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Thursday, March 21, Invited lecture

L5

STRUCTURAL BIOLOGY AT THE DIFFRACTION LIMITED SYNCHROTRON SOURCE MAX IV

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The MAX IV Laboratory is a new synchrotron in Lund, Sweden which operates two storage rings and a short-pulse facility. The MAX IV 3 GeV storage ring is the first synchrotron ring with a multi-bend achromat design providing higher photon beam brilliance and coherence.

A number of beamlines interesting for Structural Biologists is in operation or under development at this facility. Amongst these beamlines are the protein crystallography beamline BioMAX and MicroMAX, the small angle scattering beamline CoSAXS and the X-ray absorption spectroscopy beamline Balder. There are also opportunities for using imaging techniques such as m-XRF and soft-Xray STXM. The beamlines will be presented with a slight emphasis on the protein diffraction beamlines.

Friday, March 22, Session II

L6

COMPARATIVE STRUCTURAL ANALYSIS OF LECTIN FAMILY FROM *PHOTORHABDUS SPP*

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Photorhabdus is a genus of gram-negative bioluminescent bacteria living in a symbiosis with *Heterorhabditis* nematodes forming a highly entomopathogenic complex. Such a complex is used in agriculture as a nature-based insecticide. However, some members of this genus are human pathogens as well. Understanding the mechanisms that determine interaction between *Photorhabdus* and its symbionts/hosts could be highly beneficial not only in biotechnologies but also in clinical reasearch and drug development.

Cell-cell interactions are frequently mediated by sugar-binding proteins – lectins. Based on the genome analysis, there has been identified potential lectins in number of *Photorhabdus* species. Recently, we examind two of them in further details, namely PLL from *P. laumondii* (formerly *P. luminescens*) [1] and PHL from *P. asymbiotica* [2, 3]. Both lectins share the basic structural features, e.g. -barrel fold with seven blades or presence of multiple binding sites per monomer. However, despite rather high sequence similarity, some non-marginal differences were detected: oligomeric state, binding site preferences and organization. This lead us to investigate this lectin family in further details.

We analyzed several homologues of proteins PLL and PHL from *Photorhabdus spp.* We managed to prepare some of them in recombinant form and perform basic analysis, as well as solve structure of these proteins in free form and in complexes with naturally occuring saccharide lig-

ands. This research may not only reveal the differences in similar proteins from one family, but also answer the question, why single species of bacteria possess the ability to produce several similar lectins.

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1. Kumar, A. *et al* (2016) JBC, doi: 10.1074/jbc.M115.693473.
2. Jancarikova, G. *et al* (2017) PlosPathogen, doi:10.1371/journal.ppat.1006564.
3. Jancarikova, G. *et al* (2018) Chem Eur J, doi:10.1002/chem.201705853.

L7

STRUCTURAL BASIS OF HISTONE DEACETYLASE 8 (HDAC8) SELECTIVE INHIBITION

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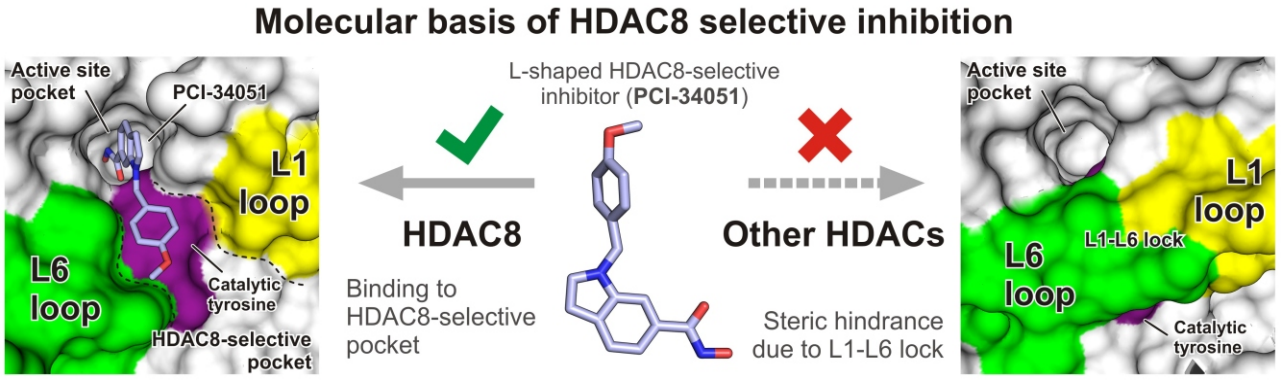
Metal-dependent histone deacetylases (HDACs) are key epigenetic regulators that represent promising therapeutic targets for the treatment of numerous human diseases. Yet, currently FDA-approved HDAC inhibitors non-specifically target most of the eleven structurally similar but functionally different HDAC isozymes, which hampers their broad usage in clinical settings. Selective inhibitors targeting single HDAC isozymes are being developed, for example HDAC8-selective inhibitors PCI-34051 and NCC-149, but precise understanding in molecular terms of their selectivity remains sparse. Here, combination of biochemical, biophysical and structural analyzes of HDAC8 inhibition reveals how selective inhibition is achieved. Our numerous crystallographic structures of HDAC8-inhibitor complexes established with a lattice-free environment at the active site provide unprecedented structural information on the binding of selective inhibitors to HDAC8. Notably, we show that HDAC8-selective inhibitors adopt a characteristic L-shaped conformation, which is required for their binding to a HDAC8-specific pocket formed by HDAC8 active site catalytic tyrosine and HDAC8 L1 and L6 loops. In other HDAC isozymes, presence of a L1-L6 lock sterically prevents L-shaped inhibitor binding. Importantly, shielding of the HDAC8-specific pocket by protein engineering markedly decreases potency of HDAC8-selective inhibitors but also affects catalytic activity. Collectively, our results unravel key HDAC8 active site structural and functional determinants important for catalysis and selective inhibition, which sheds new light on rational design of next-generation chemical probes and epigenetic drugs.

Marek M., Shaik T. B., Heimburg T., Chakrabarti A., Lancelot J., Ramos Morales E., Da Veiga C., Kalinin D., Melesina J., Schmidkunz K., Suzuki T., Holl R., Ennifar E., Pierce R. J., Jung M., Sippl W., and Romier C. (2018). Characterization of histone deacetylase 8 (HDAC8) selective inhibition reveals key active site structural and functional determinants. Journal of Medicinal Chemistry 61(22):10000-10016.



L8

NEW ANTIBODY-BASED TOOLS FOR PROSTATE CANCER IMAGING AND THERAPY

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Prostate carcinoma (PCa) is by far the most common non-cutaneous malignancy in men in countries with high standard of living. Therefore, huge efforts are invested into the search of highly specific and selective markers of PCa. Prostate-specific membrane antigen (PSMA) is a prominent PCa marker due to the established correlation between cancer progression and PSMA expression levels. Among PSMA-specific reagents developed for diagnostics and therapy antibodies are of high interest due to high affinity and selectivity for PSMA. Compared to small molecule PSMA-specific inhibitors, the use of antibodies can result in significantly lower toxicity in non-target tissues such as salivary glands. Furthermore, protein engineering techniques enable generation of small recombinant derivatives or humanized variants of antibodies favorable for *in vivo* applications.

We have recently isolated and characterized in detail two new PSMA-specific antibodies - 5D3 and 5B1, with high affinity and selectivity for human PSMA. Using cancer cell lines and a mouse PCa xenograft model we have shown that both molecules are suitable for *in vitro* and *in vivo* applications and reveal approximately 10-times higher affinity for PSMA compared to the leading second-generation antibody J591. Our results confirm the antibodies to be promising candidates for the development of new PSMA-specific diagnostic and therapeutic tools. We currently produced and characterized Fab and single chain fragments derived from antibody 5D3. Analysis *in vitro* revealed that purified recombinant fragments retain functional binding site of the original 5D3 antibody. Moreover, *in vivo* imaging experiments run in mouse model of grafted PCa confirm that produced molecules are suitable for *in vivo* applications. To uncover interaction of PSMA with 5D3 antibody in detail, structural features of PSMA-5D3 complex were mapped by H/D exchange. Based on data from H/D exchange putative binding epitope was mapped to the PSMA surface. To describe interaction between PSMA and 5D3 antibody in more detail, we are undertaking structural studies of PSMA complexes with 5D3 fragments.

L9

DESIGN AND BIOLOGICAL AND STRUCTURAL CHARACTERIZATION OF NOVEL HDAC6-SPECIFIC INHIBITORS

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Histone deacetylases also known as lysine deacetylases (HDACs or KDACs) catalyze the hydrolysis of acyl groups from -N-acetyl lysine of protein substrates. Histone deacetylase 6 (HDAC6) is a unique member of the HDAC family due to its complex domain organization and cytosolic localization. HDAC6 exerts its enzymatic activity on many non-histone substrates such as tubulin, heat shock protein 90 (Hsp90), survivin, and cortactin. It has been shown that inhibition of HDAC6 enzymatic activity can be used potentially for the therapy of several neurological conditions including peripheral neuropathies, Alzheimer`s disease, and Parkinson's disease. Unfortunately, however, currently no truly HDAC6-specific compounds exist to meet clinical needs. We have implemented in our laboratory an innovative screening cascade comprising in vitro isotype specificity testing and in vitro/in vivo toxicity and tubulin biomarker assays. Assisted by our X-ray crystallography data we designed and optimized

novel HDAC6 inhibitors bearing an alternative non-hydroxamate zinc-binding group. Such compounds have nanomolar potency and exquisite 10,000-fold selectivity for HDAC6 over other HDAC isoforms and can serve as either research tools or leads for the development of compounds used in the clinical practice.

L10

CRYSTAL GROWTH TO DATA COLLECTION MADE EASY

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Growth of well-diffracting crystals is a major bottleneck in protein crystallography. The *XP Screen* solves this problem with the Anderson-Evans polyoxotungstate $[TeW_6O_{24}]^{6-}$ (TEW) - a universal and flexible additive for protein crystallization (Figure 1a). TEW has both planar structure and high negative charge, allowing it to act as a linker between protein molecules and ultimately stabilize fragile crystal lattices [1].

Once optimal growth conditions have been established, crystals must be harvested. Harvesting stresses the protein crystals and can lead to impairment and even complete loss of diffraction quality. To solve this problem, crystals can now be grown directly on the *XtalTool* (Figure 1b). This patent-pending sample holder allows trouble-free crystal growth, any necessary crystal soaking and X-ray data collection without the need of crystal manipulation or mounting.

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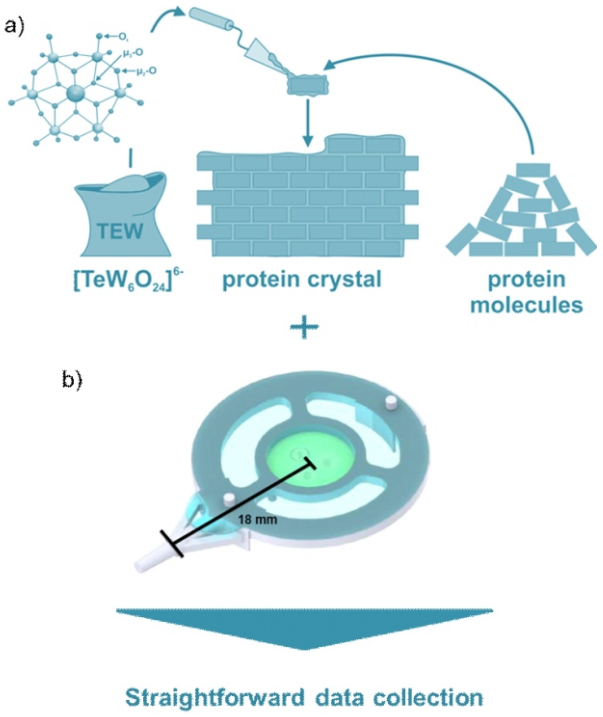

Figure 1. a) *XP Screen* is based on TEW – molecular "glue" which holds together delicate protein crystals. Figure adapted from [1], used courtesy of Prof. Annette Rompel, University of Vienna, Austria. b) The *XtalTool* is used as both cover slide and sample holder for *in-situ* data collection.