MS05-O4

High-Throughput crystallographic fragment screening for drug discovery

Alexander Metz[1], Franziska Huschmann[2], Johannes Schiebel[1], Uwe Müller[2], Manfred Weiss[2], Andreas heine[1], Gerhard Klebe[1]

1. Institute of Pharmaceutical Chemistry, Philipps-University Marburg, Marburg, Germany
2. Helmholtz-Zentrum Berlin, Institute Soft Matter and Functional Materials, Macromolecular Crystallography (HZB-MX), Electron Storage Ring BESSY II, Berlin, Germany

email: alexander.metz@pharmazie.uni-marburg.de

The key idea of fragment screening is that already a small selection of appropriate fragments (~10^3 cpds.) covers a much larger proportion of the overall chemical fragment space (~10^7 cpds.) than a typical high-throughput screening collection (10^5 – 10^6 cpds.) with respect to the drug-sized chemical space (~10^{63} cpds., *MW* < 500 Da). Moreover, in contrast to larger molecules, fragments may bypass strict steric requirements for binding, leading to high hit rates up to 20%. For the same reason fragments often find well-suited anchor positions leading to low-affinity yet highly efficient binding and making them excellent starting points for subsequent ligand design, with the inherent potential to reconstruct the larger lead- or drug-sized chemical space.

Modern semi-automated beamlines are well suited for crystallographic screening of complete fragment libraries or diverse subsets at no higher effort than most pre-screening assays.[1] In conjunction with adequately designed fragment libraries, automated data processing strategies, and optimized crystallographic methodology, this strategy routinely yields large numbers of fragment-bound structures revealing otherwise unanticipated chemotypes and interaction patterns ready to use for structure-based drug design.[2,3]

We present our fragment-screening pipeline at the BESSY synchrotron as well as results from screening the same fragment library against more than 8 diverse proteins. In addition, we present a screen of natural compound-derived fragment structures and results from following up on these with readily available fragment-superstructures suited to fit the remaining parts of the pocket. Finally, we present computational tools to elaborate and evaluate fragment derivatives, e.g. by fragment structure-based docking, also in conjunction with reaction driven de-novo design of easily accessible fragment derivatives.

The presented libraries and methods are part of the Frag2Xtal and Frag4Lead service facility for crystallographic fragment screening soon available at the semi-automated crystallographic BL14.2 at the BESSY II storage ring of the Helmholtz-Zentrum Berlin.

References:

[1] Schiebel, J. et al. (2016), ACS Chem. Biol., 11, 1693-1701.
[2] Schiebel, J. et al. (2016), Structure, 24, 1398-1409.
[3] Radeva, N. et al (2016), J. Med. Chem, 59, 9743-9759.

Keywords: Fragments, Structure-Based Drug Discovery, Computer-Aided Drug Discovery

MS05-O5

Structure-Based design of inhibitors targeting PrfA, the master virulence regulator in *listeria monocytogenes*

Elisabeth Sauer-Eriksson[1], Martina Kulén[1], Marie Lindgren[2], Christin Grundström[1], Uwe H. Sauer[1], Jörgen Johansson[2], Fredrik Almqvist[1]

1. Chemistry, Umea, Sweden
2. Molecular Biology, Umea, Sweden

email: elisabeth.sauer-eriksson@umu.se

New strategies to combat bacterial infections are essential to counteract increasing antibiotic resistance world-wide. One such alternative strategy is to target and inhibit a pathogen's virulence machinery. *Listeria monocytogenes* is a food-borne Gram-positive bacterial pathogen involved in major outbreaks every year, causing listeriosis especially among pregnant women, the immunocompromised, and other at-risk individuals. The intracellular lifecycle of *L. monocytogenes* is well studied, making it an excellent model species for the targeting of specific virulence pathways. One of the major virulence regulators is the transcriptional regulator PrfA (Positive regulatory factor A), a member of the Crp/Fnr family of regulators that bind DNA through the helix-turn-helix motif. Published data suggest that PrfA requires the binding of a co-factor, glutathione GSH, for full activity, and from the crystal structures of PrfA in complex with GSH, and in complex with GSH and its cognate DNA, the *hly* operator PrfA-box motif we revealed the structural basis for a GSH-mediated allosteric mode of activation of PrfA in the cytosol of the host cell (1). Furthermore we describe structure-guided design and synthesis of a set of PrfA inhibitors based on ring-fused 2-pyridone heterocycles (2,3). Our most effective compound decreased virulence factor expression, reduced bacterial uptake into eukaryotic cells, and improved survival of chicken embryos infected with *L. monocytogenes* compared to previously identified compounds. Crystal structures identified an intra-protein "tunnel" as the main inhibitor binding site (A_I), where the compounds participate in an extensive hydrophobic network that restricts the protein's ability to form functional DNA-binding HTH motifs. Our studies also revealed a hitherto unsuspected structural plasticity of the HTH motif. In conclusion, we have designed 2-pyridone analogues which function as site-A_I selective PrfA inhibitors with potent anti-virulence properties.

References:

[1] Hall M, Grundström C, Begum A, Lindberg MJ, Sauer UH, Almqvist F, Johansson J, Sauer-Eriksson AE. (2016) Structural basis for glutathione-mediated activation of the virulence regulatory protein PrfA in Listeria. Proc Natl Acad Sci U S A. 113(51):14733-14738.

[2] Good JA, Andersson C, Hansen S, Wall J, Krishnan KS, Begum A, Grundström C, Niemiec MS, Vaitkevicius K, Chorell E, Wittung-Stafshede P, Sauer UH, Sauer-Eriksson AE, Almqvist F, Johansson J. (2016) Attenuating Listeria monocytogenes Virulence by Targeting the Regulatory Protein PrfA.
Cell Chem Biol. 23(3):404-14.
[3] Kulén M, Lindgren M, Hansen S, Cairns AG, Grundström C, Begum A, van der Lingen I, Brännström K, Hall M, Sauer UH, Johansson J, Sauer-Eriksson AE, Almqvist F. (2018) Structure-Based Design of Inhibitors Targeting PrfA, the Master Virulence Regulator of Listeria monocytogenes. J Med Chem, in press

Keywords: Antibiotic resistance, drug design, PrfA, structure-activity relationship

MS06 molecular machines and big complexes

Chairs: Prof. Guillermo Montoya, Prof. Kristina Djinovic Carugo

MS06-O1

Unveiling (class III) transcription through integrative structural biology

Alessandro Vannini[1]

1. Division of Structural Biology, The Institute of Cancer Research, London, United Kingdom

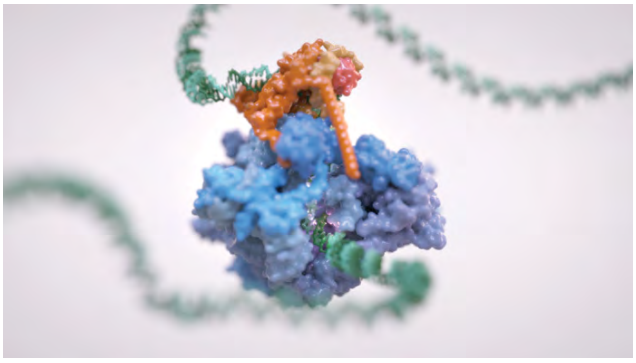
email: alessandro.vannini@icr.ac.uk

RNA Polymerase (Pol) III is the eukaryotic nuclear enzyme devoted to the transcription of essential non-coding RNAs, including the entire pool of tRNAs, the 5S ribosomal RNA and the U6 spliceosomal RNA. Yeast Pol III comprises 17 subunit and accounts for approximately 750 kDa in mass.

Initiation of gene transcription by RNA Pol III requires the activity of TFIIIB, a complex formed by Brf1, TBP and Bdp1. TFIIIB is required for recruitment of Pol III and to promote the transition from a closed to an open Pol III pre-initiation complex (PIC), a process stimulated by the activity of the Bdp1 subunit. Here we present two cryo-EM reconstruction of an open RNA Pol III PIC at 3.8Å and 3.3 Å, and a reconstruction of RNA Pol III at 3.0 Å.

The structures presented unravel the molecular mechanisms underlying RNA Pol III transcription initiation, highlighting the specific features of this highly efficient essential machinery but also the general conserved mechanisms of gene transcription initiation.

We also present the crystal structures of a vertebrate specific TFIIIB complex, containing the Brf2 subunit, a protein overexpressed in lung and breast cancers. Brf2 encompasses a redox-sensing switch, capable of turning on and off transcription of target genes in a redox dependent manner. Integrating structural and biochemical and functional data in living cells we discovered Brf2 to act as a master switch of the oxidative stress response and establish a mechanistic link between Brf2-dependent Pol III transcription and cancer.



Keywords: RNA Polymerase III, x-ray crystallography, cryo-EM