MS05-O4

High-Throughput crystallographic fragment screening for drug discovery

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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:

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MS05-O5

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

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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.

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