26th Protein Structure Determination in Industry conference

11th -13th November 2018
Versailles, France

PSDI 2018

Book of Abstracts

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PSDI 2018 - 26th Protein Structure Determination in Industry Meeting
11th – 13th November 2018
Hôtel Le Louis, Versailles Château – France

https://www.synchrotron-soleil.fr/fr/evenements/psdi-2018-26th-protein-structure-determination-industry-meeting

Scientific Committee

Tatiana Isabet	Synchrotron SOLEIL (France)
Magali Mathieu	SANOFI (France)
Alexey Rak	SANOFI (France)
Andrew Thompson	Synchrotron SOLEIL (France)

Local Committee

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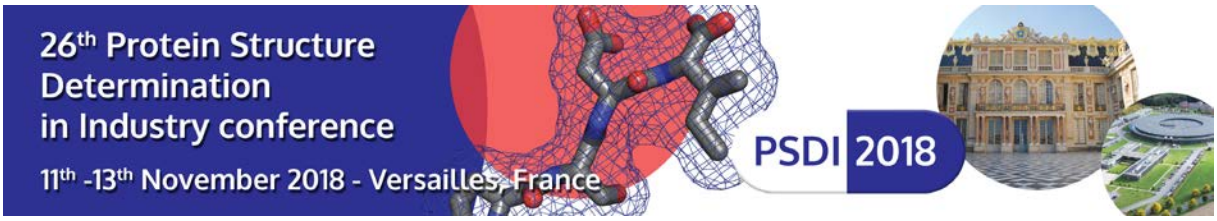


Summary

PSDI 2018
"26th Protein Structure Determination in Industry Meeting"

PRACTICAL INFORMATION

In case of emergency, please contact 112
The 112 is intended to be used:
- For any emergency requiring an ambulance, the fire department or the police when you are traveling in a European country and by the users of a smartphone.

Accommodation

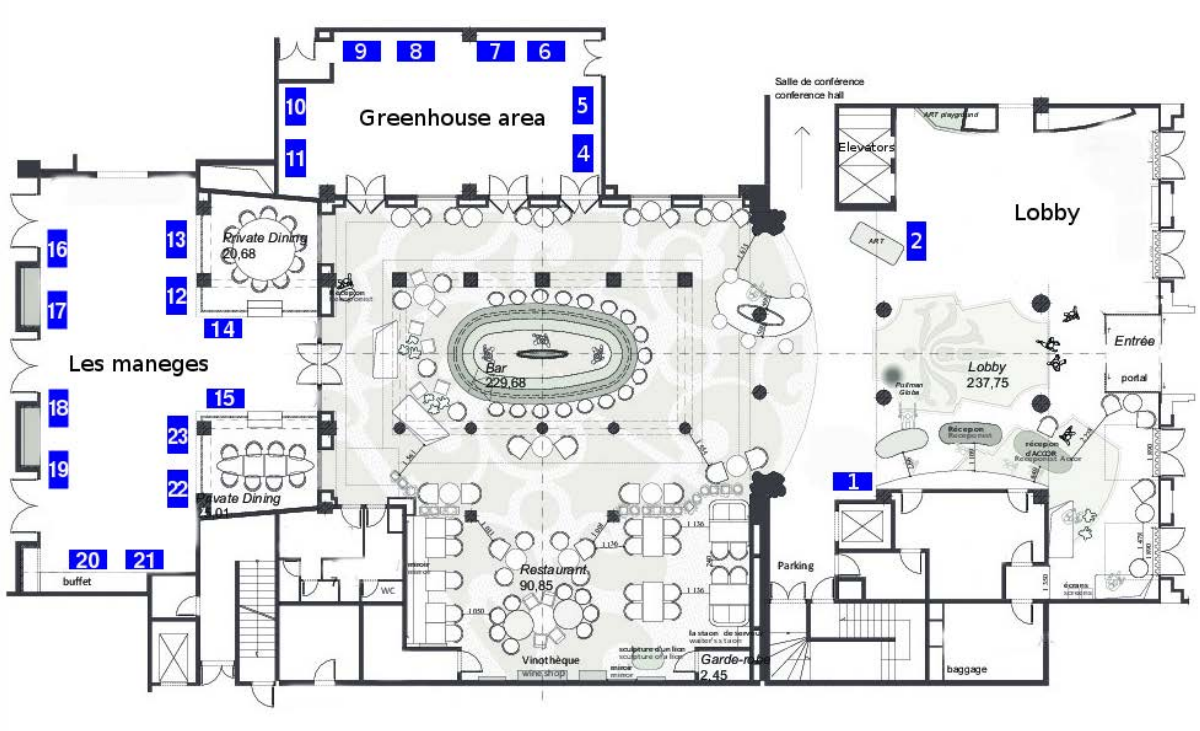
The Louis Versailles Chateau - MGallery by Sofitel hotel is a 4-star establishment of the Sofitel chain, completely renovated in 2017. It is ideally located to get to the residence of the Sun King, with only 500 meters separate the Palace of Versailles from the hotel (5 minutes by foot). We find then rooms in a purely Versailles style and high-end services. The property is a 2-minute walk from Versailles-Château-Rive-Gauche RER C Station, providing direct access to central Paris, the Eiffel Tower and Avenue Champs Elysées.

Le Louis Versailles Château – MGallery by Sofitel
2 bis avenue de Paris
78000 Versailles, France
Phone: +33 (0)1 39 07 46 46
https://sofitel.accorhotels.com/fr/hotel-1300-hotel-le-louis-versailles-chateau-mgallery-by-sofitel/index.shtml

Internet Access

Choose the ACCORHOTELS-GUEST wireless network
Open an Internet page and click on « CONNEXION »

Exhibitor booth plan



Company	Position	Company	Position
Arinax Scientific Instrumentation	10	Malvern Panalytical	6
Beckman Coulter	4	Mitegen	23
Biosaxs GmbH	18	Molecular Dimensions Anatrace	20
Bruker AXS	19	Nanotemper Technologies	9
Cordouan Technologies SAS	12	Nano Imaging Services, Inc.	21
CubeBiotech GmbH	13	Proteros Biostructures GmbH	22
Dectris LTD	5	Rigaku Europe	7
Domainex	11	Thermofisher	1
Formulatrix	14	TTP Labtech	16
Global Phasing Limited	8	Xenocs	17
Kek Japan	15	Xtal Concepts Gmbh	2

Welcome reception, 11th November, 2018

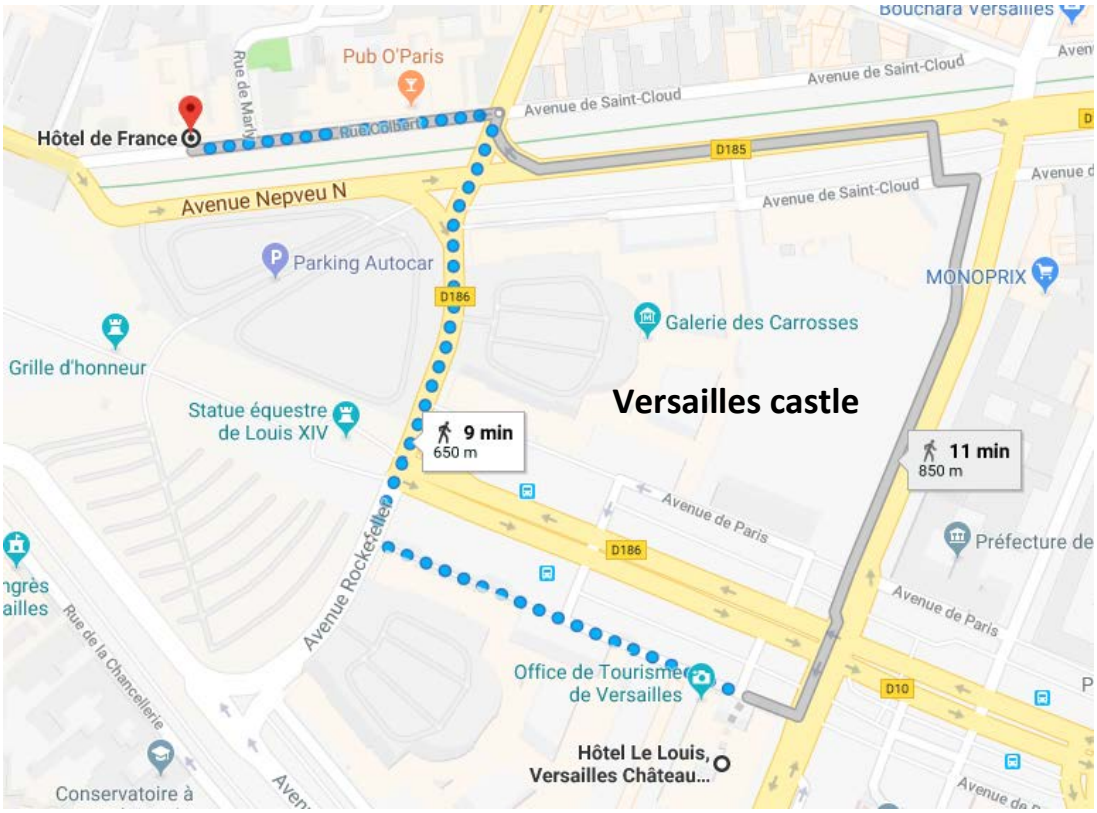
The Welcome Reception will take place on Sunday 10th November, 2017 at Hotel Le Louis Versailles Château – MGallery by Sofitel from 7:00 pm to 9:00 pm.

Conference dinner, 12th November, 2018

The conference dinner will take place on Monday 12th November at 'Les Salons de l'Hôtel de France' from 7:30 pm to 10:30 pm. An elegant and peaceful private residence.

Hotel de France
5 Rue Colbert
78000 Versailles

The bus from SOLEIL will go directly to the Hotel de France, the venue for the conference dinner. For those who will not be visiting SOLEIL, this venue is within walking distance from the Hotel Le Louis Versailles Château. Head northwest on Avenue de Paris (in the direction of the Versailles castle), then turn right on Rockefeller Avenue, turn left on rue Colbert and your destination is on the right.



The meeting dinner is sponsored by

Evotec is a drug-discovery & development solutions company offering the life sciences industry a variety of ways to access drug discovery innovation & development expertise in a time efficient manner, as stand-alone or integrated projects & have the confidence that through creative deal making & high quality alliance & project management, their programmes are in safe hands. The Company operates worldwide & has leading scientific experts, state-of-the-art technologies as well as key therapeutic expertise in multiple areas including neuroscience, pain, fibrosis, respiratory diseases, metabolic diseases, oncology, inflammation & infectious diseases.

Servier is an international and independent pharmaceutical company governed by a non-profit foundation, with headquarters in Suresnes, France. Operating in 149 countries, we have 21,700 collaborators employed worldwide and a turnover of 4.152 billion euros in 2017. Entirely independent, we are able to reinvest 25% of our total revenue (excluding generics) into Research and Development, and all profits are used for further development.

Culture and visits

Closet's frontage bluff
22 rue de Satory , 2 rue Saint Julien, 2 rue Carnot
78000 Versailles

Engrenage – Escape Game
6 bis rue Georges Clémenceau
78000 Versailles

Theater Montansier
13 rue des réservoirs
78000 Versailles

The passage of antique shops
Passage de la Geôle – rue du Baillage, 14 bis rue Baillet – Reviron and 13 rue de la Pourvoierie
78000 Versailles

The 'Domaine de Marie-Antoinette'

The gardens of Versailles and the 'Grandes Eaux Musicales' *(price : 16€)*
2 place Charost
78000 Versailles

Royal factory – stand up
2 rue Jean Houdon
78000 Versailles

Market 'Notre-Dame'
One of the largest market in Europe
Place du Marché Notre-Dame
78000 Versailles

Open every day except Monday:
Halles 'Notre-Dame': from Tuesday to Saturday from 07:00 am to 07:30 pm
'Carrés Notre-Dame' : Tuesday, Friday and Sunday morning from 07:00 am to 02:00 pm

Over 300 shops settled in the "golden triangle" of Versailles between the streets of 'la paroisse' ,' Carnot', 'Hoche' and 'Maréchal Foch'. Market-related, they are fixed, they are the heirs of the many shops that once prospered: tailors, potters, mirror makers, drapers etc.

Restaurants

Au chapeau gris *– recommanded*
7 rue Hoche
78000 Versailles

Chez Stef's *– Relaxed and warm place, French food*
12 Rue du Vieux Versailles
78000 Versailles

Gordon Ramsay at Trianon
1 boulevard de la reine
78000 Versailles

Cesar by Simone Zanoni *– Italian food*
8 avenue du Général de Gaulle
78000 Versailles

Les 4 saisons
40 rue Carnot
78000 Versailles

Banderillas *– Spanish food*
2 place Charost
78000 Versailles

L'alcôve *– Restaurant Lounge Bar*
2 bis Avenue de Paris
78000 Versailles

Le New Yor*k – American and Mexican food*
5 place Saint Antoine de Padoue
78150 Le Chesnay

Bakeries/Pastries

Maison Philippe Pele – Bakery
21 rue Carnot
78000 Versailles

Gaulupeau Pâtissier-Traiteur – Pastry
44 rue de la Paroisse
78000 Versailles

Aux pains de la Ferme – Bakery
9 rue royale
78000 Versailles

Ladurée Versailles
Château de Versailles, Place d'armes
78000 Versailles

Au Roi Soleil - traditional sweets and chocolates
46 rue de la Paroisse
78000 Versailles

You will find more information at the tourist office of Versailles:

Office de Tourisme
2 bis avenue de Paris
78000 Versailles
Phone : +33 (0)1 39 24 88 88
https://www.versailles-tourisme.com/

DISCOVER THE ESRF'S CRYO- ELECTRON MICROSCOPE

The ESRF will embark upon the construction of a new machine, the **Extremely Brilliant Source (EBS),** at the end of 2018. With performances multiplied by 100 in terms of brilliance and coherence, this new source of synchrotron radiation will offer unprecedented tools for the exploration of matter and for the understanding of Life at the macromolecular level.

While the construction is taking place, our newly-inaugurated **CRYO-EM** facility will remain operational. The facility consists of a Titan Krios microscope equipped with a GATAN K2 direct detector, GATAN imaging filter and a Volta phase plate.

We have made it very easy for you to access this state-of-the-art instrument:

- **Regular booking possible**
- **Screening facility offered to clients during its first phase of operation**
- **Pre-processing available**
- **Fully mail-in service available**
- **Real-time follow-up of the experiment through ISPYB (laboratory information management system).**

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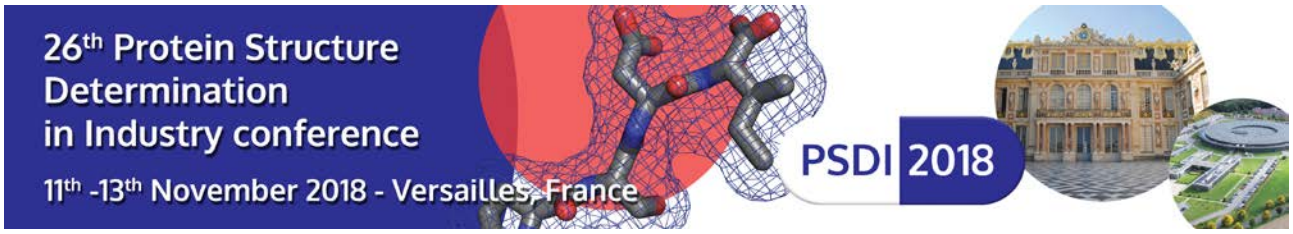
Contact us at cryoem@esrf.fr for more information
www.esrf.eu/industry



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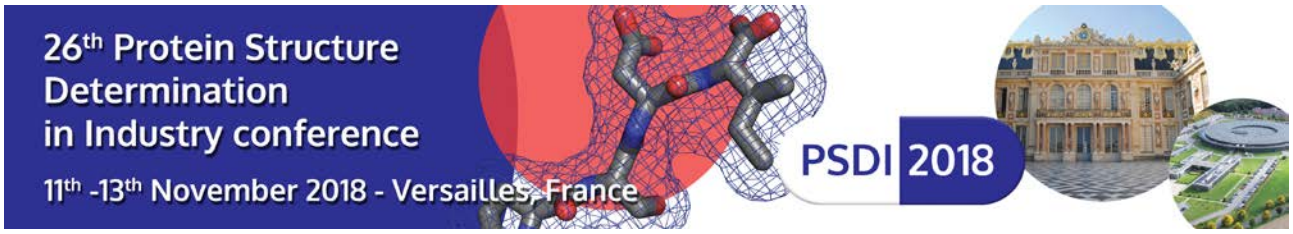
11th – 13th November 2018

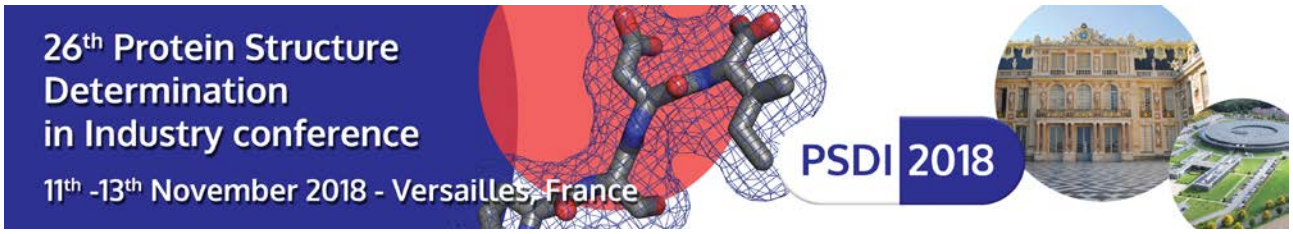
Hôtel Le LOUIS, Versailles Château - France

Programme

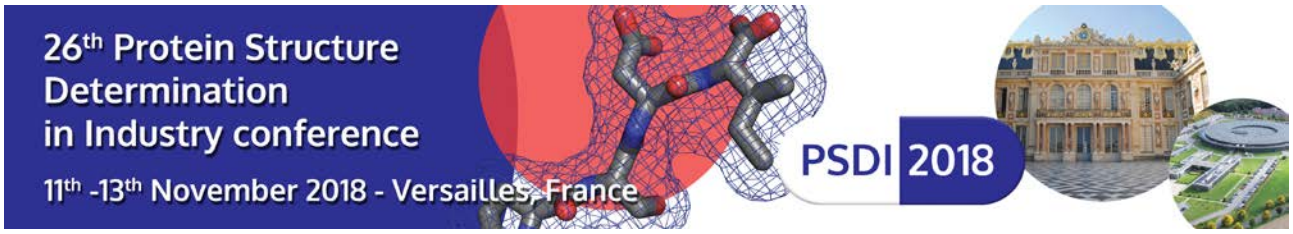
Sunday, November 11th		
14:30 - 15:15	Registration	
15:15 - 16:45	1st session of workshops, In parallel	
	Molecular Dimension/ Anatrace *Montespan room* Chair: James Gordon & Edward Pryor	**Proteros** *Maintenon room* Chair: Lars Neumann, Stephan Krapp & Claire Donat
16:45 - 17:00	*Coffee break*	
17:00 - 18:30	2nd session of workshops, In parallel	
	Nanotemper *Montespan room* Chair: Pierrick Daniel & Pierre Soule	**Global phasing** *Maintenon room* Chair: Gerard Bricogne
18:30 - 19:00	Registration	
19:00 - 21:00	Welcome reception at the Hotel LE LOUIS	
21:00 - 22:00	Keynote speaker: A Decade of GPCR Structure-Based Drug Design - Structural insights into the allosteric control of GPCR activity ***Andrew S. Doré*** *- Heptares Therapeutics Ltd., UK*	



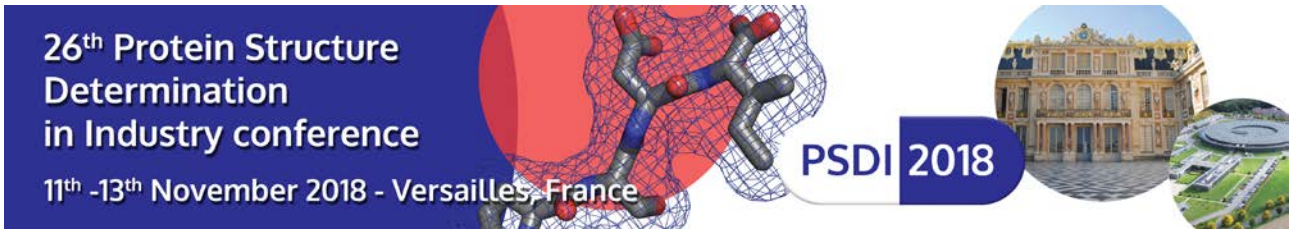
Monday, November 12th	
07:45 - 08:15	Registration
08:15 - 08:25	Welcome adress
Session #1 – Biologics *Conference room* Chair: Djordje Musil	
08:25 - 08:45	Structural biology for antibody design at Roche ***Armin Ruf*** *- Roche Innovation Center Basel, Switzerland*
08:45 - 09:05	Structural biology in the design of self-assembling protein nanoparticle vaccine antigens ***Matthew J. Bottomley*** *- GSK, Rockville, USA*
09:05 - 09:25	Anti-biopharmaceutical immunization: Analysis of anti-drug antibodies interactions ***Thomas Bertrand*** *- Sanofi R&D, Vitry-sur-Seine, France*
09:25 - 09:35	Sponsor talk : An analytical revolution: Introducing the next generation optima AUC ***Anthony Curran*** *- Beckman Coulter, High Wycombe, U.K.*
09:35 - 09:45	Sponsor talk : New direct in-situ & non-invasive technique for the monitoring of bio-pharma product in injectable syringes ***David Jacob*** *- Cordouan Technologies, Pessac, France*
09:45 - 09:55	Sponsor talk: Characterization of a novel ATP-cone driven activity regulation of ribonucleotide reductase by several techniques ***Stéphane Rouquette*** *- Malvern Panalytical, Solna, Sweden*
09:55 - 10:30	*Coffee break, Exhibitors & Networking*
Session #2 – CryoEM *Conference room* Chair: Wolfgang Baumeister	
10:30 - 11:15	Keynote speaker : Cryo-electron tomography: The promise and challenges of doing structural biology in situ ***Wolfgang Baumeister*** *- Max Plank Institute of Biochemistry, Martinsried, Germany*
11:15 - 11:35	Instruct-NL: A focus on high resolution cryo electron microscopy at NeCEN ***Ludovic Renault*** *- The Netherlands Centre for Electron Nanoscopy, Leiden, The Netherlands*



11:35 - 11:55	How Cryo-EM can push your structural biology boundaries for drug discovery? ***Hervé Remigy*** *- ThermoFisher Scientific, Eindhoven, The Netherlands*
11:55 - 12:05	Sponsor talk: Detergent-free membrane protein sample preparation for cryo-electron microscopy ***Barbara Maertens*** *- Cube biotech, Monheim, Germany*
12:05 - 12:15	Sponsor talk: Chameleon: A pico-litre dispense, self-wicking system for automated plunge freezing ***Joby Jenkins*** *- TTP Labtech, Melbourn, UK*
12:15 - 12:25	Sponsor talk : Pushing the boundaries of laboratory small angle X-ray scattering instrumentation ***Soren Skou*** *- Xenocs Nordic, Hoersholm, Denmark*
12:25 - 13:30	*Lunch break (buffet) - Exhibitors & Networking*
13:30	Departure for Synchrotron SOLEIL
Session #3 - Cutting-edge technologies *SOLEIL amphitheatre* Chair: Andrew Thompson	
14:20 - 14:40	The Salipro® system for stabilization of membrane proteins ***André Heuer*** *- Salipro Biotech AB, Stockholm, Sweden*
14:40 - 15:00	X-ray free electron laser: Opportunities for drug discovery ***Michael Hennig*** *- LeadXpro AG, Villigen, Switzerland*
15:00 - 15:10	Sponsor talk: EIGER collects best data at the Synchrotron and in the laboratory ***Marcus Mueller*** *- DECTRIS Ltd., Baden-Dättwil, Switzerland*
15:10 - 15:20	Sponsor talk: Latest innovations from Rigaku Oxford diffraction ***Mark Benson*** *- Rigaku Europe, Oxford, U.K.*
15:20 - 15:30	Sponsor talk: Structural biology research center in KEK/high energy accelerator research organization ***Mikio Tanabe*** *- KEK Tsukuba, Ibaraki, Japan*
15:30 - 16:10	*Coffee break - poster session*
16:10 - 17:20	Synchrotron SOLEIL visit
17:20 - 18:30	Round table discussion on the future of X-radiation sources in Europe
18:45	Departure for Versailles
19:30 - 22:30	*Meeting Dinner*



Tuesday, November 13th	
Session #4 - Membrane proteins *Conference room* Chair: Vadim Cherezov	
08:30 - 09:15	Keynote speaker: Dissecting GPCR structure and dynamics with X-ray lasers ***Vadim Cherezov*** *- University of Southern California, Los Angeles, USA*
09:15 - 09:35	The high-resolution structure of a metabolite sensing GPCR bound to an intracellular nanobody ***Matthias Haffke*** *- Novartis Pharma AG, Basel, Switzerland*
09:35 - 09:55	Application of microscale thermophoresis to GPCRs ***Alexey Mishin*** *- Moscow Institute of Physics and Technology, Dolgoprudny, Russia*
Session #5 - Biophysical methods *Conference room* Chair: Alexey Rak	
09:55 - 10:15	^{19}F-NMR fragment screening at SANOFI ***Magali Mathieu*** *- Sanofi R&D, Vitry-sur-Seine, France*
10:15 - 10:35	Evaluation of SPR based screening campaigns: Implications to define performance parameters and hit selection criteria ***Florian Krieger*** *- Evotec AG, Hamburg, Germany*
10:35 - 11:05	*Coffee break, Exhibitors and Networking*
Session #6 - Drug design case studies *Conference room* Chair: Armin Ruf	
11:05 - 11:25	A potent and orally bioavailable ERK1/2 series identified through fragment screening which modulates the phosphorylation and catalytic activity of ERK1/2 ***Puja Pathuri*** *- Astex Pharmaceuticals, Cambridge, U.K.*
11:25 - 11:45	Fragment screening of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and the structure-based development of potent and selective lead molecules ***Philip Day*** *- Astex Pharmaceuticals, Cambridge, U.K.*
11:45 - 12:05	Allosteric activation of striatal-enriched protein tyrosine phosphatase (STEP, PTPN5) by a fragment-like molecule ***Dennis Fiegen*** *- Boehringer Ingelheim Pharma GmbH, Biberach an der Riss, Germany*



Session #7 - Computational method & crystallization *Conference room* Chair: Gérard Bricogne	
12:05 - 12:25	Memproc: Tools for semi-automated processing of data from serial crystallography experiments ***Matthias Haffke*** *– Novartis Pharma AG, Basel, Switzerland*
12:25 - 12:45	Crystallophore, a nucleating and phasing agent ***Olivier Maury -*** *École Normale Supérieure, Lyon, France*
12:45 - 13:05	Anticipated biased condition method for macromolecular crystallization ***Fabrice Gorrec -*** *MRC Laboratory of Molecular Biology, Cambridge, U.K.*
13:05 - 13:15	Concluding remarks
13:15 - 14:00	*Lunch break (buffet)*
15:00	Visit of the Versailles Château, for those who have registered (Duration 1h30)

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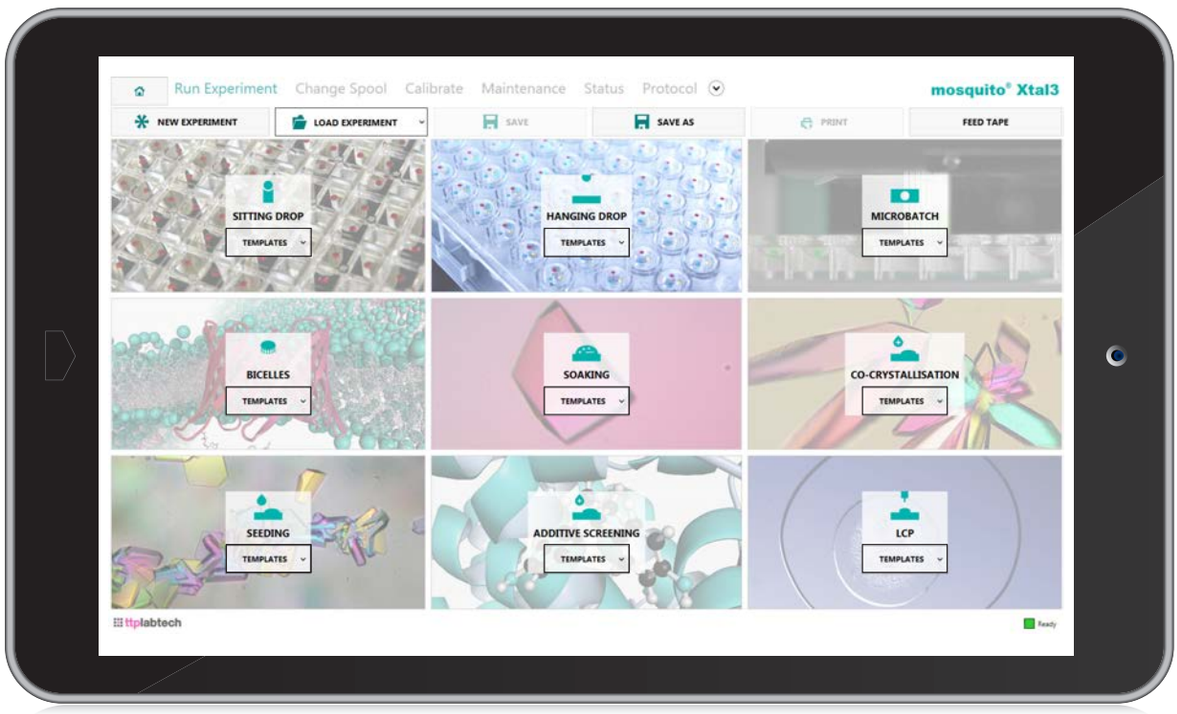

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Sunday, November 11th

Workshops 1st session	Molecular Dimension/Anatrace 'Alternative methods for protein crystal growth and detection'
	Proteros 'Strategies and technologies for the generation of drugs with innovative inhibition modes'
Workshops 2nd session	Global phasing 'The quieter revolution in X-ray crystallography: its impact on present and future industrial services in the drug discovery field'
	Nanotemper 'A quick check of protein quality that will vastly improve all protein purification and characterization workflows'
KN-01	A decade of GPCR structure-based drug design - structural insights into the allosteric control of GPCR activity *A.S. Doré*

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Workshops “Molecular Dimension/Anatrace”

November, 11th
15:15- 16:45

'Alternative methods for protein crystal growth and detection'

Growing crystals of complex human proteins for structure determination by X-ray crystallography is always challenging. This is especially the case for integral membrane proteins, yet it remains a vital part of the drug development process. While the use of many alternative screens is well established, the vast majority of trials are still carried out using vapour diffusion methods. Changing the method used for crystal growth can open up completely new areas of the phase diagram and significantly improve your chances of crystallisation. We will demonstrate the use of Laminex sandwich plates and DiffraX in situ plates, as popularly chosen for sponge phase crystallisation of membrane proteins but also for soluble proteins in applications such as serial crystallography. In addition, we will demonstrate the Crystal Former plate for microdiffusion crystallisation, which allows you to access a broad range of conditions while using smaller volumes of sample. Even where crystals of the most challenging proteins can be grown, they are often difficult to spot when scanning plates, because they are below 10 µm in size, or obscured by aggregate or precipitate. Where the conditions necessary to grow the crystals are complex, it can also be difficult to determine whether crystals are of macromolecular or inorganic origin. Use of UV-imaging and plane polarising light with the UVEX fluorescence imaging system can considerably simplify this process, ensuring you do not miss a hit condition.

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Workshops “Proteros”

November, 11th
15:15 - 16:45

'Strategies and technologies for the generation of drugs with innovative inhibition modes'

Purpose of the Workshop: The work shop will discuss strategies and technologies for drug discovery programs aiming for innovative inhibition modes, such as allosteric, PPI inhibition, covalent and extended residence time.

Previously, the drug mode-of-action for enzymatic protein targets was aimed at the catalytic center for substrate competition. Meanwhile, alternative modes of inhibitions are applied to address typical inhibitor challenges such as selectivity, efficacy and resistance formation. Less conserved interaction sites are now targeted by the means of allosteric inhibitors, which are also independent from high cellular substrate concentrations. Protein-protein-interaction inhibitors are generated that block the complex formation between the target proteins and the associated functional proteins. In addition inhibitor residence time, together with kinetic selectivity, became an important optimization parameter so that inhibitors selective for distinct activation states of enzymes can be engineered. Further on, covalent inhibitors are designed to attack specific residues within the target protein. Such recent drug discovery advancements require fundamental changes in the pool of appropriate assays, biophysics and screening tool boxes and implementation. Proteros biostructures has established stand-alone and integrated discovery service solutions that combine biochemistry with innovative biophysics and protein crystallography to facilitate both, efficient and data driven generation of innovative novel inhibitors. The Proteros drug discovery services platform enables to screen specifically for allosteric inhibitors. Assays are established to identify compounds blocking relevant protein-protein interactions.

Proteros’ high throughput binding assays allow screening for inhibitors addressing non-activated enzyme states. Proteros’ high throughput kinetic profiling provides knowledge about both binding affinity and binding kinetics against on- and off-kinase targets for all involved compounds to facilitate the rapid generation of residence time optimized inhibitors. Methods are in place that de-convolute the binding of covalent inhibitors into a) non-covalent and b) covalent contribution to escape resistance formation. Whereas protein crystallography discloses different binding modes and enables medicinal chemistry to improve the inhibitor properties. The workshop demonstrates the application and synergistic use of such different discovery service technologies for an efficient integrated drug discovery process towards innovative inhibitors.

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Workshop “Global phasing”

November, 11th
17:00 – 18:30

'The quieter revolution in X-ray crystallography: its impact on present and future industrial services in the drug discovery field'

This would survey the new instrumental resources and the associated software developments all the way down the proverbial pipeline, and analyse their transition from high-end tools for structural biology projects towards industrial services of potential interest to the pharmaceutical sector. I would invite contributors from Big Pharma, CROs and synchrotrons, and encourage discussion of what hurdles need to be overcome to fully exploit these new resources for the purposes of drug discovery.

I see this as an opportunity to respond to the current enthusiasm for cryo-EM by showing how dynamic X-ray crystallography itself continues to be, even if more discreetly than its young sibling.

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Workshop “Nanotemper”

November, 11th
17:00 – 18:30

'A quick check of protein quality that will vastly improve all protein purification and characterization workflows'

Starting with material of questionable quality for protein purification and characterization leads to irreproducible or ambiguous results. Methods such as chromatography while widely used, can also derail experiments—due to the amount of time and expertise required to perform these techniques.

We will present a new platform, the Tycho NT.6®, that swiftly identifies sample quality and relative functionality in minutes complementing and guiding purification and characterization workflows—making experimental decisions easy and quick—saving time, effort and cost downstream.

This workshop will also give you the opportunity to see real live sample-set on our new device, Tycho NT.6®. How easy-of-use it is and its value for QC in various drug discovery operations.

A Decade of GPCR Structure-based Drug Design: Structural insights into the Allosteric Control of GPCR Activity

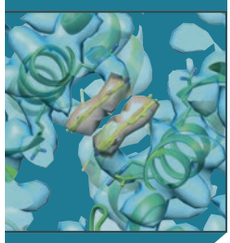
Andrew S. Doré,

Heptares Therapeutics Ltd., UK

ABSTRACT

Many of the world's top selling drugs target G protein-coupled receptors (GPCRs). The past ten years have seen an exponential increase in structural knowledge for this important and clinically relevant superfamily of membrane proteins as a result of pioneering protein engineering techniques from multiple groups globally. This, coupled to advances in crystallization methodogolies *per se*, serial crystallographic techniques such as XFEL, cryo-electron microscopy and microfocus collection techniques using conventional synchrotron radiation, has not only driven structure based drug design (SBDD) at the orthosteric site(s) of clinically relevant targets, but also uncovered a myriad of allosteric sites that may also be targeted to modulate GCPR activity across all subclasses of this superfamily encompassing a wide range of indications. As a number of NCE's developed at Heptares have entered man, the impact of the last decade of research is reaching a breakthrough point with exciting results.

NovAliX FROM SCREEN TO OPTIMIZED LEAD



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Structural Biology

Protein-small molecule co-crystals
Cryo electron microscopy
Crystallography grade protein

Characterization of interactions

Large biophysical characterization platform
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Screening

Novel scaffolds
Fragments

www.novalix.com

NovAliX

PSDI 2018
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Monday, November 12th

Session #1 - Biologics
Chair: Djordje Musil

OC-01	Structural biology for antibody design at Roche *A. Ruf*
OC-02	Structural biology in the design of self-assembling protein nanoparticle vaccine antigens *M.J. Bottomley*
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PSDI 2018
"26th Protein Structure Determination in Industry Meeting"

Session #3 - Cutting-edge technologies
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ST-09	Structural biology research center in KEK/high energy accelerator research organization *M. Tanabe*

Structural Biology for Antibody Design at Roche

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ABSTRACT

This review includes examples from more than a decade structural biology work on antibodies at Roche. It will show how structural biology contributions evolved from mere epitope determination and mechanism of action illustration to various applications in supporting the design of new antibody formats. The structural biology methods covered are X-ray crystallography, negative stain EM and cryo-EM.

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Structural Biology in the Design of Self-assembling Protein Nanoparticle Vaccine Antigens

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ABSTRACT

Introduction: Vaccines are one of the most effective ways to improve global human health and are believed to save 2-3 million lives per year. Nevertheless, many diseases are not yet preventable by vaccination and certain groups (e.g. the elderly, pregnant women, neonates) remain especially vulnerable. Compared to earlier vaccines containing killed or live-attenuated pathogens, modern sub-unit vaccines are safer but tend to be less immunogenic. Therefore, a key research question is: How to increase vaccine immunogenicity without reducing safety or tolerability? Here, a case study of the *Neisseria meningitidis* surface protein NadA3, an antigen included in the multi-component meningococcal serogroup B vaccine (4CMenB), is presented to show how structural biology can inform the design of self-assembling protein nanoparticle antigens with enhanced immunogenicity.

Methods: Molecular biology, biochemistry and biophysics methods were used to clone, screen and select antigen candidates for structural studies. X-ray crystallography was used to determine the structure of an N-terminal fragment of NadA3, which enabled the design of thermo-stabilized forms. Nanoparticles displaying NadA3 were designed *in silico* and the corresponding purified antigens were used to immunize mice. Protective immunogenicity was evaluated using a standard serum bactericidal assay.

Results: The crystal structure of NadA3 (residues 24-170) was determined at 2.45 Å resolution, revealing a trimeric head-on-stalk elongated structure, with two unexpected regions of undecad coiled-coil motif. Structure-guided engineering was performed to raise thermostability by over 15 °C and indeed the crystal structure of stabilized NadA3 revealed increased packing contacts. Surface plasmon resonance studies of NadA3 binding to human cell surface receptor LOX1 or to recombinant human Fabs obtained following vaccination with 4CMenB revealed the presence of key functional regions and protective epitopes in the NadA3 head region. With the aim of increasing immunogenicity, a ferritin-based self-assembling protein nanoparticle was designed to display 24 copies of NadA3 (eight trimers), oriented such that the key epitopes project to engage with the immune system. The ferritin-NadA3 nanoparticle antigen demonstrated increased protective immunogenicity in mice.

Conclusions: A rational structure-based approach, guided by the identification of key epitopes that induced protective antibodies in humans, was used to inform the design of an immunogenic nanoparticle antigen. This result and others that have recently emerged in the literature will be discussed. Self-assembling protein nanoparticles displaying multi-copy arrays of oriented antigens are a very promising strategy for the design of effective vaccines.

Anti-biopharmaceutical Immunization: Analysis of Anti-drug Antibodies Interactions

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ABSTRACT

Around 80 antibody drugs are approved by regulatory agencies for therapeutic use and the market is expected to exceed $100 billion by 2022 (1). While the format for these “new” drugs shows a clear advantage in a number of chronic pathologies compared to more classic small-molecule approaches, a proportion of patients treated with these antibody drugs might develop an immunization reaction, therefore limiting the efficacy for the antibody drugs and leading to adverse effects. As part of the IMI ABIRISK consortium (Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK), our laboratory has been involved in improving the understanding of the immunization mechanisms observed in a selection of patients for a panel of antibody marketed drugs.

In order to understand if a common epitope is responsible of the patients’ immunization, we have been investigating a number of Fab complexes from Anti-Drug Antibodies (antibodies from patients raised against antibody drugs) with biophysical approaches, supported by structural biology data.

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An Analytical Revolution: Introducing the Next Generation Optima AUC

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ABSTRACT

The Optima AUC is the latest innovation from Beckman Coulter and offers significant advantages as an analytical ultracentrifugation platform over the predecessor ProteomeLab. Besides the plethora of advancements in user experience on the new platform, the Optima AUC's optical, thermal, and drive systems have also been upgraded significantly, creating an overall better experience to the end-user in terms of data quality and ease of use. A battery of applications from proteins and nanomaterials to viral vectors and lipid-based nanoparticles have already been assayed on the new instrument and the results are very positive in terms of data quality, reproducibility, and capabilities, especially in the case of multi-wavelength experiments. Here, insights are provided to highlight the advantages of the new instrument as well as proof-of-concept data on the aforementioned applications.

New Direct In-situ & Non-invasive Technique for the Monitoring of Bio-pharma Product in Injectable Syringes

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ABSTRACT

Aggregation of proteins and active principle ingredients (API) in injectable biopharmaceutical products remains a major concern impacting the stability and usability of a product. Indeed, Protein aggregation can occur during all stages of the lifetime of a protein therapeutic, including expression, refolding, purification, sterilization, shipping, storage, and delivery processes [1, 2]. The mechanism of protein aggregation is still not well understood; but it is known that certain manufacturing stages like formulation composition, presence of microbial or vial contaminants during cell culture, and storage influence the risk of chemical degradation, which increases the risk of physical degradation and the formation of aggregates. In a context of more and more stringent international health regulations about the control of biopharmaceutical products, the in-situ monitoring of the denaturation and degradation process of therapeutic proteins during production and storage can be a key competitive advantage for manufacturers and researchers.

We present here the **first practical demonstration of a contactless in situ nano-particle size measurement of bio-pharma injectable suspension directly into a syringe.** Demonstration of the technique is achieved on a commercial bio pharma product stored in a hermetically sealed glass syringe. Comparison of measurement results between originators and bio-similar are presented. In conclusion, by eliminating sample batching steps, the new DLS set up based on an innovative optical fiber remote head is opening up new fields of application to particle size measurement systems, in particular for the **in-situ monitoring of protein aggregation in biopharmaceutical injectable products**. This new setup could also be used to monitor in real-time nano-particle synthesis Kinetic in various type of reactor configuration (double jacket glass reactor, high pressure &high temperature Super Critical CO2 autoclaves, microwave reactors, micro fluidic chips, etc.) or for instrumental coupling [3].

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Characterization of a Novel ATP-cone Driven Activity Regulation of Ribonucleotide Reductase by Several Techniques

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ABSTRACT

Ribonucleotide reductases (RNRs) are key enzymes in DNA metabolism, providing the only known pathway for the biosynthesis of deoxyribonucleotides (dNTPs), the immediate precursors for DNA synthesis and repair. Class I RNRs are active complexes which consist of a large catalytic subunit and a smaller radical-generating subunit. Allosteric mechanisms control substrate specificity and overall activity of these enzymes. In RNRs, the activity master-switch, the ATP-cone, has been found exclusively in the catalytic subunit. In two class I RNR subclasses whose catalytic subunit lacks the ATP-cone, we instead discovered ATP-cones in the radical-generating subunit.
We employed several complementary biophysical techniques: ITC, DLS, DSC, multi-detection SEC and X-ray crystallography, in combination with activity assays to investigate the allosteric regulation of these unusual RNRs. We performed global fitting of ITC data using AFFINImeter to analyze complex protein-ligand interactions. We explored how binding of nucleotide effectors affected the oligomeric state and as a consequence the activity of the enzyme.
We demonstrate that the ATP-cone in the *Leeuwenhoekiella blandensis* radical-generating subunit regulates activity via quaternary structure induced by binding of nucleotides. ATP induces enzymatically competent dimers, whereas dATP induces non-productive tetramers, resulting in different holoenzymes. The tetramer forms by interactions between ATP-cones, shown by a 2.45 Å crystal structure.

Cryo-electron Tomography: The Promise and Challenges of Doing Structural Biology *in situ*

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ABSTRACT

Traditionally, structural biologists approach cellular complexity in a reductionist manner by characterizing isolated and purified molecular components. This 'divide and conquer' approach has been highly successful, as evidenced by the impressive number of entries in the PDB. However, awareness has grown in recent years that only rarely can biological functions be attributed to individual macromolecules. Most cellular functions arise from their acting in concert. Hence there is a need for methods developments enabling studies performed *in situ*, i.e. in unperturbed cellular environments. *Sensu stricto* the term 'structural biology *in situ'* should apply only to a scenario in which the cellular environment is preserved in its entirety. Cryo-electron tomography has unique potential to study the supramolecular architecture or 'molecular sociology' of cells. It combines the best structural preservation that is physically possible to achieve with the power of three-dimensional high resolution imaging.

Instruct-NL: A Focus on High Resolution Cryo Electron Microscopy at NeCEN

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ABSTRACT

The Instruct-ERIC consortium consists of eleven-member countries and nine research centres, which allow open access to high-end structural biology technologies and services across Europe, with funding available to researchers from member countries. www.instruct-eric.eu
Instruct-NL is both the Dutch national Instruct centre and a community encompassing all main areas of structural biology research within the Netherlands.

Instruct-NL consists of several partners and covers a wide range of structural biology techniques. The Bijvoet Centre of Utrecht University contributes biomolecular NMR spectroscopy, native mass spectrometry and proteomics, computational structural biology and protein crystallography. The Protein Facility of the Netherlands Cancer Institute provides access to biophysical characterization of proteins and The Netherlands Centre for Electron Nanoscopy (NeCEN) at Leiden University offers access to two Thermo Fisher Titan Krios microscopes for cryo Electron Microscopy (cryo-EM).

In this presentation, we will focus on the NeCEN cryo-EM facility.

The Netherlands Centre for Electron Nanoscopy (NeCEN) is the open access research facility for high-resolution cryo-EM in The Netherlands. NeCEN offers research institutes and companies, both Dutch and international, access to advanced cryo-EM infrastructures and expertise.

NeCEN offers a variety of services ranging from training, sample preparation and screening to high resolution data collection and image processing.

The core service provided to our users is high throughput data collection on two state of the art Titan Krios transmission electron microscopes. Our two Titan Krios microscopes give unique possibilities to perform a very broad range of experiments; Cs corrected atomic resolution single particle acquisition, phase plate tilt series acquisition, STEM and diffraction applications. NeCEN's experienced EM scientists, in combination with the advanced and highly automated hardware, allow the user to get the most out of their sample and microscope time. An on-the-fly processing and reporting has been developed to ensure the best data quality is collected and the data is uploaded live to our fast-online servers so that the user can download the data live and start data processing right away.

For challenging samples, we can help during buffer optimization and sample screening. For this, a basic equipped wet-lab and dedicated entry level screening TEM are available on site.

To broaden its portfolio, NeCEN recently acquired an Aquilos cryo-FIB SEM. With this machine it is now possible to prepare cryo-Lamellae of biological materials for high resolution cryo-Electron Tomography.

In addition to assistance in sample preparation, imaging and data processing, NeCEN is also meant as a training facility for structural biologists. Therefore, together with CNB-CSIC in Madrid and with Thermo Fisher Scientific, NeCEN started an initiative to organize an in-depth cryo-EM school. An extensive 9-week pilot was held at NeCEN at the beginning of 2017. The new version of the course consists of online lectures about the theory of cryo-EM single particle analysis and 4-weeks of hands-on practicals supervised by 2 dedicated expert teachers including 3-days of introductory image processing in the cloud with Scipion framework by CNB group. Participation is limited to 6 persons per course to ensure enough Krios practice time per participant. For more information contact NeCEN directly (www.NeCEN.nl).

How Cryo-EM can Push your Structural Biology Boundaries for Drug Discovery?

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ABSTRACT

Cryo-Electron Microscopy (Cryo-EM) is increasingly adopted by the pharmaceutical industry to decipher mechanism of drug-target interactions for drug discovery. Cryo-EM is undergoing a similar development as protein crystallography was undergoing about two decades ago. Cryo-EM yields atomic structures of macromolecular complexes, without the need for crystalline sample. There is a strong demand to implement improvements to feed the Cryo-EM structural analysis pipeline and improve its throughput. During this presentation, promising results will be shown, followed by the introduction of new tools aiming at improving the Single Particle Analysis process efficiency. Finally, various options to access the Cryo-EM technology will be presented, whether you are unexperienced or an expert, or whether you need a full in house solution or to outsource your Cryo-EM activities.

Detergent-free Membrane Protein Sample Preparation for Cryo-electron Microscopy

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ABSTRACT

Cube Biotech is an experienced service provider focusing on expression, purification and stabilization of membrane proteins as critical drug targets. Our customers inquire sample preparation for many different applications like assay development, immunization of membrane proteins to generate antibodies and especially sample preparation for Cryo-electron microscopy. For all these different applications, a non-detergent based stabilization is a prerequisite to reduce the adverse effect of detergents on membrane proteins.

We at Cube Biotech focus on Nanodisc as an exciting alternative for stabilizing membrane proteins and offer a broad Nanodisc portfolio. This covers different Nanodisc diameter, scaffolds from different species like human, mouse, rat and most recently alpaca to generate nanobodies and scaffolds with site specific biotinylation. Beside a scaffold made of protein, we also use synthetic scaffolds for solubilization and stabilization to allow for flexibility in projects.

Depending on applications, membrane protein expression is either done in insect cells or in Hek293 cells using the BacMam system. In addition, we offer an E.coli based cell-free expression system is a perfect match with pre-assembled nanodisc to avoid detergent.

Chameleon: A Pico-litre Dispense, Self-wicking System for Automated Plunge Freezing

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ABSTRACT

Protein structure determination is fundamental to the understanding of function, providing context for both individual proteins and within large biological complexes. Recent developments in the field of cryoEM have automated and streamlined the data collection and analysis processes, while direct electron detectors and their associated improvements in resolution have now become standard equipment in many imaging facilities. Advances in microscopes, data collection strategies and computational approaches have shifted the bottleneck to cryoEM sample preparation, which remains highly challenging and a barrier to broader adoption of the method beyond traditional cryoEM laboratories.

To facilitate this adoption, ease-of-use improvements, such as reducing manual grid handling and variability between grid freezing sessions, are necessary. In addition, reduction in the sample volume necessary for preparation of multiple grids will enable this technique to be used with difficult to acquire samples. And the decreased time the sample spends on the grid prior to freezing may lessen the challenges of preferential orientation caused by interactions with either the support film or the air-water interface. Together, these advances will enable consistent structure determination for broad ranges of targets.

The development of a blotting-free approach to cryoEM sample preparation has been previously described as Spotiton [1-2]. The newest version of this instrument, Chameleon, uses picolitre amounts of sample applied to self-blotting grids, allowing for automated rapid vitrification and removing the need for blotting or evaporation to create a thin film prior to freezing.

We provide here a development update on the Chameleon instrument and associated self-wicking grids. We highlight recent data from collaborating groups using samples from a variety of target classes.

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Pushing the Boundaries of Laboratory Small Angle X-ray Scattering Instrumentation

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ABSTRACT

Biological small angle x-ray scattering (SAXS) is an established technique for characterizing biological macromolecules in solution and is becoming increasingly popular especially in drug development and pharmaceutical sciences.
Highly complementary to high-resolution techniques such as NMR, MX and EM, SAXS provides detailed structural information on a very wide range of molecule sizes with almost no sample preparation. Being completely non-intrusive, SAXS is also a powerful tool for verifying models and studying protein function in their natural environment as well as combining structural information obtained with other techniques.
Where SAXS on biological samples once mainly required the intensity of synchrotron x-ray facilities, several recent technological advances have now facilitated use of biological SAXS in the home laboratory on an entirely new level than previously possible. State-of-the-art instruments can provide publication grade results in a few minutes on even very small molecule sizes with a level of automation that rivals the best synchrotron SAXS facilities.
This opens the technique not only to routinely daily use for both structure characterization and validation, but also for high-throughput screening of sample interactions and buffer conditions, which can greatly increase research productivity.
Apart from a very short introduction to biological SAXS, the latest in laboratory instrumentation will be presented together with examples of both applications and data, including size exclusion chromatography SAXS (SEC-SAXS), once only practically feasible on synchrotrons for most samples.

The Salipro® System for Stabilization of Membrane Proteins

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ABSTRACT

More than 60% of all current drug targets are membrane proteins. However, structural and functional studies of membrane proteins are however limited by their poor stability outside the native membrane environment.

We present the lipoprotein nano-membrane system (Salipro) that allows for the reconstitution of membrane proteins into a lipid environment [(1)]. Membrane proteins embedded in Salipro display an increased stability, retain functionality and fold in a native lipid environment [(2,5,6)]. The Salipro system also enables the direct extraction of a target protein from the native membranes.

The Salipro system is applicable for NMR and SAXS studies and enables high resolution cryo-EM structures in a lipid environment [(6,7)]. Recent publications highlight the advantage of the Salipro system over standard methods (detergents, amphipols, nanodiscs), especially for high-resolution cryoEM [(3,4)]. They further demonstrate that the Salipro system enables reconstructions of membrane proteins beyond 3.8 Å and enables the visualization of lipids at the protein-lipid interface.

In addition, the Salipro system allows for indirect labeling of the membrane protein with tags and fluorophores on the Salipro scaffold, enabling interesting research opportunities in the field of single-molecule studies and fluorescence cryo-electron microscopy.

In conclusion, membrane proteins retain their functionality and fold by the Salipro approach, in an entirely detergent-free environment. This enables novel structure determination approaches by cryo-EM as well as drug development for viral, human and bacterial targets.

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X-ray Free Electron Laser: Opportunities for Drug Discovery

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ABSTRACT

Past decades have shown the impact of structural information derived from complexes of drug candidates with their protein targets to facilitate the discovery of safe and effective medicines. However, membrane protein drug targets like ion-channels, transporters and GPCRs still represent a significant challenge. Recent developments in single particle cryo-electron microscopy have significantly improved the options to derive structural information for ion-channels and complexes of GPCRs with G protein. LeadXpro is a structure based lead discovery company focusing on challenging membrane protein drug targets, including G-protein coupled receptors (GPCRs), ion channels and transporters.

Advances in serial crystallography are a pre-requisite to use the unique properties of X-ray Free Electron Laser (XFEL) with unmet peak brilliance and beam focus, which allows successful structure determination from smaller and weakly diffracting crystals. Serial crystallography at synchrotron has already been shown to be instrumental for structure determination and here we present an example in which a GPCR structure was solved using such a method. To further capitalize on the XFEL advantage which allows the capturing of dynamic processes of drug molecule binding and associated conformational changes with great impact to the design of candidate drug compounds, innovations in crystal sample delivery for the X-ray experiment, data collection and processing methods are required and some recent developments will be shown.

In August 2018, the SwissFEL facility was used for the very first biostructure experiments. We performed successfully the structure determination of a GPCR at the ALVRA beamline using the LCP jet and the brand-new Jungfrau 16M detector.

Acknowledgement: We thank all colleagues from PSI for contribution to the work and Heptares Therapeutics for protein supply.

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EIGER Collects Best Data at the Synchrotron and in the Laboratory

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ABSTRACT

Drug development relies on structural information about the biological target to model ligands and optimize interactions. Best X-ray diffraction data are obtained with EIGER Hybrid Photon Counting pixel detectors from DECTRIS. These detectors are effectively noise-free and remarkably fast. Direct detection of single X-ray photons in 75 µm pixels ensures highly accurate data.

Experiments at the Swiss Light Source showed that EIGER X series detectors record data of higher quality than does PILATUS3, the previous gold standard (1). The key to the higher quality lies in the smaller pixel size, which increases the signal to noise of sharp reflections, and the continuous read-out technology, which increases the benefit of ultrafine-phi slicing. A few examples of recent results from synchrotrons (2, 3, 4) will put these findings into context.

The advantages of EIGER technology are also available for laboratory applications. EIGER R 1M and 4M are mega-pixel HPC detectors that turn diffractometers into powerful platforms for all kinds of crystallography. I will show diffraction data collected from crystals with an exceptionally large unit cell on an EIGER R 4M. The quality of data collected with an EIGER R 1M at room temperature is high enough to phase several test proteins from the anomalous signal of intrinsic sulfur atoms.

EIGER allows crystallographers to make the best use of precious beamtime at synchrotrons and of laboratory X-ray sources. The detectors can lead to better X-ray structures more quickly and provide optimal starting points for drug development projects.

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Latest Innovations from Rigaku Oxford Diffraction

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ABSTRACT

Since the formation of Rigaku Oxford Diffraction in 2015 a new diffractometer range under the **Synergy** name has been launched.

I will review the **Synergy** range including the latest innovations and accessories.

Structural Biology Research Center in KEK/High Energy Accelerator Research Organization

M. Tanabe, N. Matsugaki, Y. Yamada, M. Hikita, N. Shimizu, N. Adachi, M. Kawasaki, T. Moriya, M. Senda, F. Yumoto, N. Igarashi, R. Kato and T. Senda

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ABSTRACT

Structural biology research center (SBRC) in the Institute of Materials Structure Science (IMSS), KEK/High Energy Accelerator Research Organization has promoted research and development of a field of structural biology using synchrotron beamlines at Photon Factory (PF). SBRC operates 5 protein crystallography (PX) and 3 small angle X-ray scattering (SAXS) beamlines in PF. All beamlines have been open for both academic and industrial researchers.

The recent developmental focuses in SBRC have been Native-SAD phasing and high-throughput Bio-SAXS analysis. A PX beamline, BL-1A has been specially designed for Native-SAD data collection. High-speed and reliable Native-SAD or MR-SAD data collection and now associated protocol are available at the wavelength above 2.7 Å. Collected data are smoothly processed by structure determination pipeline we developed.

The solution scattering is a complemental method with crystallography to analyze dynamics of proteins in the solution. A robust high-throughput sample exchanger, SEC/SAXS, SEC-MALL SAXS data collection system have been established, and now fully automated at a SAXS beamline BL-15A2. 192 samples in 16 hours can be measured without human's intervention.

Not only synchrotron beamlines, SBRC also installed 200kV Cryo-EM (Talos arctica) this year, and try to extend research support by hybrid structural biology approaches. SBRC in particular has given high priority to the establishment of user friendly automation and high-throughput system from protein preparation, crystallization to structure determination to understand important biological questions.

The presentation will highlight the recent developments and the plan for future of SBRC, and how we can contribute our industrial users to be successful.

PSDI 2018
"26th Protein Structure Determination in Industry Meeting"

Tuesday, November 13th

Session #4 - Membrane proteins
Chair: Vadim Cherezov

KN-03 Dissecting GPCR structure and dynamics with X-ray lasers
V. Cherezov

OC-08 The high-resolution structure of a metabolite sensing GPCR bound to an intracellular nanobody
M. Haffke

OC-09 Application of microscale thermophoresis to GPCRs
A. Mishin

Session #5 - Biophysical methods
Chair: Alexei Rak

OC-10 ^{19}F-NMR fragment screening at SANOFI
M. Mathieu

OC-11 Evaluation of SPR based screening campaigns: Implications to define performance parameters and hit selection criteria
F. Krieger

Session #6 - Drug design case studies
Chair: Armin Ruf

OC-12 A potent and orally bioavailable ERK1/2 series identified through fragment screening which modulates the phosphorylation and catalytic activity of ERK1/2
P. Pathuri

OC-13 Fragment screening of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and the structure-based development of potent and selective lead molecules.
P. Day

OC-14 Allosteric activation of striatal-enriched protein tyrosine phosphatase (STEP, PTPN5) by a fragment-like molecule
D. Fiegen

Session #7 - Computational method & crystallization
Chair: Gérard Bricogne

OC-15 memproc: Tools for semi-automated processing of data from serial crystallography experiments
M. Haffke

OC-16 Crystallophore, a nucleating and phasing agent
O. Maury

OC-17 Anticipated biased condition method for macromolecular crystallization
F. Gorrec

Dissecting GPCR Structure and Dynamics with X-ray Lasers

Vadim Cherezov

University of Southern California, Los Angeles, USA

ABSTRACT

G Protein-Coupled Receptors (GPCRs) are versatile cellular gatekeepers that regulate the majority of physiological processes in the human body and have been targeted by a large share of pharmaceutical drugs. Structural studies of GPCR superfamily have been enabled a decade ago by multiple breakthroughs in technology that included receptor stabilization, crystallization in a membrane environment, and microcrystallography. The recent emergence of X-ray free electron lasers (XFELs) has further accelerated structural studies of GPCRs and other challenging macromolecules by overcoming radiation damage and providing access to high-resolution room temperature structures and dynamics using micrometer-sized crystals. This talk will summarize key technology advancements and major milestones of GPCR research at XFELs, and provide a brief outlook on future developments in the field.

The High-resolution Structure of a Metabolite Sensing GPCR Bound to an Intracellular Nanobody

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ABSTRACT

G-protein coupled receptors (GPCRs) can recognize diverse ligands, ranging from lipids and peptides to neurotransmitters and metabolites and trigger intracellular signaling pathways via coupling to G-proteins or arrestins[1].

About 35% of all currently marketed drugs target GPCRs, which is no surprise given their implications in many diseases[2,3]. With the implementation of new technologies for expression, purification, stabilization and crystallization in the past 10 years, structure based drug discovery on GPCRs became feasible and dozens of structures have been reported to date[4-6]. However, we still lack important high-resolution structural information for a vast number of GPCRs, limiting the possibilities to design and develop efficient and safe drugs for these highly valuable targets.

Here we report the 2.1 Angstrom crystal structure of a novel metabolite sensing class A GPCR bound to a nanobody in the inactive confirmation. The nanobody binds to the intracellular side of the receptor and acts as a negative allosteric modulator. We identified a hydrophobic pocket close to the proposed orthosteric ligand binding site, which potentially could be explored to obtain novel selective and high-affinity antagonists for this receptor.

We found a secondary binding site occupied by a 2,5-Hexanediol molecule at the TM7/Helix8 interface. Comparison with other GPCRs reveals structural similarities but also significant differences in an allosteric ligand binding site close to TM2/3/4, which could provide a starting point for the elucidation of new chemical entities targeting this metabolite sensing class A GPCR.

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Application of Microscale Thermophoresis to GPCRs

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ABSTRACT

G protein-coupled receptors (GPCRs) constitute the largest protein superfamily in the human genome with over 800 unique members. GPCR-mediated signaling pathways play a key role in all essential physiological systems as well as many pathophysiological conditions. GPCRs are important drug targets, with over 30% of all prescribed drugs on the market acting on these receptors. Here we report our attempts to apply the microscale thermophoresis technology (MST) to studies of GPCR interactions with their ligands.

This method is simple conceptually, does not require the use of radioactive or fluorescently labeled ligands, and also have prospects for scaling, which is important for high-throughput ligand screening. While simple in execution, the method requires substantial optimization of experimental conditions and protocols for increasing the signal detection sensitivity. Additionally, GPCRs are low expressing and highly unstable membrane proteins, with a substantial difficulty in achieving a sufficient yield of properly folded purified protein. Wild-type receptors are generally unstable and prone to aggregation, and, therefore, require using proper stabilization strategies, such as stabilizing mutations and fusion partners, suitable membrane-mimicking environment, and optimized protocols for protein expression and purification.

The work was supported by the Russian President Grant for Governmental support of Young Scientists (project no. MK-5184.2018.4)

^{19}F-NMR Fragment Screening at SANOFI

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ABSTRACT

Fragment screening has proven very efficient in providing new chemical material for structure-based drug design approaches. Ligand-observed ^{1}H NMR is one of the methods routinely used, as it is especially suited to detecting low affinity binders. It nevertheless has some drawbacks, for instance the number of false positives, due to the relatively large ligand concentration used for screening.

^{19}F NMR presents many advantages for fragment screening, amongst them sensitivity, simplicity and speed. We have set-up a new fluorine-containing fragment library and have carried out ^{19}F fragment screening successfully on several targets. We will present our process and some of the results we obtained by ^{19}F-NMR fragment screening.

Evaluation of SPR Based Screening Campaigns: Implications to Define Performance Parameters and Hit Selection Criteria

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ABSTRACT

Screening of large compound collections is one major strategy of pharmaceutical companies to fuel their pipeline with new drug candidates. Besides screening with functional, biochemical or cellular assays there is an increased demand for the identification of protein binders using biophysical screening assays. Surface Plasmon Resonance (SPR) based ligand binding assays with their throughput, low protein target consumption, ability to discriminate between the specific and non-specific interactions and quantitative (K_D) hit validation offer an attractive solution for the identification of protein binders. Though, some examples of screening campaigns using SPR based binding assays are present in the literature[1,2], however, parameters describing assay robustness and pharmacological sensitivity are rarely discussed[3]. Based on comparison of results obtained from various SPR based screening campaigns conducted at Evotec we evaluate factors critical for development and validation of robust SPR assays. Performance parameters such as assay sensitivity, accuracy, reproducibility or selectivity as well as hit selection criteria are discussed and compared to parameters used in classical screening campaigns with functional, biochemical or cellular assays.

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A Potent and Orally Bioavailable ERK1/2 Series Identified Through Fragment Screening Which Modulates the Phosphorylation and Catalytic Activity of ERK1/2

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ABSTRACT

The Ras-Raf-MEK-ERK pathway is activated through mutations in Ras or Raf in approximately one third of cancers. Discovery of B-Raf and MEK kinase inhibitors has led to effective treatments for tumours driven by activating mutations in B-Raf, but resistance is known to emerge via increased ERK1/2 signalling. This has prompted the development of direct inhibitors of ERK1/2, several of which are in early clinical trials. The majority of clinical ERK1/2 inhibitors are ATP competitive, blocking ERK1/2 catalytic phosphorylation of downstream substrates such as RSK, but do not modulate phosphorylation of ERK1/2 by MEK. Published and in-house structure-based studies on the pERK1/2 modulating inhibitor SCH772984 suggested that it induces a conformational change in the glycine-rich loop of ERK2. We hypothesized that this change might affect the phosphorylation of ERK1/2.

A fragment-based campaign using multiple screening methods, including high throughput crystallography and biophysical assays, was started to identify and develop novel, orally bioavailable inhibitors which elicit a similar conformational change and also modulate the phosphorylation of ERK1/2. Progressive rounds of structure-guided optimization led to an understanding of the structure determinants required in an inhibitor to induce the desired conformational change. These efforts, together with iterative optimization in a screening cascade that included measurement of pRSK and pERK levels and anti-proliferative activity in RAS and BRAF mutant cells, led to the discovery of a novel series of pERK modulating ERK1/2 inhibitors.

The lead compound has low nanomolar potency in ERK1/2 biochemical and cell proliferation assays in both BRAF and RAS mutant cell lines. An excellent kinome selectivity profile and good pharmacokinetics resulted in robust anti-tumour activity upon oral dosing in a range of sub-cutaneous xenograft models, including the mutant BRAF colorectal line Colo205, providing a promising basis for further optimization towards clinical pERK1/2 modulating ERK1/2 inhibitors.

Fragment Screening of Lipoprotein-associated Phospholipase A_2 (Lp-PLA_2) and the Structure-based Development of Potent and Selective Lead Molecules

P. Day *et al*

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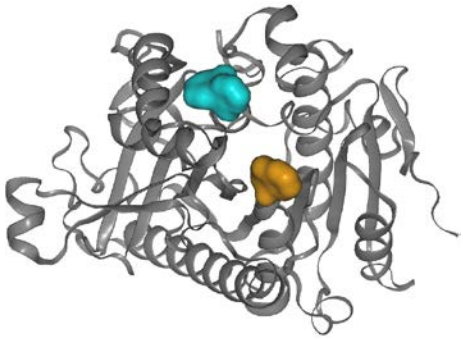
ABSTRACT

Lp-PLA_2 is a plasma enzyme that is predominantly bound to low density lipoprotein and that produces pro-inflammatory stimuli by hydrolyzing oxidation damaged phophatidylcholine[1]. Inhibition of Lp-PLA_2 has been suggested to be a useful strategy for the treatment of atherosclerosis[2],dementia[3] and diabetic edema[4].
We used our fragment-based technology (Pyramid™) to screen for novel fragments that bind to Lp-PLA_2. Fragments bound throughout the active site, mapping the entire substrate binding site[5]. Subsets of these fragments either induced the formation of a small pocket remote from the site of catalysis or formed a covalent bond with the catalytic serine residue. We subsequently used structure-guided design to develop potent lead molecules from these novel fragments.



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Allosteric Activation of Striatal-enriched Protein Tyrosine Phosphatase (STEP, PTPN5) by a Fragment-like Molecule

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ABSTRACT

Protein tyrosine phosphatase non-receptor type 5 (PTPN5, STEP) is a brain specific phosphatase that regulates synaptic function and plasticity by modulation of N-methyl-d-aspartate receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) trafficking. Dysregulation of STEP has been linked to neurodegenerative and neuropsychiatric diseases, highlighting this enzyme as an attractive therapeutic target for drug discovery. Selective targeting of STEP with small molecules has been hampered by high conservation of the active site among protein tyrosine phosphatases. We report the discovery of the first small molecule allosteric activator for STEP that binds to the phosphatase domain. Allosteric binding is confirmed by both X-ray and 15N NMR experiments, and specificity has been demonstrated by an enzymatic test cascade. Molecular dynamics simulations indicate stimulation of enzymatic activity by a long-range allosteric mechanism. To allow the scientific community to make use of this tool, we offer to provide the compound in the course of an open innovation initiative.

The corresponding article has recently been published in J. Med. Chem.
https://pubs.acs.org/doi/10.1021/acs.jmedchem.8b00857

memproc: Tools for Semi-automated Processing of Data from Serial Crystallography Experiments

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ABSTRACT

In the past decade, many synchrotrons around the world have implemented microfocus beamlines with a beam size of 10µm or less, allowing data collection and structure solution of very challenging proteins despite limited crystal size[1]. The implementation of fast direct detectors in conjunction with highly intense microfocus beams made it possible to collect data from hundreds of microcrystals in serial crystallography experiments in a short time[2,3].

In these cases, it is necessary to merge datasets from dozens or hundreds of crystals to obtain a complete, high-quality dataset. However, the data analysis, selection and merging of so many datasets remains a challenging task. Not only is the data processing time consuming, but it is important to remove data, which have been affected by radiation damage or other pathologies in order to obtain the best quality datasets.

We developed a suite of tools called *memproc*, which allow to process hundreds of datasets in parallel on a high performance cluster utilizing *autoProc*[4] in a short time. By providing a summary of data quality indicators for each dataset, the user can selectively omit bad data from subsequent iterative data processing steps and maximize data quality.

Scaling and merging is handled by *aP_scale* and feedback to the user in form of correlation coefficient heat maps and other data quality indicators helps to select the best datasets for the final scaling and merging step. Anisotropy analysis by *staraniso*[5] as part of *aP_scale* in our *memproc* tools allows to retain the maximum of useful data for structure refinement in *Buster*[6].

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Crystallophore, a Nucleating and Phasing Agent

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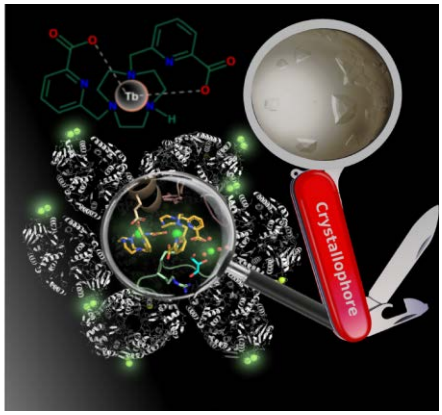
ABSTRACT

With 90% of the structures present in Protein Data Bank, crystallography is one of the methods of choice for obtaining structural information on proteins. However, the method still suffers from the two inherent bottlenecks: obtaining single crystals and solving the phase problem. The crystallization of proteins thus remains the major obstacle and it is considered that approximately one purified protein over three will lead to exploitable crystals, despite the exploration of hundreds of physico-chemical conditions allowed by automatic crystallization screening. Another approach is the use of nucleating agents (heterogeneous nucleation) to lower the energy required to form the crystalline germ such as Molecular Imprinted Polymers [1] or porous materials [2].

We recently proposed a new molecule, called *Crystallophore* (Xo4), which is a cationic lanthanide complex built from a macrocycic ligand. This unique soluble additive combine exceptional phasing properties and can act as a straightforward nucleating agent [3-5]. We will present the results obtained on about fifteen proteins which show that Xo4 induces unique crystallization conditions, avoid twinning problem and promotes new crystalline stacks thanks to a versatile network of supramolecular interactions. We will also show that Crystallophore can also be exploited to solve the phase problem due to the strong anomal scattering properties of the central lanthanide ion.

By adding its nucleating properties to its phasing abilities, Tb-Xo4 is an excellent additive improving the crystallization reproducibility and crystal quality, opening great opportunity in FBDD or serial crystallography for instance and will surely engage a new revolution in the macromolecular crystallography field as the seleno-methionine labeling did few decades ago.



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Anticipated Biased Condition Method for Macromolecular Crystallization

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ABSTRACT

Crystallization is an essential step for determining macromolecular structures at atomic resolution with X-ray crystallography. Quality diffraction crystals obtained from purified samples of proteins, RNAs, DNAs and their complexes help structural biologists to better understand metabolism and enable drug discovery. However, novel samples are increasingly challenging to produce and the right conditions to later obtain useful crystals are unpredictable. Approaches to increase the yield of macromolecular crystals are hence urgently required. In this perspective, the Anticipated Biased Condition method was developed. This method is based on an unusually early selection of the initial conditions producing leads. This, to proceed with follow-up optimization screens with the same sample as the one investigated initially. The underlying goal is to bypass the reproducibility issues induced by the samples, which are typically hard to reproduce and unstable. In addition, optimization is facilitated by extracting the selected conditions from the plate to bias the same screen formulation and protocol that were used initially. The efficiency of the Anticipated Biased Condition method is demonstrated with optimizing the yield of useful crystals for three test samples of interest for drug discovery.

PSDI 2018
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Poster Session

PO-01	Structure analysis of Dipeptidyl peptidases DPP8 and DPP9 reveal inhibitor- and substrate binding mode and cooperativity *S. Krapp*
PO-02	MetalJet source for high-throughput screening in the home laboratory *E. Espes*
PO-03	The MORPHEUS III protein crystallization screen: at the frontier of drug discovery *F. Gorrec*
PO-04	Sanofi Cryo-EM *J. Batchelor*
PO-05	Using structure-based methods for hit finding in the real and virtual worlds *W. Savory*
PO-06	Pronounced affinity loss of Gal-3 inhibitors at mouse versus human Gal-3 due to the absence of a critical alanine residue in the mouse Gal-3 binding site *A. Mac Sweeney*
PO-07	Protein domain trapping: High throughput protein engineering to enable biophysics and structural biology based drug discovery *D. Pogoryelov*

Structure Analysis of Dipeptidyl Peptidases DPP8 and DPP9 Reveal Inhibitor and Substrate Binding Mode and Cooperativity

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ABSTRACT

Human Dipeptidyl peptidases 8 and 9 are serine proteases cleaving their substrates at the amino-terminus mainly post-proline. DPP8 is located in the cytosol and DPP9 locates to the cytosol and nucleus. Both proteins are relevant in immune response and cancer [1].
Enzymatic activity of DPP8 and DPP9 can be modulated by specific small molecule inhibitors like 1G244 or peptides like SLRFLYEG [2], [3].
We solved the dimeric structures of DPP8-apo, DPP8-SLRFLYEG, DPP9-apo and DPP9-1G244 revealing a disorder-order transition of a 26aa segment (R-segment) upon ligand binding, essential for enzyme activity in the closed state. The observed structural transition upon ligand binding translates into cooperativity, which is confirmed by enzymatic functional data [4].
The Interaction of DPP8 and DPP9 with SUMO1 has been confirmed by pull down assays and SPR. We propose a DPP8/9-SUMO1 interaction model, where the R-segment and a mutation-based identified segment (SUBA region) in DPP8/9 are essential in complex formation.

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MetalJet Source for High-throughput Screening in the Home Laboratory

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ABSTRACT

High-end x-ray diffraction techniques such as small molecule crystallography, macromolecular crystallography and non-ambient crystallography rely heavily on the x-ray source brightness for resolution and exposure time. As boundaries of technology are pushed forward samples are becoming smaller, weaker diffracting and less stable which put additional requirements on ever brighter sources. With bright enough compact sources, time resolved studies can be achieved even in the home laboratory. Traditional solid or rotating anode x-ray tubes are typically limited in brightness by when the e-beam power density melts the anode. The liquid-metal-jet technology (MetalJet) has overcome this limitation by using an anode that is already in the molten state thus e-beam power loading above several megawatts per mm are now feasible.

Over a decade ago the first prototypes of liquid-metal-jet x-ray sources were demonstrated. These immediately demonstrated unprecedented brightness in the range of one order of magnitude above current state-of-the art sources [1-3]. Over the last years, the liquid-metal-jet technology has developed from prototypes into fully operational and stable X-ray tubes running in more than 50 labs over the world. X-ray crystallography has been identified as a key application for the x-ray tube technology, since this application benefits greatly from small spot-sizes, high-brightness in combination with a need for stable output. To achieve a single-crystal-diffraction (SCD) platform addressing the needs of the most demanding crystallographers, multiple users and system manufacturers has since installed the MetalJet x-ray source into their SCD set-ups with successful results [4].

This contribution reviews the evolvement of the MetalJet technology specifically in terms of stability, lifetime, flux and brightness and its applicability for pushing boundaries of high end SCD supported by recent user data. We also present recent possibilities to achieve cost effective solutions attainable for a wider application range. Finally, we discuss details of the technology with a focus on the fundamental limitations and its abilities to free up synchrotron time by efficient home laboratory screening.

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The MORPHEUS III Protein Crystallization Screen: At the Frontier of Drug Discovery

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ABSTRACT

The novel samples required to study biological mechanisms at the atomic level are increasingly challenging to produce and crystallize. Besides, there are additional difficulties to make X-ray crystallography amenable to the discovery of drug compounds (1). Subsequently, innovations which enhance the yield of useful crystals are urgently required. The original MORPHEUS (2) and its first follow-up MORPHEUS II (3) have proven to be very efficient protein crystallization screen in the long term for a broad variety of protein samples. The main ideas behind the formulation of MORPHEUS III were the same as for MORPHEUS I and II, with essentially:

- Increasing the chances of crystal nucleation and growth by integrating mixes of additives that can act as stabilizers, cross-linkers, *etc.* (4).
- Systematic approaches to select the reagents and formulate the screen, including the integration of ligands found in the Protein Data Bank (PDB).
- Enhancing pragmatic ways to facilitate the steps towards structure solution, notably with cryoprotected conditions.

The novelty in MORPHEUS III is the integration of small drug-like compounds as additives (average MW = 248 Da). To some extend, the approach can be compared to fragment-based lead discovery (5). In this perspective, MORPHEUS III is at the frontiers of drug discovery. The primarily aim however is still to obtain useful crystals from a broad variety of novel samples.

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Sanofi Cryo-EM

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ABSTRACT: TBD

Using Structure-based Methods for Hit Finding in the Real and Virtual Worlds

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ABSTRACT

Domainex performs fully-integrated structure-based drug design programs. We show how we use structural information to find small-molecule ligands by both virtual screening (LeadBuilder) and fragment screening (FragmentBuilder). These technologies will be illustrated with a case study on the lysine methyltransferase enzyme, G9 (also known as EHMT2).

Firstly, a proprietary crystal structure of G9a bound to peptide was used to set up LeadBuilder, our virtual screening platform to identify small molecule starting points for drug discovery programs. LeadBuilder has two key elements: the first is our in-house 'NICE' curated database of commercially-available lead-like compounds; the second is a proprietary two-stage virtual screening protocol based on searches against multiple Target Site Pharmacophore Models (TSPM), followed by docking into the protein target site. Typically 500-1500 virtual hits are then acquired and tested in an appropriate biochemical assay. This approach has a much better success rate than HTS, and delivers highly-developable hit compounds that enable an accelerated hit to lead process.

We also report a fragment-based drug design (FBDD) approach using Microscale Thermophoresis (MST) - an affinity-based technique - to screen a library of low molecule weight compounds ('fragments'). FBDD has become widely used as an alternative to traditional high throughput screening (HTS). Since fragment hits have relatively weak affinity, they are often identified using biophysical techniques such as NMR, SPR, DSF, ITC, or X-ray crystallography. MST measures the movement of fluorescently-labelled molecules in temperature gradients created by laser within microliter-volume glass capillaries. The thermophoretic movement of a molecule is determined by its size, charge, and hydration shell. Ligand binding affects these properties, resulting in changes in the thermophoretic characteristics of the molecule. These changes can be used to derive dissociation constants (Kd) within minutes. This method offers a number of benefits for FBDD, notably its fast, efficient and precise ability to characterise fragments with a low number of false positives and false negatives, whilst using very small amounts of protein.

Out of a library of 320 fragments, we identified 17 fragments hits against G9a by single-shot screening at 1 mM concentration (5.3% hit rate) using MST; whereas screening the same library with Differential Scanning Fluorimetry (DSF) and AlphaScreen, afforded only one hit with each of the techniques. We used NMR Saturation-Transfer Difference (STD) to confirm hits and X-ray crystallography to obtain structural information on the positioning of the fragment hits for hit-to-lead development. This highlights the advantages of MST for working with ternary systems, which can be difficult using some other biophysical techniques such as ITC and DSF.

Pronounced Affinity Loss of Gal-3 Inhibitors at Mouse versus Human Gal-3 due to the Absence of a Critical Alanine Residue in the Mouse Gal-3 Binding Site

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ABSTRACT

Galectin-3 (LGALS3; Gal-3) inhibition represents a promising therapeutic strategy in multiple human diseases. Human and mouse Gal-3 differ by one amino acid (human: alanine146; mouse: valine160) in an area that interacts with the F-phenyl-moiety of competitive Gal-3 blockers such as TD139. We hypothesized that this single amino acid difference is the reason for the pronounced affinity loss of TD139 and related compounds at mouse Gal-3 when compared to human Gal-3. We tested whether the exchange of valine for alanine at position 160 in mouse Gal-3 would lead to human-like affinities of TD139 and related compounds. Using competitive binding assays we could show that mouse Gal-3^{V160A} displays human-like affinities for TD139 and related compounds while retaining its affinity towards the natural ligand N-acetyl-lactosamine. Subsequently, analysis of compound-Gal-3 complexes (human, mouse and V160A) by X-ray crystallography was performed to rationalize the critical roles of alanine / valine in inhibitor binding. In conclusion, humanization of mouse Gal-3 with respect to binding affinity to TD139 and related Gal-3 inhibitors can be achieved by the V160A single amino acid exchange. Knock-in mice carrying the mouse Gal-3^{V160A} mutation should allow the in vivo analysis of Gal-3 inhibitors under conditions of human-like high target binding affinity.

Protein Domain Trapping: High Throughput Protein Engineering to Enable Biophysics and Structural Biology Based Drug Discovery

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ABSTRACT

With increasing emphasis on novel targets or attacking recognized targets with novel mechanisms, the rate limiting step in small molecule drug discovery often becomes the availability of protein in an appropriate, well-behaved form. Standard methods for generating well-behaved constructs are far too slow and sample only a very limited fraction of the great number of possibilities. ZoBio has developed a system, called Protein Domain Trapping, to screen millions of protein variations for those that express high levels of soluble, well-behaved protein with the desired biological activity. Initial mapping studies require only 4-6 weeks.

PDT is based on the split GFP complementation system. First, a library of different sized DNA fragments is created from the desired gene. Using ZoBio's proprietary know-how, the focus can be placed on any portion of, or the entire gene. The library is ligated into a custom expression vector where the sequence for the 11th beta strand of the green fluorescent protein (GFP) is fused to the c-terminus. Next, the library is transformed into an E. coli host that expressed GFP beta strands 1-10. If and only if, the protein derived from the target is expressed in a soluble form, the 11th beta strand will bind to GFP1-10 and generate the fluorophore. Interesting clones are picked, sequenced and mapped back to the target. If the required portion of the target is present in the clones, the complementation is performed in vitro providing confirmation of the solubility of the protein in cell lysate and information on the aggregation state. These data are used to further filter the clones for intermediate scale expression and purification studies. Case studies illustrating the power and efficiency of the process will be provided. The PDT approach also has the advantage of selecting well behaving protein domains of a pharmaceutical target that otherwise might be difficult for structural biology approaches like X-ray crystallography