New Frontiers in Structure-Based Drug Discovery

International Conference

Convitto della Calza, Florence - Sep 23-25, 2019

https://www.nfsdd.eu/

Invited Speaker

Patrizia Aducci
Michelle Arkin
Alessio Ciulli
Stephen Fesik
Mike Hann
Birthe Kragelund
Ora Schueler-Furman
Jan Steyaert
Ed Tate
Rebecca Wade

Organization

Michael Sattler
Christian Ottmann
Eva Schlosser
Laura Bassani

Local Organisation

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The conference *"New Frontiers in Structure-based Drug Discovery"* as well as the programs AEGIS and TASPPI are funded by the EU Horizon 2020 framework program under the Marie Skłodowska-Curie Grant Agreement no. 675555 and Grant Agreement no. 675179

AEGIS Accelerated Early stage drug IS covery | TASPPI

New Frontiers in Structure-Based Drug Discovery

International Conference

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New Frontiers in Structure-Based Drug Discovery

International Conference

Welcome

Dear colleagues

It is our great pleasure to welcome you to the international conference "***New Frontiers in Structure-Based Drug Discovery",*** at the Convitto della Calza in Florence, Italy. We are grateful to the positive resonse by our internationally renowned distinguished keynote speakers. We expect a very exciting meeting with stimulating discussions during poster sessions and breaks with participants from academia and industry. There was an overwhelmingly positive response from invited speakers and participants.

The conference is organized by the two European Marie Skłodowska-Curie Innovative Training Networks (ITN) AEGIS (Accelerated Early Stage Drug Discovery) and TASPPI (Targeted small-molecule Stabilisation of Protein-Protein Interactions). Both consortia trained young researchers in the past four years in early stage stage drug discovery, using structure and fragment-based drug discovery targeting novel and challenging targets, including protein-protein interactions. The aim of both ITNs was to develop and apply innovative tools to explore novel targets with a focus on rare and neglected diseases. Results from the work of the 28 fellows will also be presented during conference, accompanied by lectures by internationally highly recognized experts in this field.

We wish you a great and stimulating meeting, and are looking forward to exciting and vivid discussions and your comments and suggestions.




Michael Sattler **Christian Ottmann** **Eva Schlosser** **Laura Bassani**

Organizers of the conference

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Exhibitor

	**Instruct-ERIC Hub** Oxford House Parkway Court, John Smith Drive Oxford OX4 2JY **Francesca Morelli** +44 (0) 1865 98 86 39 admin@instruct-eric.eu

General Information

Venue

Convitto della Calza

Piazza Della Calza, 6

50125, Florence – Italy

Phone: +39 055.222287

calza@calza.it

Registration of **New Frontiers in Structure-Based Drug Discovery** will take place in the **Chiostro** (cloisters) at the entrance of Pontevecchio Room.

All talks will take place at **Pontevecchio Room**.

Poster Session will take place in the Chiostro.

How to get to Convitto della Calza

Public transport (subway, trains, trams or buses)

From Florence airport to city centre:

T2 tramway connection between the Florence airport and the City Centre (Stop at Alamanni-Stazione);

From Florence train station S.M. Novella:

by foot it is approx 2 km;

by bus nr. 11 (Direction La Gora - from Stazione Scalette bus stop to Porta Romana bus stop)

Taxi

Taxi Florence **+39 (0)55 4293** or

+39 (0)55 4242

A taxi ride from the airport to *Convitto della Calza* takes ca 30 min and costs approx. 30 €.

Registration

The registration desk is located in the Chiostro and will be open.

The registration desk will be open:

Monday,	Sep 23	09:00 – 18:00
Tuesday,	Sep 24	09:00 – 18:00
Wednesday,	Sep 25	09:00 – 14:00

Help, information - important phone numbers

Conference office:

Lorenzo Maggiorelli +39 335 7487016 (during registration hours)

Convitto della Calza, Segretaria Congressuale:

Rachele Taddeucci +39 (0)55 2306140

Organizers:

Laura Bassani +39 3920589344

Eva Schlosser +49 15165519496

Emergency numbers

Carabinieri **112**

State police **113**

Fire brigade **115**

Ambulance **118**

(It is not necessary to dial any country or area code for these numbers.)

Municipal police **+39 (0)55 3285** - line open 24 hours a day - only for absolute emergencies: serious accidents, immediate danger or serious risk to public health

+39 (0)55 3283333 - line open 24 hours a day - any request for assistance or to request the presence of the municipal police

Internet

WLAN access is available at the conference location. Details will be provided on-site.

Coffee, lunch, dinner

Coffee and soft drinks will be provided during the conference breaks outside the Pontevecchio Room.

During the poster session on Monday evening, drinks will be available.

Light Lunches will be offered in the chiostro.

Gala Dinner on Tuesday evening will be served in the Chiostro and the Loggia.

Posters

Posters will be displayed during the entire conference in the Chiostro. Presenters are requested to be at their poster during the scheduled poster sessions on Monday and Tuesday evening.

Poster numbers correspond to the page number of the poster abstract in this booklet.

Posters can be mounted from Monday 10:00 on and should be removed by Wednesday 14:00.

Poster Prizes

There will be three poster prizes awarded supported by the ***sponsors*** of the New Frontiers of Structure-Based Drug Discovery.

Exhibitions

Exhibitions of our sponsors and the exhibitor are located in the Chiostro.

Invited Speakers

Patricia Aducci *Fusicoccin and 14-3-3 proteins, a long story from plant pathology to drug design.*	Università di Roma, Italy
Michelle Arkin *Chemical Biology Approaches to Understanding the VCP/p97 Protein Interaction Network*	University of California San Francisco, USA
Alessio Ciulli *Targeted Protein Degradation with Small Molecules: How PROTACs work*	University of Dundee, UK
Stephen Fesik *Cancer Drug Discovery Using Fragment-Based Methods and Structure-Based Design*	Vanderbilt University, USA
Mike Hann *'PhABits – shining new light on fragment approaches for assessing target tractability'*	GlaxoSmithKline, UK
Birthe Kragelund *Disordered Protein Complexes*	University of Copenhagen, Denmark
Ora Schueler Furman *Peptide as leads for targeted inhibition of interactions – How free, how determined?*	Hebrew University, Israel
Jan Steyaert *From Nanobodies to Megabodies for applications in cryo-EM*	VIB, Brussels, Belgium
Edward Tate *Fragment-guided discovery of an irresistible antiviral protein-protein interaction inhibitor*	Imperial College London, UK
Rebecca Wade *Computational Approaches to Protein Dynamics and Binding Kinetics for Drug Discovery*	HITS & Heidelberg University, Germany

Program

Monday, Sep 23, 2019	*Key note lectures: 35+5 min, Talks: 15+5 min, Flashtalks: 2 min*
12:45	**Welcome** **Michael Sattler, Christian Ottmann**
	SESSION 1 – Structure- and Fragment-based Drug Discovery **Chair:** ***Christian Ottmann***
13:00	**Keynote lecture: Stephen Fesik** *Cancer Drug Discovery Using Fragment-Based Methods and Structure-Based Design*
13:40	**Francesco Bosica** *Mg^{2+}-assisted small-molecule stabilisation of 14-3-3 Protein-Protein Interactions.*
14:00	**Barak Akabayov** *Development of antibacterial agents that target the ribosomal PTC of M. tuberculosis*
14:20	**Christian Griesinger** *Modulation of aggregating proteins studied by NMR and beyond in neuro- and cellular degeneration*
14:40	**Madita Wolter** *Drug Discovery at the Boundary of Cancer and Infection*
15:00	**Keynote lecture: Mike Hann** *'PhABits – shining new light on fragment approaches for assessing target tractability'*
15:40	*Coffee Break*
	SESSION 1 – cont'd **Chair:** ***Tomáš Obšil***
16:10	**João Neves** *Nuclear Magnetic Resonance guided screening applied to the discovery of modulators of 14-3-3 Protein-Protein Interactions*
16:30	**Stefan Hoerer** *Allosteric Activation of Striatal-Enriched Protein Tyrosine Phosphatase (STEP, PTPN5) by a Fragment-like Molecule*
16:50	**Keynote lecture: Edward Tate** *Fragment-guided discovery of an irresistible antiviral protein-protein interaction inhibitor*
17:30	**Flash Talks**
18:45 - 20:30	**Poster Session 1** with drinks

Program

Tuesday, Sep 24, 2019	
	SESSION 2 – Intrinsically Disordered and Amyloidogenic Proteins Chair: ***Isabelle Landrieu***
09:00	**Keynote lecture: Birthe Kragelund** *Disordered Protein Complexes*
09:40	**Orgeta Zeijneli** *A VHH directed against tau as a novel therapeutic approach in tauopathies*
10:00	**Carlos Camacho** *Neuroprotection by small molecule inhibition of disordered peptide and syntaxin interaction*
10:20	*Coffee Break*
	SESSION 3 – Chemical biology, targeting & new concepts Chair: ***Alex Dömling***
10:50	**Keynote lecture: Alessio Ciulli** *Targeted Protein Degradation with Small Molecules: How PROTACs work*
11:30	**Felix Hausch** *Selective FKBP51 inhibitors enabled by transient pocket binding*
11:50	**Markella Konstantinidou** *Discovery of proteolysis targeting chimeras for leucine-rich repeat kinase 2 (LRRK2)*
12:10	**Nikolaos Sgourakis** *New tools for the analysis of polyclonal T cell repertoires using chaperone-mediated peptide exchange*
12:30	**Valeria Napolitano** *Inhibition of glycosomal protein import: the sweet death of Trypanosoma*
12:50	Lunch
	SESSION 3 – cont'd Chair: ***Alex Dömling***
14:20	**Keynote lecture: Michelle Arkin** *Chemical Biology Approaches to Understanding the VCP/p97 Protein Interaction Network*
15:00	**João Encarnação** *Bioengineering subunit B from Shiga Toxin 1 for intracellular drug delivery*
	SESSION 4 – Peptides, natural products and non-small molecules Chair: ***Andrew Wilson***
15:20	**Keynote lecture: Ora Schueler-Furman** *Peptide as leads for targeted inhibition of interactions – How free, how determined?*
16:00	**Helen Boyd** *Identification of a novel cyclic peptide that disrupts the homodimerization of the E3 ubiquitin ligase IDOL*

16:20	*Coffee Break*
	SESSION 4 – cont'd **Chair:** ***Andrew Wilson***
16:50	**Françoise Ochsenbein** *Rational Design of Peptides and Foldamers inhibiting a New Epigenetic Target in Cancer*
17:10	**Grzegorz Dubin** *Macrocycle mediated inhibition of PD-1/PD-L1 immune checkpoint*
17:30	**Katiuscia Pagano** *Design of antiangiogenic agents targeting Fibroblast Growth Factors-2/Tyrosine-Kinase Receptor*
17:50	**Federica De Leo** *Diflunisal targets the HMGB1/CXCL12 heterocomplex and blocks immune cell recruitment*
18:10	**Xiao-Ling Cockcroft** *New Paradigm for Finding Natural-product-like KRAS Inhibitors*
18:30	**Keynote lecture: Patricia Aducci** *Fusicoccin and 14-3-3 proteins, a long story from plant pathology to drug design.*
19:10 - 20:30	**Poster Session 2**
20:30 - 23:00	**Gala Dinner**

Wednesday, Sep 25, 2019	
	SESSION 5 – Computational Drug Discovery Chair: ***Helena Danielson***
09:00	**Keynote lecture: Rebecca Wade** *Computational Approaches to Protein Dynamics and Binding Kinetics for Drug Discovery*
09:40	**Marcus Gastreich** *Debunking a myth - Can σ-Holes Really Drive Affinity?*
10:00	**Alessandro Paiardini** *Identification of small-molecule inhibitors of the Aurora-A/TPX2 complex*
	SESSION 6 – Structural biology, biophysics & methods Chair: ***Michael Nilges***
10:20	**Sebastian Guenther** *Serial Crystallography for Ligand-Binding Studies*
10:40	**Charlotte Softley** *Placing fragments with lanthanide tags using paramagnetic NMR*
11:00	*Coffee Break*
	SESSION 6 – cont'd Chair: ***Michael Nilges***
11:30	**Claire Munier** *Small molecule stabilization of the GR 14-3-3 protein-protein interaction (PPI)*
11:50	**Daisy Paiva** *Because structure means function - Conformational changes in proteins measured with switchSENSE®*
12:10	**Charlotte Hodson** *Expanding Crystallographic methods for Fragment-based Drug Design*
12:30	**Keynote lecture: Jan Steyaert** *From Nanobodies to Megabodies for applications in cryo-EM*
13:10	*Lunch*
14:40	**Announcement of Posterprizes** **END/FAREWELL** Michael Sattler, Christian Ottmann
14:50 - 16:50	**Workshop BioSolveIT**

Abstracts

Oral Talks

SESSION 1

Structure- and Fragment-based Drug Discovery

Cancer Drug Discovery Using Fragment-Based Methods and Structure-Based Design

S. Fesik[1]

[1] *Vanderbilt University School of Medicine, Nashville, Tennessee, USA*

Cancer is a devastating disease that affects the lives of almost everyone, and its effective treatment still remains an important unmet medical need. In order to discover new cancer drugs, we are applying fragment-based methods and structure-based design to identify and optimize small molecules that inhibit highly validated cancer targets. Although many of these targets are technically challenging and thought to be undruggable, fragment-based methods offer several advantages over more conventional approaches which suggest that it may be possible to achieve success. In this presentation, examples will be given of how this methodology can be used to discover small molecules that bind to highly validated but technically challenging cancer targets.

Mg^{2+}-ASSISTED SMALL-MOLECULE STABILISATION OF 14-3-3 PROTEIN-PROTEIN INTERACTIONS

F. Bosica[1], G. O'Mahony[1], A. Gunnarsson[2], S. Andrei[3], C. Ottmann[3,4]

[1] *Medicinal Chemistry, Cardiovascular Renal and Metabolism, Innovative Medicines and Early Development Biotech Unit, AstraZeneca Gothenburg, Mölndal, Sweden*

[2] *Biophysics, Discovery Science, Innovative Medicines and Early Development Biotech Unit, AstraZeneca Gothenburg, Mölndal, Sweden*

[3] *Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, Eindhoven, The Netherlands*

[4] *Department of Chemistry, University of Duisburg-Essen, Essen, Germany*

Protein-Protein Interactions (PPIs) are central to most biological processes, making them attractive targets for modulation with small-molecules. PPI stabilisation, as opposed to PPI inhibition, is a yet relatively unexplored field in medicinal chemistry. 14-3-3 proteins are adapter proteins regulating the function of many client proteins via protein-protein interactions (PPIs), and represent a structurally well-characterised model system for the medicinal chemistry study of PPI stabilisation. Stabilisation of 14-3-3 PPIs offers a means of modulating the activity of the often classically-undruggable 14-3-3 binding partners.

Fusicoccin A (Fc-A) is a complex natural product stabiliser of 14-3-3 PPIs but is not suitable as a starting point for medicinal chemistry exploration, so small molecule lead matter is sought. Using the 14-3-3:ERα PPI as a model system, we have developed robust assays (FP and SPR) for compound screening and testing. Starting from the known racemic 14-3-3 PPI stabiliser Pyrrolidone1 (Pyr1) we have identified non-natural product, small molecule 14-3-3:ERα stabilisers with improved activity over Pyr1.

Testing of Pyr1 enantiomers in our FP assay showed that only one enantiomer contributes to PPI stabilisation activity. Protein- and ligand-based NMR experiments confirmed the active enantiomer as a true hit and identified the compound binding site as the Fc-A binding site. An X-ray crystal structure of the Pyr1 active enantiomer ternary complex with 14-3-3:ERα was obtained, unambiguously assigning the *(R)* absolute configuration, confirming the NMR-deduced binding site and identifying key ligand-protein interactions that contribute to *(R)*-Pyr1 binding. In addition, a magnesium ion was observed to chelate to the vinylogous carboxylate of *(R)*-Pyr1, potentially leading to conformational restriction of the ligand. The contribution of this chelation to binding affinity was confirmed in FP, with Mg^{2+} titration indicating that high [Mg^{2+}] affords a ~25-fold increase in apparent stabilisation activity. *(R)*-Pyr1, but not Fc-A, was also found to stabilise the 14-3-3:CaMKK2 complex, paving the way to the understanding of the drivers of selectivity. The Pyr1 scaffold is accessed via a multicomponent reaction, SAR expansion has been conducted and results will be presented.

In conclusion, through a rational medicinal chemistry approach, we have confirmed Pyr1 as a suitable chemical starting point for development of PPI stabilisers and have made progress towards improved PPI stabilisation activity.

Development of antibacterial agents that target the ribosomal PTC of *M. tuberculosis*

B. Tam1, G. Wagner2, B. Akabayov1

[1] *Department of Chemistry, Ben Gurion University of the Negev, Be'er Sheva, Israel*
[2] *Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, U.S.A.*

We have developed new lead compounds that target the ribosomal peptidyl transferase center (PTC) of *M. tuberculosis*, a pathogenic bacterium that kills more than 1.5 million people worldwide every year. For this purpose, we used a fragment-based screening workflow in which the first step was the novel exploitation of NMR transverse relaxation times (T_2) to identify fragment molecules that bind specifically to RNA hairpin 91 in the ribosomal PTC of *M. tuberculosis*. This initial screening was followed by computational optimization of the fragment molecules into larger molecules with drug-like properties. Specifically, a virtual filtration followed by a high-throughput docking procedure yielded drug-sized molecules. A machine-learning model predicted two molecules that exhibited IC_{50} values superior to that of chloramphenicol, an antibiotic drug that acts on the ribosomal PTC.

Modulation of aggregating proteins studied by NMR and beyond in neuro- and cellular degeneration

S. Ryzanov[1,2], L. Antonschmidt[1,2], R. Dervisoglu[1], M. Wegrzynowicz[3], K. Runge[1,2], A. Martinez Hernandez[4,5], Manikam S Saravanan[6], Dana Bar-On[7], H.Y. Agbemenyah[4], S. Shi[8], A. Fischer[4], G. Eichele[5], S. Becker[1], R. Benz[9], M. Zweckstetter[1,2], J.A. Killian[6], J. Höppener[10], A. Leonov[1,2], U. Ashery[7], M.G. Spillantini[3], A. Giese[8], L. B. Andreas[1], and C. Griesinger[1,2]

[1] *Dept. for NMR-based Struct. Biology, MPI for Biophysical Chemistry, Germany*
[2] *DFG-Center for the Molecular Physiology of the Brain, Göttingen, Germany*
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[5] *Genes and Behavior Dept., MPI for Biophysical Chemistry, Göttingen, Germany*
[6] *Membrane Biochemistry and Biophysics - Utrecht University, The Netherlands;*
[7] *Department of Neurobiology, George S. Wise Faculty of Life Sciences and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel*
[8] *Center for Neuropathology and prion research, LMU, Munich, Germany*
[9] *Jacobs University of Bremen, Germany*
[10] *Department Laboratory for Translational Immunology, UMC Utrecht, The Netherlands*

In Parkinson's disease (PD), α-Synuclein aggregates to Lewy bodies, which are connected to neuronal dysfunction and death, similar to Aβ and tau in Alzheimer's (AD), prion protein in Creutzfeldt Jacob and IAPP in Type II diabetes mellitus (T2DM). Using structural biology derived predictions [1], α-Synuclein is shown to form non-toxic intrinsically disordered monomers and non-toxic fibrils, while immediate toxicity is exerted by oligomers [2]. Prevention of formation of these toxic oligomers by small molecules, specifically anle138b, observed in vitro and in vivo [3] using ultracentrifugation or superresolution imaging [4], specifically anle138b, leads to neuroprotection and restoration of functionality of the neurons in all mentioned diseases, specifically in PD [3,4], MSA [4], AD based on tau [6] or A β_{42} overexpression [7] and T2DM [8]. Biophysical characterization of anle138b with target proteins will be discussed [6,9] in solution and in membranes with NMR spectroscopy.

[1] Bertoncini, CW. et al. (2005). PNAS, 102 (5): 1430-1435.
[2] Karpinar, DP. et al. (2009). Embo Journal, 28 (20): 3256-3268.
[3] Wagner, J. et al. (2013). Acta Neuropathol. 125, 795-813; Levin, J. et al. (2014). Act. Neuropath. 127, 779-780; Giese, A. et al. (2010). WO/2010/000372; Giese, A. et al. (2017). WO2017/102893.
[4] Wegrzynowicz, M. et al. (2019). Acta Neuropath. doi.org/10.1007/s00401-019-02023-x.
[5] Heras-Garvin, A. et al. (2019). Mov Disord 34, 255-263.
[6] Wagner, J. et al. (2015). Act. Neuropath.130, 619-631.
[7] Hernandez, M. et al. (2018). EMBO Mol. Med. 10, 32-47.
[8] Hoppener, J. et al. (in preparation).
[9] Deeg, A.A. et al. (2015). Biochim. Biophys. Act. 1850 (9), 1884-1890; Reiner, A.M. et al. (2018). Biochim. Biophys. Acta Gen. Subj. 1862, 800-807.

Drug Discovery at the Boundary of Cancer and Infection

M. Wolter[1], D. Valenti[1,2], J. Filipe Neves[3], S. Srdanović[4], Y. Higuchi[6], A. Wilson[4,5], L. Levy[2], T. Genski[2], L. Brunsveld[1], I. Landrieu[3], D. Tzalis[2] & C. Ottmann[1]

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[3] *UMR 8576 CNRS-Lille University, Lille, France*
[4] *School of Chemistry, University of Leeds, Leeds, UK*
[5] *Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK*
[6] *The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Japan*

NF-κB is one of the most important transcriptional mediators of inflammatory responses in auto-immune diseases and cancer, qualifying this protein complex as a high-value target for drug discovery. However, despite significant drug discovery efforts, no efficient direct small-molecule modulator of NF-κB function has been developed to date. The adapter protein 14-3-3 has been reported to negatively regulate NF-κB by facilitating its cytoplasmic sequestration. Here, we report the high-resolution crystal structures of two 14-3-3-binding peptide motifs of the NF-κB subunit p65 in complex with 14-3-3. A natural product derivative – DP-005 – stabilizes the inhibitory p65/14-3-3 complex and binds to a pocket in the direct interface between the p65 peptide and 14-3-3. The X-ray crystal structure of the ternary complex permitted identification of an important lysine residue in this interface that could be targeted by covalent fragments. Fragments comprising an aldehyde function covalently bind this lysine as established through crystallographic analyses and provide starting points for the development of a NF-κB/14-3-3 stabilizer with anti-inflammatory potential.

‘PhABits – shining new light on fragment approaches for assessing target tractability'

M. Hann[1]

[1] *Medicinal Sciences and Technologies, GSK Medicines Research Centre, Stevenage, UK*

Novel targets are increasingly selected for drug discovery based on genomic and genetic data, but triaging which of these are the most viable for intervention by small molecules is essential at the outset of a drug discovery project if resources are not to be wasted on non-productive screening efforts. This talk will focus on approaches (both in silico and experimental) that we are exploring to help objectively assess the tractability of potential targets in the early stages of drug discovery[1]. In particular, I will discuss the use of Photo Activatable Bits (PhABits) as an extension of Fragments methodology, based on the pioneering results of Ben Cravatt’s group[2].

[1] Brown K.K. et al. (2018) MedChemComm. 14;9(4):606-613.
[2] Parker, G.G. et al. (2017) Cell. 168, 527–541e29.

Nuclear Magnetic Resonance guided screening applied to the discovery of modulators of 14-3-3 Protein-Protein Interactions

JF. Neves[1], D. Valenti[2], FX. Cantrelle[1], SA. Andrei[3], FA. Meijer[3], DL. Santo[4], L. Brunsveld[3], T. Obšil[4], L.M. Levy[2], D. Tzalis[2], LG. Milroy[3], C. Ottmann[3], X. Hanoulle[1], I. Landrieu[1]

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[2] *Medicinal Chemistry, Taros Chemicals GmbH & Co. KG, 44227 Dortmund, Germany*
[3] *Dpt.t of Biomedical Engineering and Institute for Complex Molecular Systems, Technische Universiteit Eindhoven, Eindhoven, the Netherlands*
[4] *Dpt. of Physical and Macromol. Chem., Faculty of Science, Charles University, Prague, Czech Republic.*

14-3-3 proteins are ubiquitous adapter proteins that exert their biological functions through the modulation of the activity of hundreds of proteins. This remarkable interactome makes 14-3-3 proteins influent actors in many cellular events and, by consequence, in several pathologies [1].

We employ different approaches in order to obtain modulators of 14-3-3 PPIs. On one hand we used Fragment-Based Drug Discovery in order to obtain new 14-3-3 binding scaffolds with low molecular weight that can be further optimized with the aim of stabilizing or inhibiting different 14-3-3 PPIs. On the other hand, based on the crystal structure of 14-3-3 bound to the Tau peptide surrounding $pSer_{214}$, a rational design of small peptidomimetic inhibitors of the 14-3-3/Tau PPI has been accomplished [2,3].

For both strategies, NMR spectroscopy was the technique selected for the screening of the compounds. Since this technique allows the detection of the binding of hits with mM range affinity, it is very suitable for a fragment screening campaign [4]. Moreover, if one employs protein-based methods, NMR can be used to determine the binding site of the hits. This method is not only suitable for the screening of modulators of one single target, but it can also be used to study the effect of a compound on the interaction between two full-length proteins in solution.

On the scope of the Fragment-based approach, 785 fragments were accessed in 157 cocktails of 5 for binding to 14-3-3 by both ligand-based NMR methods and protein-based NMR methods. Our screening allowed the identification of some hits that are being accessed for the modulation of different 14-3-3 PPIs. On the scope of the peptidomimetic inhibitors for the 14-3-3/Tau PPI, our NMR based assay showed that these compounds inhibit the binding of 14-3-3 to phosphorylated Tau in a concentration dependent manner by binding exclusively to 14-3-3 [3].

In this work, we were able to identify fragments and peptidomimetic compounds able to bind 14-3-3 and to modulate 14-3-3 PPIs. Our future efforts will be focused on the optimization of these molecules in order to obtain potential therapeutic options

[1] J.F. Neves et al (2019), Biomol NMR Assign., 13(1), p.103.
[2] L.G. Milroy et al (2015), Angew. Chem. Int. Ed., 54, p.15720.
[3] S.A. Andrei et al (2018), ACS Chem. Neurosci., 9, p.2639.
[4] M. J. Harner et al (2013), Journal of Biomol. NMR, 56, p.65.

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

C. Tautermann,[1] F. Binder,[1] F. Büttner,[2] C. Eickmeier,[1] D. Fiegen,[1] U. Gross,[1] M. Grundl,[1] R. Heilker,[2] S. Hobson,[3] S. Hoerer,[1] A. Luippold,[2] V. Mack,[3] F. Montel,[1] S. Peters,[1] S. Bhattacharya,[4] N. Vaidehi,[4] G. Schnapp,[1] S. Thamm,[2] and M. Zeeb[1]

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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-hydroxy5-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 ^{15}N 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.

Fragment-guided discovery of an irresistible antiviral protein-protein interaction inhibitor

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My group develops chemical biology approaches to identify and validate potential drug targets, and in this talk I will discuss recent work in our labs on fragment discovery and recombination approaches. I will also present an example in which we recombined fragment-like HTS hits to enable structure-guided discovery of ultrapotent inhibitors of N-myristoyltransferase (NMT), an enzyme which undertakes post-translational modification of hundreds of proteins in all eukaryotic organisms, through a conserved protein-protein interaction (PPI) site. This work has led to pharmacological validation of this target in malaria, and the validation of human (host) NMT as a novel 'irresistible' target across a wide range of viruses including the common cold virus, poliovirus, foot-and-mouth disease virus, pox viruses and HIV.

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SESSION 2
Intrinsically Disordered and Amyloidogenic Proteins

Disordered Protein Complexes

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Intrinsically disordered proteins (IDPs) (or –regions (IDRs)) are functional while existing in broad ensembles of near iso-energetic conformations. Despite their lack of tertiary structure, IDPs are involved in communication with other molecules forming associations ranging from binary, discrete complexes to large multicomponent assemblies. Similar to globular proteins their complexes serve structural, functional and regulatory roles, but due to their dynamic nature, they expand the types of association possible, further enabling functional regulations by very different mechanisms. The fast dynamics characteristic of IDPs may persist in their complexes and the degrees of disorder within the complex can therefore vary greatly. We have been exploring the role of disorder in cellular control processes including pH homeostasis, cytokine signalling, transcriptional regulation, and DNA metabolism, combining NMR spectroscopy with other biophysical methods as SAXS, neutron diffraction, single-molecule FRET and cell biology [1–4]. In one end of the scale we observe folding-upon-binding forming nearly globular-like complexes with little disorder while at the other end, disorder may persist and results in complexes where both binding partners stay disordered in high-affinity binding [3]. Still, the kinetics combined with higher order complex formation allows regulation on biologically relevant timescales. Between these extremes, a continuum of dynamic complexes is possible. The characterisation and functional decoding of dynamic complexes challenges the methodological toolbox, but NMR spectroscopy continues to be a critical contributor in the understanding of disorder dependent biology.

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A VHH directed against tau as a novel therapeutic approach in tauopathies

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Tauopathies, including Alzheimer's disease (AD), are neurodegenerative diseases characterized by the accumulation of aggregated tau into the brain cells of the patients. Tau is a soluble, unfolded microtubule-associated protein that regulates cytoskeletal dynamics of neurons in the central nervous system. Under pathological conditions, tau becomes abnormally phosphorylated and aggregates into filamentous brain inclusions. Although the mechanisms leading to the pathological tau species are not clearly understood, different molecular features have been identified as involved in the aggregation process, including the identification of the peptide motif PHF6 (306-VQIVYK-311) that compose the nuclei of tau aggregation. One of the new highly promising therapeutic approaches towards tauopathies is the immunotherapy directed against tau. This strategy has shown good outcomes in tauopathies and AD mouse models. Further, clinical trials targeting different tauopathies such as progressive supranuclear palsy (PSP) and AD are ongoing. Here, we use VHHs (or nanobodies, Variable Heavy-chain of the Heavy-chain-only-antibody), for targeting Tau and preventing its aggregation. In partnership with Hybribody Company, a synthetic phage-display library of VHHs was screened against recombinant full-length Tau protein. The epitopes recognized by the selected VHHs, were defined using Nuclear Magnetic Resonance (NMR) spectroscopy. A VHH targeting an epitope in the microtubule binding domain of tau, which corresponds to the nuclei of tau filaments, was selected. Further, yeast two-hybrid was performed to increase its biochemical properties. The encouraging results of inhibitory effect towards tau aggregation both *in vitro* and in a cellular model of seeding with this VHH raised the hopes for a potential novel therapy in tauopathies.

Neuroprotection by small molecule inhibition of disordered peptide and syntaxin interaction

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Therapeutic options to prevent, halt, or ameliorate neurodegenerative disorders remain critical areas of unmet medical need in spite of three decades of aggressive research efforts. Central to this challenge is the fact that many neurological diseases are driven by proteins with highly flexible and unstructured domains that bind multiple targets, making both their mechanistic characterization and their pharmacological targeting exceedingly difficult. One such protein is the delayed rectifier potassium channel Kv2.1, which mediates a well-characterized neuronal cell death cascade via the interaction between its disordered cytoplasmic C-terminus domain and syntaxin 1A (syntaxin). Here, we developed a novel translational strategy to predict key interactions between syntaxin and a disordered peptide of Kv2.1, finding that key interactions were shared with Mammalian UNCoordinated-18 (munc18). Based on a virtual screening of 28M compounds, we discovered the first small molecule Kv2.1-syntaxin antagonist that improves cultured cortical neuron survival by suppressing the enhancement of Kv2.1-mediated currents. We validated that cpd5 selectively displaces Kv2.1 syntaxin-binding peptides and munc18, without affecting either synaptic or neuronal intrinsic properties in brain tissue slices at a neuroprotective concentration. Collectively, our findings validate a novel target for neuroprotection, as well as a novel pipeline to mimic disordered peptides.

SESSION 3

Chemical biology, targeting & new concepts

Targeted Protein Degradation with Small Molecules: How PROTACs work

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Bivalent degrader molecules (also termed PROTACs) target proteins for degradation through recruitment to E3 ligases. PROTACs are a revolutionary new modality class with therapeutic potential. Formation of a ternary complex between the degrader, the ligase and the target leads to the tagging by ubiquitination and proteasomal degradation of the target protein.

In 2015, we disclosed MZ1, a potent degrader made of a ligand we had previously discovered for the E3 ligase von Hippel-Lindau (VHL), and a pan-selective ligand for the BET proteins Brd2, Brd3 and Brd4. We made the unexpected but fascinating observation that MZ1 induces preferential degradation of Brd4 over Brd2 and Brd3 - despite engaging BET proteins with the same binary affinity. This demonstrated a now well-established feature of PROTACs: they can achieve a more narrow degradation profile in spite of broad target engagement. Our co-crystal structure of a PROTAC ternary complex (VHL:MZ1:Brd4) illuminated the role of cooperative molecular recognition inducing de novo contacts to form a stable ternary. Our work is revealing the structural basis and guiding principles of PROTAC degradation selectivity and mode of action.

Selective FKBP51 inhibitors enabled by transient pocket binding

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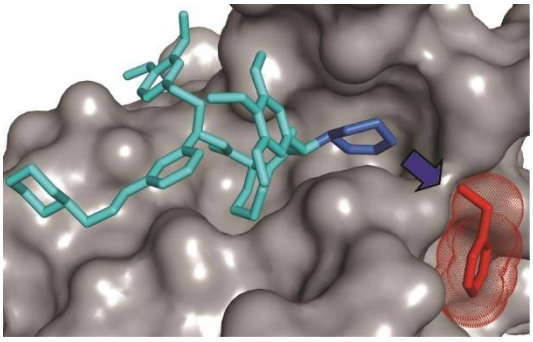
The FK506-binding protein 51 (FKBP51) plays a key role in human stress biology and contributes to major depression, obesity and chronic pain. Drug discovery for FKBP51 has been hampered by lack of selectivity against the highly homologous functional counter-player FKBP52. Here, we present the discovery of SAFit2, the first potent and highly selective inhibitor of FKBP51[1]. SAFit2 achieves selectivity for FKBP51 by stabilizing a transient conformation that much less favorable for FKBP52. This otherwise invisible transient binding pocket can be detected by NMR studies to preexist as a very low populated minor conformation in the apo state[2]. By using SAFit ligands, we demonstrate that selective inhibition of FKBP51 enhances neurite elongation in neuronal cultures and improves neuroendocrine feedback and stress-coping behavior in mice[1,3,6]. Furthermore, SAFit2 ameliorated inflammatory pain-induced disabilities[4], diet-induced obesity[5], and alcohol-seeking behavior[7]. Our findings show how high selectivity can be achieved in the conserved class of FKBP proteins by exploiting differences in conformational dynamics. The resulting ligands allowed to validate FKBP51 inhibition as a novel pharmacological treatment option for depression, obesity and chronic pain.



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Discovery of proteolysis targeting chimeras for leucine-rich repeat kinase 2 (LRRK2)

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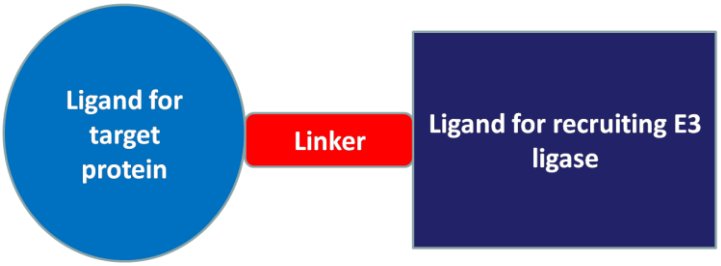
Proteolysis targeting chimeras are hetero-bifunctional molecules, consisting of a ligand that recruits an E3 ligase and a ligand interacting with the protein of interest. The two ligands are connected via a linker. The recognition of the ligand by the E3 ligase initiates the ubiquitination process that eventually leads to the degradation of the complex. Thus, the protein of interest is not inhibited, but degraded and this has certain advantages.[1,2]



Our protein of interest is the leucine-rich repeat kinase 2 (LRRK2), which has drawn considerable attention regarding its role in the pathogenesis of Parkinson's disease.[3] We have chosen two low nanomolar, highly selective and brain-penetrating known inhibitors of LRRK2 and modified them in order to attach the linkers and the ligand for recruiting the E3 ligase, which in this case is Cereblon. A small library of LRRK2 – PROTAC was synthesized and their ability to act as degraders was evaluated by Western blots showing clear dose-dependent POI degredation.

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New tools for the analysis of polyclonal T cell repertoires using chaperone-mediated peptide exchange

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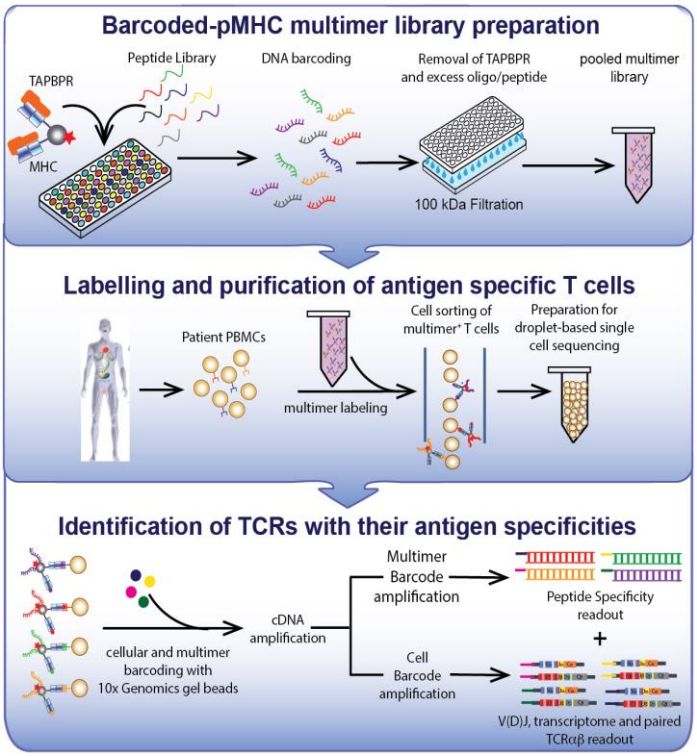
Peptide exchange technologies are essential for the generation of pMHC-multimer libraries, used to probe highly diverse, polyclonal TCR repertoires. Using the molecular chaperone TAPBPR, we present a robust method for the capture of stable, empty MHC-I molecules which can be readily tetramerized and loaded with peptides of choice in a high-throughput manner. Combined with tetramer barcoding using multi-modal cellular indexing technology (ECCITE-seq), our approach allows a combined analysis of TCR repertoires and other T-cell transcription profiles together with their cognate pMHC-I specificities in a single experiment.



Inhibition of glycosomal protein import: the sweet death of Trypanosoma

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Chagas' disease is a parasitic condition caused by *Trypanosoma cruzi*. According to the Word Health Organization (WHO) disease burden estimates, it is the first among parasitic diseases in the Americas, accounting for nearly 5 times as many disability-adjusted life years lost as malaria. Only two drugs are currently available which suffer from toxicity, limited efficacy and increasing resistance. Therefore, many efforts are address to the identification of new macromolecular drug targets and strategies for the treatment of Chagas' disease. The compartmentalization of glycolysis - the only source of energy in Trypanosomatids – in specialized organelles, so-called glycosomes, is a unique feature of Trypanosomes. Recent studies demonstrated that inhibiting glycosomal protein import selectively kills the parasites. The translocation machinery of the glycosomal enzymes requires the concerted action of Peroxin (PEX) proteins. Using a structure-based drug design approach we developed small molecule inhibitors of selected PEX proteins. A HTS of a large library of compounds was performed to identify the hits that were further evaluated and optimized to obtain molecules with trypanocidal activity. X-ray structures had a central role in this work since they allowed to characterize the binding interactions at the molecular level and explain SAR (structure-activity relationship) profiles. Our results provide solid ground for further development of glycosomal protein import inhibitors as drugs against Trypanosomiasis.

Chemical Biology Approaches to Understanding the VCP/p97 Protein Interaction Network

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Valosin containing protein (VCP, p97, CDC48) is a member of the ATPases Associated with diverse cellular Activities (AAA+) family of molecular machines. Due to its central role in protein homeostasis and membrane remodeling, VCP is an exciting therapeutic target for cancer and neurodegenerative diseases. In particular, point mutations in VCP cause a degenerative disease called multisystem proteinopathy 1 (MSP1). The rich biology of VCP warrants careful investigation to understand which processes are homeostatic, and which are modulated in disease states. VCP's diverse activities are governed by a set of 'adaptor' proteins and ubiquitin-processing enzymes, which together regulate VCP's enzymatic activity, substrate recognition, and subcellular localization. Despite the centrality of the VCP/adaptor network to proteostasis, the specific functions and ubiquitin substrates of most adaptor proteins are not well defined.

During ATP hydrolysis, VCP undergoes major conformational changes that alter the binding affinity of adaptor proteins. MSP1 mutations, positioned at the interface between the N and D1 domain, increase conformational dynamics. MSP1 cells display derangements that suggest both gain and loss of proteostatic functions, and it is hypothesized that MSP1 mutations lead to dysregulation of the protein-protein interaction network. Significant gaps in knowledge include a) the effect of VCP conformation and dynamics on adaptor binding, b) unique functions and substrates of adaptor/VCP complexes, and c) the cellular outcome of blocking one adaptor-VCP complex.

This presentation will describe the efforts of an interdisciplinary team from several universities, focused on developing inhibitors of this complex molecular machine. Using small-molecule discovery approaches, we have modulated VCP enzymatic function through multiple mechanisms, and are exploring how inhibition affects diverse functions. In parallel, a protein-engineering approach aims to modulate protein-protein interactions and protein conformation directly. Together, these complementary methodologies will shed light on the complex cellular functions of VCP/p97 and may lead to design of effective therapeutics.

Bioengineering Subunit B from Shiga Toxin 1 for intracellular drug delivery

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Improving binding specificity is one of the main goals in drug discovery and optimization, especially in biologicals as antibodies. In solid tumours antibody uptake depends on an efficient penetration and retention in the targeted tissue. Shiga toxin is a protein that acquired characteristics as an intestinal pathogen, able to penetrate tissue and stable in different physiological environments. Shiga toxin can internalize into cells due to the nontoxic homopentameric 38.5 kDa B-subunit (STxB) that binds specifically to the CD77/Gb3 receptor of mammalian cells. Once it binds to Gb3 at the cell surface, STxB is rapidly internalized. In specific human cancers, as lymphomas and colorectal carcinomas, the receptor Gb3 is strongly expressed. For this reason, StxB is a putative vehicle for transporting biomolecules into specific cancer cells, both for solid and liquid tumours.

Modifying StxB to deliver drugs or biomolecules can change its binding and internalization capacity. Real-time cell-based assays (RT-CBA) can provide information about binding properties such as affinity and kinetics. LigandTracer® (Ridgeview Instruments AB, Sweden) technology allow us to measure the binding of a drug or a protein to a receptor expressed on living mammalian cells in real-time, providing valuable information about the dynamics and underlying mechanism of the molecules in a living system.

In this study, LigandTracer® Green was used at different temperatures for understanding and confirming the binding of StxB modified proteins on the surface of cancer cells, internalization and transport. We could observe that coupling small compounds to the primary amines of StxB slightly change the nanomolar affinity interaction with its natural receptor, but still able to internalize. However, a quite surprising finding was the study of the fusion of StxB with the protein eGFP. The fusion on the C-terminal of StxB, did not affect at all the binding interaction of StxB with its receptor, and the 38,5 kDa subunit was able to carry 5 eGFP proteins into cells.

RT-CBA hence provided a better characterization and understanding of the binding of StxB modified constructs and internalization capacity to living cancer cells.

SESSION 4

Peptides, natural products and non-small molecules

Peptide as leads for targeted inhibition of interactions – How free, how determined?

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Peptide-mediated interactions play major regulatory roles in the cell. They are often transient and tightly regulated by their context. These interactions are dominated by short sequence motifs that are often embedded in unstructured regions of a protein. Peptides, and their peptidomimetic derivatives, have also been suggested as excellent starting points for the development of drugs targeted to a specific interaction. To accomplish such aims, they should be stable compounds and strong binders. How, and when, can these opposite qualifications required from a peptide be matched?

Our group develops accurate approaches for the characterization, modeling and manipulation of peptide-mediated protein interactions. I will describe an overview of our protocols for (1) the modeling of protein-peptide structures (Rosetta FlexPepDock and PIPER-FlexPepDock), (2) the structure-based identification of substrates of domains that read, write and modify peptides (FlexPepBind), and the design of peptide-based inhibitors of interactions (FlexPepDesign). I will highlight specific applications and draw a general picture of the identified basic features and qualifications that characterize successful peptidic interactions.

Identification of a novel cyclic peptide that disrupts the homodimerization of the E3 ligase IDOL.

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E3 ligases are intracellular proteins that are involved in the ubiquitination of target proteins via lysine residues (usually K48 or K63). Upon ubiquitination target proteins undergo trafficking to the proteasome for subsequent degradation. There are over 600 E3 ligases spanning a diverse range of target proteins, tissue localisation and specificity. The E3 ligase inducible degrader of the LDL receptor (IDOL) is a RING type E3 ligase containing both a RING and FERM domain. It is involved in the ubiquitination of the cytoplasmic tail of the LDL receptor (LDLr) leading to it's subsequent degradation. Inhibition of IDOL leads to an increase in LDLr levels resulting in an increase in LDL cholesterol (LDLc) uptake into cells and thus a decrease in circulating LDLc levels. We have generated IDOL knock out mice to validate this mechanism in vivo. We have identified a novel cyclic peptide and demonstrated enyme inhibition in vitro. This is the first chemical tool for IDOL to date and opens up opportunities for chemical agents targeting this E3 ligase.

Rational Design of Peptides and Foldamers inhibiting a New Epigenetic Target in Cancer

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Anti-silencing function 1 (ASF1) is a conserved H3-H4 histone chaperone involved in histone dynamics during replication, transcription and DNA repair [1]. Overexpressed in proliferating tissues including many tumors, ASF1 emerged as a promising therapeutic target [2,3]. We combined structural, computational, and biochemical approaches to design peptides and foldamers that inhibit the ASF1-histone interaction [4,5]. Starting from the structure of the human ASF1-histone complex, we developed a rational design strategy combining epitope tethering and optimization of interface contacts to identify a potent peptide inhibitor with a dissociation constant of 3 nM [6]. We then assessed the functional capacity of these ASF1 competitive peptide inhibitors to impair cancer cell proliferation in vitro, and cancer growth in vivo using mouse allograft models. The results validate ASF1 as a valid therapeutic target. We next exploited the high resolution structure of the ASF1-peptides complexes as templates to derive peptidomimectis [7] and urea-based foldamers that closely mimic the inhibitory peptides. These peptidomimetic foldamers efficiently bind ASF1 and show improved stability in biological media.

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Macrocycle mediated inhibition of PD-1/PD-L1 immune checkpoint

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Targeting the PD-1/PD-L1 immune checkpoint with monoclonal antibodies has recently revolutionized cancer treatment for some patients. Due to inherent difficulty of targeting protein-protein interactions with small molecules, the development of orally available inhibitors is staggering behind. Here we report a macrocyclic peptide inhibitor of PD-1/PD-L1 interaction. We show that the inhibitor binds to PD-L1 thus preventing its interaction with the receptor. We provide a crystal structure of the macrocycle inhibitor in complex with PD-L1. By offering a detailed description of the interface of the macrocyclic probe and the target protein the structure facilitates rational design of true small molecule inhibitors of the PD-1/PD-L1 immune checkpoint.

Design of antiangiogenic agents targeting Fibroblast Growth Factor-2/ Tyrosine-Kinase Receptor interactions

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Fibroblast growth factor-2 (FGF2) plays a major role in angiogenesis, the process of generating new capillary blood vessels from pre-existing ones, an important natural phenomenon used for healing and reproduction. In healthy tissues the body controls angiogenesis by producing a precise balance of growth and inhibitory factors. Pathological angiogenesis underlies a wide range of diseases, including cancer, and FGF2 thus represents a target for anti-angiogenic therapies. FGF2 needs to set up a productive ternary complex with the tyrosine-kinase receptors (FGFRs) and the heparan sulphate proteoglycans (HSPG) to exert its pro-angiogenic activity. Natural and synthetic molecules, able to interfere with HSPG/FGF2/FGFR interaction, have been designed starting from endogenous inhibitors of angiogenesis, such as Long Pentraxin-3 and Thrombospondin-1. We demonstrated, by a combination of NMR and MD approaches, that they regulate angiogenesis through different mechanisms, including binding and perturbing the dynamics of the complex, through direct and allosteric mechanisms. Their effective action was evaluated through in vitro and in vivo studies [1-4]. The mechanism of action of rosmarinic acid, an antiangiogenic natural compound capable of targeting FGF/FGFR complex, has been widely characterized by NMR to identify the common hot spots allosteric modulation at the basis of antiangiogenic action.

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Diflunisal targets the HMGB1/CXCL12 heterocomplex and blocks immune cell recruitment

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Extracellular HMGB1 triggers inflammation following infection or injury and supports tumorigenesis in inflammation-related malignancies. HMGB1 has several redox states: reduced HMGB1 recruits inflammatory cells to injured tissues forming a heterocomplex with CXCL12 and signaling via its receptor CXCR4; disulfide-containing HMGB1 binds to TLR4 and promotes inflammatory responses. Here we show that Diflunisal, an aspirin-like nonsteroidal anti-inflammatory drug (NSAID) that has been in clinical use for decades, specifically inhibits *in vitro* and *in vivo* the chemotactic activity of HMGB1 at nanomolar concentrations, at least in part by binding directly to both HMGB1 and CXCL12 and disrupting their heterocomplex, as proved by NMR and MST. Data-driven molecular models of Diflunisal bound to HMGB1 or CXCL12 have been experimentally validated via mutant approach. Importantly, Diflunisal does not inhibit TLR4-dependent responses.

The ability of Diflunisal to selectively interfere with the HMGB1/CXCL12/CXCR4 inflammatory axis offers unprecedented structural/functional insights into the anti-inflammatory activity of this small molecule as a NSAID. These insights also show that protein-protein interactions within the HMGB1-CXCL12 heterocomplex are druggable with high specificity and selectivity.

Our findings clarify the mode of action of Diflunisal and open the way to the rational design of functionally specific anti-inflammatory drugs.

New Paradigm for Finding Natural-product-like KRAS Inhibitors

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Natural products have proven to be a rich source of molecular architectures for drugs. Here we described a new paradigm in natural product screening which uncovered eight new natural product scaffolds for KRAS - the most frequently mutated oncogenic driver in human cancers with hitherto no treatment. The paradigm combines aspects of four established approaches, virtual screening, natural products annotation by chemoinformatics approach, fragment-based screening, SAR by NMR-HSQC and structure-based drug discovery, to overcome the limitations in traditional natural products approaches. Using our approach and the first crystal soaking system of the active form of KRASG12D, the protein-ligand X-ray structures of a tricyclic indolopyrrole fungal alkaloid and an indoloisoquinolinone have been successfully elucidated. The wealth of natural product KRAS hits discovered provides fruitful ground for the optimization of highly potent, natural-product based inhibitors of the active form of oncogenic RAS. This new paradigm for screening natural products holds promise for other “undruggable” targets.

Fusicoccin and 14-3-3 proteins, a long story from plant pathology to drug design.

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The fungal phytotoxin fusicoccin (FC) was isolated from the fungus *Phomopsis amygdali*, a pathogen of almond and peach plants more than 50 years ago. FC, a glycosylated diterpene, was first recognized as a wilt-inducing phytotoxin able to irreversibly open stomata of higher plants. Intensive biological and biochemical studies have elucidated its value as a tool in plant physiology, since the toxin binds to a protein complex formed by the master enzyme of plant ion transport, the plasma membrane H^+- ATPase and regulatory 14-3-3 proteins, thereby activating a number of physiological and biochemical processes of any higher plant.

In the last 20 years' molecular studies clarified details of the mechanism of proton pump stimulation, consequence of the fusicoccin-mediated irreversible stabilization of the complex between the H^+-ATPase and activatory 14-3-3 proteins.

The molecular details of the very peculiar interaction between H^+-ATPase and 14-3-3 proteins have shown that FC specifically accommodates into a pocket at the interface between the two proteins; the pocket is generated by interaction of a C-terminal sequence on the proton pump and a unique 14-3-3 consensus sequence, known as mode III binding and a much less common than canonical mode I and II consensus motifs of 14-3-3s.

FC became in recent years a tool in pharmacological research, since both plants and animals possess a number of potential mode III 14-3-3 clients that are known to be involved in a wide array of physiological and/or pathological processes. Its ability to be a very specific stabilizer molecule is the ideal starting point for the development of a family of structurally related drugs able to selectively tune 14-3-3 interaction with their targets, I will highlight the main milestones of this story and the contribution of our research group.

SESSION 5

Computational Drug Discovery

Computational Approaches to Protein Dynamics and Binding Kinetics for Drug Discovery

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The dynamic nature of protein structures and the different types of protein binding pocket dynamics [1] provide challenges and opportunities for ligand design. We have developed TRAPP, a toolbox of computational approaches to identify TRAnsient Pockets in Proteins for ligand design [2,3]. I will present recent developments in TRAPP to identify pocket conformations with high druggability. Protein binding site flexibility is one of the factors that can affect drug-target binding kinetics [4]. Growing evidence that the efficacy of a drug can be correlated to target binding kinetics has led to the development of many new methods aimed at computing rate constants for receptor-ligand binding processes [5], see also: kbbox.h-its.org. Here, I will describe our studies to explore the determinants of structure-kinetic relationships and to develop computationally efficient methods to estimate drug-target binding kinetic parameters. I will introduce our τ-random acceleration molecular dynamics simulation (τRAMD) method to compute relative residence times [6] and discuss how machine learning analysis of τRAMD trajectories [7] and the application of Comparative Binding Energy (COMBINE) Analysis [8] can be used to decipher the determinants of drug-target residence times.

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Debunking a Myth — Can σ-Holes Really Drive Affinity?

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Over the past years, σ-holes [1] (the localized electron deficiency of polarizable halogen atoms leading to favorable electronic interactions with Lewis bases) have experienced vivid discussions and broad published awareness. Some drug researchers have recently started to incorporate the halogen binding concept into their rationalizing of lead optimization [2].

In this talk we will shine light on the fine difference between *correlation* versus *causation*, and — using a multitude of examples — we will analyze the impact of these clearly physical, electronic effects on binding affinity.

Whereas most electron structure calculations quantify σ-hole interactions in an *in vacuo* context, it is important to note that solvation and desolvation play an additional, very important role in the definition and thus calculation of binding affinities in a drug design context.

We will balance the effect of water versus σ-holes onto substrate and drug binding using affinity measurements that shall be compared to both an empirical, logP-based model [3] and advanced quantum chemical computations. A broad geometric analysis of complexes in the PDB using a recently developed academic tool [4] supports the assumption that the overall energetic contributions are almost negligible in an aqueous environment, and that the expected geometries are only very rarely found in protein-ligand crystal structures. Conclusions and consequences for rational design shall be discussed.

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[2] a) Hardegger et al. (2011). Angew. Chem. Int. Ed. 50, 314 –318 (and references therein).
b) Derek Lowe in http://blogs.sciencemag.org/pipeline/archives/2013/01/17/halogen_bonds.
c) Lam et al. ACS National Meeting, Org Div abs. 58, Aug 16th, 2009.
[3] a) Reuleck, I. et al. (2008). ChemMedChem 3, 885 – 897.
b) Schneider et al. (2013). J. Comput.-Aided Mol. Des. 27, 15-29.
c) HYDE in SeeSAR v7, BioSolveIT GmbH, St. Augustin, Germany, 2017.
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Identification of small molecule inhibitors of the Aurora-A/TPX2 complex

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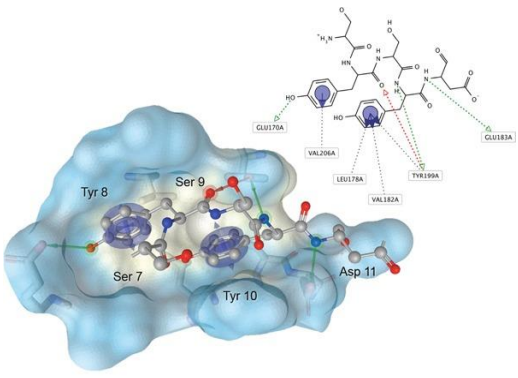
Aurora-A kinase (AurkA) is a key cell division regulator that controls the correct assembly of a bipolar mitotic spindle and the fidelity of chromosome segregation. AurkA overexpression is associated with genomic instability and aneuploidy, and is frequently observed in cancer. Accordingly, competitive inhibitors targeting AurkA activity at the ATP-binding site are being investigated for therapeutic purposes. Despite promising pre-clinical data, these molecules display moderate effects in clinical trials and incomplete selectivity. As an alternative approach, protein-protein interaction inhibitors targeting AurkA and its activator TPX2 can be exploited to achieve increased specificity of action. In this study, a virtual screening of small molecules led to the identification of 25 potential inhibitors of the interaction between AurkA and TPX2. In vitro experiments confirmed that 4 hits bind AurkA in the low micromolar range and compete for TPX2 binding. Immunofluorescence assays showed that 2 compounds also yield lowered AurkA activity and spindle pole defects in cultured osteosarcoma cells. Structure-based optimization of the initial hits led to compounds with improved potency and drug-likeness. The identified protein-protein interaction inhibitors of the AurkA/TPX2 complex might represent lead compounds for further development towards pioneering anti-cancer drugs and provide the proof-of-concept for a new exploitable strategy to target mitotic kinases [1].



Pharmacophore hypothesis of the Aurora-A/TPX2-7-11 interaction

Residues 7-11 of human TPX2 (balls-and-sticks) were identified as a hot spot of interaction and used to derive a PH (arrows for projections and centroids) at a shallow hydrophobic groove at the N-terminal lobe of Aurora-A (surface). Green arrow, hydrogen-donor feature; red arrow, hydrogen-acceptor feature; blue arrow/centroid, hydrophobic/ aromatic interaction. Exclusion volumes are not shown.

[1] Asteriti, I. A. et al. (2017). Oncotarget. 9, 32117-32133.

SESSION 6

Structural biology, biophysics & methods

Serial Crystallography for Ligand-Binding Studies

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The last decade has seen the advent of X-ray free electron lasers (XFELs) and third- and fourth-generation synchrotrons that provide highly brilliant X-rays enabling novel types of experiments. The extremely intense X-rays at these facilities are a two-edged sword. On one side they enable diffraction experiments from very small samples (microcrystals), on the other side these small samples quickly suffer from the high, absorbed X-ray doses and collection of complete data sets from single crystals becomes impossible. These facts led to the development of serial crystallography first at XFELs and later also at synchrotrons. Here the exposed crystals are continuously replaced by new crystals.

Sample-delivery systems for serial crystallography can be broadly divided in liquid (or viscous) jet-based systems and fixed-target systems. While jet-based systems currently offer the fastest sample-exchange speed, they suffer from inefficient sample and beamtime usage (low ratio of recorded diffraction patterns/total amount of recorded images). Moreover, jets have a tendency to clog, further reducing their efficiency. Fixed-target delivery systems have the advantage of full control over sample exchange rate, therefore enabling an adjustment to the specific demands of the X-ray source. Subsequently, much higher hit-rates can be achieved and sample consumption is drastically reduced. Our group has developed micro-pattered silicon chips as sample-holders [1]. Here crystals are placed on a silicon membrane, all liquid is subsequently removed by blotting through the pores of the membrane and the “naked” crystals remain on the chip surface. Now the chip can either be flash-cooled for cryogenic data-collection or the crystals can be maintained in a humidified environment for data-collection at room-temperature. The use of naked crystals without the use of any sealing materials like additional foil leaves the crystals available for further manipulation, for example by laser excitation or addition of ligands.

The chips offer several advantages for ligand-binding studies. Ligand solutions can be directly applied to “naked” crystals at the highest concentration as no diluting crystallant is present. Furthermore, the crystals do not need to be individually transferred as they remain on the chip surface. Lastly, crystals can be directly grown on the chips, thus completely removing the need to manipulate the crystals, which is especially important for fragile crystals [2]. We will present several case studies exemplifying the applicability of the chips for ligand-binding studies. In addition, we present the latest development of the chips for data collection at room-temperature at any diffraction source without the need for a dedicated humidifying system.

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Placing fragments with lanthanide tags using paramagnetic NMR

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Paramagnetic NMR using lanthanide binding tags has proven to be very useful for structural characterization of proteins and strongly bound ligands in slow exchange. This is based on the distance and orientation dependence of pseudocontact shifts (PCS) induced by the paramagnetic center. A combination of NMR titrations and binding kinetics from other techniques such as AlphaScreen™ can be used to tackle the challenge of using this approach with molecules binding in fast exchange, for example fragment molecules in drug discovery programs, to characterise the binding pose. This could aid in fragment placement for key drug development projects where crystallisation or soaking fragments is difficult, as is often the case in early stage drug discovery. We use DOTA-M8 [1] and vinyl-dipicolinic acid [2] to determine the binding site of a small fluorinated fragment that binds in fast exchange to *Trypanosoma cruzi* PEX14 N-terminal domain using Lutetium, Ytterbium and Thulium. This is a medically relevant target in the treatment of Chagas Disease, prevalent in South America: interruption of the Protein-Protein Interface (PPI) between this and PEX5 has been shown to lead to death of the parasite [3] but soaking and co-crystallisation with fragments has proved challenging.

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Small molecule stabilization of the GR 14-3-3 protein-protein interaction (PPI)

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Classically drug targets have been single proteins, e.g. enzymes or GPCRs. In the search for new targets, however, it is necessary to go beyond these established groups and look into new opportunities. With 650 000 PPI in human cells, the ability to modulate these interactions would potentially significantly enlarge the “drugable genome”.

14-3-3 proteins are a family of seven highly conserved regulatory proteins and have been reported to interact with the glucocorticoid receptor (GR), a nuclear receptor which functions as a ligand dependent transcription factor, and modulate its activity [1]. Different reports however have ascribed both positive and negative regulatory roles to GR/14-3-3 interactions [2,3]. Given the importance of GR agonists in medicine it is of great interest to understand the role(s) of these interactions and to study their modulation.

The interaction between GR and 14-3-3 has been studied; peptides corresponding to the most promising putative 14-3-3 binding sites of GR have been synthesized. A 31-mer diphosphopeptide, from GR ligand binding domain (LBD), was identified with Kd 18 – 30 nM (assay dependent). MINK1 and ROCK1 where pinpointed by a kinase screen to phosphorylate the two promising sites. GR LBD phosphorylated by MINK1 was shown to interact with 14-3-3 by far-western blot. Cell assays on full length GR are ongoing.

Stabilization of the GR/14-3-3 interaction has been investigated; the identified binding site peptides have been crystallized with 14-3-3. About 8K AZ compounds were screened to obtain plausible stabilizers which will be used as tool compounds in future cell assays.

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Because structure means function - Conformational changes in proteins measured with switchSENSE®

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Function, activity, and interactions of proteins crucially depend on their three-dimensional structures and the relative arrangement of individual protein domains. switchSENSE is a novel method to measure the binding kinetics and Stokes radii of proteins on a chip. The technology uniquely provides information on protein conformation as well as analyte binding kinetics in the same measurement and under native conditions. This allows for complex mode-of-action investigations and opens new perspectives for hit validation through in-depth information on protein conformation, oligomeric state, and stability.

The proteins of interest are immobilized onto DNA-functionalized gold microelectrodes on the biosensor surface using generic conjugation methods. The fluorescently labeled nanolevers are actuated by alternating electric fields and are used to sway the proteins through the solution close to the surface at high-frequencies. The speed of this oscillatory movement is observed in real-time by fluorescence energy transfer. As the speed of the protein motion depends on its hydrodynamic drag, it can be directly converted to the protein's Stokes diameter and thus provides quantitative and easy-to-interpret means for the analysis of protein size and shape. The method is particularly sensitive for sizes in the range of 1 – 10 nm, encompassing peptide and protein targets in the kDa to MDa molecular weight range. As the signal due to the target protein's conformational change can be distinguished from a pure analyte binding event, analyte binding and target structural changes can be screened for simultaneously.

Here, we demonstrate the range of applicability of this technique by presenting examples for the analysis of conformational changes and allosteric regulation in small proteins – such as kinases and STING – as well as for large proteins, including transglutaminase and the insulin receptor protein. Additionally, we show the monomerization kinetics of TNFα in real-time by changes in protein friction. Taken together, switchSENSE proves to be a useful tool not only for determination of absolute Stokes radii, but titration and flow experiments can yield K_D values and kinetics (on- and off-rates) parameters for analyte to target binding.

Expanding Crystallographic methods for Fragment Based Drug Design (FBDD)

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FBDD at Astex begins with high throughput crystallographic fragment screening. These fragments are then developed further using structure-guided drug design, ultimately leading to a clinical compound.

Our fragment library consists of compounds with an average heavy atom count (HAC) of 12, allowing chemical space to be sampled efficiently. A typical crystallographic fragment screen identifies 'hits' binding to 'hot-spots' on a target protein. To further explore and exploit protein ligand-binding sites for drug discovery we have developed a MiniFrag library. MiniFrags are very small fragments (HAC<8) which are soaked into crystals at ultra-high concentrations. The MiniFrags have enabled us to identify novel 'hot spots' as well as 'warm spots' to expand existing ligand binding sites on target proteins. I will present our findings from our MiniFrag screens and discuss how MiniFrags and other new technologies are enhancing the FBDD process at Astex.

O'Reilly et al. (2019). Drug Discovery Today. 24, 5, 1081-1086.

From Nanobodies to Megabodies for applications in cryo-EM

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Nanobodies (Nbs) are highly popular and versatile tools for structural biology. Here we report the development of megabodies, whereby Nbs are rigidly grafted into selected protein scaffolds to increase their molecular weight while retaining the full antigen binding specificity. The megabody design principles are applicable to other scaffolds without size limitations and expand cryo-EM analysis to proteins that are small and/or display preferential orientation in ice, two major factors that limit the resolution of reconstructed density maps. Such megabodies have been instrumental to solve the first structures of the human heteropentameric GABAA receptor in complex with well-known drugs [1].

[1] www.steyaertlab.eu

POSTER ABSTRACTS

Structure based development of inhibitors of *P. falciparum* 6-pyruvoyltetrahydropterin synthase and adenylosuccinate lyase as novel antimalarials and development of highly selective insecticides against malaria infected mosquito, *A. gamb*.

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Before we commenced our first SBDD work, a proof of the concept of our approach that mimics a SBDD but established on an off the shelf drug, 6-diazo-5-oxonorleucine (DON), was implemented in Plaimas et al. (PMID: 24121016). However, DON is rather toxic and mutagenic, and hence can only be applied with care but the results obtained have been successful on one of the enzymatic sites listed in Fatumo et al. (PMID: 18313365), and it demonstrated that the computational method worked as designed. Benefits from this kind of work include that we may be able to produce novel antimalarial drugs, whose biological mode of action can be determined accurately (PMID: 21515412). Our first SBDD effort combined the effectiveness of the interdisciplinary approach to develop drugs against malaria at the RBC by first building a much robust reconstructed network models for *P. falciparum*. Using the techniques in Fatumo *et al* (PMID: 21515412) and further analysis, we arrived at an enzymatic site (PFF1360W) with 3D structure in the PDB. Over this site, we have completely built a SBDD platform that first produced 22 antimalarial drug-like substances. We have performed successfully further two rounds of re-synthesis and preclinical tests. Simultaneously, we have identified further fifteen based signaling pathways and transcription factors novel drug targets against the deadly malaria parasite at the RBC. One of these sites (PFB0295w) has a 3D structure in the PDB for *P. vivax*. Using homology modelling, we derived one for P. fal. and have started the construction of a SBDD (Oduselu et al. (PMID: 31391779)). In another work funded by the German Science Foundation (aka DFG) project KO-3678/5-1, since 2018, we have started to use our experiences to design and develop highly selective insecticidal against the vector, *A. gambiae.* Three fifth of the newly World Bank Funded Covenant Applied Informatics and Communication African Centre of Excellence. (CApIC-ACE) is committed to fully establishing our drugs and insecticides development against the deadliest parasite at the human RBC and in the mosquito, *A gambiae* using extensively SBDD approach.

Development and implementation of a novel NanoBIT-based biosensor for monitoring Hippo pathway activity

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The Hippo signaling pathwayis involved in organ size control, cell differentiation and survival. It plays a pivotal role in tissue homeostasis and acts as a tumor suppressor. The core components of the pathway include upstream kinases MST1/2 and LATS1/2, co-activators YAP/TAZ and DNA binding proteins TEADs. Phosphorylation on serine residues of TAZ (Ser89) and YAP (Ser127) promotes binding to 14-3-3 that leads to their cytoplasmic sequestration and eventual degredation, thus preventing transcription of the target genes associated with proliferation and anti-apoptosis (CTGF, Cyr61, ANKRD1, etc.) .The pathway is dysregulated in various cancers, which has brought interest in research of interacting pathways, stimuli and proteins that orchestrate phosphorylation status of the Hippo pathway effectors YAP/TAZ.

We have developed a new system for monitoring interaction between 14-3-3 and TAZ in intact cells. NanoLuc® Binary Technology (NanoBiT) is a method based onprotein-fragment complementation assay (PCA) with NanoLuc® luciferase split into two subunits Large BIT (LgBIT) and Small BIT (SmBIT). Subunits are fused to 14-3-3 and TAZ. When they are in proximity, they form a functional enzyme, which in the presence of the cell permeable substrate furimazine produces bright luminescent signal. The system allows intracellular detection of protein-protein interactions in real time, thus it makes it a powerful tool for screening fast acting modulators. Despite the availability of the multiple technologies based on structure complementation this system provides unique features. Relatively small size of the subunits LgBIT (18 kDa) and SmBIT (11 amino acids) and flexible linker do not compromise protein function. Another feature is that subunits do not self-associated (Kd =190 µM) and reversible interactions can be monitored.

In order to identify modulators of the Hippo pathway carefully selected in-house kinase inhibitors were screened and hits further assessed.

Ligandability of 14-3-3/SOS1 complex to bind a drug-like molecule

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Activation of Ras-MAPK signaling regulates essential cellular functions; its deviant Ras activation machinery is found in approximately 30% of all human cancers (i.e. pancreatic cancer). Son of sevenless homolog 1 (SOS1) is an important protagonist of this pathway that plays a key-role in aberrant cell proliferation and differentiation. The formation of the complex of the 14-3-3 protein and SOS1 with 14-3-3 proteins modulates SOS1 activity in Ras-MAPK signaling and it would represent a key-process to downstream the deviant Ra-MAPK signaling. To better understand how this interaction actually occurs, we analyzed the 14-3-3/SOS1 protein-protein interaction (PPI) by different biochemical assays and reported the high resolution crystal structure with a 13mer peptide motif of SOS1 phosphorylated at position 1161 bound to 14-3-3ζ to provide structural information about the binding mechanisms behind this PPI complex a 13-mer motif of SOS1. These structural and functional insights are important for the evaluation of this PPI interface for small-molecule stabilization as a new starting point in drug discovery for modulating the Ras-Raf-MAPK pathway. In particular, the identification of specific compounds such as small molecules that are able to stabilize the binding of SOS1 to 14-3-3 might offer a new therapeutic strategy to increase the negative feedback control in RAS-RAF-ERK-MAP kinase cascade in the treatment of tumor cells. For this purpose, a NMR-fragment based screening of 1100 fragments was performed. Two promising HITS have been found; related biophysical assays and X-ray crystallography are on-going.

P(O)ME$_2$-CONTAINING BUILDING BLOCKS FOR DRUG DESIGN

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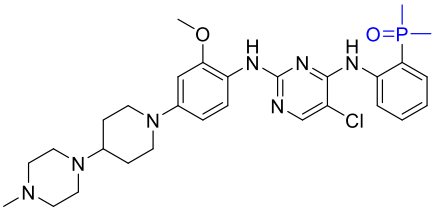
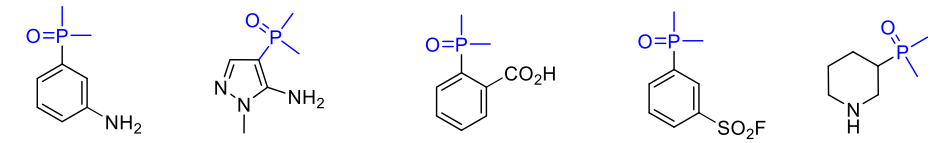
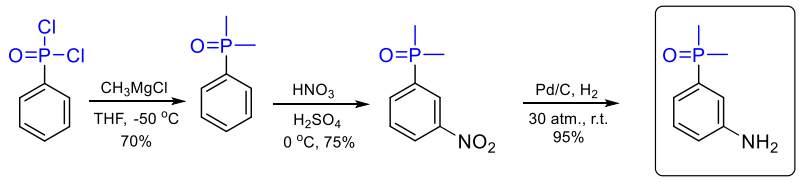
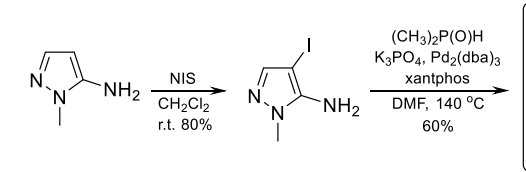
Phosphine oxides belong to a chemical class seldom employed in drug design. However, the FDA-approval of *Brigatinib* drug (ARIAD Pharm.) in 2017 may further inspire application of this unique functional group in medicinal chemistry. The highly ionic P=O bond imparts a number of important drug-like properties, including decreased lipophilicity, increased aqueous solubility, H-bond acceptor ability, and high metabolic stability. Herein we have designed and synthesized a library of phosphine oxide derivatives for drug design.

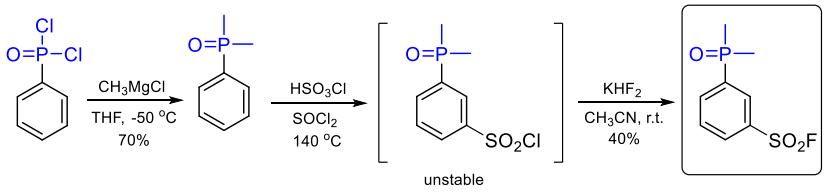


Anti-cancer drug **Brigatinib** (Alunbrig)
ARIAD Pharm.
2017









NMR-based fragment placement using paramagnetic relaxation enhancement from a soluble spin label

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Fragment-based drug discovery is an essential strategy in the development of new clinical candidates. Both NMR and crystallography-based fragment screening can be applied to detect binding. A challenge in early-stage drug discovery is to acquire enough structural information on fragment placement in order to drive compound development. Crystallography may provide such information. However, achieving crystallisation and diffraction with weakly binding fragments is challenging. Also, unless high resolutions are obtained, fragment position and pose may be uncertain. The high concentrations required for crystallographic fragment screening may also lead to non-specific binding. Paramagnetic NMR is an alternative approach and can provide orientation information between a paramagnetic centre and a bound ligand.[1]

Here, we use a small, soluble, hydrophobic nitroxyl to paramagnetically probe the protein surface. We demonstrate that this compound can target the active site of our model system, IMP-13 metallo-β-lactamase, through protein-detected PREs (paramagnetic relaxation enhancements). Metallo-β-lactamases are a significant challenge in antibiotic resistance, showing activity against all major classes of β-lactam antibiotics by hydrolysing the β-lactam ring.[2] Development of drugs against these enzymes is important in the fight against antibiotic resistance. PREs between our soluble paramagnetic nitroxyl and novel fragments, selected in screening against IMP-13, can be used to position these ligands relative to the paramagnetic compound. This can provide important structural information early in fragment-based drug discovery programmes, without the requirement for protein modification e.g. incorporating a bound tag.

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Filling in the gaps: geometric complementarity and its role in ligand-protein interaction prediction.

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In chemoinformatics, recent developments in the application of deep-learning methods have brought significant advances in *de novo* compound generation, advanced target-prediction, and retrosynthetic route recommendations [1,2]. Each of these aims share a common requirement: that we should have good representations of our entities in the first instance, whether small- or macro-molecule. Current representations are often dependent on features pertinent to one, but not the other. They rarely perform well over the entire range of molecular size and complexity. Here, we utilise the known importance of geometric complementarity between ligands and their targets [3], and develop methods for the rapid generation and analysis of shape-based fingerprints. These incorporate geometric information from the local atomic environment through description of their fractal dimension [4].

We developed these fingerprints to be suitable for any molecule type. They provide a means for conducting alignment-independent 3D-similarity searching, and a molecular representation, which is a natural, consistent, and easily-stored input for the deep-learning method. In a retrospective analysis, our fingerprints outperformed existing gold-standard methods on published benchmark datasets. A prospective study utilising this approach resulted in the selection of novel, nanomolar to low-micromolar activators and inhibitors for seven different targets of therapeutic interest. We trained deep-learning models on geometric descriptions of 14,000 ligand-target pairs to examine to what extent their interactions can be predicted using fractal dimension fingerprints. Insights from such models could be used to rapidly screen virtual libraries based on an inferred pattern of on-target-likeness, including in those cases where the target has not yet been structurally resolved.

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Small molecule modulation of 14-3-3 binding to Usp8 and Amot130

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Targeted modulation of protein–protein interactions (PPIs) is an attractive concept for the development of novel therapeutic strategies in many diseases. The family of 14-3-3 proteins is involved in a wide range of cellular processes and it represents an interesting platform for studying small molecule PPIs modulation. Several hundred protein partners of 14-3-3 have been described and many of them are implied in diseases where the stabilization of the protein-protein interaction would be therapeutically beneficial. This would be the case of the interaction of 14-3-3 with Usp8 and Amot130. Impairment of 14-3-3/Usp8 binding has been shown to exert a significant role in the pathology of Cushing's disease and might play a key function in driving the constitutively activated EGFR signalling and ACTH production observed in pituitary tumours. The 14-3-3/Amot130 interaction has been implied in the transmission of the Hippo signalling, in a process that involves binding of the ubiquitin ligase atrophin-1 interacting protein (AIP) 4 and promotion of ubiquitination and degradation of the yes associated protein (YAP).

In order to assess the possibility of modulating these interactions using small molecules, we have analysed the 14-3-3/Usp8 and 14-3-3/Amot130 interactions using different biophysical assays and solved the high resolution crystal structures of their binding motifs in complex with 14-3-3. Fragments libraries have been screened using biophysical techniques and X-ray crystallography and some initial hits have been identified. The structural information obtained with the elucidation of the ternary complex (14-3-3/peptide/fragments) are in the process of being used to guide the chemical optimization of the compounds, aiming to achieve an optimal binding affinity.

Novel purification strategies enabling structure determination of large, labile multi-subunit biological assemblies and drug discovery

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Recent high-throughput whole-genome functional studies have revealed that proteins do not act in isolation within cells, but are incorporated into biological assemblies consisting in average of 5 – 10 proteins. Thus, these biological assemblies are the functional units that ensure the homeostasis of living cells and perform all cellular activities required for survival. When drug discovery programs are pursued with isolated proteins, they do not take this vital essential feature into account, which might be one reason for the low hit-rate of drug-discovery programs.

On the other hand, recent developments in structural biology, most notably the improvement in the structure determination by single particle cryo- electron microscopy (cryo-EM), the availability of X-ray free electron lasers (XFELs), ever increasing sensitivity in Nuclear Magnetic Resonance (NMR) experiments and diffraction-limited synchrotron light sources for X-ray crystallography have moved the bottleneck in high-resolution structure determination of large biological assemblies to the biochemical preparation of high-quality samples. However, precisely the biochemical purification of such large, labile assemblies remains a formidable challenge and often fails when strategies suitable for single biomolecules are adapted to larger complexes, due to the dissociation of such samples resulting in structural heterogeneity. To overcome this pitfall novel purification strategies as reported herein will be necessary.

Here, I will present the development of chromatography-free purification strategies, which enable the purification of large biological assemblies in high-yield and high-quality. The strategies reported here have enabled the structure determination of proteasomes and fatty acid synthases at unprecedented (high-) resolution, allowed the discovery of novel subunits, allowed the elucidation of new enzyme- and inhibition-mechanisms and opened up new venues for drug discovery. The revised views of the functional mechanisms of the large macromolecular complexes also allow for the biotechnological manipulation of the biological assemblies to perform novel chemistry.

Type I and II BIR domains inhibitors in cancer therapy

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Inhibitors of apoptosis proteins (IAPs) constitute a family of conserved proteins whose over-expression enhances cell survival and resistance to anticancer agents. IAPs are E3 ligases that ubiquitylate different substrates for the regulation of NF-κB; furthermore, they sequester caspases to prevent apoptosis. IAPs interactions occur through type I and type II BIR (Baculovirus IAP repeat) domains.

Type II BIR domains inhibitors. Smac-mimetics (SM), mimicking the N-terminal tetrapeptide of Smac-DIABLO, have been shown to sensitize cancer cells to apoptosis. SM interact with type II BIR domains of IAPs, thus relieving caspases from X-linked IAP (XIAP) inhibitory activity and leading to cellular IAPs (cIAPs) auto-ubiquitylation and proteasomal degradation. Using the information achieved through 3D structural analysis of the complexes between XIAP BIR3 and our first synthesized 4-substituted SM (named SM5 and 10 [1]), we generated a library of monovalent and divalent SM compounds with *in vitro* low nM affinity values for the BIR3 and/or BIR2-BIR3 domains of XIAP, cIAP1 and cIAP2, and good cytotoxicity properties against selected cancer cell lines [2]. However, although SM are currently promising candidates for cancer therapy, some cancer cell lines present SM-resistance due to renewed cIAP2 activity and re-activation of NF-κB. We thus decided to explore alternative mechanisms that can be exploited to interfere with IAP-involving signaling in NF-κB regulation.

Type I BIR domains inhibitors. IAPs-mediated regulation of NF-κB signaling is based on the formation of different protein-protein complexes, regulating ubiquitin-dependent signal transduction cascades. Type I BIR domain from different IAPs has been recognized as a pivotal platform for the assembly of such complexes. The surface of type I BIR domains (X- and cIAP-BIR1) has been thus analyzed to identify hot-spots for the relevant protein-protein interactions. Virtual docking, using libraries of commercially available compounds (LOPAC, ChemBridge, Drug-Bank), allowed the selection of hits able to impair *in vitro* BIR1-based complexes with low μM affinities (i.e.NF023 [3]). 3D-structural analysis of the protein-ligand complexes obtained and a structure-activity relationship analysis, allowed the optimization of specific and selective drug candidates. Treatment of cancer cell cultures with the selected compounds will verify their effects on the modulation of IAPs-dependent signaling. Preliminary data obtained on MDA-MB-231 (human breast adenocarcinoma) cell-based experiments, showed that one of the selected compounds (cmp2) has a significant effect on cell viability, more pronounced in combination with TNF, suggesting the involvement of NF-κB pathway in its mode of action.

In conclusion targeting type I and II BIR domains represent a promising strategy for the development of new lead compounds affecting different aspects of the apoptotic pathways.

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P11 at PETRA III: A Versatile Beamline for High-Throughput and Serial Crystallography

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The Bio-imaging and Diffraction Beamline P11 at PETRA III in Hamburg is dedicated to structural investigations of biological samples from atomic to micrometer length scales. The beamline provides two experimental endstations: an X-ray crystallography experiment open to users since 2013 [1] and a scanning X-ray microscope currently under construction. The flexible X-ray optics allow for tailoring the beam properties to the experimental requirements. A first mirror system located in the optics hutch is used for generating a secondary source at 65.5 m downstream from the X-ray source. With this, a parallel beam can be generated which is ideally suited for structure determinations from large unit cell systems, such as large molecular complexes [2]. The KB system installed in the experimental hutch can be used for refocusing the secondary source in order to generate a highly intense microbeam with more than 1.3 × 10^{13} ph/s in a 4 × 9 μm^2 (v × h, FWHM) focal spot at the crystallography experiment. This allows for the investigation of microcrystals and the application of novel data collection routines, such as serial crystallography [3-6]. The P11 crystallography endstation can be operated between 5.5 and 28 keV and provides full SAD/MAD capability. Energy and beam size changes can be easily realized by the users within a few minutes. The endstation is equipped with a high precision single axis goniostat. Crystals can be rapidly exchanged in less than 20 s using an automatic sample changer equipped with an in-house designed cryogenic sample gripper and a large capacity storage Dewar, providing space for 23 uni-pucks (368 samples). Together with the Pilatus 6M detector in place, P11 allows for high-throughput crystallography and is ideally suited for industrial applications, such as e.g. fragment screening. For spring 2020 an upgrade of the crystallography endstation is planned. It will include the implementation of a new Roadrunner goniometer [6] which will allow for conventional (rotation series) and serial crystallography (fixed targets). In addition, the Pilatus 6M detector will be replaced by a faster EIGER2 16M and a new on-axis microscope for better sample visualization of micrometer-sized crystals will be installed.

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NUFIP2, a new cofactor, which promotes recognition and regulation of *ICOS* mRNA by Roquin binding

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In T cells, the paralogous proteins Roquin-1 and Roquin-2 repress post-transcriptionally target mRNAs of co-stimulatory factors, like ICOS and Ox40. Roquin interacts with CCR4-CAF1-NOT de-adenylation and mRNA decapping complexes after binding on the 3'UTR of the mRNAs and induce their decay. The Roquin proteins are essential for the immune cell function and postnatal survival of mice. Furthermore, the so-called sanroque mutation in the ROQ domain of murine Roquin leads to severe autoimmunity.

Because there was little knowledge of modulatory cofactors of Roquin-induced mRNA decay, an siRNA screen was performed. NUFIP2 (Nuclear FMRP Interacting Protein 2), an unstructured protein, was identified as a cofactor of Roquin-mediated ICOS repression. Using Surface Plasmon Resonance, we showed that Roquin binds directly and with high affinity to NUFIP2. In parallel, we showed that both Roquin-1 and NUFIP2 bind to tandem stem-loops, each of them separately, but also stronger together to the ICOS and Ox40 using EMSAs. Moreover, quantitative RT-PCR, after treatment with actinomycin D, showed that the downregulation of target transcripts was lower in cells with knocked down NUFIP2, indicating that NUFIP2 cooperates with Roquin to induce *ICOS* mRNA decay. All these data confirmed that NUFIP2 is a cofactor that contributes to mRNA target recognition by Roquin. Structural studies on the cooperative interaction of Roquin and NUFIP2 are underway to better understand their interaction.

Investigating HuR–small molecule interactions via STD NMR and molecular modelling. Towards the discovery of new HuR binders.

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RNA-binding proteins (RBPs) play a key role in regulating post-transcriptional processes, and their complexes with RNA are involved in various dysfunctions (*i.e.*, inflammation, neurodegeneration and cancer). In the last few years, the interesting question has been posed of whether they could be exploited as therapeutic targets with clinical relevance.[a]

Intrigued by this concept, the research efforts of our group in this study field have been aimed at the identification of small molecules endowed with the ability to modulate protein–RNA interactions, with a special focus on the ELAV/Hu (embryonic lethal abnormal vision) protein family, in particular HuR. In fact, HuR has been reported to be highly abundant in several cancers, and upregulated and dysregulated in cancer cells; indeed, it could either be a marker for malignancy or have an oncogenic role in numerous tumor systems. Therefore, HuR is considered a promising candidate target for governing gene regulatory mechanisms by developing compounds able to modulate the stability of its complexes with RNA.[b,c]

By applying a structure-based ligand design strategy we identified diverse scaffolds, and we synthesized the most interesting structures. [d] In the present work, we report on the synthesis and binding mode elucidation of novel and previously identified HuR ligands. The chiral resolution of the most interesting compounds was performed and their binding to HuR assessed according to a STD (saturation transfer difference) NMR and *in silico* combined approach. The information thus obtained represent the basis to further develop these structures aimed at developing small molecules able to act on the stability of HuR–RNA complexes to modulate gene expression.

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Design of new paramagnetic tags for NMR Spectroscopy: A new strategy to yield fast, selective and irreversible tagging of protein

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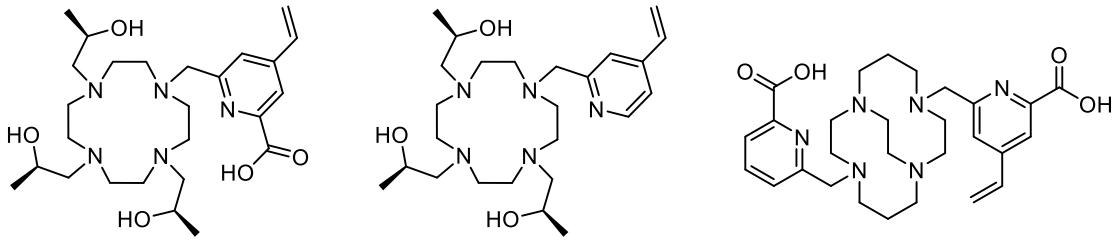
Paramagnetic restraints have been used in Biomolecular NMR for the last three decades in order to elucidate and refine structures but also to characterize protein/ligand interactions. Pseudo Contact Shifts (PCS) are the most commonly used restraints, consisting in measuring a shift between a paramagnetic species and a diamagnetic reference, whose magnitude depends on the distance from the paramagnetic centre. A common technique to generate such restraints consists in the attachment of Lanthanides ions to the protein via a Lanthanide-Binding-Tag (LBT).

In order to design such LBTs, it is important to consider the efficiency and stability of the conjugation, the geometry of the complex (conformational exchanges and coordination) and the chemical inertness of the ligand. Here we describe a photocatalyzed thiol-ene reaction for the cysteine-selective paramagnetic-tagging of proteins. We designed three LBTs with vinylpyridine moieties which were attached to model protein GB1 and medicinally relevant *tc*Pex14 in a fast and irreversible fashion. One of those vinyl-pyridine containing ligand is a cross-bridged cyclam, introduced here as a new class of highly rigid and inert tags for NMR. Those three ligands yielded medium to large tensors with different Lanthanides and were carefully characterised via NMR and Relaxometry. The influence of the coordination geometry on the tags' properties was studied. We believe those findings are relevant for the future development of Lanthanide-Binding-Tags.



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Custom Scoring Aurora-A with SMINA

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In 2002, Sir Philip Cohen predicted that protein kinases would become 'the drug targets of the 21st century'. So far, kinases have lived up to this expectation, in fact protein kinases have emerged as one of the most successful families of drug targets.

Actually, Virtual Screening (VS) studies in the kinase field, are hampered by the fact that many specific inhibitors are not fully selective for a single target, due to the high structural similarity of the ATP-binding site of kinases.

The aim of this work is to explore strategies to improve the effectiveness of VS on kinase proteins. As a paradigm, we focused on Aurora A kinase. The Aurora kinases are implicated in a variety of hematological and solid cancers, because of the essential role in mitosis and cell cycle regulation. In recent years, Aurora kinases have proved popular cancer targets and many inhibitors have been developed. The majority of these clinical candidates are multi-targeted, rendering them inappropriate as tools for studying Aurora kinase mediated signaling.

Our starting point was a structural analysis of 24 Aurora-A crystal structures complexed with different ligands. Starting from this dataset and using data derived from PubChem, we benchmarked the performance of the default scoring function (SF) of the popular Vina docking tool to identify true binders out of a set of decoys, obtained by DUDE. These decoys are selected to be chemically dissimilar from the provided active compounds. Thus, we used the RDkit library to generate 3D conformations from the 2D SMILES obtained by DUDE. The docking performance was analyzed using the receiver operating characteristic (ROC) curves, which represent the plot of false positive rate versus the true positive one. By analyzing the ROC curves for docking performance against the various Aurora-A structures, we were able to simultaneously evaluate our docking protocol and the most appropriate receptor structure to be used for VS purposes. Moreover, in addition to the default scoring of VINA, we developed a custom SF that was parametrized using the previously described benchmark. To this end, we made use of logistical regression with backward variable selection, to fit docking scores to activity data. We show that the obtained custom SF that was tailored on Aurora-A outperformed the original VINA custom scoring. Finally, an *ad-hoc* set of heuristic rules on receptor's side-chains flexibility was conceived during the analysis to further improve the docking performance of Aurora-A inhibitors.

The protocol used to derive this custom SF and the obtained heuristic rules can be easily adopted and made generalizable to whole human kinome.

Structure-based design and bench-to-bedside translation of precision detection of cancer at 1Å

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Advances in precision molecular imaging promise to transform our ability to detect, diagnose and treat disease such as cancer. Here, we describe the complete bench-to-bedside process of structure based designing, bioengineering, and validating a novel protein scaffold that selectively recognizes integrin $\alpha_v\beta_6$ with single-digit nanomolar affinity. We solve its 3D structure by NMR spectroscopy and x-ray crystallography, and validate leads in preclinical models of cancer with 3 different radiolabels ([^{64}Cu]DOTA, [^{68}Ga]NODAGA, [^{18}F]FP). We show its ability to detect multiple cancer patients including lung, cervical, and pancreatic cancer, and we evaluate the lead tracer's safety, biodistribution, and pharmacokinetics in healthy human volunteers. These newly developed drug and diagnostic scaffolds have potential utility in multiple cancers that are associated with overexpression cell-extracellular matrix components stimulating mitogenic signal transduction. Significantly, these results indicate that structure-based design will have broad clinical application in detecting/diagnosing multiple indications, monitoring the efficacy of multiple therapeutics, as well as in staging both cancer and pulmonary fibrosis.

Introducing the CSP Analyzer: a Novel Machine Learning-based Application for Automatic Analysis of Bidimensional NMR Data in Fragment-Based NMR Screening

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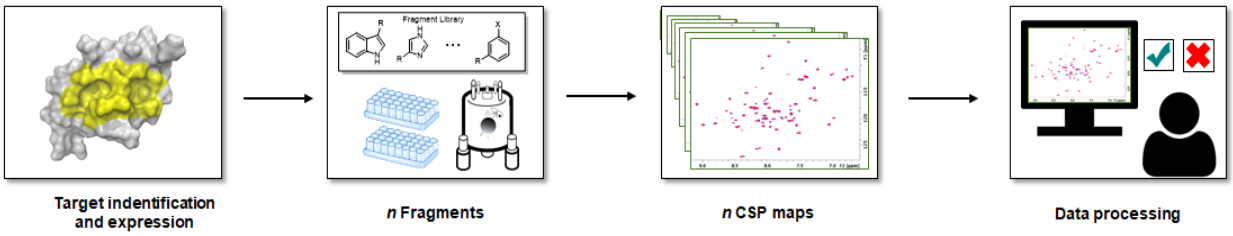
Recently, NMR-based screening, especially Fragment-Based Drug Discovery has become progressively more important in early-stage drug discovery. The necessity to quickly compare hundreds of multidimensional spectra has not been answered so far. While automatic analysis tools were developed for automated 1D NMR data analysis, automatic 2D NMR multidimensional analysis is impeded by the fact that titration experiments are needed to assign peaks to each protein amino acid in order to track the Chemical Shift Perturbations (CSPs) in the spectrum of each fragment screened. Computational tools are available that simplify the tracking of CSPs in 2D NMR titration spectra analysis but, to the best of our knowledge, no software package among these is capable of tracking CSPs of many hundred 2D spectra without peak assignment. We present a novel and fast approach to the holistic analysis of multiple 2D HSQC spectra based on advanced Machine-Learning-driven statistical discrimination: the CSP Analyzer software package. The CSP Analyzer features a C# designed frontend (as proof of concept, the software has been written to work with Windows OSes) that is interfaced to a Python ML classifier. The software allows rapid evaluation of 2D



screening data from large number of spectra without the need of assignment. It also prevents user-introduced bias to the evaluation.

2-oxo-pyridine derivatives abrogate inflammation in LPS-stimulated BV2 microglial cells

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Many evidences suggest the involvement of the endocannabinoid system (ECS) in the modulation of neuroinflammatory disorders whose common feature is an hyperactivation of microglia, responsible for the secretion of many reactive species, including cytokines, chemokines, glutamate, prostanoids and free radicals that cause oxidative stress. The ECS, and in particular the CB2 receptor (CB2R), has been reported to be an attractive target for managing microglial-derived neuroinflammation since its modulation might have beneficial effects for specific symptoms and for the slowdown of disease progression.

In a research program aimed at obtaining ligands of cannabinoid receptors (CBRs), a series of 1,2-dihydro-2-oxo-pyridine-3-carboxamide derivatives variously substituted on central nucleus was synthesized. From these studies, the novel multi-target modulator **FM-6b** (Figure 1) was selected for its high binding affinity with Ki in the nM range and for its agonist behaviour at both CBRs (data not published). Moreover, very recently, our group reported the synthesis and the biological evaluation of **EC-21a** (Figure 1) as the first synthetic CB2R positive allosteric modulator (PAM). In order to evaluate the ability of these compounds to interfere with microglial activation, we analysed the ability of **FM-6b** and **EC-21a** to modulate the release of pro- and anti-inflammatory cytokines in lipopolysaccharides (LPS)-activated mouse BV2 microglial cells, either alone and in combination. Our results showed:

- **FM-6b** induced CB2R-mediated anti-inflammatory effect. In detail, **FM-6b** significantly decreased the pro-inflammatory cytokines (IL-1β and IL-6) and significantly increased the release of anti-inflammatory cytokines (IL-4 and IL-10). This effect resulted reverted in the presence of the CB2R antagonist SR144528, confirming **FM-6b** action on CB2R.
- **EC-21a** did not present a significant ability to modulate the cytokine release. This behaviour is in accordance with the allosteric modulator activity of **EC-21a**.
- the co-treatment with **FM-6b** and **EC-21a** enhanced the anti-inflammatory effect of **FM-6b.** This data confirmed that **EC-21a** is an allosteric modulator, since allosteric ligands are able to modulate the affinity and/or efficacy of specific orthosteric ligands by binding topographically distinct allosteric sites.

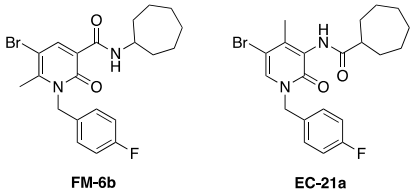

Figure 1. Chemical structure of **FM-6b** and **EC-21a**.

The interesting activity presented by these compounds in LPS-stimulated BV2 microglial cells indicates that their combination might represent a promising therapeutic approach for mitigating neuroinflammation and neurodegeneration.

Conjugation of c(CGisoDGRG) with succinimide-based linkers improves affinity to avb3, without promoting its allosteric activation

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The isoDGR sequence has emerged as an integrin binding motif that has been successfully employed as tumor vasculature-homing device for the targeted delivery of drugs and diagnostic agents to tumors. We have previously shown that coupling c(CGisoDGRG) with a common drug conjugating linker (sulfosuccinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate, SMCC) markedly improves the peptide affinity and selectivity for v 3, an integrin α β over-expressed in the tumor vasculature. Here, by combining NMR, computational and biochemical methods, we provide a structural rationale for the enhanced binding affinity. In particular, we show that the improved binding is mainly ascribable to the interactions engaged with the receptor by the succinimide ring present in the chemical linker, suggesting that polar contacts outside the classical isoDGR motif actively contribute to the pharmacophoric features required for optimal interactions with αvβ3.

In addition, we demonstrate that various peptides containing the isoDGR sequence embedded in different molecular scaffolds and coupled to SMCC, do not induce αvβ3 allosteric activation, working as true integrin antagonists. Here, we introduce the novel concept that allosteric inhibition is an intrinsic property of the isoDGR motif, which is independent from its flanking residues and from the molecular scaffold harboring the motif. Importantly, this feature is preserved after conjugation to other chemical moieties, thus enlarging the field of application of this pure integrin antagonist sequence. These results suggest that isoDGR could represent a valid alternative to RGD, a widely used tumor-homing, tripeptide with integrin partial agonist proper - ties, and that it could be profitably exploited for the rational design of efficient and safe tumor- homing agents for diagnostic and therapeutic applications, devoid of adverse integrin activating side effects.

AGV01-1260, a new potent and selective ERK inhibitor to treat MAPK-dependent cancers

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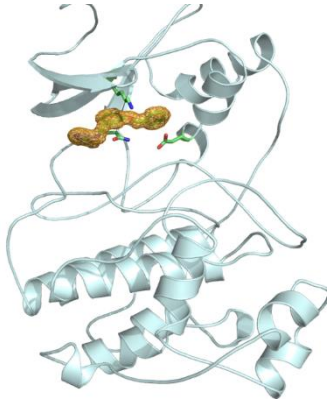
RAS/RAF/MEK/ERK pathway plays a major role in cell proliferation, growth and survival. This signaling is over-activated in several human tumors. Thus, RAS mutations occur in almost 30% of all cancers such as in pancreatic (90%), colorectal (45%) and lung (35%) cancers (1). Likewise, RAF mutations accounts for 7% of cancers and are frequently observed in melanomas (22-72%), thyroid (36-53%) and ovarian (30%) cancers (2, 3).

Proteins of this MAPK pathway, BRAF and MEK, have been targeted to block tumor growth and have proven clinical efficacy. However, resistance ultimately appears with current targeted therapies (4, 5). Thus, it seems essential to develop new therapeutic options to treat MAPK-dependent cancers. Moreover, most resistance to RAF and MEK inhibitors induces ERK reactivation, through different mechanisms such as MEK and NRAS mutation, BRAF and COT amplifications (6). Therefore, ERK inhibitors may be a method to overcome those resistance mechanisms to RAF and MEK inhibitors.

AGV Discovery and its academic partners, Inserm and Montpellier University, have identified a new original series of small molecules that inhibit ERK kinase activity by using a Fragment-Based approach. The company currently possesses a potent and selective inhibitor with an IC_{50} lower than 1nM for ERK2. Preclinical candidate named AGV01-1260 shows a strong anti-proliferative activity in a broad range of MAPK-dependent cell lines with no cytotoxicity in MAPK-independent cell lines and in healthy PBMC. This candidate is orally bioavailable with a strong efficacy in a BRAF melanoma xenograft model. AGV01-1260 shows high synergy with reference treatments including chemotherapies, targeted therapies and anti-apoptotic inhibitors in several in vitro models. This candidate also shows a promising in vitro toxicity profile.

The presentation will show the process to discover AGV01-1260 and it is activity on in vitro and in vivo models.



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Do All Roads Really Lead to Rome? *Learnings from Comparative Analysis of Fragment Binding using SPR, NMR, and X-Ray Crystallography*

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There are several biophysical methods developed to rapidly identify weakly binding fragments to a target protein, but which method is less likely to produce false positives and false negatives? Having a reliable cascade of screening methods to be used as pre-screens prior to labor-intensive X-ray crystallography appears on first sight extremely beneficial. This would allow the filtering of compounds as the screening progresses so that only the most promising hits remain. But which of these methods should start the screening cascade? In this work, we screened various sets of fragment libraries against three different proteins; namely TGT (tRNA guanine transglycocylase), PEX14 (peroxin 14), and EP (endothiapepsin), to investigate whether different screening methods will reveal similar collections of putative binders. The screening of a 96-fragment library against TGT by SPR, NMR, and X-ray gave no overlapping hit rates which could be explained by several factors such as exclusion of fragments from individual screens due to technical reasons. Additionally, X-ray allowed the detection of specific binders that may be too weak binders to be detected by SPR and NMR. In another study with PEX14, the unsuccessful soaking of NMR fragment hits into various crystal forms of a mutated target prompted us to study the crystal packing. It revealed not only a tight crystal packing, but also that one important binding site was tightly blocked by its own crystal mate. This highlights the importance of choosing the appropriate mutations and analyzing crystal packing before embarking on soaking trials. Successful crystallization of the wild type PEX14 revealed free binding pockets which enabled the soaking of a lead compound and obtaining a crystal structure of the complex at 1.8 Å. This proves the feasibility of using this crystal form for subsequent soaking experiments. As for EP, a 361-fragment library was previously screened by X-ray crystallography and 71 fragment hits were detected. These 71 hits were rescreened in a follow-up study for a second time with STD NMR and WaterLOGSY. Compared to a previously performed STD NMR screen applying slightly different conditions, the second STD NMR detected almost double the amount of hits as the initial screen, and the WaterLOGSY screen had the highest correlation to the X-ray hits. The 361-fragment library was also screened using SPR resulting in a hit rate of 34% and an overlap of 11% with the X-ray hits - the highest correlation between screening methods reported by us thus far. The detailed comparative analysis of these findings include the importance of using deuterated water in STD NMR, and the phenomena of active site fragment displacement by use of so-called reporter ligands.

Discovery of new compounds potentially targeting 14-3-3 proteins in trypanosomatid parasites

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Trypanosomatid parasites are responsible for some of the most important neglected tropical diseases, including African trypanosomiasis (also known as sleeping sickness) caused by two subspecies of *Trypanosoma brucei* (*T. brucei*) and Chagas disease which is caused by infection with *Trypanosoma cruzi* (*T.cruzi*). Altogether, affect an estimated 1 billion people around the world and collectively cause over 150 000 deaths per annum. Currently, there are no vaccines for these diseases and the available drugs are far from ideal, making the need for novel drug targets and the development of new, effective drugs even more urgent. [1,2]

In early drug discovery, different approaches can be used to finding new active and safe compounds. Here, we report a new successful pathway for the identification of promising hits in the early stages of drug discovery projects. Our strategy aimed to "Recycling/repurposing Non-Active Compounds" (RNACs) belonging to an in-house library. These molecules were designed and synthesized toward a specific target, but due to their lack of activity, they were deprioritized as inactive compounds.

A compound named KLDS47 showed significant potency against *T.cruzi* with low cytotoxicity against mammalian host cells. KLDS47 was originally developed inside the Botta's group as part of a set of molecules designed to be inhibitors of the human 14-3-3, some of them demonstrated activity. KLDS47 was inactive, nevertheless, due to the structural similarities, it was possible to consider that 14-3-3 proteins of *Trypanosoma* could be the potential target of this compound. [3]

A set of 40 analogues of KLDS47 were designed, synthesized, and assessed as potential inhibitors of 14-3-3 proteins on the parasites. A deeper investigation was carried out in order to validate the mode of action of the compounds. Our working hypothesis is that these new compounds may bind to the Tb14-3-3 and inhibit the interaction with PP1.

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Targeting APOBEC3B by fragment-based drug discovery.

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Cancers are caused by somatic mutations; however, there is presently a focused attention on understanding the biological processes generating these mutations. Mutations from 30 cancer classes have been analysed, and strikingly a signature was associated with the APOBEC mutational pattern[1]. APOBEC3B appears more likely involved than other members of the APOBEC family[2]. APOBEC3B is a multi-domain protein member of the APOBEC family, a class of enzymatic cytosine deaminases characterized by at least one zinc-dependant catalytic domain[3]. The biochemical reaction induced by APOBEC3B is cytosine to uracil (C-to-U) deamination of single stranded DNA (ssDNA), a base transition that is not hereditarily encoded[4]. This mutational process is believed to be fuelling tumour growth, immune escape and cancer therapy resistance[5]. The development of an efficient APOBEC3B inhibitor is therefore essential. Here we describe our fragment-based drug discovery (FBDD) effort to identify APOBEC3B inhibitors. Crystal structures of protein-ligand complexes were obtained, revealing new binding sites. Their relevance has yet to be investigated.

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Hit-identification and -optimization using target directed dynamic combinatorial chemistry for the anti-infective target DXS

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Antibiotic resistance is one of the biggest threats to humankind.[1,2] This global problem is aggravated by bacteria developing new resistance mechanisms and the emergence of extremely drug-resistant strains of the pathogens. In this alarming situation, novel targets for which inhibitors with an unprecedented mode of action can be developed are urgently required.

Our study aims at the development of selective and potent inhibitors of the important and underexplored anti-infective target DXS. This enzyme from the 2C-methyl-D-erythritol 4-phosphate pathway (MEP-pathway) is entirely absent in humans but is essential for medically relevant pathogens (e.g., *Plasmodium falciparum, Mycobacterium tuberculosis, Pseudomonas aeruginosa, and methicillin-resistant Staphylococcus aureus*). Despite substantial efforts dedicated to the discovery of inhibitors for DXS, to date, very few active compounds have been reported and none of them fulfil the requirements as an ideal candidate for further development. To address these issues and maximise the chances of success, we are using target-directed dynamic combinatorial chemistry (tdDCC) as hit-identification strategies for the first time for DXS. To expand the structural diversity and obtain potent and selective inhibitors of DXS, we designed the dynamic combinatorial library for acyl hydrazone formation. Different heterocyclic hydrazides and aldehydes were chosen based on calculated estimated affinity using SeeSAR for all possible acyl hydrazone products. Biochemical evaluation of several hit compounds amplified in three rounds of tdDCC experiment against *M. tuberculosis* DXS afforded inhibitors with IC_{50} in the range of 30 – 190 μM.[3] Use of tdDCC for hit optimisation showed interesting improvement in inhibitory potency of the hits. Further improvement of the activity by more tailor-made DCC-library and by SAR of hits obtained is underway.

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Structure-based pharmacophore virtual screening identifies first-in-class antagonists of Spire-FMN2 interaction

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Spire is a multi-domain protein that belongs to the family of actin nucleators. Experimental evidence suggests that Spire contributes to the function of invadosomes, an actin-rich structures that supports the invasion of cancer cells through extra cellular matrix (ECM) [1]. Spire cooperates with formin FMN2. The interaction between proteins is mediated by short, highly conserved amino acid sequence located at the very C-terminus of FMN2 (FSI) which binds to KIND domain of Spire. The exact role of Spire-FMN2 complex is not fully understood. In contrast to Arp2/3 complex [2] and formins [3], a different types of actin nucleators, chemical modulators of Spire are not available, what significantly hampered the interrogation of its function in cells. To expand the chemical toolbox of actin nucleators inhibitors we sought to identify compounds that impair the Spire-FMN2 interaction.

Herein we used crystal structure of Spire in complex with FMN2 (PDB: 2YLE) to design a 3D pharmacophore mimicking the binding mode of three key residues of FSI peptide to the pocket on the KIND domain of human Spire. The resulted pharmacophore search using ZINCPharmer server led to the identification of 1573 compounds which were subsequently docked to KIND domain of Spire. The top-ranked compounds were experimentally tested in thermal shift assay and by monitoring binding to ^{15}N-labeled KIND domain using ^{1}H-^{15}N heteronuclear correlation NMR spectroscopy. Despite the relatively weak affinity to target protein (in mM range), the identified compounds represent first-in-class Spire inhibitors and their optimization should result in more potent binders.

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Cubanes for Drug Design

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In 2016, chemists showed that replacing a benzene ring in the neurotropic compound Leteprinim with a skeleton of cubane beneficially affected activity and water solubility of the parent compound (Figure. 1) [1]. Since then the cubane-containing building blocks are gaining high popularity in drug discovery projects, as mimics for the benzene ring [2-4].

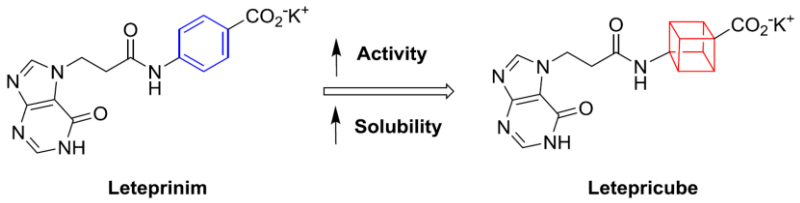
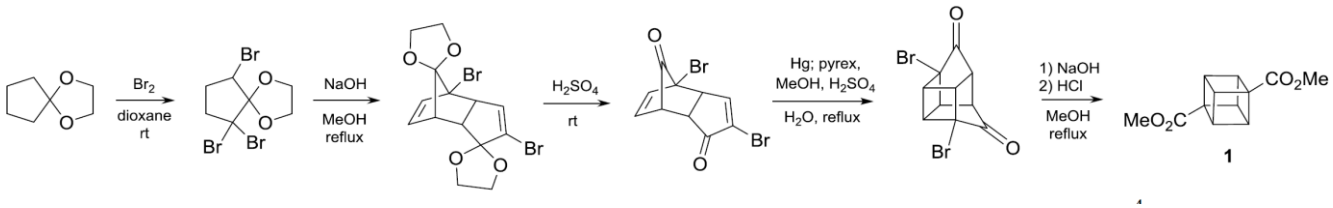
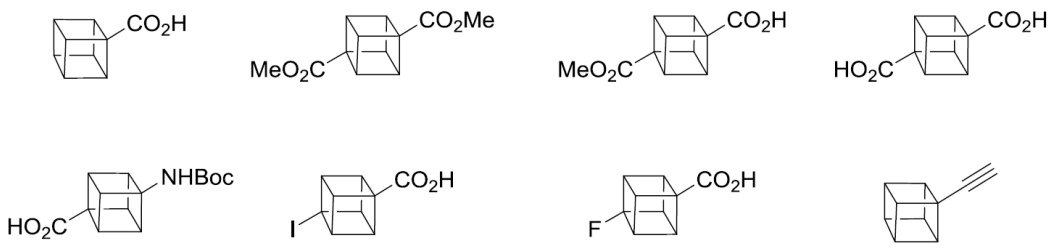


Fig. 1 Modification and improvement of activity of Leteprinim drug.

We synthesized cubane-1,4-diester 1 in 100 g scale following the literature protocol (Schemes 1) [4], and used it for the synthesis of diverse cubane-containing building blocks (Schemes 2).



Scheme 1. Literature synthesis of cubane-containing compound [1]



Scheme 2. A library of cubane-containing building blocks for drug discovery programs

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Discovery and Synthesis of Highly Selective Inhibitors of Class II Phosphoinositide 3-Kinase

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Phosphoinositide 3-kinase (PI3K) is one of the prime kinase targets in drug development in cancer as PI3K signaling is one of the most frequently deregulated pathways in cancer [1]. Tumor activating mutations have been found in PIK3CA encoding the p110α isoform of PI3K, a direct target of the Ras oncogene, and in the tumor suppressor PTEN, an enzyme that hydrolyses phosphatidylinositol 3,4,5-trisphosphate [2]. While specific class I PI3K inhibitors have recently entered clinical trials in oncology (four FDA-approved drugs and more than 30 clinical candidates) [3], specific inhibitors of class II PI3Ks have not been discovered yet. Notwithstanding, in the last decade, strong evidence has been accumulated that they may play important roles in glucose transport, insulin signaling, clathrin-mediated endocytosis, cell migration, cell growth, and survival [4,5]. Therefore, identifying highly selective inhibitors of class II phosphoinositide-3-kinases (PI3K-C2) is key to unravel their role as future's drug discovery targets.

Given the high sequence homology among the class II congeners, it is highly challenging to develop selective inhibitors that only bind to class II PI3Ks, in particular in the absence of structural information of the protein. We have identified 2-thiopteridinones as potent inhibitors of α-isoform of class II PI3K (PI3K-C2α) by screening about 37,000 compounds using two different assays (ADAPTA® and ADP-GloTM). Further structure-activity relationship studies led to the discovery of several class II PI3K inhibitors with nanomolar activity. Kinase profiling of three lead compounds with a panel of 117 diverse kinases as well as 22 lipid kinases showed no off-target kinase inhibition beyond class II PI3K. Notably, one of the lead structures showed nanomolar inhibition towards PI3KC2α and >100-fold selectivity over the other isoforms, PI3K-C2β and PI3K-C2γ.

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Stabilization of 14-3-3/p53 protein-protein interaction

A. Kuusk[1,2], R.G. Doveston[3], S. Andrei[2], J.F. Neves[4], K.B. Rodriguez[5,6], H. Chen[1], H. Boyd[7], C. Ottmann[2,5]

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14-3-3 proteins are adapter proteins which are involved in protein – protein interactions with more than 500 binding partners. The tumor suppressor protein, p53, plays an important role in cell cycle arrest and apoptosis and is often mutated in human cancers. 14-3-3 proteins are positive regulators of p53. Their binding to the C-terminal domain of p53 protects the tumor suppressor protein from MDM2-dependent degradation thus preserving its important activity in the cell. Therefore, the discovery of small-molecules which could stabilize this protein – protein interaction would be a significant strategic development in the search for new cancer medicines. We have identified a small molecule stabiliser of this interaction which opens the door for a new mechanism to target p53. Using X-ray crystallography, biophysical methods and in vitro assays we have built a robust suite of assays to further our understanding of this protein – protein interaction and to find new small molecule stabilisers.

Structural studies of 14-3-3 complexes with peptides containing 14-3-3 binding motifs of protein kinase CaMKK2

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14-3-3 protein family isoforms, known as scaffold proteis and expressed in all eukaryotic cells, influence diverse cellular processes through binding and interacting with a wide range of proteins. 14-3-3 binding and interactons have been demostrated, with several intra-cellular Kinases through homo- and heterodimerization, to be crucial in many disease and essential for many physiological processes in differentiated cells.

Calmadulin is an intracellular receptor for Calcium, controlling the downstream pathways of the CaMK cascade to contribute and maintain the Metabolic homeostasis.

CaMKK2, mentrioned in this poster, is a member of Ser/Thr Kinase Family, necessary to the maintenance of organism energy homeostasis. Indeed CaMKK2 deficiencies seem to protect mice from diet-induced obesity, glucose-intolerance and Insulin resistance.

CaMKK2 is activated by the increased affinity and binding with Ca/CaM, activating in turn, by phosforiltion, CaMKIV, to regulate sympathetic tone, and AMPK, to regulate appetite and gluconeogenesis.

CaMKK2 contains two PKA phosphorilation sites in Ser 100 and S 511 that bind 14-3-3.

The interaction of 14-3-3 with CaMKK2 can, not only, directly inhibits CaMKK2 activity, but also blocks the dephosphorilation of Thr108, an inhibitory PKA phosphorilation site, downregulating serveral signaling pathways.

In this study, I'm showing the evident fisiological interaction between 14-3-3 G and the synthetic peptides containing the N-terminal 14-3-3 binding motifs of CaMKK2, using Fluorescence Polarizzation Assay and Protein Crystallography.

The Crystal structures of these two complexes, between 14-3-3 and 14-3-3 binding motifs of CaMKK, showed that both phosphopeptides interact with the amphipathic groove of 14-3-3 according to other 14-3-3 complexes. In addiction, in the fist model, the interaction between the side chain of Gln at the position +2 from S100 peptide and the phosphate group appears to change the direction of the polypeptide chain, leaving the potential cavity for small-molecule binding empty.

Considering our data this interaction might be stabilized by small-molecule compounds, like fusicossin, to inhibit CaMKK2 activity for treatment of metabolic-related diseases.

Evolutionary divergent PEX3 is essential for the glycosome biogenesis and survival of the trypanosomatid parasites

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Trypanosomatid parasites cause African sleeping disease, Chagas disease and Leishmaniasis that affect about 18 million people worldwide. Here we report the identification of the highly divergent trypanosomal PEX3, a single docking factor for PEX19-bound peroxisomal membrane proteins and the master regulator of peroxisome biogenesis. The Trypanosomatid PEX3 shows very low level of conservation and its identification was made possible by identifying of the PEX19-interacting proteins and screening of secondary structure homology. The trypanosomal PEX3 localises to glycosomes and directly interacts with the cytosolic membrane protein receptor PEX19. RNAi studies revealed that PEX3 is essential and that its depletion results in mislocalisation of glycosomal proteins and rapid death. Comparison against human PEX3 disclosed differences on the parasite protein that might be accessible for drug development. The absolute requirement for biogenesis of glycosomes and its structural distinction from its human counterpart make PEX3 an interesting drug target for development of novel therapies against trypanosomiasis. The identification paves the way for future drug development targeting PEX3, and for the analysis of additional partners involved in this crucial step of glycosome biogenesis.

FragMAX: X-ray crystallography-based Fragment Screening at MAX IV

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The drug discovery process is heavily depended on the identification of hits that can be chemically developed into leads. Improving a biological property of ligands may require details about the binding mode of the ligand in the target protein. Few methods can give accurate atomic information as X-ray crystallography, and recent improvements in data acquisition and processing increased the throughput to enable its use in early stages of drug discovery projects.

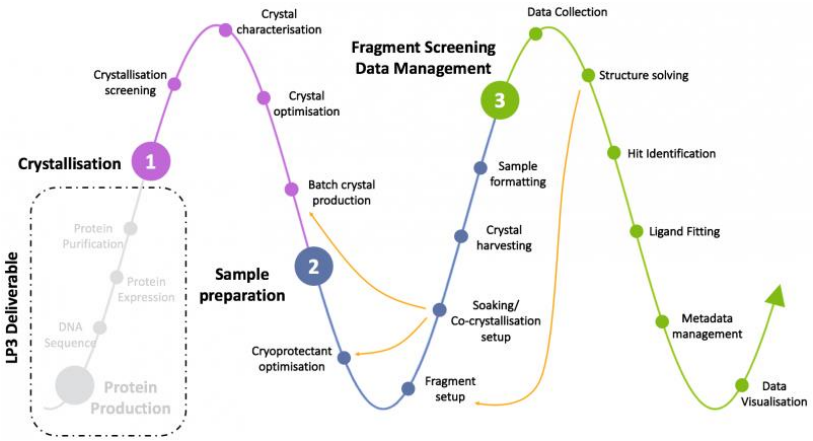

Fig. 1 *FragMAX platform entry points overview*

FragMAX is the fragment screening platform at MAX IV's macromolecular crystallography beamline BioMAX. Our platform offers support for users to prepare samples (soakings, fishing and organising), collect and process data. An original WebApp is being developed at MAX IV allowing users to interact with our HPC environment, allowing massive data processing and visualisation from a simple and intuitive interface, requiring minimal knowledge of command line tools. FragMAX access scheme allows multiple entry points depending on project demands, from full support since cryo optimisations to only data collection with custom fragment library, providing maximum flexibility for both industrial and academic users. This project is done in collaboration with Lund Protein Production Platform (LP3), AstraZeneca and SARomics Biostructures.

In our current stage of development, we ran four commissioning projects whereas one using a custom fragment library. More information about FragMAX and our web-app is available at BioMAX webpage (www.maxiv.se/biomax).

14-3-3 regulation of the autophagy-initiating kinase ULK1

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14-3-3 proteins play key roles in cell signalling pathways by docking pairs of phosphosites at specific motifs on hundreds target proteins, thus have been implicated in the regulation of cellular processes including enzyme activation, cell-cycle control, apoptosis, and metabolism. The human 14-3-3-binding phosphoproteome is highly enriched in ohnologues and bioinformatic analyses suggest that the autophagy-regulating protein kinases ULK1 and ULK2 are such proteins. We are investigating whether differential regulation of ULK1 and ULK2 sister ohnologues by 14-3-3s might explain how autophagy can be controlled by diverse stimuli. ULK1 is an autophagy initiating kinase that is essential for phagophore formation, working in complex with ATG13, FIP200 and ATG101. Its known regulators include the kinases mTOR and AMPK, and indeed AMPK has previously been shown to stimulate 14-3-3 binding to ULK1. We do not know if 14-3-3 binding to ULK1 is essential for autophagy, or if other signalling pathways differentially regulate 14-3-3 binding to ULK1 and ULK2. Here, we first confirmed the AMPK-dependent interaction of 14-3-3 with ULK1 and found that though multiple compounds could activate AMPK, only the novel pan beta AMPK activator MK-8722 robustly stimulated ULK1 phosphorylation at Ser556 to induce 14-3-3 binding. This suggests ULK1 may be under isoform-specific AMPK regulation. We also analysed other signalling pathways using a panel of kinase inhibitors and activators that have been shown to regulate 14-3-3 interactions with other proteins. Preliminary data suggest that AMPK is not the only kinase involved, as IGF1 (implicating Akt) and H89 (which inhibits kinases including cAMP-dependent kinases) also stimulate 14-3-3 binding to ULK1. We will unravel the mode and mechanism of 14-3-3 binding in these instances and determine the consequences in autophagy.

The Power of Fragments: FBLD approach to investigate proteins structure

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Fragment-based lead discovery (FBLD) is a well-established method to investigate protein structure. In particular, protein X-ray-crystallography is a powerful approach to characterize the binding modes of weakly binding fragments as a versatile starting point for lead optimization. Owing to their low molecular weight, fragments are able to bind to known pockets acting as starting points for the search and development of new chemical scaffolds as well as they are able to allow exploring unknown pockets or cavities impossible to reach with the standard ligands of usual average-size dimensions.

Considering the importance that these small molecules are exhibiting in the field of drug discovery lately, it is of fundamental importance to have a fragment library ready to use. Even more convenient is to have a general purpose library designed “at hand” and validated on a broad range of proteins belonging to different classes. Such library is available from Jena Bioscience and it was developed in a joint project between the HZB MX-group at BESSY II (AG Weiss) and the Institute of Pharmaceutical Chemistry, University of Marburg (AG Klebe). It consists of 96 entries chosen for chemical diversity and it have demonstrated a mean a hit rate of 11% across a sample of 7 validated proteins.

This library was the starting point to investigate target proteins such as Farnesyl Pyrophosphate Synthase (FPPS) from *T.brucei* and *T.cruzi*. It is a homodimer involved in the mevalonate pathway. Through the inhibition of this key enzyme it might be possible to treat protozoan parasite disease such as Chagas diseases caused by *Trypanosoma cruzi.* Prior to crystallographic screening it is of crucial importance to optimize the crystals quality and to produce a well-established protocol suitable for soaking experiments.

The crystallographic screening allowed us to explore the so-called “allosteric pocket” and hypothesize, during the first step of fragment optimization, chemical groups essential for the binding. All soaked crystals have a resolution better that 2.5 Å and due to this good resolution, we could suggest a plausible binding mode of the fragment hits within the FPPS motion.

NMR structural and functional studies of the yeast telomerase

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Telomerase is a ribonucleoprotein complex responsible for the elongation of telomeric repeats and maintenance of genome integrity. Its hyperactivation provides a characteristic signature for the majority of cancers, while its down-regulation is associated with ageing and several early cell death related pathologies. In this light modulation of telomerase activity is an attractive route of finding new therapeutics. The lack of structural information hampers development of telomerase modulators.

Here we present the NMR study of structures and functions of two proteins from yeast *Hansenula Polymorpha* telomerase complex: TEN-domain of telomerase catalytic subunit [1] and Est3. Both proteins are essential for enzymatic activity *in vivo.* High-resolution NMR structures have been solved and interactions with oligonucleotide and protein binding partners have been studied. Obtained results provide new insights on the molecular mechanism of telomerase action.

The work is supported by Russian Science Foundation grant 18-73-00068.

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Fragment-based drug discovery targeting *Trypanosoma brucei* FPPS: Discovery of new binding sites

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Trypanosoma brucei (*T. brucei*) is the causative agent of the Human African Trypanosomiasis (HAT), a neglected disease, with a high demand for safer and more efficient new drugs. The bone disease drugs, nitrogen-containing bisphosphonates have been shown to block *T. brucei* parasitic growth by inhibiting farnesyl pyrophosphate synthase (FPPS), but they exhibit undesired pharmacokinetic properties for non-bone indications. In a reported fragment-based approach on human FPPS, several fragment binders were detected in a previously unknown allosteric site. Hence, the high unmet medical need combined with the discovery of a potential new target site prompted a fragment-based drug discovery approach to identify non-bisphosphonate binders on *T. brucei* FPPS.

Fragment screening was performed by NMR and X-ray crystallography. Initially, 2000 fragments were screened by 1D-NMR and fragment hits were further validated and characterized in protein-observed 2D-NMR resulting in 44 validated fragment hits for *T. brucei* FPPS. Fragment hits were subjected to crystallization experiments to identify the exact binding mode. Additionally, a 500 fragment library was screened by X-ray using the automatic crystal harvesting pipeline at EMBL Grenoble. Most notably, the application of the PanDDA software was essential and enabled the identification of weak fragment binders in X-ray structures. The combined two screening approaches identified five fragments of novel chemotype in the active site, four fragments in the previously unknown allosteric site, and five fragments in additional binding sites. First SAR-by-archive and catalogue was performed to optimize the binding affinity of identified fragments and a crystal structure of a fragment analogue of an active site binder was solved with the fragment bound in the allosteric site.

Medicinal chemists in the 90s have spent years trying to get away from the bisphosphonate chemotype, without success. The identification of non-bisphosphonate active-site FPPS binders and fragment binders in a previously unknown allosteric site show the power of fragment-based approaches and thereby paves the way for future studies aiming to identify high-affinity non-bisphosphonate inhibitors for *T. brucei* FPPS with pharmacokinetic properties that are suitable for parasitic indications.

A modified Chromogranin A derived Peptide is a Potent Dual Ligand for Integrins αvβ6 and αvβ8

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[2] *Istituto di Chimica del Riconoscimento Molecolare, Milan, Italy*
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Integrins αvβ6 and αvβ8 are emerging targets for the tailored delivery of diagnostic and/or therapeutic agents to various types of epithelial cancers [1,2]. Recently, chromogranin A, a neurosecretory RGD-containing protein that regulates wound healing and tumour physiology, has emerged as natural ligand of αvβ6 [3]. Combining heteronuclear 2D-NMR STD methods, docking calculations and competition experiments we have unveiled the structural determinants of the selective interaction of a peptide derived from human chromogranin A (**1**) with αvβ6. Rational mutation of (**1**) and its chemical modification, resulted in a new serum stable compound (**2**) with high affinity and bi-selectivity for integrins αvβ6 and αvβ8. Thus, **2** might represent a new potent molecule for the dual targeting of αvβ6 and αvβ8. Compound **2** has also proven useful for nanoparticle functionalization and delivery to cancer cells, holding promise for future diagnostic and/or therapeutic applications.

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Hitting on the move: targeting intrinsically disordered protein states of the MDM2-p53 interaction

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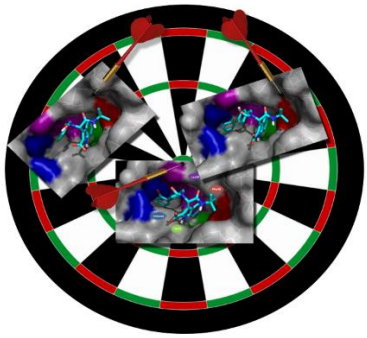
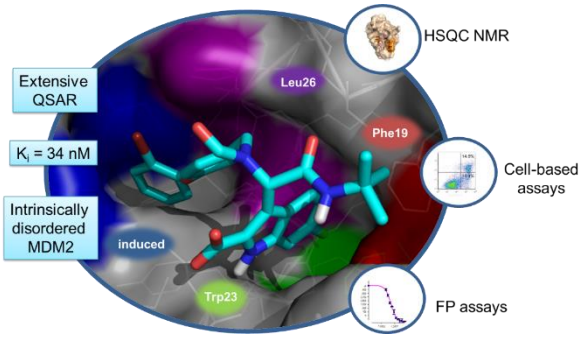
Intrinsically disordered proteins are an emerging class of proteins without a folded structure and currently disorder-based drug targeting remains a challenge. p53 is the principal regulator of cell division and growth whereas MDM2 consists its main negative regulator. The MDM2-p53 recognition is a dynamic and multistage process that amongst other, employs the dissociation of a transient α-helical N-terminal ‘‘lid’’ segment of MDM2 from the proximity of the p53-complementary interface. Several small molecule inhibitors have been reported to inhibit the formation of the p53-MDM2 complex with the vast majority mimicking the p53 residues Phe19, Trp23 and Leu26.

Herein, we describe the transit from the 3-point to 4-point pharmacophore model stabilizing this intrinsically disordered N-terminus by increasing the binding affinity by a factor of 3. We performed a thorough SAR analysis, including chiral separation of key compound, evaluated by FP and 2D NMR. Finally, p53-specific anti-cancer activity towards p53-wild-type cancer cells was observed for several representative compounds.





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Structural view upon the complex formation between the 14-3-3 protein and caspase-2

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Caspase-2 is an apical protease responsible for the proteolysis of cellular substrates directly involved in mediating apoptotic signaling cascades. Caspase-2 activation is inhibited by phosphorylation followed by binding to the scaffolding protein 14-3-3, which recognizes two phosphoserines located in the linker between the caspase recruitment domain and the p19 domains of the caspase-2 zymogen. However, the structural details of this interaction and the exact role of 14-3-3 in the regulation of caspase-2 activation remain unclear. Moreover, the caspase-2 region with both 14-3-3-binding motifs also contains the nuclear localization sequence (NLS), thus suggesting that 14-3-3 binding may regulate the subcellular localization of caspase-2. Here, we report a structural analysis of the 14-3-3ζ:caspase-2 complex using a combined approach based on small angle X-ray scattering, NMR, chemical cross-linking, and fluorescence spectroscopy. The structural model proposed in this study suggests that phosphorylated caspase-2 and 14-3-3ζ form a compact and rigid complex in which the p19 and the p12 domains of caspase-2 are positioned within the central channel of the 14-3-3 dimer and stabilized through interactions with the C-terminal helices of both 14-3-3ζ protomers. In this conformation, the surface of the p12 domain, which is involved in caspase-2 activation by dimerization, is sterically occluded by the 14-3-3 dimer, thereby likely preventing caspase-2 activation. In addition, 14-3-3 protein binding to caspase-2 masks its NLS. Therefore, our results suggest that 14-3-3 protein binding to caspase-2 may play a key role in regulating caspase-2 activation. This work was supported by the Czech Science Foundation (Project 17-00726S).

Smidova, A et al. (2018) FEBS J. 285:4196-4213

Tackling Trypanosomiasis; targeting glycosomal protein import as a means to battle this neglected morbidity

J. D. J. Olmos[1], V. Napolitano[2,3], D. Lenhart[2], M. Dawidowski[4], G. Popowicz[2*], G. Dubin[1*]

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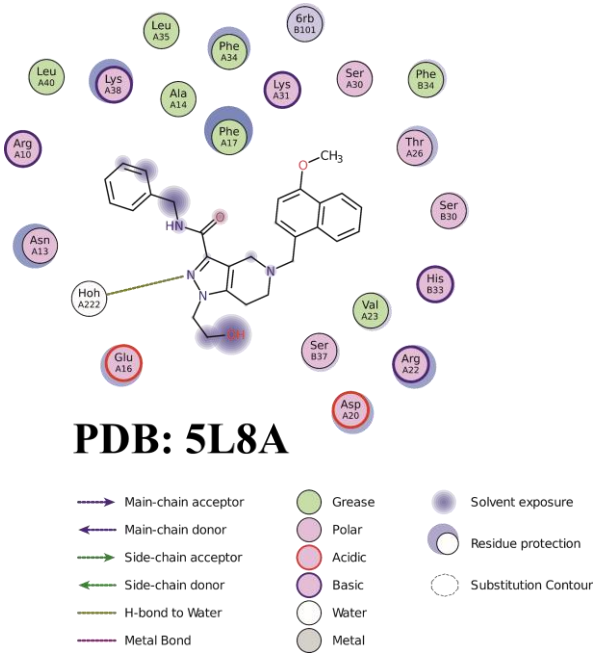

The morbidity and lethality of Trypanosomiasis is considerable. Blocking the glycosomal protein import at the molecular level selectively kills Trypanosoma parasites by targeting peroxins (PEX), which are indispensable components of the translocation machinery for glycosomal matrix enzymes. X-ray structures elucidate and characterise the binding interactions at the molecular level of PEX proteins to explain SAR (structure-activity relationship) profiles. A recent structure provides promise for further development of peroxin targeting compounds as drugs against Trypanosomiasis. In this structure, *Trypanosoma brucei brucei* peroxisomal protein number 14 is elucidated with an included inhibitor. Phenylanaline and tryptophan pockets are filled by the methoxynaphthyl and phenyl moieties, respectively. Interestingly and peculiarly, one bound water molecule forms one or two hydrogen bonds with the ligand (depending on the calculation and the amount of solvent present). Interestingly and curiously, this water molecule is present consistently in all the complex structures of inhibitors tested indicating that this interaction is of, apparently, critical importance. This particular interaction was not predicted by docking calculations, which explains lack of consistency with predicted models and the actual crystal structure. Attempts to displace the water molecule with polar elements yielded inactive compounds, indicating that the water is very strongly bound to the protein. Critically, this peculiar water molecule interacts with Asn^{31}, a residue that is specific to *Trypanosoma* and distinct from human peroxisomal protein 14. Therefore, this interaction may in fact contribute to ligand specificity and possibly, enhance inhibitory capacity of the ligands.

Dawidowski et al. (2017). Science. 355, 1416–1420.

Validation of tcFPPS as a fragment-based lead discovery target

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Fragment-based lead discovery (FBLD) is one of the most promising approaches of the last decade when looking for new chemical scaffolds and (possibly) allosteric sites. The major challenge in the use of fragment molecules is the very weak affinities and often non-specific interaction with the target, especially if not correctly folded. Here we described a strategy for facilitating SPR-biosensor driven fragment-based discovery of novel leads targeting the parasitic enzyme *Trypanosoma cruzi* farnesyl pyrophosphate synthase (tcFPPS). tcFPPS is part of the mevalonate pathway, validated target for trypanocidal molecules since responsible of the synthesis of essential components in the parasite. A panel of orthogonal biophysical methods (Circular Dichroism, Dynamic Light Scattering, nanoDifferential Scanning Fluorimetry, DSF) have been applied to confirm the produced enzyme to be folded, stable and in form of the dimer. A novel real time coupled enzymatic assay using luciferase for pyrophosphate detection was developed and used to confirm tcFPPS activity. A 90 fragments library was screened using two biophysical methods - DSF and SPR - with a slight overlap between the initial hits. Control experiments were run in the absence of tcFPPS co-factor (Mg^{2+}), in order to validate both methods. The SPR biosensor-based fragment library screening resulted in the identification of five selected fragments. Binding efficiency was then determined and used for hits ranking. The overall work resulted in the validation of the structural and functional properties of the produced enzyme and procedures for identifying weak hits, despite the lack of a reference compound for validation of the screening. Structural information about the fragment hits might then be used to guide compound optimization.

Innovative inhibition strategy against functional structural transitions of essential pathogenic factors.

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This study describes the design of novel modulators of specific nicotinic acetylcholine receptors (nAChRs) to target addiction, and of inhibitors of two essential malaria enzymes: *Plasmodium vivax* subtilase SUB1, required for parasite egress and *Plasmodium falciparum* lactate dehydrogenase, critical for parasite metabolism.

Herein, we describe a multidisciplinary approach to target α5 containing nAChRs (α5* nAChRs) to tackle addiction as a link was found between a single nucleotide polymorphism in α5 cytoplasmic domain and the smoking phenotype [1]. We apply a fragment-based approach using an AChBP engineered chimera, which structure was solved in complex with the first known α5 ligands. This structure and two comparative modeling models were used to perform *in silico* screening of a tailored nAChR fragment library. Docking protocols were adapted for nAChRs. A cation-π interaction definition was introduced in the FlexX software and side chain flexibility was allowed in the binding site. An interactive pipeline was developed for the analysis, clustering and visualization of the virtual screening (VS) results. About fifty molecules have produced binding signal by NMR (STD studies). Five of them have been confirmed. Ongoing structural and binding studies will be followed by electrophysiological activity characterization on concatemeric nAChRs. Results will help to build deep neural networks models to assess and improve the prediction power of VS and will be enriched by calculation of the transition path [2.3.4] between resting and active full atomistic models and cavity analysis [5].

Our efforts to target malaria subtilase *Pv*SUB1, focus on the design of reversible covalent inhibitors. We have performed covalent docking simulations of potential peptide candidates and studied peptide cyclization. Several candidates have been synthesized and tested, showing activity in the low micromolar range and co-crystals have been obtained.

For the malaria lactate dehydrogenase (*Pf*LDH), the design is based on NADH analogs with reported inhibitory properties. We have built and screened a combinatorial library *in silico* to find inhibitors that could bridge the cofactor and the substrate binding site, while avoiding affecting the human isoenzyme.

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Novel active site and allosteric site binders identified for farnesyl pyrophosphate synthase of Trypanosoma cruzi

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Trypanosoma cruzi (*T. cruzi*) is the causative agent of Chagas disease, which is considered a neglected disease. Medication for this disease is based on empirically discovered drugs with low efficacy, difficulties in administration and severe side effects. The development of a safe and efficient drug is therefore urgently needed.

Farnesyl pyrophosphate synthase (FPPS) is a key enzyme in isoprenoid biosynthesis. The parasite is dependent on isoprenoids, such as ergosterol, as they cannot be acquired by other mechanisms. Bisphosphonates (BPs) are active site directed FPPS inhibitors. They are used in the clinic as drugs for bone diseases due to their ideal pharmacokinetics in targeting bone tissue. They can also combat *T. cruzi* flagellates but are not ideal to treat Chagas disease. Several non-BP inhibitors that bind to an allosteric pocket were found for human FPPS by fragment based screening (FBS). More recently it was shown that the product of FPPS, farnesyl pyrophosphate (FPP) can bind to this pocket and locks the enzyme in an open and inactive state.

Encouraged by these findings, we started our investigations by FBS against *T. cruzi* FPPS. Screening and validation of 1806 fragments by NMR spectroscopy revealed 118 diverse fragment hits. Counter screening against human FPPS and *T. brucei* FPPS, the causative agent of African sleeping sickness, showed selectivity of the fragments at this early stage of screening. To enable follow up by X-ray crystallography, a crystallization system was set up that yielded apo-crystals of *T. cruzi* FPPS with a diffraction limit of around 1.6 A. A number of 72 fragments were employed to soaking experiments that resulted in two structures. One ligand was active site directed and the other binding to the homodimer interface. The major break trough was achieved by FBS by X-ray crystallography at the XChem facility in Harwell, UK, and the HTXlab in Grenoble, France. In total 1113 data sets were collected and analyzed using the statistical analysis tool Pan-Dataset Density Analysis (PanDDA). More than 50 hits with non-bisphosphonate scaffolds were obtained. Binding sites were distributed all over the protein, including the active site, the allosteric site, the homodimer interface, sites on the surface and a new site in close proximity to the active site.

In summary, we found active site binders of a novel scaffold and discovered the first allosteric site binders for *T. cruzi* FPPS. Both will deliver starting points for medicinal chemistry. Thus, the herein reported findings will give new impulses in drug discovery for Chagas disease.

PDBe-KB aggregated views: Small molecules in their biological contexts

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The Protein Data Bank in Europe - Knowledge Base (PDBe-KB; https://pdbe-kb.org) is a community-driven resource managed by the Protein Data Bank in Europe (PDBe; https://pdbe.org), collating functional annotations and predictions for structure data in the PDB archive. Our latest data access service is the aggregated view for proteins (https://pdbe-kb.org/proteins), which provides all the available structural information related to full-length proteins, including annotations of domains, ligand-binding sites, interaction interfaces and other functional residues.

The next upcoming aggregated view will focus on small molecule ligands and will provide the biological and structural context of drugs, cofactors, reactants and other small molecules, frequently found in the PDB entries. These pages will enable greater understanding of the interactions and functions of small molecules in the PDB and will help to support the efforts of researchers involved in drug discovery.

Similarly, to the aggregated views for proteins, all the information displayed on the web pages will be readily accessible over REST API and as downloadable files. Visualization of the aggregated data will be through reusable web-components which may easily be integrated into 3rd party solutions. These small-molecule centric aggregated views will be available in early 2020.

Chemogenomic Profiling of Human and Microbial FK506-Binding Proteins

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Human FK506-binding proteins (FKBPs) play an important role in various diseases like depression [1], obesity [2] and chronic pain [3]. FKBPs are also highly conserved in bacteria (as macrophage infectivity potentiator, Mip) and increase infectivity in some pathogens [4], altogether making FKBPs an interesting drug target. Ligand development proved to be difficult and many FKBPs still lack useful druglike ligands [5]. To explore the scope and potential of C^5-substituted [4.3.1]-aza-bicyclic sulfonamides as broadly appliccable FKBP inhibitors, we developed a new synthesis method for the bicyclic core scaffold and used it to prepare a FKBP- and Mip-focused library [6]. This allowed us to perform a systematic structure-activity-relationship analysis across key human FKBPs and microbial Mips, yielding highly improved inhibitors for all the FKBPs studied. The best FKBP and Mip ligands showed promising antimalarial, antilegionellal and antichlamydial properties in cellular models of infectivity, suggesting that [4.3.1]-aza-bicyclic sulfonamides could be a novel class of anti-infectives.

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Structural and biochemical studies for the development of new NADPH oxidase inhibitors/modulators as a pathway to cancer therapy

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Reactive Oxygen Species (ROS) is associated to cancer biology, from cell transformation, proliferation, invasion or tumor survival. ROS production is also involved in oncogene expression through mitogenic action, and in the mechanism of many if not most anticancer drugs. NADPH-oxidases (NOXs) (Figure 1) are the only known enzymes whose sole function is ROS generation and are known to modulate pathways that induce and sustain cell proliferation. This notion is exemplified by the vital role of NOX4 in the development of the pancreatic ductal carcinoma, possibly the deadliest cancer. NOX4 is also investigated as target for fibrosis and its overexpression has been associated to tumour progression in lung cancer cells, renal cell carcinoma, colorectal cancer, melanoma, and ovarian cancer. Recently, our research group has been successful in accomplishing the first crystal structure of the cytosolic dehydrogenase (DH) (PDB: 5o0x) and trans-membrane (TM) (PDB: 5o0t) domains of NOX using a bacterial ortholog which is highly similar (40% sequence identity) to human NOX5. The first phase of the project relies on a bacterial enzyme as a model NOX system to provide a general understanding about catalysis and druggability. By investigating human NOX4, we translate this knowledge to a human isoform deeply involved in cancer. Accordingly, our current efforts rely on the study and validation of known NOX inhibitors and the development of new and effective isoform-specific NOX inhibitors and allosteric modulators. These ligands can be useful tools to help expression, purification, and crystallization of the TM-DH core subunit by stabilizing its structure. As a result, this work will help understanding the biology of ROS signalling and establish NOXs as potential targets in chemotherapeutic treatment with a focus on NOX4 and its roles in carcinogenesis.

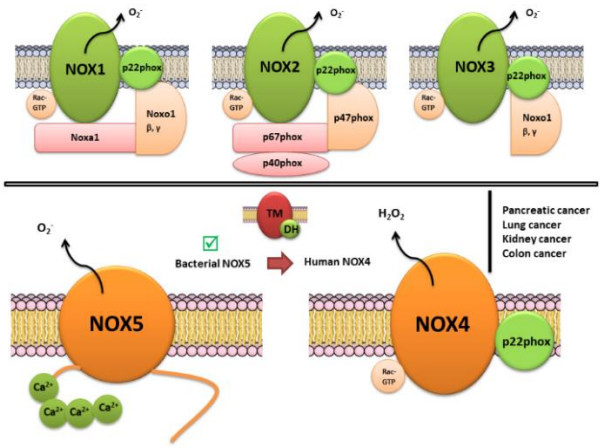

Figure 1 - The NADPH oxidase family comprises 7 isoenzymes coded by different genes: NOX1-5 and DUOX1 and 2 (not depicted).

Protein Interference Assay (PIA) Enables the Validation *in vivo* of New Drug Target Against Malaria and the Discovery *in Vitro* of the First Class of Fragments Acting at the Oligomeric Interface

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Malaria is a parasitic disease caused by four different species of gender *Plasmodium* and *P. Falciparum* encodes for the most lethal one, causing 435 000 people a year, the majority of whom are children. Despite the fact that the most effective remedies remain those based on chloroquine derivatives and natural extracts of A*rtemisia annua*, the appearance of treatment-resistant strains encourages the validation of new drug targets. Here we present the discovery of the first compound able to bind to the oligomeric interface of the cytoplasmic malate dehydrogenase (*Pf*MDH), a key enzyme for the energy metabolism and the downstream synthesis of the purines and pyrimidines during the erythrocytic phase of the infection. In our previous studies we showed that oligomeric surfaces have the advantages of high specificity and binding affinity between the cognate partners, thereby they could successfully be utilized in drug target validation both *in vivo* [1] and *in vitro* [2]. Thanks to a robust platform of high throughput screening (HTS) established at the Helmholtz-Zentrum München, 1500 fragments were screened by STD-NMR and then validated by x-ray crystallography at Giotto biotech, we were able to solve the crystal structure of a 2-phenylthiazole derivative in complex with *Pf*MDH. Scorpion scores analysis reveals that several hydrophobic interactions occur between the fragment and apolar residues and thermal shift assay shows that the fragment decreases the stability of the tetramer in solution, thus encouraging further chemical optimization towards its full disruption by small molecule approach (manuscript in preparation).

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Characterization of 14-3-3/SLP76 Protein-Protein Interaction and its modulation by Small Molecules

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Protein-Protein Interactions (PPIs) are known to play key roles in most biological processes [1]. Their modulation by small molecules through stabilization rather than inhibition is a relatively new approach and it had not been followed systematically in the past [2]. SLP76 (Lymphocyte Cytosolic Protein 2) is an adaptor protein that orchestrates signal transduction downstream of T-Cell Receptors (TCRs). As an adaptor protein, it recruits pivotal signaling proteins to the cell membrane, therefore mediating the formation of a multimolecular signaling complex. The assembly of the complex contributes to multiple cellular events resulting in the regulation of the immune response [3, 4]. A recent study, reveals that the immune response is attenuated by negative regulation of TCR signaling, in which SLP76 undergoes ubiquitination and subsequent proteasome degradation upon HPK1 (Hematopoietic Progenitor Kinase 1) phosphorylation and 14-3-3 binding [5, 6]. This novel model has been chosen as the entry point to address TCR signaling regulation. The stabilization of the 14-3-3/SLP76 PPI by small molecules could lead to a desired therapeutic effect in autoimmune diseases.

We have designed a short phosphorylated synthetic peptide, pS376, derived from the SLP76 sequence. With a combination of biophysical techniques and X-ray protein crystallography we have demonstrated that SLP76 is a genuine 14-3-3 binding partner. Then, to better mimic the 14-3-3/SLP76 interaction, a larger SLP76 construct, SLP76 SH2, was purified to develop an HTRF assay to screen for small molecule stabilizers. Potential hits were obtained from the UCB diversity deck. To validate these hits and assess their specificity, they were counter-screened against comparable but different PPI systems. Finally, the remaining hits were further validated by STD-NMR and SPR experiments. The more promising molecules will be used in structural studies with the goal of validating them as genuine stabilizers of the interaction between 14-3-3 and SLP-76.

Understanding and modulating PPIs in the p53 pathway

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Protein-Protein Interactions (PPIs) are of remarkable significance given their role in the vast majority of cellular processes.[1] p53 is known as “the guardian of the genome” due to its involvement in DNA repair, inhibition of cell proliferation and cell cycle regulation in general. The activation of p53 is triggered by various stress signals, inducing a negative-feedback loop that mediates levels of p53.[2] The structurally similar proteins, hDMX and hDM2 bind to a transcriptional domain of p53, inhibiting its function in cell cycle repair or apoptosis. p53 is additionally degraded by hDM2-mediated ubiquitination, which is amplified by formation of hDMX/hDM2 heterodimers.[3] Positive regulators of this cycle are dimeric hub 14-3-3 proteins and prior cellular studies have shown both hDMX[4] and hDM2[5] undergo phosphorylation, which is recognized by these phospho-binding 14-3-3 proteins. Stabilizing or inhibiting interactions of hDMX and hDM2 with 14-3-3 might promote increased or decreased levels of cellular p53 respectively. Firstly, to fully characterize these interactions, a peptide-based approach employing biophysical techniques (FA, ITC) was used to determine the binding affinity between hDMX/hDM2 and 14-3-3. In addition, X-ray crystallography was used to obtain structural information on the hDMX/14-3-3 and hDM2/14-3-3 interfaces. Finally, we sought to find a small molecule that would modulate this pathway by employing small-molecule and fragment screening along with a protein templated fragment ligation approach. This study provides better understanding of the role that 14-3-3 and hDMX/hDM2 play in the p53 pathway, shining light on a novel approach to modulate PPIs in the p53 pathway.

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Targeting Acute Myeloid Leukemia with Functionalized Biocompatible Nanoparticles

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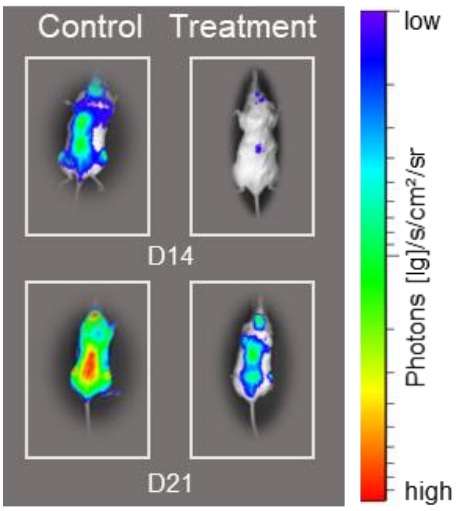
Acute myeloid leukemia (AML) is characterized by proliferative blast cells populating bone marrow, blood, and other tissues. The malignancy is associated with an unfavorable prognosis. Particularly, elderly patients suffer massively from the standard chemotherapy and have a 5-year survival of only 4%. Hence, new therapeutic agents with higher specificity and lower general cytotoxicity are urgently required.

We have discovered that amino-functionalized polystyrene nanoparticles (PS-NH2) inhibit mTOR in leukemia cells. Accordingly, PS-NH2 inhibit proliferation and induce G2 cell-cycle arrest in three myeloid leukemia cell lines. Besides, PS-NH2 trigger apoptosis in leukemia xenografts *in vivo*. At the molecular level, PS-NH2 also inhibit downstream targets of mTOR, such as Akt and p70 ribosomal S6 kinase 1 followed by overexpression of the cell-cycle regulator p21Cip1/Waf1 and degradation of cyclin B1. In leukemia cells, PS-NH2 elicit autophagy followed by activation of caspase 3 and subsequent induction of apoptosis. By contrast, upon exposure primary macrophages do not exhibit activated mTOR signalling and proved to be relatively resistant to the PS-NH2-induced toxicity. *In vivo*, PS-NH2 also exhibited cytotoxicity on THP-1 xenografts on the chorioallantoic membrane of chick eggs (CAM).

We created biocompatible gold nanoparticles, which were characterized by various physico-chemical methods. Similar to PS particles, amino-functionalized gold nanoparticles (Au-NH2) proved to be highly cytotoxic towards AML cell lines. Likewise, Au-NH2 induce cell death in primary human leukemia cells derived from various AML patients and reduce their colony-forming potential, whereas normal hematopoietic cells remain unaffected by the treatment with Au-NH2. In agreement with the in vitro data, Au-NH2 exhibited antileukemic efficacy against primary human AML xenografted into mice. Importantly, systemic Au-NH2 treatment was not associated with any detectable adverse effects in treated mice.



Thus, these engineered nanoparticles hold great promise as novel nanotherapeutics for treatment of acute myeloid leukemia independent of its cytogenetic profile.

PEX14-PEX5 interaction inhibitors as new drug strategy against Leishmaniasis

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Neglected Tropical Diseases (NTDs) comprise 17 infectious parasitic diseases affecting more than 1 billion people worldwide, mostly in the developing countries. Three of these diseases are caused by the protozoan parasites of the family trypanosomatidae *Trypanosoma* and *Leishmania*. Over 20 million people have been infected with one of these parasites, which lead to ~80,000 deaths per year. Currently used drugs suffer several drawbacks, which include severe toxicity and side effects, emergence of drug resistant strains and difficulty to administer. Hence there is urgent need for development of new affordable drugs and identification of novel drug targets.

Here we focused on the identification of *Leishmania* PEX14 inhibitors. Using structure-based drug design, we developed high-affinity small molecule inhibitors of the glycosomal matrix protein import by targeting PEX5-PEX14 interaction. We show that these inhibitors can inhibit *Leishmania* PEX14-PEX5 interaction using *in vitro* assays. The inhibitors disrupt glycosomal matrix protein import leading to mislocalisation of glycosomal enzymes to the cytosol. Unregulated glucose phosphorylation in the cytosol depletes cellular ATP, accumulates glucose metabolites to toxic levels and kills the parasites. Our data suggest that these inhibitors serve as leads for development of novel therapy against Leishmaniasis.

Fragment-based approach applied to the discovery of protein-protein interaction stabilisers

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Protein-protein interactions (PPIs) are constituents of numerous biological pathways and offer therapeutic intervention points into different pathologies such as cancer[1], inflammation[2], neurodegenerative[3] and metabolic diseases[4]. The Taros' fragment collection was selected for developing small molecules able to stabilize the complexes of 14-3-3 protein and its partners. Merging of natural compounds and known scaffolds from drug discovery campaigns inspired the new cores' design. This approach produced an interesting Nicotine-like fragments set (Figure 1) that showcases a new concept in fragment design named "SAR by Biocores". To date, the fragment collection offers approx. 1.230 fragments and presents ample opportunities for expansion. Differential scanning fluorimetry, X-ray crystallography and NMR-based techniques have been applied during the primary screening and led to the identification of novel hits binding to different 14-3-3 complexes. These novel binders represent an important starting point for future medicinal chemistry-based fragment evolution campaigns.

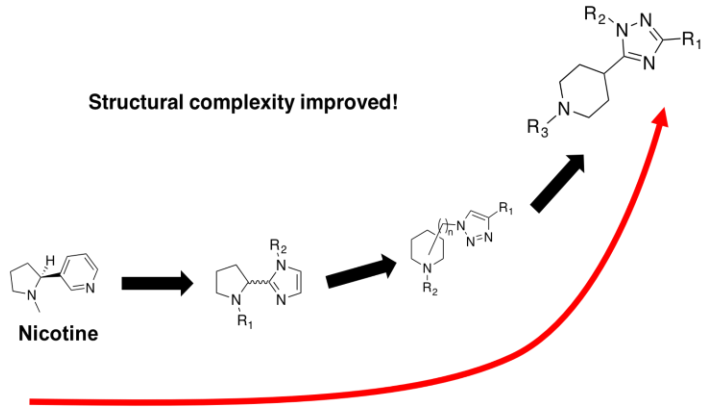

Figure 1. Nicotine-like fragment structural evolution.

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From target characterisation to novel inhibitor discovery – A biophysical way

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Enzymes are of highest interest as potential targets for novel drug discovery. Up to date enzyme inhibitors represent around half of all drugs that are currently available on the market. A good understanding of their roles within diseases as well as their mechanism of action is crucial for well-directed drug discovery.

With approximately two million fatalities each year tuberculosis is one of the top ten diseases causing death all over the globe. The increasing number of multiple drug-resistant and extensively drug-resistant cases urgently requires the development of new antibiotics against the tuberculosis-causing agent *Mycobacterium tuberculosis*.

Our work focuses on new inhibitor discovery against mycobacterial UMP kinase. Catalysing the reversible phosphorylation of UMP to UDP, it has a key role in the pyrimidine pathway and is essential for cell growth and survival. Not having a counterpart in human further makes it a promising anti-infective target.

To reach the overall aim of new inhibitor discovery we need to unravel the protein characteristics. To get detailed information on ligand affinities, ITC and SPR were the methods of choice. Furthermore, we solved the structure of the protein in complex with its natural negative effector UTP by X-ray crystallography and state-of-the-art cryo-EM. For hit identification we performed a screening campaign of a small-fragment library via thermal shift assay and further validated the hits by NMR.

A combination of these methods has provided more detailed insights into the allosteric regulation of the enzyme and to the identification of first fragment binders. This work forms the basis for future hit-to-lead processing.

“Open sesame”: fragments crack open a cryptic pocket on a challenging protein-protein interaction target

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Disulfide bond formation protein A (DsbA) is a thiol-disulfide oxidoreductase enzyme which catalyzes disulfide bond formation in the periplasm of Gram-negative bacteria. DsbA facilitates folding of multiple virulent factors that bacteria use for host cell manipulation, host colonization and spread. Bacteria lacking a functional DsbA display reduced virulence, increased sensitivity to antibiotics and diminished capacity to cause infection.[1] This protein is indispensable for the pathogenesis of critical human pathogens such as *Escherichia coli*, *Salmonella Typhimurium* and *Pseudomonas aeruginosa* that are becoming increasingly resistant to antibiotics.[2] The critical role of DsbA in bacterial virulence makes it an attractive drug target to combat multi-drug-resistant (MDR) bacteria.

DsbA comprises a classical CXXC redox active site (X=any amino acid) that catalyzes disulfide bond exchange. The active site is flanked by an extended hydrophobic groove, which binds to various unfolded substrates. Previously we carried out a fragment screening campaign against *Escherichia coli* DsbA (*Ec*DsbA) and identified the first inhibitors that bind to the hydrophobic groove and inhibit *Ec*DsbA activity *in vitro* and cell-based assays.[3,4] By exploiting an array of biophysical/biochemical tools (NMR, SPR, X-ray crystallography and *in vitro* assays), two chemical classes of inhibitors, phenythiazole and diaryl ether, were optimized in a structure-based approach in this work. 200+ analogues were synthesized and 100+ co-structures were solved by X-ray crystallography, but only limited affinity gain was obtained. This shows that the hydrophobic groove is highly difficult to target. More recently through structural, biochemical and NMR dynamics studies we postulated a cryptic pocket on *Ec*DsbA that could be exploited for inhibitor development. A second fragment screen campaign using smaller and more polar fragments allows the identification of binders for the predicated cryptic pocket. These fragment hits showed promising structure-activity relationship (SAR) and higher binding affinity. More importantly, binding of fragments to the cryptic pocket inhibit *Ec*DsbA oxidation activity both *in vitro* and in cell-based assay. This work shows that identification of cryptic sites on proteins can convert poorly druggable or undruggable targets into druggable targets, potentially expanding the druggable proteome.

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Development and Exploitation of Tools to Study Protein-Protein Interactions

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A key problem in life-sciences research is to understand protein-protein interactions (PPI) with molecular and temporal resolution. In this presentation we will describe our latest efforts to develop tools to analyse PPIs and map them using photo-activated peptide/protein labelling and cross-linking chemistry. We have developed a suite of 'tag and transfer' diazirine-based cross-linking reagents [1]. Diazirines are ideal cross-linking groups because upon excitation with UV light, they generate highly reactive carbenes capable of indiscriminate insertion into proximal bonds (Fig. 1a) [2]. Here we will exemplify the power of these new reagents for structural proteomics analyses. In the second part of the presentation, focusing on the helix mediated NOXA-B/MCL-1 PPI [3], we will describe efforts to develop peptides conjugated to ruthenium (II) (tris)chelates as reagents that can perform photo-activated traceless protein-labelling reactions [4] – a necessary first step in being able to label proteins in live cells without abrogating their normal function.

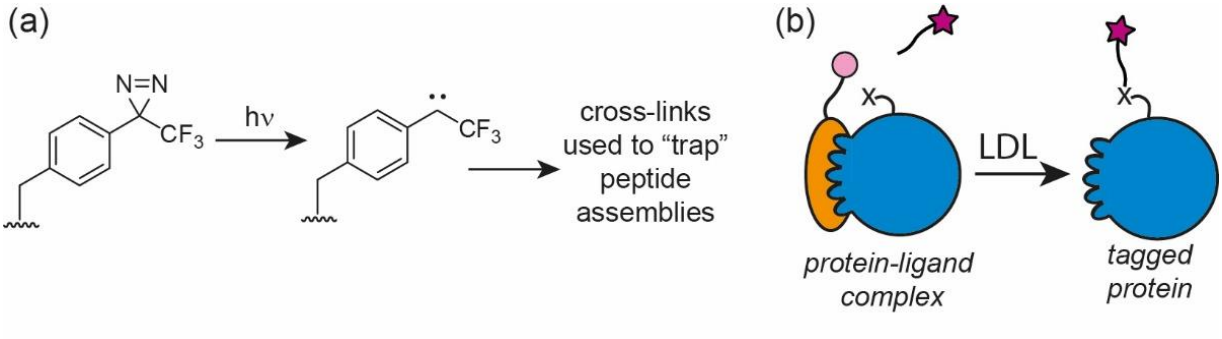

Figure 1: *Diazirines for PIC (a) UV activated carbine formation (b) schematic depicting the use of photo-activated probes to tag proteins.*

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Establishing the tRNA methyltransferase TRMT2a as a novel drug target for treatment of PolyQ diseases

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Huntington's disease (HD) belongs to the family of Polyglutamine (PolyQ) diseases. These neurodegenerative disorders share an expanded CAG repeat stretch in their coding region that is translated into expanded PolyQ tracts in the disease-linked protein. In the case of HD, the Huntingtin protein harbors the pathologically elongated polyQ tract. Presence of polyQ Huntingtin causes neuronal decline predominantly in basal ganglia. Loss of these neurons result in the cardinal symptoms of HD: jerky movements and cognitive decline. Until now, no widely applicable, approved HD therapy is available.

An RNAi screen in *Drosophila melanogaster* has shown that the inhibition of tRNA-methyltransferase homolog A (TRMT2a) reduces PolyQ-induced aggregate formation and toxicity in yeast, flies and HEK cells (Aaron Voigt's lab), establishing TRMT2a as a potential novel drug target.

TRMT2a is a tRNA methyltransferase that converts uridine to 5-methyl uridine at position 54 in tRNAs. The protein is predicted to contain an RNA recognition motif (RRM) and a catalytic domain (CD). In our lab we achieved to purify and crystallize the predicted RRM domain of TRMT2a. With this structure in hands an *in silico* drug screen was performed using molecular simulation and chemoinformatics (Giulia Rossetti's lab) resulting in a set of potential RRM-TRMT2a inhibitors. Using biophysical techniques such as ITC and SPR, binding properties of those inhibitors to the RRM-TRMT2a were tested. Functionally, the inhibitory effect of those compounds on the enzymatic activity of TRMT2a was dissected with a newly established methyltransferase assay.

Focused Library Generator: Case of Mdmx Inhibitors

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We present a Focused Library Generator that is able to draw from scratch new molecules with desired properties. The Generator was trained on the ChEMBL [1] database and simultaneously it was embedded with docking [2] and QSAR [3] IC_{50} rewards. Next, the transfer learning technique was used to switch the Generator on producing “new” Mdmx inhibitors that are a promising class of anticancer drugs. Thousands of drug-like compounds could be produced in this way and then followed by Lilly medicinal chemistry filters [4]. Besides, pharmacophore screening [5], and molecular dynamics (MD) simulations [6] were used to filter further the output molecules. Finally, we got several promising hits with equivalent or even better predicted binding free energies and IC_{50} values compared to known Mdmx inhibitor. The implementation of the Focused Library Generator is available on http://github.com/bigchem/online-chem.

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Fighting beta-lactamase activity using a combination of known and novel small molecule inhibitors

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In recent decades, antibiotic resistance has emerged as a significant risk to public health. Resistance to all known classes of antibiotics, including the drugs of last resort, the carbapenems, has now emerged. Carbapenems are members of the beta-lactam class, the most commonly and clinically relevant antibiotic class used in treatment of gram-negative and gram-positive bacterial infections and are now being rendered useless by beta-lactamases synthesized by bacteria.

Our work focuses on elucidating the structure and dynamics of the active site of beta-lactamases and discovering low molecular weight inhibitors which bind and inhibit the activity of the enzyme. We screened a diverse fragment library using NMR methods to find hits. Selected molecules were assayed to characterize inhibitory activity and then co-crystallized to elucidate fragment location, binding mode and to guide possible further development. For the first time, several clinically relevant antibiotics were also crystallized with IMP-13, giving us vital information on the availability of chemical space around the active site and its use in the context of developing fragment screen hits. Protein dynamics studies using NMR and molecular dynamics simulations show that metallo-beta-lactamase IMP-13 shows high L1 loop flexibility crucial for substrate recognition and processing. This is supported by crystal structures apo-state IMP-13, demonstrating different loop conformations, as well as in complex with hydrolyzed carbapenems.

Utilizing the variety of obtained results, we hope to develop potent new molecules targeting beta-lactamase activity, to aid in the fight against antibiotic resistance.

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