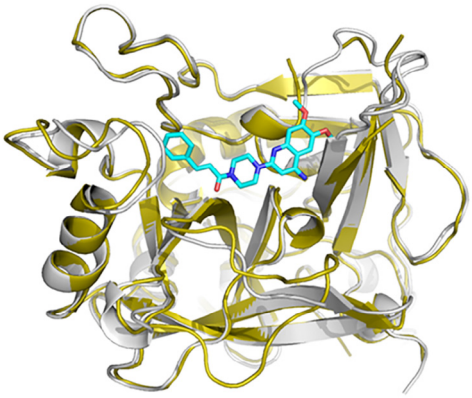
Discovery and optimization of inhibitors of the complement system

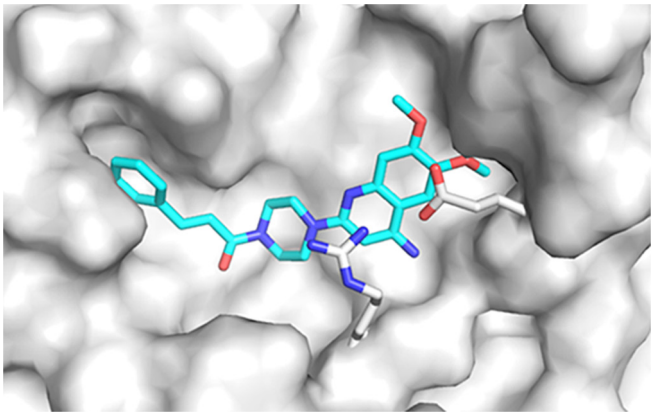
Aengus Mac Sweeney[1)], Holger Sellner[2)]

[1)]*Actelion Pharmaceuticals Ltd,*
Gewerbestrasse 16, 4123 Allschwil, Switzerland.

[2)]*Novartis Institutes for Biomedical Research,*
Novartis Campus, 4056 Basel, Switzerland.

The complement system is one of the major defense mechanisms of the innate immune system composed of the classical pathway (CP), the lectin pathway (LP), and the alternative pathway (AP). There is strong scientific evidence for AP involvement in diseases such as age-related macular degeneration (AMD) or paroxysmal nocturnal hemoglobinuria (PNH). We report on the discovery and structure guided optimization of low-molecular weight inhibitors of complement factors B and D, as well as preclinical testing of the resulting potent and selective compounds.





Discovery of Allosteric Modulators of the α7 Nicotinic Acetylcholine Receptor

Radovan Spurny[1)], Sarah Debaveye[1)], Ana Farinha[1)], Ken Veys[2)], Ann Vos[2)], Thomas Gossas[3)], John Atack[4)], Sonia Bertrand[5)], Daniel Bertrand[5)], U. Helena Danielson[3),6)], Gary Tresadern[2)] and Chris Ulens[1)]

[1)]Laboratory of Structural Neurobiology, KU Leuven, Leuven, B-3000, Belgium

[2)]Janssen R&D, Beerse, B-2340, Belgium

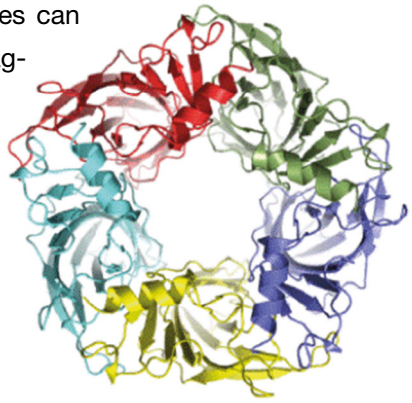
[3)]Beactica, 754 50 Uppsala, Sweden

[4)]Translational Drug Discovery Group, University of Sussex, BN1 9QJ Brighton, United Kingdom

[5)]HiQscreen, Geneva, 1222 Vésenaz, Switzerland

[6)]Department of Chemistry, Uppsala Biomedical Center, Uppsala University, SE-751 23 Uppsala, Sweden

Novel allosteric binding sites were identified in a homologue of the α7 nicotinic acetylcholine receptor by surface plasmon resonance-based fragment screening in combination with X-ray crystallography and electrophysiology. The α7 nicotinic acetylcholine receptor belongs to the family of pentameric ligand-gated ion channels and is involved in fast synaptic signaling. In this study a recently identified chimera of the extracellular domain of the native α7 nicotinic acetylcholine receptor extracellular domain and acetylcholine binding protein (AChBP), termed α7-AChBP was used. Allosteric fragments were discovered by a screening set-up experimentally designed to identify allosteric ligands by using orthosteric ligands as competitors. Five fragments were found in three different allosteric binding sites that were mapped by X-ray crystallography. Using electrophysiological recordings on the human α7 nAChR it was demonstrated that each of the three allosteric sites can modulate receptor activation. This work presents an innovative fragment screening strategy to discover allosteric ligands and a structural framework for understanding the molecular recognition at different allosteric binding sites in the α7 nAChR. This paves the way for future development of novel allosteric modulators with therapeutic potential.



Reference: Spurny *et al.* (2015) *PNAS*, 112(9):2543–2552.

Inhibition of the membrane-embedded Microsomal prostaglandin synthase-1

Tove Sjögren[1)], Johan Ulander[2)], Stefan Geschwindner[1)], Margareta Ek[1)], Patrik Johansson[1)]

[1)]Discovery Sciences, AstraZeneca R&D Mölndal, S-43183 Mölndal, Sweden

[2)]CVMD Innovative Medicines, AstraZeneca R&D Mölndal, S-43183 Mölndal, Sweden

The lack of experimental data on protein-ligand interactions within the lipid bilayer has caused both drug discovery and an understanding of lipid-protein interactions to lag behind. Microsomal prostaglandin E2 synthase-1 is one of the key enzymes responsible for the production of prostaglandin E2, a powerful mediator of inflammation. We here present the high resolution crystal structures of mPGES-1 both alone and in complex with a number of potent inhibitors of PGE2 biosynthesis. In contrast to neighboring enzymes the mPGES-1 structure was found to exhibit a small cytoplasmic domain, shielding the solvent access of the active site. This indicates that the PGH2 substrate as well as other ligands need to enter the binding pocket from within the lipid bilayer. The structures combined with biochemical and biophysical data highlight some general features and distinct challenges associated with lead generation towards proteins with lipid-embedded binding sites, including water droplet-formation, diffusion of ligands and membrane-protein promiscuity.

Future impact of biostructural and biophysical approaches on drug discovery

Hans-Joachim Böhm

Drug Discovery has delivered many outstanding therapies that have revolutionized the treatment of serious diseases. Computational, biostructural and biophysical approaches have contributed significantly to this success.

The increased impact of these methods is due to i) rapid access to 3D structural information on the target protein, ii) new approaches to analyze and assess protein-ligand interactions, and iii) new approaches to make the large amounts of available structure-property data actionable.

Nevertheless, several important challenges remain, such as tackling protein-protein interactions, targeting certain cell types or organs and developing new strategies to avoid off-target toxicity. I will present recent work trying to address these issues and will provide an outlook about the future impact of biostructural and biophysical approaches.

Hsp 90 as a model system to explain the kinetics of the interaction of small molecules

Frech Matthias

Merck Serono, Darmstadt, Germany

Analysis of binding kinetics and thermodynamics of DPP-4 inhibitors and their relationship to structure

Gisela Schnapp and Herbert Nar

Boehringer Ingelheim Pharma GmbH & Co. KG

Dipeptidylpeptidase 4 (DPP-4) is a N-terminal dipeptidyl exopeptidase that exists as both a membrane-bound protein and a soluble form in plasma. DPP-4 inhibitors (DPP-4i, gliptins) have clinical benefit in patients with type 2 diabetes by generating increasing levels of glucose-lowering incretin hormones, like glucagon-like peptide-1 (GLP-1).

Kinetic and thermodynamic profiling of preclinical stage compounds has recently received increasing attention in pharmaceutical research. The notion is that this information can be used in compound prioritization and that optimization of binding kinetics rather than affinity would be the better alternative in many drug discovery projects.

We show here kinetic and thermodynamic data for the currently marketed DPP-4i sitagliptin, vildagliptin, saxagliptin, linagliptin, alogliptin and teneligliptin and discuss them in the context of available structural information.The compounds exhibit relatively fast, electrostatically driven on-rates. Their slow off-rates are due to distinct modes-of-action (reversible covalent vs. non-covalent). Binding of all gliptins is strongly enthalpy driven.

Case study on CDK8/CycC: Structure guided fragment evolution towards long residence time

Lars Neumann[1)], Elisabeth Schneider[2)], Robert Huber[2)], Klaus Maskos[1)]

[1)]*Proteros Biostructures GmbH, D-82152 Martinsried, Germany*

[2)]*Max-Planck-Institut für Biochemie, D-82152 Martinsried, Germany*

Despite the fact that residence time is an increasingly appreciated optimization parameter in drug discovery, molecular understanding of binding kinetics in order to facilitate efficient generation of compounds with tailor made residence time values remains a challenge. We have used the target CDK8/CycC to demonstrate one strategy how fast binding fragments can be evolved to long residence time compounds by combination of structural biology and kinetic profiling. Deep pocket binding fragments with fast binding kinetics were identified by a fragment screening campaign. After structure based examination of the binding mode, the fragments were gradually extended in order to establish an increasing number of compound-protein contacts. These contacts were systematically analyzed for their impact on residence time.

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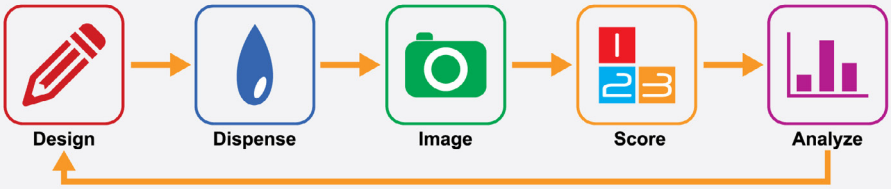
Protein Crystallization Automation



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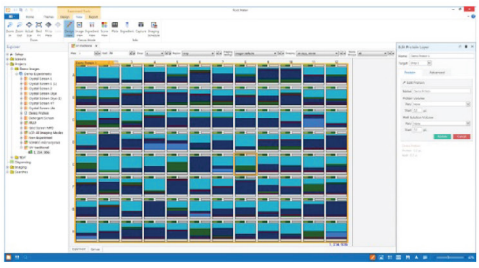

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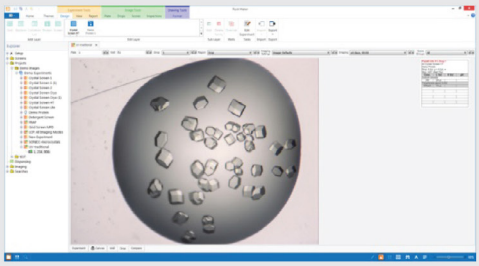

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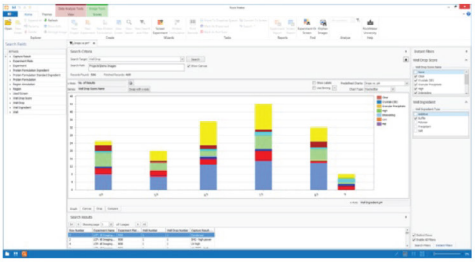

Biophysical approaches for hit finding and evaluation at Bayer: Discovery and characterization of the SMYD2 chemical Probe BAY-598

Simon Holton

Bayer Pharma AG

Target binding thermodynamics and kinetics are important parameters in our Lead Generation and Optimization process at Bayer. The demand for such data has steadily increased over the years. A biophysics platform with sufficient throughput for the biophysical evaluation during hit generation and validation, as well as lead optimization was established.

The application of these techniques is presented as part of a chemical probe development program for the discovery and characterization of aminopyrazoline-based inhibitors of SMYD2, a protein which catalyzes the methylation of p53-K370.

Structure determination using X-Ray FELs

Henry Chapman

Center for Free-Electron Laser Science
DESY / Universität Hamburg
Notkestrasse 85
22607 Hamburg, Germany

The pulses from X-ray free-electron lasers are a billion times brighter than the brightest synchrotron beams available today. When focused to micron dimensions, such a pulse destroys any material, but the pulse terminates before significant atomic motion can take place. This mode of “diffraction before destruction” yields structural information at resolutions better than 2 Angstrom, from proteins that cannot be grown into large enough crystals or are too radiation sensitive for high-resolution crystallography. This has opened up a new methodology of serial femtosecond crystallography that yields radiation damage-free structures without the need for cryogenic cooling of the sample. The method has begun to yield new structures and has the potential to increase the rate at which structures can be solved. Ultrafast pump-probe studies of photoinduced dynamics in proteins or other materials can also be studied. Irreversible reactions can be studied, synchronised with the short pulses, with new sample being constantly replenished. We have yet to reach the limit of the smallest samples that can be studied this way, and many innovations indicate the feasibility of single molecule diffractive imaging.

Strengths of Single Particle Cryo-EM

Stefan Raunser

Max-Planck-Institute of Molecular Physiology
Department of Structural Biochemistry
Otto-Hahn-Str. 11
44227 Dortmund

Recent advances in cryo-EM and the potential of the technique for industrial drug discovery.

Understanding random crystal screening with microseeding – how new strategies can improve productivity

Patrick Shaw Stewart and Stefan Kolek

Douglas Instruments Ltd, Hungerford, UK

Random Microseed Matrix-Screening (rMMS), where seed crystals are added automatically to random crystallization screens, is a significant recent breakthrough in protein crystallization [1]. During the eight years since the method was published, understanding of the theoretical advantages of the method has increased [2 - 4], and several important practical variations on the basic method have emerged. Important variations that will be discussed include combining seeds from several hits [5], the best methods of selecting hits to optimize [2], and cross-seeding targets with crystals of homologous proteins [6]. We will also present an approach that allows the method to be applied to the crystallization of membrane proteins in LCP.

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[1] D'Arcy, Allan, Frederic Villard, and May Marsh. "An automated microseed matrix-screening method for protein crystallization." Acta Crystallographica Section D: Biological Crystallography 63.4 (2007): 550-554.

[2] Shaw Stewart, Patrick D., et al. "Random microseeding: a theoretical and practical exploration of seed stability and seeding techniques for successful protein crystallization." Crystal Growth & Design 11.8 (2011): 3432-3441.

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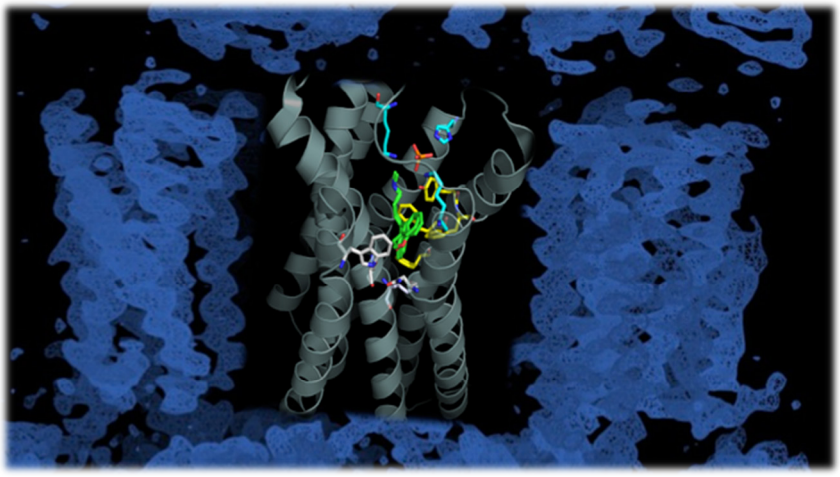
Structure Determination of Membrane Proteins The Present and the Future

Isabel Moraes

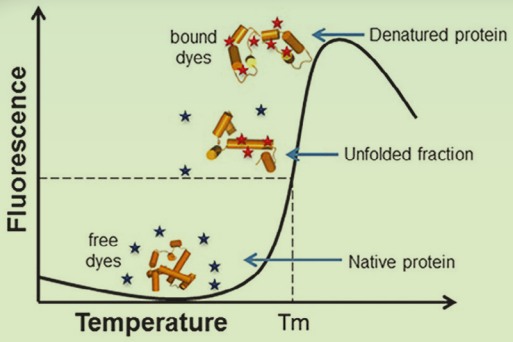
The Membrane Protein Laboratory at Diamond Light Source

Membrane proteins are important pharmaceutical targets as they are associated wit many diseases. Currently approximately 60% of the available drugs target membrane proteins of which G protein-coupled receptors (GPCRs) and ion channels constitute the largest groups. The knowledge of their atomic structure is critical in drug discovery and thus to the welfare of our global society. The advent of genomics and proteomics initiatives combined with high-throughput technologies, such as automation, miniaturization, integration and third-generation synchrotrons, has enhanced membrane protein structure determination rate. Yet the growth of membrane protein crystals suitable for X-ray diffraction studies amazingly remains a fine art and a major bottleneck in the field. It is often necessary to apply as many innovative approaches as possible. Here it is presented the latest methods and strategies in membrane protein structure determination.



JBScreen Thermofluor

Two systematic protein stability screens based on FUNDAMENTAL and SPECIFIC variables



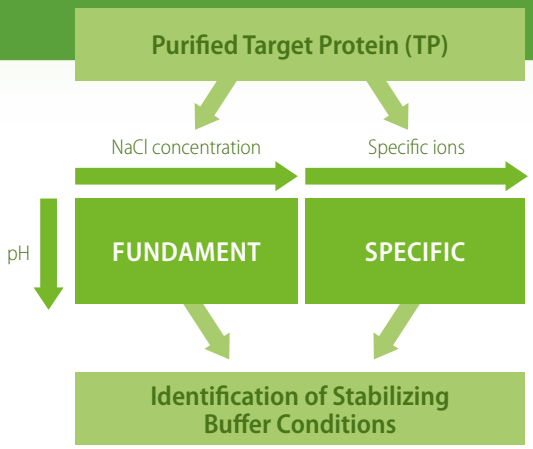
Identification of a buffer environment that enhances the protein's stability and homogeneity is crucial for the success in protein purification, characterization and crystallization[1-3].

The protein melting temperature (Tm), determined by monitoring the protein unfolding in a temperature-dependent manner, is used as a reporter to assess protein thermostability. The higher the Tm, the higher is the thermostability of the protein in that specific environment.

JBScreen Thermofluor is the first protein stability screen that reveals the effect of only one parameter at once. Based on Super Buffers[4], the entire pH range from 4.0 to 10.0 is screened with one and the same buffer system thereby keeping the chemical environment constant.

JBScreen Thermofluor FUNDAMENT allows screening the pure pH effect at different ionic strengths without interfering additive effects, that would occur when changing the buffer system. pH and ionic strength are FUNDAMENTAL factors that influence the ***whole*** **protein molecule**.

JBScreen Thermofluor SPECIFIC screens for specific high-scoring mono-, di- and trivalent cations derived from the pdb.[5] These specific factors affect **energetically important *hot spots* on the protein**.



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JBScreen Thermofluor SPECIFIC HTS TSA for protein stability	CS-333	93 solutions (0,5 ml each)	227,00

References:

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Fragment screening in crystals comes of age: 500 soaks in a week at Diamond I04-1

Frank von Delft[1),2),3)], Jose Brandao-Neto[1)], Patrick Collins[1)], Alice Douangamath[1)], Tobias Krojer[2)], Romain Tallon[2)], Jia Tsing Ng[2)], Anthony Bradley[2)], Nicholas Pearce[2)], Oakley Cox[4)], B. Marsden[2)], P. Brennan[2),4)]

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[4)]Target Discovery Institute (TDI), Nuffield Department of Medicine, University of Oxford, Oxford OX3 7FZ, UK

Fragment-based lead discovery is now a well-established as a powerful approach to early drug or lead discovery: since small (<250Da) compounds ("fragments") tend to bind relatively promiscuously, hits can be readily identified by screening against comparatively small compound libraries (100s-1000s). What remains challenging is that hits typically bind weakly: not only must the screening technique be sufficiently sensitive, but potency can only be achieved through considerable synthetic elaboration. Historically, the most sensitive primary screening technique of all, direct observation in crystal structures, has been too challenging to be achievable by but a few labs world-wide. Equally, no consensus has yet emerged on systematic strategies for synthetic follow-up.

Now, beamline I04-1 at Diamond Light Source has established X-ray screening as a routine medium-throughput experiment with a capacity of up to 500 crystals/day, the whole experiment (soaking to dataset) doable within a week. The facility is open to Diamond users since April 2015, with dedicated weekly beamtime. The highly streamlined process includes image recognition for crystal targeting, soaking by acoustic dispensing, robot-assisted harvesting, unattended X-ray data collection, automatic data integration, and pan-dataset electron density analysis for detecting hits. The technology was developed as a joint research project with the Protein Crystallography group of the SGC at Oxford University, and has been validated on a series of diverse targets, all of which have yielded hits.

Moreover, a "poised" fragment library has been developed that provides clear and robust routes to first-shell follow-up: combined with new algorithms for prioritizing compounds, the ultimate ambition is to establish how potency can be achieved cheaply from very limited initial experiments.

Flying through optimization screening with dragonfly

Paul Thaw

Product Manager – Crystallography
TTP Labtech Ltd. Melbourn Science Park, Melbourn, UK.

Protein crystal optimization is vital to ensure high quality X-ray diffraction data for the solving of high resolution structures. This process involves the set-up of a series of complex screening combinations where the ratios of the individual components identified from primary crystallization studies are varied.

In order to reduce the effort and tedium of this time consuming process, TTP Labtech have designed **dragonfly®** for crystallization screening as an addition to their successful mosquito® liquid handling portfolio.

dragonfly is a liquid handler for simple, fast and accurate crystal screen optimization with unsurpassed reproducibility. dragonfly's positive displacement technology ensures highly accurate dispensing, from 0.5 µL up to 4 mL, across a wide range of viscosities. Its rapid plate preparation is uniquely combined with non-contact dispensing to ensure zero cross-contamination. Dispense resolution is 0.1 µL, allowing very fine gradients to be created.

The partner software – dragonfly designer has been designed from the ground up to be an easy-to-use gradient design tool which is stand alone and provided without restriction on user numbers to significantly reduce ongoing costs.

In this talk we will review both the technology behind the dragonfly and real world examples of speeding up successful crystallization projects.

Advancements in Automated Imaging: Trace Fluorescence Labeling and SONICC

Jochen Müller-Dieckmann

PhD, Formulatrix.

Fast, reliable protein crystal identification has long been the bottleneck in the crystallography workflow. While enhancing throughput, automated high-resolution imaging with visible light is insufficient for positively identifying crystalline material in varying drop conditions. UV imaging addresses part of the issue, but is highly dependent on the amount of tryptophan inherent in the sample. In this presentation, we introduce Trace Fluorescence Labeling, a protocol that overcomes this obstacle, and its most complimentary imaging method, Second Order Non-Linear Imaging of Chiral Crystals (SONICC).

Trace Fluorescence Labeling overcomes the inherent tryptophan dependency required for successful UV imaging by labeling 0.1% of a sample with a variety of fluorescent dyes in a quick and simple process. In addition to providing a significantly enhanced signal to noise ratio, Trace Fluorescence Labeling can reveal the presence of different components within the multi-subunit components of a crystal. However, different labels can require different wavelengths for proper imaging. SONICC enables users to quickly change between various excitation wavelengths required by different dyes, while simultaneously offering the most reliable imaging to identify chiral crystals on the market. The presentation is complete with image examples from the field.

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Reassessing Rigaku Oxford Diffraction's HomeLab Rotating Anode X-ray Sources

Joseph D. Ferrara

Rigaku Oxford Diffraction

In this brief presentation I will review the properties of X-ray generators and highlight the benefits of the well characterized and reliable Rigaku MicroMax-007HF and FR-X. Both generators provide the same flux at both ports at the same time making them ideal for large facilities. As such they can be configured with state-of-the-art automated single crystal data collection systems and solution scattering systems.

Review of AFITT for automatically fitting ligand to density, refitting protein side chains and optimizing ligand conformations in BUSTER and PHENIX

G.L. Warren

OpenEye Scientific Software Inc., Santa Fe, NM, USA

We will present the algorithm AFITT. This algorithm automatically identifies potential ligand electron density, generates high quality bioactive conformations, adiabatically fits complimentary conformations into the identified density and generates a small molecule force field (MMFF94) derived dictionary file. We will review the features in AFITT for fitting ligand conformations to density, automatically refitting protein side chains and the provided helper files for optimizing ligand conformations in two refinement packages BUSTER and PHENIX. We present data showing that the use of the helper files dramatically improves ligand conformations as assessed by force field energy and bond and angle value deviations from small molecule crystal data.

The Oryx Range by Douglas Instruments: Special Tools for Crystallization

Presented by: Hilary McNeill
Co-Authors: Patrick Shaw Stewart and Stefan Kolek

Douglas Instruments Ltd, Douglas House, Westfield Farm, East Garston, Berkshire, RG177HD.

This presentation will cover the recently developed features of the Oryx robots for screening and quick-and-easy optimization in protein crystallization. Optimization options range from simple 2-D grids to multivariate experimental design (up to 7-D) with reservoir-filling for up to 24-wells. Our new 'Additive Scatter' script will also be described.

Dispensing with the Oryx involves minimal waste, with a dispensing range from 100+100nl to as large as 8+8µl drops. For aqueous dispensing, the Oryx range now includes a combined Microbatch and Sitting Drop Vapour Diffusion script and cover slide dispensing for Hanging Drop experiments, conveniently dispensing up to 5 drops per slide. For oil dispensing, the LCP option is available.

The presentation will briefly explain how random microseeding (rMMS) works well with the Oryx dispensing system, assuming that you have some initial crystal hits to make the seed stock from and why Douglas Instruments continues to recommend it as part of the Protein Crystallizer's routine workflow.

Lead Discovery at the Cutting Edge of Epigenetic Targets

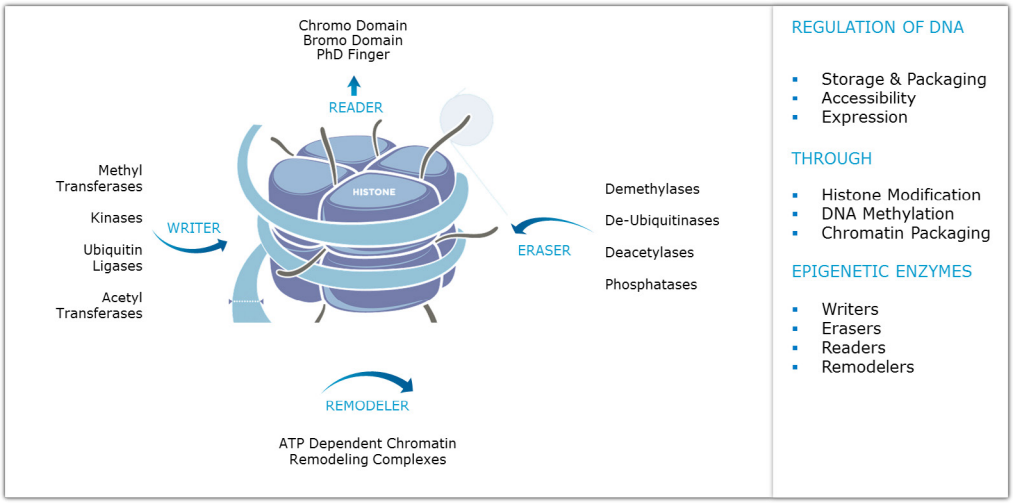
Torsten Hoffmann, Adrian Schomburg and Robert van der Burgh

Proteros biostructures GmbH, D-82152 Martinsried

There is an ever-growing body of evidence that epigenetic changes in gene expression play a profound role in normal development as well as in disease manifestation and progression. Reversing those changes resembles a fundamentally new approach in drug discovery for therapeutic interventions.

To target these highly challenging epigenetic mechanisms, we at Proteros utilize our proprietary nucleosomal epigenetic assay technologies (NEAT™). The patent protected nucleosome preparation and screening process of NEAT™ provides access to fully homogenous and scalable screening materials with uniquely introduced and tailored histone modifications.

Together with Proteros' well-established technology platform for structure-based ligand design, Medicinal Chemists are now further enabled to leverage the full potential of lead discovery against novel epigenetic targets.



Schematic representation of a nucleosome core and its manipulation through epigenetic enzymes

Discussion of new tools and techniques for membrane protein purification and crystallization.

Edward Pryor

Anatrace

Significant strides have been made in the field of membrane protein structural biology since the deposition of the first structure for this class 30 years ago. Despite the substantial growth and tremendous success, bottlenecks persist throughout the membrane protein workflow, including expression, solubilization, purification and crystallization. For almost as long, Anatrace Products has been a trusted resource, providing a large portfolio of detergents, lipids, and tools to the membrane protein research community. To further support the community, Anatrace has begun an expansion in its life sciences division to deliver best-in-class, forward-thinking tools enabling membrane protein research and, in particular, membrane protein crystallization. This expansion has been spurred by the acquisition of Microlytic North America, based on a shared vision to improve output of the crystallization workflow in general and with a special focus on small and large membrane protein research groups, alike.

Technological advances in rational drug design

Andreas Förster

on behalf of DECTRIS Ltd.

Rational drug design critically depends on structural information on the biological target to model ligands and optimize interactions. DECTRIS has developed the EIGER R series of Hybrid Photon Counting pixel detectors to be effectively noise-free and remarkably fast. Highly accurate data can be collected efficiently, making best use of precious beamtime at synchrotrons and laboratory X-ray sources. DECTRIS EIGER R series detectors can lead to better X-ray structures more quickly and provide optimal starting points for rational drug design."

If the dog won't hunt: Advanced mutagenesis strategies for crystallization of recalcitrant targets.

Artem Evdokimov

HarkerBIO LLC, Buffalo NY

The success of macromolecular crystallization experiment is traditionally determined by three key meta-factors: the quality and composition of the sample, the extent of crystallization space sampled (i.e. screening), and the intrinsic crystallization propensity of the target. Unfortunately the third factor is quite often dominant – and it is also the hardest one to manipulate. This short presentation summarizes some of the strategies for the rational design of crystallization-enhancing mutations that are employed to dramatically change protein crystallization behavior and elicit successful crystallization of difficult targets.

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Latest Developments in Home-Lab Macromolecular Crystallography

Vernon Smith

Bruker AXS GmbH, Oestliche Rheinbrueckenstrasse 49, 76187 Karlsruhe, Germany.

Recent Developments in x-ray instrumentation in the *D8 VENTURE* Systems have provided an opportunity to redefine their role in the SBDD workflow.

The introduction of the novel *METALJET* X-Ray source technology enables smaller x-ray beams of higher intensity to be produced than was previously possible with existing technologies. New *Active Pixel Sensor* technology finally makes available home-lab detectors that combine a large area, high sensitivity and low noise and fast readout for continuous data collection.

In situ crystallography has become popular at synchrotrons as a method to identify targets quickly and efficiently. The *ISX Stage* is designed to be mounted on the *KAPPA* stage and is compatible with any source. Being fully motorised and software controlled, it enables automated high-throughput screening and data collection from crystals *in situ*.

Discovery of kinase inhibitors binding outside the ATP site

Sandra W. Cowan-Jacob

Novartis Institutes for Biomedical Research
CH-4056 Basel, Switzerland

The potential of targeting novel pockets on kinases has been discussed for several years now. It is hoped that such compounds will be more selective, have different chemical properties, provide an avenue for dealing with resistance and lead to alternate functional readouts compared to inhibitors targeting the catalytic site. However, even though the idea of targeting other sites on kinases is not new, there is relatively little being reported. In this presentation the challenges of identifying these “allosteric” inhibitors will be discussed, and two examples will be presented where fragment-based screening and structure-based drug design were used to identify potent and selective inhibitors; one of which is in the clinic and the other which is a probe suitable for target validation experiments.

Structure and Biophysics: Fragment-based discovery of the first known inhibitors of PHGDH

Alex Milbradt[1)], Gareth Davies[1)], Kevin Embrey[1)], Jon Renshaw[1)], David Hargreaves[2)], Joe Patel[3)], Scott Cowen[3)], Nathan Fuller[3)]

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[2)]AstraZeneca, Discovery Sciences, Building 310, Cambridge Science Park, Milton Road, Cambridge, CB4 0WG, United Kingdom
[3)]AstraZeneca, Discovery Sciences, 35 Gatehouse Drive, Waltham, MA 02451, USA

Studies have linked the enzyme 3-phosphoglycerate dehydrogenase (PHGDH) to in vivo tumourgenesis in aggressive breast tumours. PHGDH utilises the cofactor NAD+ to convert 3-phosphoglycerate to 3-phoshohydroxypyruvate, the rate limiting step in the synthesis of serine. A number of tumours have an increased metabolic flux of glucose to lactate, with a large proportion of glycolytic carbon being diverted into the serine and glycine metabolism through PHGDH. Given the role of PHGDH in potentially driving tumour proliferation and tumourgenesis, we explored the druggability of this unprecedented target. Our hit finding strategies included an X-ray screen of an AstraZeneca fragment library. Multiple ligand efficient binders were identified, and the rich structural output enabled structure-based design strategies to greatly improve binding affinity in both biophysical and biochemical assays. The pivotal role of biophysics and structure in the optimization of an indole fragment hit into a series of nM inhibitors will be presented.

Discovery of BAY 85-8501, a Novel and Highly Potent Induced-Fit Binder of Human Neutrophil Elastase for Pulmonary Diseases

F. von Nussbaum, S. Anlauf, M. Delbeck, H. Gielen-Hertwig, A. Harrenga, D. Karthaus, D. Lang, V. Li, K. Lustig, D. Meibom, J. Mittendorf, M. Schäfer, S. Schäfer, J. Schamberger

Bayer Pharma AG

Human neutrophil elastase (HNE) is a key protease for matrix degradation. High HNE activity is observed in inflammatory diseases. Accordingly, HNE is a potential target for the treatment of pulmonary diseases such as chronic obstructive pulmonary disease (COPD), acute lung injury (ALI), acute respiratory distress syndrome (ARDS), bronchiectasis (BE), and pulmonary hypertension (PH). HNE inhibitors should reestablish the protease–anti-protease balance.

First potent elastase inhibitors described were biologicals. First small molecule inhibitors were reactive acylators or transition state mimetics. In general, selectivity is a high hurdle for serine protease inhibitors.

Starting from a sub-micromolar quinoline inhibitor that was found in a high-throughput screen, various chemical core modifications finally led to dihydropyrimidinones as a novel lead structure class with frozen bioactive conformation. Further chemical optimization of physicochemical parameters and metabolic stability yielded orally active compounds with favorable pharmacokinetics in rodents and dogs. Finally, a novel potent and selective class of small molecule hNE inhibitors was discovered. E.g. BAY 85-8501 showed picomolar in vitro potency versus hNE and high selectivity towards 21 serine proteases. These favorable characteristics are based on an induced-fit binding mode, allowing for tight interactions with the S2 and the S1 pocket. BAY 85-8501 showed in vivo activity in rodent animal models related to PH and ALI.

Structure of the kinase domain of TGFβR2

Andrew Tebben[1)], Max Ruzanov[1)], Mian Gao[1)], Dianlin Xie[1)], Susan Kiefer[1)], Chunhong Yan[1)], John Newitt[1)], Liping Zhang[1)], Kyoung Kim[1)], Hao Lu[2)], Lisa M. Kopcho[2)], Steven Sheriff[1)]

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The cytokine TGF-β consists of three isoforms that act pleiotropically on epithelial, endothelial, and hematopoietic cells to promote hemostasis, immunomodulation, extracelluar matrix deposition, and tumor suppression through differentiation, activation, and regulation of proliferation. TGF-β becomes a tumor promoter when cancer cells become insensitive to TGF-β growth suppression and overexpress TGF-β, which creates a microenvironment where tumor cells can grow unchecked, but surrounding and immune cells are inhibited. TGF-β signals through two transmembrane serine/threonine kinase receptors, TGFβR1 and TGFβR2. While the structure of the kinase domain of TGFβR1 has been known for over 15 years, to date no structure of the TGFβR2 kinase domain has been reported. The TGFβR2 kinase domain is extremely soluble and we found that a surface entropy reduction strategy was necessary to obtain crystals. A construct that yielded crystals that diffract to high resolution (<2 Å) contains mutations to Ala of 6 charged residues in a patch on the surface of the C-lobe of the TGFβR2 kinase domain. The apo structures and structures of staurosporine binding are compared for TGFβR1 kinase domain, TGFβR2 kinase domain, and a chimeric TGFβR1 kinase domain, where 7 active site residues have been mutated to their TGFβR2 equivalents.

Discovery and optimization of small-molecule inhibitors of transforming growth factor β receptor type I (TβRI)

Christiane Amendt, Bertram Cezanne, Paul Czodrowski, Dieter Dorsch, Ulrich Grädler, Hartmut Greiner, Per Hillertz, Günter Hölzemann, Alfred Jonczyk and Djordje Musil

Merck Serono R&D, Merck KGaA, Frankfurterstr. 250, D-64293 Darmstadt

TGF-β signalling regulates diverse cellular processes, including cell proliferation, differentiation, apoptosis, cell plasticity and migration. Its dysfunctions can result in various kinds of diseases, such as cancer and tissue fibrosis. TGF-β signalling is initiated by the binding of TGF-β to its serine and threonine kinase receptors, the type II (TβRII) and type I (TβRI) receptors on the cell membrane. Ligand binding induces formation of the receptor hetero-complex, in which TβRII activates TβRI by phosphorylation. Activated TβRI phosphorylates SMAD2 and SMAD4, which dissociate from the receptor to form a complex with SMAD4. The complex is subsequently translocated to the nucleus where it modulates the transcription of the TGF-beta-regulated genes.

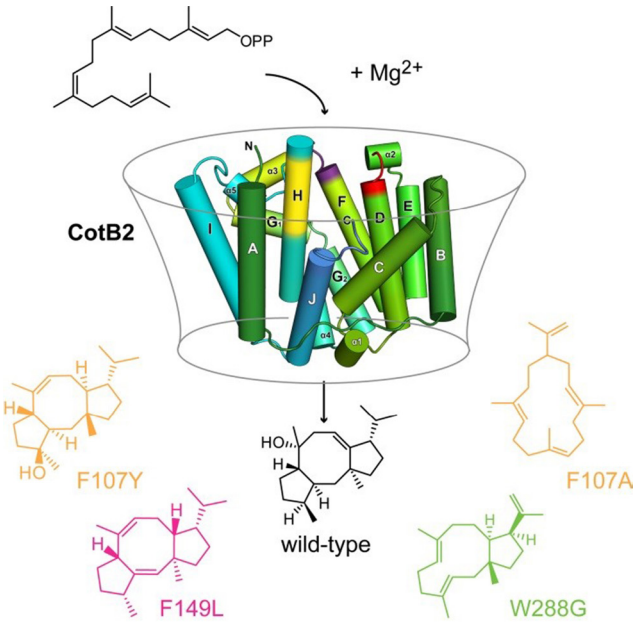
Since modulation of TGF-β receptor activity is a critical step in TGF-β signalling regulation, we focused on inhibiting TβRI kinase activity by small molecule ligands. Selected fragments as well as ligands from several optimization series were crystallized in complex with TβRI kinase domain to guide rational discovery of TβRI inhibitors. Results from fragment screening and from several hit and lead discovery and optimization cycles will be presented.

Production of macrocyclic diterpenes in bacterial hosts

Loll, Bernhard & von Moeller, Holger

moloX GmbH, Takustr. 6, 14195 Berlin, Germany

Sesqui- and diterpenes are a diverse class of secondary metabolites predominantly derived from plants and prokaryotes. Properties of these natural products encompass anti-tumor, antibiotic, and even insecticidal activities. Therefore they are interesting commercial targets for the chemical and pharmaceutical industry. Due to their structural complexity these compounds are more efficiently accessed by metabolic engineering of microbial systems than by chemical synthesis. Here we report, the first crystal structure of a bacterial diterpene cyclase CotB2, that cyclase that catalyzes the cyclization of linear geranylgeranyl diphosphate to tri-cyclic cyclooctat-9-en-7-ol. Subsequent oxidation by two cytochrome P450 monooxygenases leads to bioactive cyclooctatin. Plasticity residues that decorate the active site of CotB2 have been mutated, resulting in alternative mono-, di- and tri-cyclic compounds that show bioactivity. Now, we have successfully incorporated CotB2 in a downstream element of a microbial production platform in E. coli that allows for production of compounds.



Optimising and driving synchrotron experiments as a third party

Gerard Bricogne,
Claus Flensburg, Peter Keller, Wlodek Paciorek & Clemens Vonrhein

Global Phasing Ltd, Sheraton House, Castle Park, Cambridge CB3 0AX, UK

The ever-increasing speed of MX beamline instrumentation is leading to ever-stronger emphasis being placed on brevity of execution as the main design goal for data collection protocols, often to the exclusion of other criteria that would aim at achieving higher data quality. This can be counter-productive, especially, but not only, for phasing experiments.

Global Phasing, among others, has been interested in bucking that trend by creating combined capabilities for the fast design of optimal strategies and the direct supervision of their execution on an actual beamline. Our approach has been to aim for a full “third-party design and control” capability rather for than separate add-on programs that would need to be invoked by local software on each specific group of beamlines running under given control software.

Progress in implementing this ambitious paradigm will be presented, along with plans and collaborative links for disseminating this work to European and US synchrotrons.

Recent developments in *Phaser*

Randy J Read

Cambridge Institute for Medical Research, Department of Haematology, University of Cambridge, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, UK

Traditional algorithms in macromolecular crystallography were based on intuitive use of the properties of the Patterson function (e.g. finding the positions of heavy atoms, solving structures by molecular replacement), or on minimising the differences between observed and calculated diffraction data with least-squares methods. In the last two decades, most of these algorithms have been adapted to use the principle of maximum likelihood, which has the great advantage of taking proper account of the effects of errors in the data and the models. Our program Phaser uses likelihood as a very sensitive target for the solution of structures by molecular replacement (especially relevant when the model is poor or comprises only a small fraction of the asymmetric unit) as well as for phasing by single-wavelength anomalous diffraction (SAD).

There is a significant, though not prohibitive, computational cost to the increased rigour of the likelihood targets, so one recent focus in Phaser has been to make it more efficient. It turns out that, for molecular replacement, it is possible to predict the signal that will be obtained from the size and estimated RMS error of any particular model. This allows us to optimise the search order for different components, and even to choose which resolution limit should be sufficient for a successful search. Other enhancements allow multiple copies of a model to be placed simultaneously, instead of working through a full tree search with one copy placed at a time.

Another focus of effort is on dealing with frequently encountered pathologies. By working out the statistical effect of the presence of translational non-crystallographic symmetry (tNCS), we have been able to solve some structures that eluded previous versions of Phaser and other programs. We are currently implementing algorithms that deal much better with very weak intensity data, encountered for the systematically weak data arising from tNCS or severe anisotropy, or even from pushing the resolution limit of poorly diffracting crystals.

Finally, we are aiming to apply our algorithms at the interface between macromolecular crystallography and the exciting new developments with cryo-EM: using cryo-EM reconstructions to solve crystal structures (as done for the Cascade complex of the CRISPR system) or providing an alternative algorithm to dock crystal structures into EM images.

Using Coot tools for protein-ligand complex model validation

Paul Emsley

MRC-Laboratory of Molecular Biology, Cambridge

A number of tools related to handling of ligands have been added to Coot [1,2] in recent years - these include 2D depictions, ligand binding pocket layout and, most recently, a ligand scoring system - which will be the focus of this presentation.
Coot incorporates a number interface to external tools (CCP4's Refmac, Molprobity's probe and reduce and the CCDC's Mogul) to generate score for protein ligand complexes. This scoring system has been applied to models (with data) from the PDB. The details of the ligand density fit score and distortion score and the application to one's own complex structure will be discussed.

[1] "Features and Development of Coot" Emsley P, Lohkamp B, Scott W, Cowtan K, (2010) Acta Cryst. D. 66, 486-501
[2] "Handling ligands with Coot" Judit É. Debreczeni, Paul Emsley (2012) Acta Cryst. D, 68, 425-430

Second-harmonic generation (SHG) as a sensitive, real-time probe of protein structure

Josh Salafsky

Biodesy

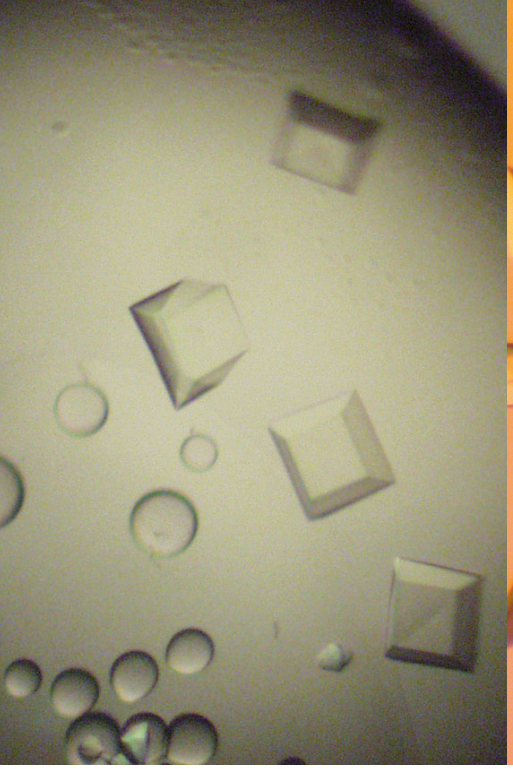
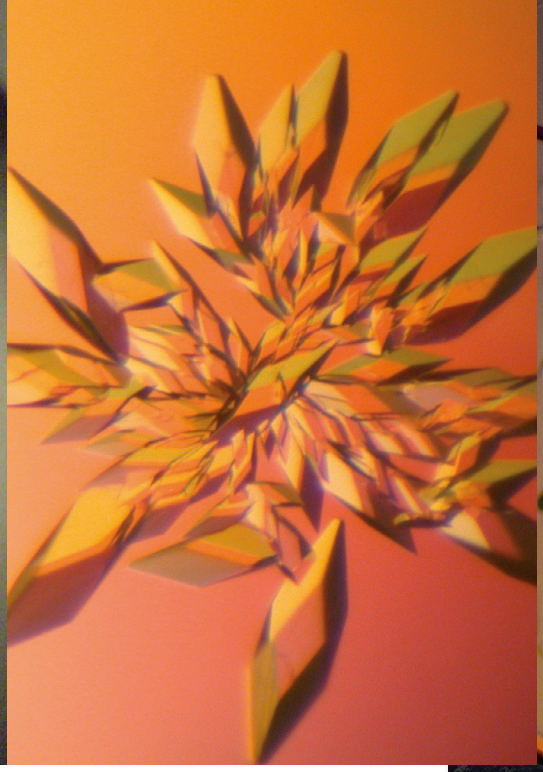
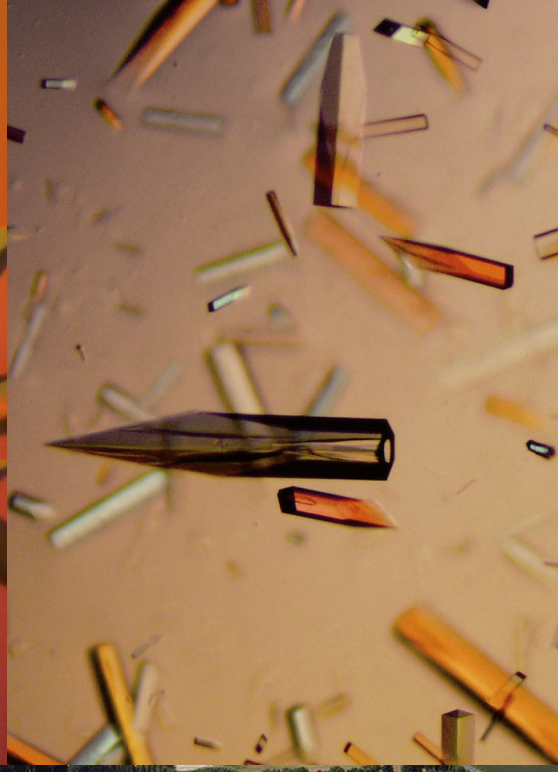
Second-harmonic generation (SHG) is emerging as a highly sensitive probe of protein structure and conformation in solution and in real-time (1,2). To date we have built conformational assays for more than 30 protein targets. Here I briefly review the technique and present data from a number of studies. SHG easily distinguishes between protein conformations and thus bins ligands by mechanism. For example, SHG assays of kinase proteins, both KD and full-length, produce clear and different responses in conformation upon binding type-I, II or III inhibitors. SHG provides information that can be directly linked to functional, cell-based data. Throughput is on the order of 1000's of measurements/day, and a variety of assay formats can be developed to rapidly identify and characterize allosteric or orthosteric binders. As conformational changes determine protein function, SHG provides crucial structural data in a native-like environment.

1. Small molecules detected by second-harmonic generation modulate the conformation of monomeric alpha-synuclein and reduce its aggregation in cells

Moree, B.; Yin G.; Lázaro, D.F.; Munari, F.; Strohäker, T.; Giller, K.; Becker, S.; Outeiro, T.F.; Zweckstetter, M. and Salafsky, J. *The Journal of Biological Chemistry.* **(2015).**

2. Protein conformational changes are detected and resolved site specifically by second-harmonic generation

Moree, B.; Connell, K.; Mortensen, R.B.; Liu, C.T.; Benkovic, S.J. and Salafsky, J. *Biophysical Journal. 109,* 806 – 815 (2015).

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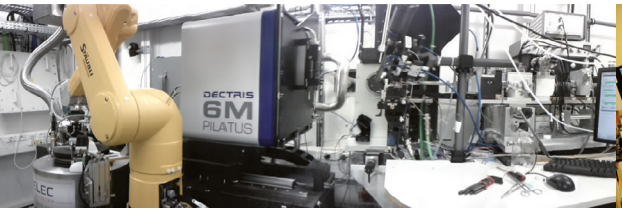


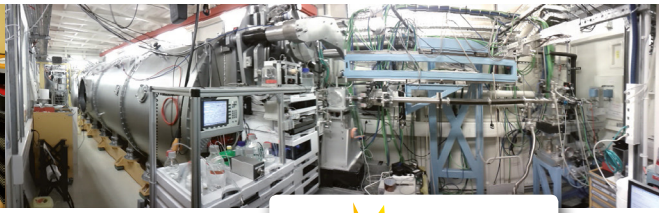

Contact Tatiana Isabet (Industrial liaison scientist for life science and health applications)
at the PSDI 2015 or tatiana.isabet@synchrotron-soleil.fr

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New tools and techniques at the service of drug discovery at ESRF

S. Monaco-Malbet[1], *on behalf of the ESRF/EMBL Joint Structural Biology Group, European Synchrotron Radiation Facility, 71 Avenue des martyrs, CS40220, 38043 Grenoble cedex 9, France.*

This talk will focus on the range of tools that ESRF has recently implemented on the structural biology beamlines of ESRF including completely automatic, high performance diffraction data collection[1] for fragment screening on robust crystal systems (MASSIF-1, mail-in service); the collection of diffraction data from small crystals that cannot be aligned manually in the X-ray beam; multi-crystal diffraction data collection[2], treatment and analysis from systems where only tiny (< 10mm, largest dimension) crystals of a system under study are available. A vision for future ESRF facilities for structural biology (*in situ* data collection, the provision of micron-sized beams for serial crystallography) will also be presented.

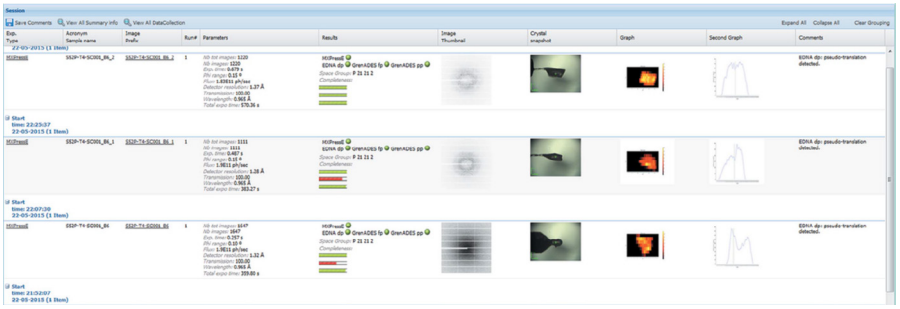


Figure 1: ISPyB reporting of results from completely automatic diffraction data collection carried out in ESRF beamline MASSIF-1.

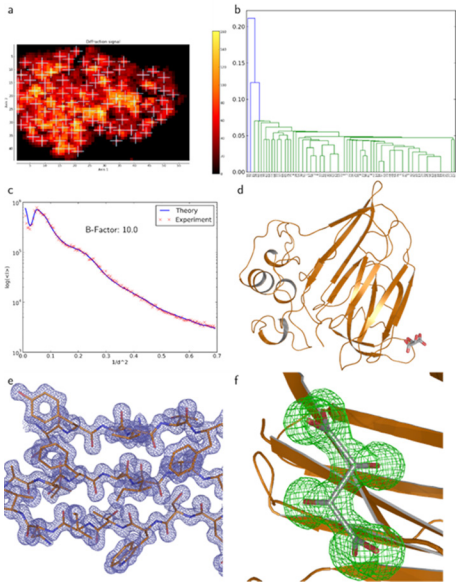


Figure 2: The multi-crystal workflow available at all ESRF MX beamlines.

[1] M.W. Bowler et al., (2015). J. Synchrotron Rad. 22, doi:10.1107/S1600577515016604;

[2] U. Zander et al., (2015). Acta. Cryst., D71, in press

Why do we need biologics structures, especially antibody-antigen complex structures? Structure-guided antibody engineering and humanization

Guy Georges,

Roche, Penzberg, Germany

Antibody discovery and engineering importance for pharmaceutical industries has grown significantly following Biotech's pioneering work over the last 2 decades. During the past few years, modeling software companies have developed their own tool for antibody modeling; all taking advantage of a huge structural knowledge existing in the protein database (PDB). The number of structures containing an antibody VH-VL motif was 1830 on July 1st 2015. But modeling relies on experimental data; the antibody repertoire diversity is around 10 billion and specific maturation extends it to 10^{13}.

First, an antibody structural database, dissected in heavy and light chains, and in frameworks and CDRs (Complement Determining Regions) segments, comprises a numbering system that enables novel features in searching templates during modeling. Secondly, a modeling tool is automatically producing models of high quality at a very low computational cost; a benchmark analysis reveals that our modeling tool systematically better performs than all other published ones. The template search focusses on structurally relevant portions, e.g. CDR3H split in ascending and descending portion from the loop peak, and an accurate prediction of the relative VH-VL orientation ensure the model quality.

The VH-VL orientation prediction is also used to predict the CDR grafting success during humanization. Quantitative correlations between VH-VL orientation and affinity to antigen will be presented. Finally and for the very first time, a technology based on neighborhood search is used to predict the position of side chains; this represents a fantastic add-on on the classical homology modeling (sequence-based) approach.

Despite excellent progress in antibody modeling, a great need to understand much more deeply the rules governing the specific recognition of antigen remains acute. Consequently, the biologics community is constantly looking for opportunity to increase the number of complex-structure in the public domain, i.e. the PDB, or to at least make available unpublished structures from much less relevant candidates or/and stopped projects.

Biologics structure based rational design, validation and characterization

Presenter – Alexey Rak
Thi-Lan-Anh Le, Saskia Villinger, Anke Steinmetz, Alain Dupuy, Alexey Rak

Structure-Design-Informatics, LGCR at Sanofi R&D, 13,
Quai Jules 94403 Vitry sur Seine FRANCE - for all the authors

Biologics modality represents substantial part of Pharmaceutical companies portfolio nowadays. Structural biology and biophysical methods can improve time and cost-effectiveness in biologics research through the application of rational structure based approaches. Examples of structural based biologics design, optimization and characterization in Sanofi laboratories will be presented and discussed.

Antibodies and NCEs Opportunities at the Interface

Tom Ceska and Alastair Lawson

UCB Celltech, 216 Bath Road, Slough, UK, SL1 3WE

Structure-based antibody engineering

Alexey Teplyakov, Galina Obmolova, Thomas Malia, Jeffrey Luo, Gary L. Gilliland

Janssen R&D, 1400 McKean Road, Spring House, PA 19477, USA.

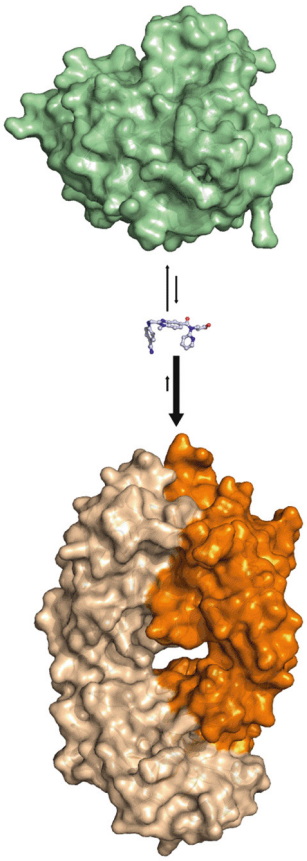
Detailed and accurate structural information obtained by X-ray analysis builds a solid base for rational antibody engineering. Crystal structures of the Fab fragment in the unbound state and in complex with the antigen provide complementary pieces of information. The complex structure describes the antibody-antigen interactions and is of prime value for affinity maturation of the antibody. On the other hand, the Fab structure in the unbound state describes the conformation of the antigen-binding loops, CDRs, and may be useful in antibody humanization and for evaluating the risks of post-translational modifications. Comparison of the two structures often reveals the regions of conformational mobility or points of tension that may be the focus of antibody optimization. Selected examples illustrate various aspects of structure-based antibody engineering at Janssen R&D.

Specific Antidotes For Dabigatran: Structure-Guided Affinity Optimisation and Functional Characterisation

Herbert Nar

Boehringer Ingelheim GmbH & Co. KG, Biberach, Germany

Dabigatran etexilate is a direct thrombin inhibitor and widely used as an anticoagulant for the prevention of stroke in patients with atrial fibrillation (1 & 2). Despite clear advantages in efficacy and safety above traditional treatment options, anticoagulation therapy with dabigatran is associated with a risk of bleeding.



Here, we present data on the identification, humanization and in vitro pharmacology of an antibody fragment for dabigatran, idarucizumab. The X-ray crystal structure of dabigatran in complex with the antidote reveals many structural similarities of dabigatran recognition compared to thrombin. By a tighter network of interactions, the antidote achieves an affinity for dabigatran that is ~350 times stronger than its affinity for thrombin. Despite the structural similarities in the mode of dabigatran binding, the antidote does not bind known thrombin substrates and has no activity in coagulation tests or platelet aggregation. In addition we demonstrate that the antidote rapidly reversed the anticoagulant activity of dabigatran in vivo in a rat model of anticoagulation. This is the first specific antidote for a next-generation anticoagulant which may become a valuable tool in patients that require emergency procedures (3).

Further, we present a distinct, novel antibody fragment against dabigatran that was identified in a search for backup clinical candidates to idarucizumab. By structure-guided protein design we were able to improve the affinity and residence time of the original FAB significantly. The optimized mutant exhibits prolonged and more effective neutralization of dabigatran's anticoagulative effects in vivo compared to the parental antibody fragment (4).

References:

(1) van Ryn, Joanne; Goss, Ashley; Hauel, Norbert; Wienen, Wolfgang; Priepke, Henning; Nar, Herbert; Clemens, Andreas, Frontiers in Cardiovascular and Smooth Muscle Pharmacology (2013), 4, 12-19

(2) Hauel, Norbert; Clemens, Andreas; Nar, Herbert; Priepke, Henning; van Ryn, Joanne; Wienen, Wolfgang, The Discovery of Dabigatran Etexilate, in Analogue-based Drug Discovery III, First Edition. Edited by Janos Fischer, C. Robin Ganellin, and David P. Rotella. (2013) Wiley-VCH Verlag GmbH & Co. KgaA, p. 243-267

(3) Schiele, Felix; van Ryn, Joanne; Canada, Keith; Newsome, Corey; Sepulveda, Eliud; Park, John; Nar, Herbert; Litzenburger, Tobias, Blood (2013), 121(18), 3554-3562

(4) Schiele, Felix; van Ryn, Joanne; Litzenburger, Tobias; Ritter, Michael; Seeliger, Daniel; Nar, Herbert, mAbs (2015) 7:5, 871

Novel Mcl-1 Antibody Assisted Crystal System: supporting a DNA encoded library hit finding strategy targeting Protein-Protein Interactions.

David Hargreaves

AstraZeneca, Cambridge

Mcl-1 is an anti-apoptotic protein (Bcl-2 family). Tumors with elevated Mcl-1 levels display Mcl-1 dependence. While Mcl-1 inhibition is a high value target, the long shallow binding groove of Mcl-1 has proven difficult to drug, with many literature-reported compounds possessing off-target activities which clouds the understanding of the biological function of Mcl-1.

This talk will cover the production of a novel antibody mediated Mcl-1 crystal system which provided key structural information for a DNA encoded library screening hit finding campaign. The discovery, structure, validation and SAR of a novel series of diaminopyridines as Mcl-1 antagonists are described.

Structural Biology in Vaccine Research

Matthew Bottomley

GSK Vaccines, Siena, Italy.

Advances in structural biology have dramatically increased the ability to characterize the atomic structures of protein antigens, providing new opportunities for the design of vaccines that were so far impossible. This presentation describes work on two different antigens (the Neisseria meningitidis Factor H Binding Protein and the Respiratory Syncytial Virus F protein) where the protein structure enabled the development of novel vaccine candidates. The implications for structure-based design of future vaccine antigens will be discussed.

Poster Abstracts

Design and Utilisation of a Poised Fragment Library to Discover Inhibitors of PHIP(2), an Atypical Bromodomain

Oakley Cox

University of Oxford, TDI, NDM Research Building, Old Road, OX3 7DQ, Oxford, UK

Oakley B. Cox[1),2),3)], Tobias Krojer[2)], Patrick Collins[3)], Romain Talon[2)], Anthony Bradley[2)], Octovia Monteiro[1),2)], Oleg Fedorov[1),2)], John Spencer[4)], Frank von Delft[2),3),5)] and Paul E. Brennan[1),2)]

[1)]*Target Discovery Institute (TDI), Nuffield Department of Medicine, University of Oxford, Oxford OX3 7FZ, UK.*

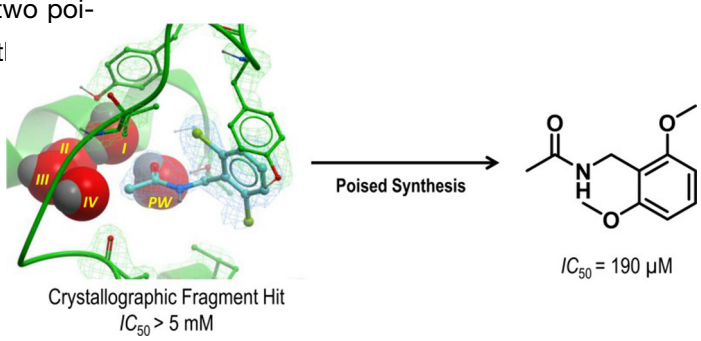
[2)]*Structural Genomics Consortium (SGC), University of Oxford, Oxford OX3 7DQ, UK.*

[3)]*Diamond Light Source (DLS), Harwell Science and Innovation Campus, Didcot, OX11 0DE, UK.*

[4)]*Department of Chemistry, School of Life Sciences, University of Sussex, Brighton, BN1 9QJ, UK.*

[5)]*Department of Biochemistry, University of Johannesburg, Aukland Park 2006, South Africa.*

Research into the chemical biology of bromodomains has been driven by the development of acetyl-lysine mimetics. The ligands are typically anchored by binding to a highly conserved asparagine residue. Atypical bromodomains, for which the asparagine is mutated, have thus far proven elusive targets, including PHIP(2) whose parent protein, PHIP, has been linked to disease progression in diabetes and cancers. The PHIP(2) binding site contains a threonine in place of asparagine, and solution screening have yielded no convincing hits. By combining the sensitivity of X-ray crystallography, used as the primary fragment screen, with rapid follow-up synthesis we have identified the first reported hit compounds of PHIP(2) with measurable IC_{50} by AlphaScreen competition assay. The use of a chemically-poised fragment library allowed hits to be readily modified by parallel chemistry both peripherally and in the core. The follow-up libraries of two poised fragment hits improved potency into t sub-mM range while showing good ligand efficiency and detailed structural data.



Crystallographic Fragment Hit
IC_{50} > 5 mM

IC_{50} = 190 µM

Combinatorial Approaches to Identify and Validate Small Molecule Inhibitors using Biophysical Techniques

Selina Carl, Katharina Klat, Florian Krieger, Julia Pflaumenbaum, Julian Plaga, Martin Prahm, Katja Unger, Dennis Wegener, Halina Wojtowicz, Patrick Zielinski

Evotec AG
Manfred Eigen Campus
Essener Bogen 7
22419 Hamburg (Germany)

Biophysical methods have become attractive screening techniques in drug discovery to both discover primary hits, as well as to validate hit compounds identified through conventional biochemical or cellular assays. At Evotec we are developing an assay platform focussing on the application of various biophysical techniques for progression of hit compounds in crystallographic studies including surface plasmon resonance (SPR), differential scanning fluorimetry (DSF) and microscale thermophoresis (MST). The application of combinatorial approaches of biophysical techniques additionally allows for the verification of several biophysical parameters such as IC_{50}, K_D, k_{on}, and k_{off} which may guide chemical development of a ligand series. We present several examples for the rigorous application of combined approaches resulting in the identification of mode of action of hit compounds and successful nomination of hit compounds for crystallography studies.

Three ways to active, stable, wild-type membrane proteins

Barbara Maertens[1)], Erik Henrich[2)], Frank Bernhard[2)], Jan Kubicek[1)], Roland Fabis[1)]

[1)]Cube Biotech GmbH, Monheim, Germany,
[2)]Institute for Biophysical Chemistry, University Frankfurt, Germany

Membrane proteins are of key relevance for pharmaceutical research, but due to their biophysical properties, it is still difficult to prepare high quality samples for structural and functional analyses. Mutations have been widely used to increase the stability of membrane proteins, but at the risk of compromising activity and structure.

We developed three methods to prepare wild-type, active membrane proteins:

(1) Enrichment of active membrane proteins by protein-specific affinity chromatography, based on interaction with a specific ligand. Inactive or partially folded protein fractions are removed. It was possible to measure agonist binding activity and to determine the KD of a human GPCR by SPR.

(2) Target-specific optimization of lipids to support activity and structure. A significant increase of agonist binding activity was detected after optimization of the lipid composition in a human GPCR.

(3) Reconstitution of the nascent membrane protein into nanodiscs during a cell-free protein expression reaction, providing a “near-natural” surrounding of the transmembrane regions. We developed a screening platform that was successfully applied to >30 of human GPCRs and transporters. Expression success rates were >90%, activity measurements are in progress.

Structural Analysis of Proteins by Electron Microscopy

Anette Schneemann, Joyce J. Sung, Jeffrey A. Speir, Sean K. Mulligan, Joel Quispe, Kathy On, Bridget Carragher, Clinton S. Potter

NanoImaging Services, Inc., San Diego, CA USA

Cryo transmission electron microscopy (cryoTEM) is a well established method for analyzing the structure of biological macromolecules. The resolution of cryoTEM maps was limited to the (sub)nanometer range until recently but development of direct electron detectors and improvements in image processing software have led to a so-called "resolution revolution" in the EM field (Kühlbrandt W, Science 343:1443). Several protein structures have now been solved at near atomic resolution by cryoTEM and many more are likely to follow. Here we illustrate the power of the method using a cryoTEM structure of the 20S proteasome from Thermoplasma acidophilum solved at 2.8Å using single particle reconstruction methods (Campbell M, Elife 4, e06380). The electron density map revealed a level of detail not previously observed for cryoTEM structures. Secondary structural elements of the polypeptide backbone as well as amino acid side chain conformations and ordered water molecules were clearly resolved. The results suggest that cryoTEM is a viable alternative to X-ray analysis for high resolution structure determination, particularly for proteins that are difficult to crystallize or produce in sufficient quantity. They also establish cryoTEM as a suitable method for drug discovery and development.

While high resolution 3D structure determination by cryoTEM is often the primary interest, low resolution 2D projection images can also provide a wealth of information that may be difficult to obtain using other methods. This is illustrated in two examples that address a range of issues in structure-function relationships of antibodies. Different imaging and analysis techniques were employed to probe specific aspects in each case: (i) Negative staining and 2D class averaging were used to visualize and characterize chicken IgY, a type of antibody whose structure remains poorly characterized. In contrast to current hypotheses, which predict IgY to be a relatively rigid molecule, we find that it is highly flexible and comparable to IgG in this regard. As expected, its Fc region is longer than that of IgG given the presence of an extra constant domain in this part of the molecule. (ii) Alignment and 2D class averaging of mammalian IgM, combined with selective masking and sub-classification, revealed that the five antibody monomers are arranged asymmetrically around a central hub. This, too, is in contrast to prevailing models which show the monomers to be symmetrically arranged. These results demonstrate that TEM is well suited to address a range of issues in protein structure and function that may be refractory to other experimental methods.

The facilities for structural biology at synchrotron SOLEIL

Tatiana Isabet, Leonard Chavas, Javier Perez, William Shepard, Andrew Thompson

Synchrotron SOLEIL L'orme des merisiers 91192 Gif sur Yvette, France

Since 2008, the SOLEIL synchrotron, located on the plateau of Saclay near Paris, has been open to scientists from both academic labs and industrial companies. A strong structural biology activity is based primarily on the two beamlines dedicated to bio-crystallography (PROXIMA 1 and 2) and the beamline for small angle X-ray scattering, SAXS (SWING).

PROXIMA 1 and 2, have a complementary design in view of collecting the best diffraction data from a wide variety of samples. The philosophy and approach at SOLEIL is to allow for high quality experiments to be performed, and consequently favoured over high-throughput and automated applications. This will to collect the best data possible was the starting point in the construction of our beamlines, with for instance optimisations aiming at reducing background noise, collecting with various protocols such as helicoidal scans, grid scans, inverse beams, full implementation of kappa goniometry etc. One SOLEIL staff member is dedicated to industrial users, helping them to collect the best data as quickly as possible.

PROXIMA 1, supervised by Leonard CHAVAS, operates at 450mA stored current with world-class stability and high flux (1.4 x 1E12 ph/s @ 8keV). The beamline is equipped with a full kappa goniometry, a pixel detector (Pilatus-6M, 25Hz) to speed up data collection, and operates using a monochromatic beam with a tunable energy in a range between 5 - 15 KeV, which covers most of the commonly exploited edges for anomalous experiments. A CATS sample changer robot is also implemented on the beamline, configured for sample cassettes (UniPuck 1 format) giving a sample capacity of 48 samples without entering the X-ray hutch. We recently further improved the quality of the data collected through the implementation of a capillary / pinhole hence reducing air diffusion and scattering to unreached levels. Coupled with a newly designed beamstop, this allows to the user to obtain exceptionally low background noise.

PROXIMA 2, supervised by William SHEPARD, is a microfocus beamline (5x3 microns2) equipped with a microdiffractometer (MD2) and will shortly (October 2015) be equipped with an EIGER 9M pixel detector, well adapted to microfocus beam, applications. The beamline also has a full coverage of energies between 5 and 15 keV.

SWING is a beamline where half the beamtime is devoted to Bio-SAXS, with a flux of 5.10^{12} photons/s at 12 keV and a typical beam size of 400 µm x 80 µm (FWHM). A 17 x 17 cm^2 AVIEX CCD detector is positioned on a three axes translation stage within a large chamber under primary vacuum, allowing for virtually immediate distance modification, from 0.6 to 8 m. Absolute intensity measurements are achieved using reference samples and calibrated intensity monitors. The routinely available range of q ($q=4\pi \sin\theta / \lambda$) using the two extreme distances is 1.6 10^{-3} – 2.8 $Å^{-1}$.

An in vacuum thermostated Quartz capillary cell permits precise and reproducible measurements of proteins in solution. A SEC HPLC device directly coupled to the SAXS measurement cell is permanently installed for online purification. Medium throughput data collection can be achieved using an automated injector, with a typical sample volume of 20 µl. Switching between the two modes is immediate. A friendly graphical application, Foxtrot, allows for online and offline data reduction.

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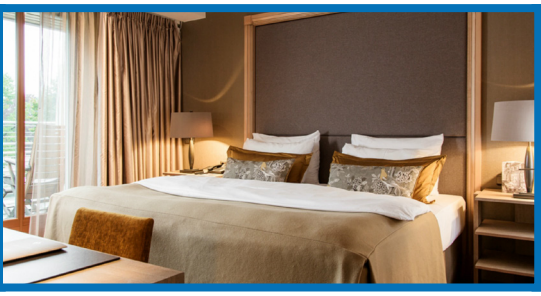
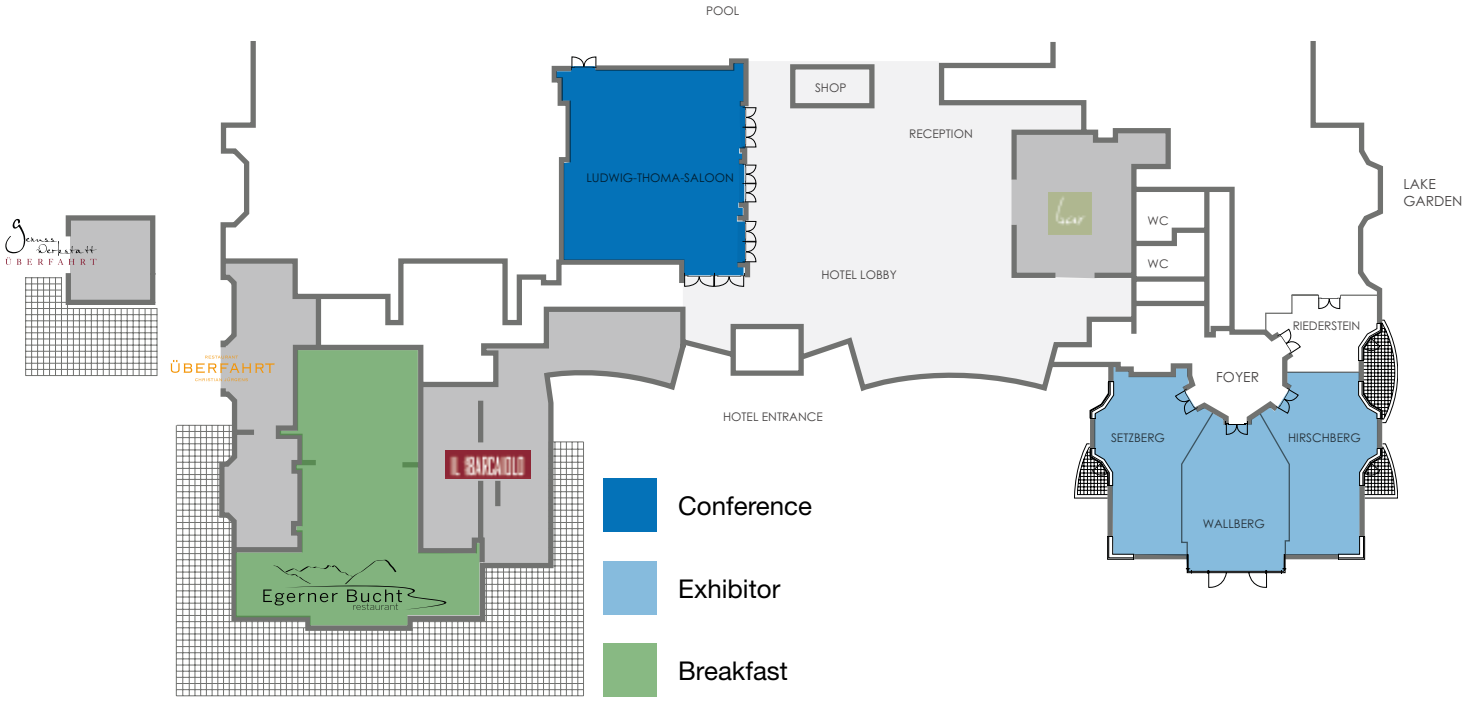


Floor Plan – Hotel



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„Let‘s hear it for hospitality“ or as the bavarians say: „Ein Prosit der Gemütlichkeit“!

Shuttles to Conference Dinner

Please find below the time table for shuttle to the Conference Dinner at „Herzogliches Bräustüberl Tegernsee“.
The dinner will be held on November 9, at „Herzogliches Bräustüberl Tegernsee“, about 10 min drive from the Althoff Seehotel Überfahrt.

Conference Dinner – Monday, November 9	
18.30	Departure to Conference Dinner
19:15–22:45	Dinner at Herzogliches Bräustüberl Tegernsee
22:00	Bus 1 departure back to the hotel
22:30	Bus 2 departure back to the hotel
22:50	Bus 3 departure back to the hotel
Dresscode:	Casual

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Munich – M´unique

The City of Munich holds a unique position in Germany with all the prerequisites for authentic and exciting incentives and motivational programs.
With its world-famous „Gemütlichkeit“ (coziness) and colorful traditions, its wealth of art and culture, excellent infrastructure and unparalleled location at the foot of the Bavarian Alps, it is the perfect destination.

Things to do

Go to the heart of the city of Munich, Marienplatz and join the audience for a performance of the famous Glockenspiel. Thirty-two mechanically moved figures re-enact scenes of Munich‘s history.

Climb up the 306 steps to the tower of St.Peter‘s (called „Alter Peter“) and enjoy the best overwiev of the city. St.Peter‘s is Munich‘s oldest church.

Feel the atmosphere of Munich‘s beer halls at the famous Hofbräuhaus drinking a Maß Beer (= 1 litre of white beer) and eating Weißwurst.

Tegernsee

If there is a place in the world where everything is still in order, it's the Tegernsee Valley. This is where cattle graze on lush green meadows under white and blue skies at one of the cleanest lakes in Bavaria. Where traditions are fostered and traditional costumes worn with pride. This is where you will find a reason to celebrate and things to do all year round.

Tegernsee / Rottach-Egern

Style, elegance and good living are what the attractive and lively town of Rottach-Egern is famous for. Many well-known painters, poets and composers were inspired by the bustling cultural life at the southern end of the lake. Today, guests from all over the world savour the energetic and exclusive atmosphere around the Egerner Bay, where zeitgeist and tradition mingle. Haute cuisine and historical Bavarian guest houses, luxury hotels and cosy holiday farms, fancy boutiques and the picturesque lakefront all invite visitors to create their very own holiday experience. This diversity is also reflected in the range of cultural and sports activities available.

History of PSDI Meetings

PSDI 1 - 1993 - Zeneca, United Kingdom
PSDI 2 - 1994 - SmithKline Beecham, United Kingdom
PSDI 3 - 1995 - GlaxoWellcome, United Kingdom
PSDI 4 - 1996 - Pfizer Sandwich, United Kingdom
PSDI 5 - 1997 - Zeneca, United Kingdom
PSDI 6 - 1998 - Roche Welwyn, United Kingdom
PSDI 7 - 1999 - SmithKline Beecham, United Kingdom
PSDI 8 - 2000 - Aventis, France
PSDI 9 - 2001 - GlaxoSmithKline, United Kingdom
PSDI 10 - 2002 - AstraZeneca, United Kingdom
PSDI 11 - 2003 - Medivir, Sweden
PSDI 12 - 2004 - Pfizer, United Kingdom
PSDI 13 - 2005 - Astex, United Kingdom
PSDI 14 - 2006 - Novartis, Italy
PSDI 15 - 2007 - ESRF, France
PSDI 16 - 2008 - GlaxoSmithKline, United Kingdom
PSDI 17 - 2009 - Roche, Basel, Switzerland
PSDI 18 - 2010 - Diamond Light Source, Randolph Hotel Oxford, United Kingdom
PSDI 19 - 2011 - AstraZeneca, Mölndal, Sweden
PSDI 20 - 2012 - Sanofi, Chateau de Montvillargenne, France
PSDI 21 - 2013 - PSI Expose, Hotel Astoria Lucerne, Switzerland
PSDI 22 - 2014 - MERCK, Hotel Miragem, Lisbon/Cascais, Portugal

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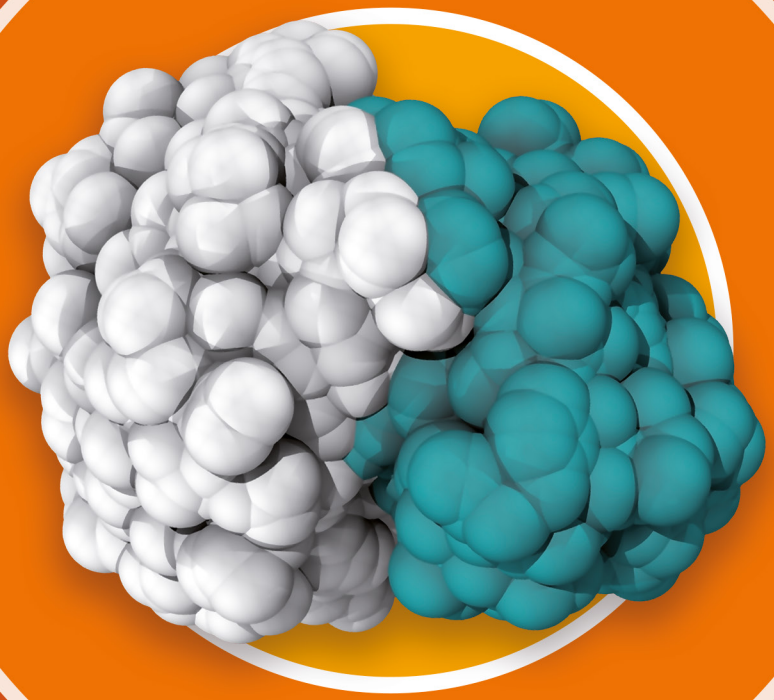
BIOSAXS

Small angle X-ray scattering from pharmaceutical formulations to study

- Solubility/stability at various conditions
- Assembly and folding state
- Interactions and complex formation
- Oligomerization equilibria
- Effects of ligand binding
- Size and shape



Describe the problem
Send the samples
Receive the answers

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Notes

Notes