From genes to lead molecules: a workflow tuned for drugging challenging therapeutic targets using fragment-based drug discovery

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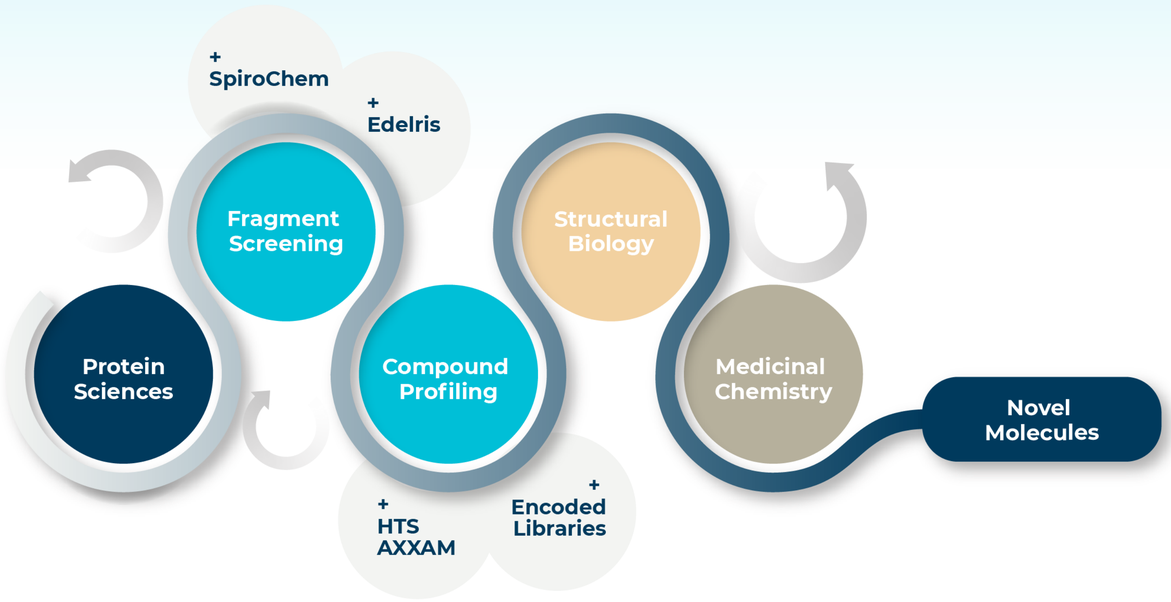
FBDD at ZOBIO

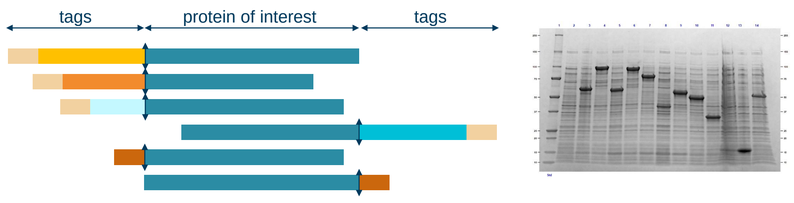


There are many novel popular drug targets, originating from several target families with hardly any literature available. Facing the lack of information and the diversity of the targets, robust and reproducible production of high-quality protein is a critical first step in enabling drug discovery.
At ZoBio, we have developed a platform of biophysical technologies to support fragment-based drug discovery (FBDD), where we screen our library of small (< 300 Da) molecules against the target.
Our workflow encompasses all steps of an FBDD campaign: primary hit identification using our proprietary NMR-based TINS and SPR fragment screening technologies, confirmation and validation of the binding site using both NMR and X-ray crystallography, and medicinal chemistry.

Construct design & protein expression

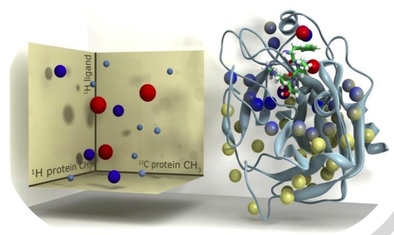
Our workflow starts with multipurpose construct design for protein expression in *E. coli* or mammalian cells. We are using literature information and bioinformatics to decide on domain boundaries and build a set of fusion constructs with solubility- and affinity tags. Keeping downstream applications in mind, we include immobilization tags and protease sites to release the native protein/domain at the end.

Sampling constructs and expression conditions



We screen our constructs for soluble overexpression in *E. coli* or mammalian cells. For this we employ different strains, culture media, temperatures and duration.

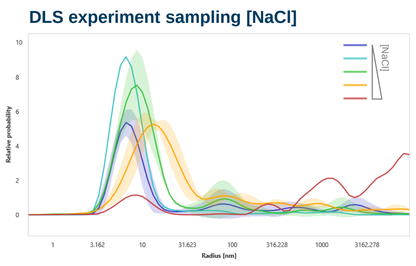
To enable protein-ligand interaction studies and structure determination by NMR, we routinely prepare isotopically labeled proteins. To overcome the size limitations of NMR and to reduce the complexity of NMR spectra, we routinely use triple labeled (^{15}N, ^{13}C, ^{2}H) protein together with selective methyl labeling.



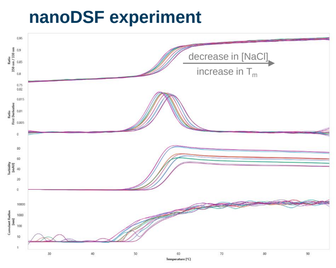
Protein purification & buffer optimization

To develop robust purification protocols, we use state-of-the-art chromatography systems. Starting with several constructs in parallel in our feasibility phase, we quickly determine which constructs to scale up to enable screening and structural biology.

DLS experiment sampling [NaCl]

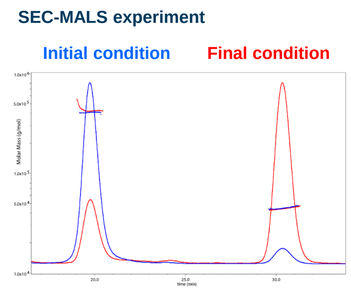


nanoDSF experiment

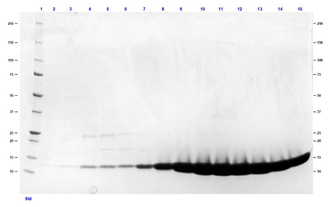


Choosing the right conditions can have a dramatic effect on the NMR spectra. Precisely controlling the oligomeric state at higher concentrations is the most critical parameter.

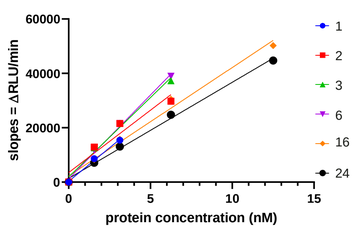
SEC-MALS experiment



SDS-PAGE gel

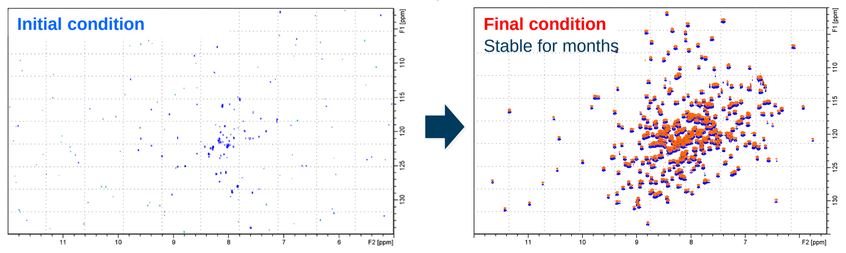


Kinase activity assay



We rigorously check protein activity and have implemented iterative cycles of protein stability and oligomeric state assessment by nanoDSF/DLS, SEC-MALS and NMR. As a result, we are sure that we work with the right conditions for each protein and record meaningful data.

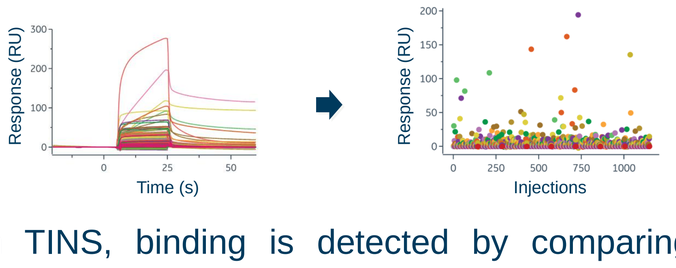
NMR spectra



Screening & structural biology

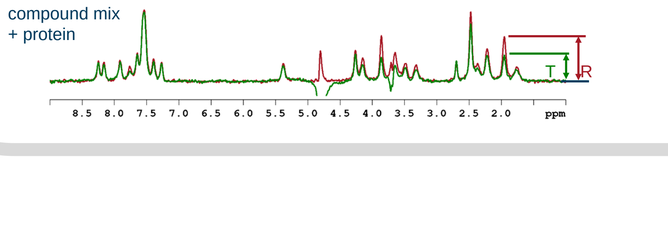
We screen our library of fragments against the target using surface plasmon resonance (SPR) or target immobilized NMR screening[1] (TINS).

SPR fragment screen



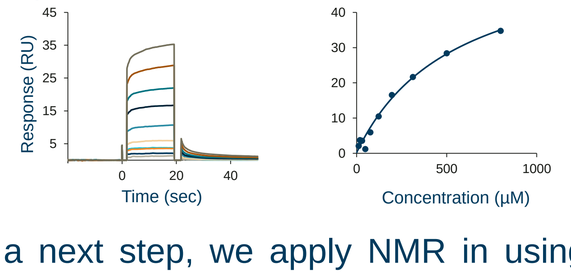
In TINS, binding is detected by comparing spectra of compound mixtures in the presence of a target (T) to a reference sample (R).

TINS traces

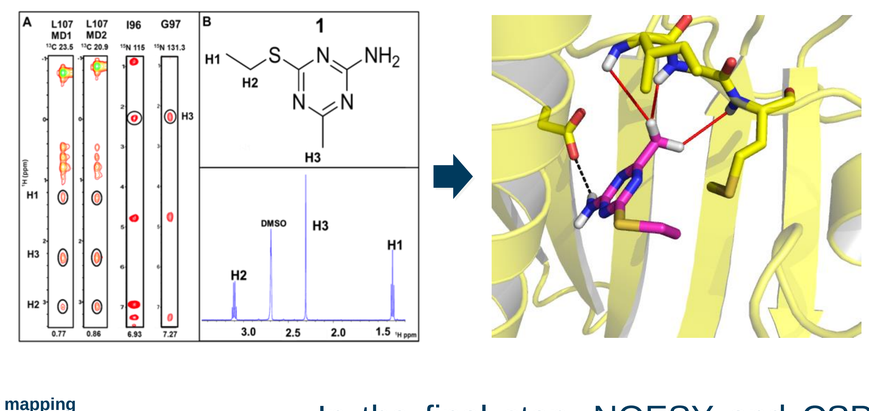


After the initial screen, potential hits are validated in orthogonal assays and their affinity is evaluated.

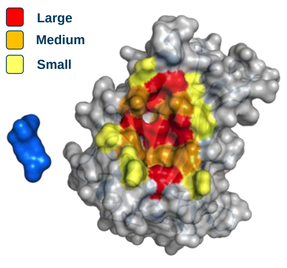
SPR compound titration



In a next step, we apply NMR in using semi-quantitative chemical shift perturbation (CSP) data to determine the binding site of the ligand in solution; and later to filter the calculated structures. The CSP study also serves to find the optimal ligand-to-protein ratio and to check for a 1:1 binding model.



CSP mapping



In the final step, NOESY and CSP data are used as input for the program HADDOCK[2] to calculate, cluster and score a set of high-resolution ligand-protein structures[3].

1. Vanwetswinkel, *et al.*, Chem. Biol., (2005) 12:207-216
2. De Vries, *et al.*, Nature Protoc. (2010) 5:883
3. Shah, *et al.*, J. Med. Chem. (2012) 55:10786

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