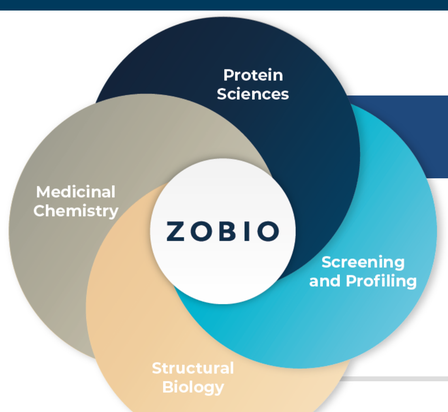
Tailor-made Structural Biology at ZoBio

Finding the right molecule for your target

Inês Justo, Marc Morgan, Wolfgang Kölmel, Misbha Ud Din Ahmad, Nicola Pontillo, Charis Georgiou, Eiso AB, Johan Hollander, Stephan Theisgen, Balasubramanian Harish, Marta Carneiro, Gregg Siegal



ZoBio's Drug Discovery Platform

From gene to structure and beyond. ZoBio offers a powerful, integrated pipeline including: protein engineering/production, a diverse fragment collection for hit discovery, NMR and SPR assays for fragment screening/compound characterization, all of which are supported by **a platform optimized for Structure Based Drug Design**. Uniquely, our structural biology capabilities include **X-ray crystallography** as the primary driver, complemented by **NMR** and **Cryo-EM**. This approach makes for a far more robust discovery engine and **increases the chance of finding the right molecule for your target.**

X-ray Crystallography and Hit Discovery

Protein Engineering and Production

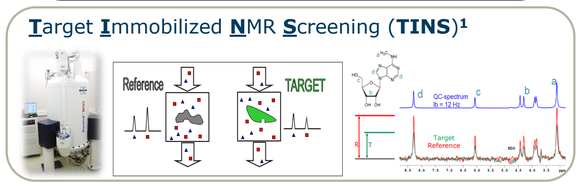
Construct design, cloning, expression (*E.coli* and HEK cell), purification, biochemical and biophysical characterization

Fragment Library

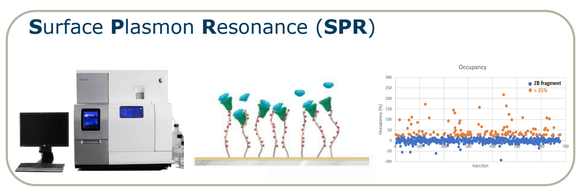
Proprietary library of ~2000 fragments or external libraries

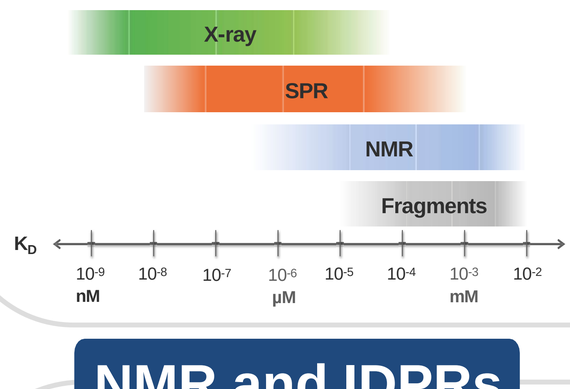
Hit Discovery and Characterization

Target **I**mmobilized **N**MR **S**creening (**TINS**)[1]



Surface **P**lasmon **R**esonance (**SPR**)





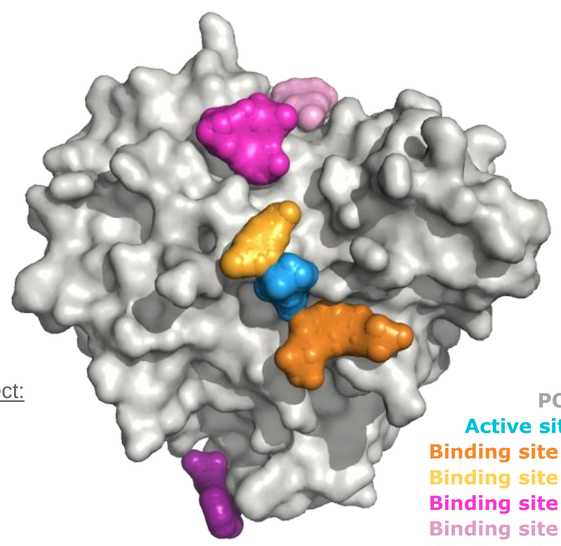
Hits

Fragments, covalents, DELs, HTS…

Tailored approaches for each project:

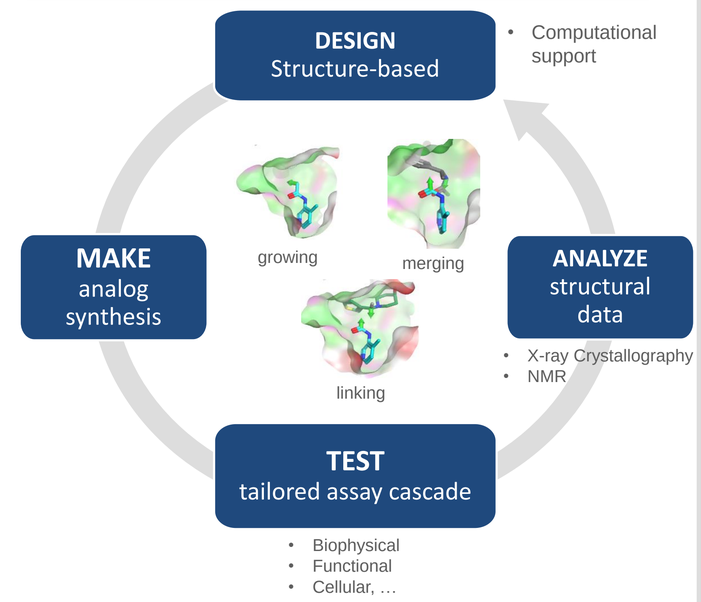
- Alternative co-solvents
- Hybrid co-crystallization and soaking schemes
- Heavy atom/halogen analogues

Hit Validation



1 - 5 chemotypes

Hit Evolution



Fully integrated high throughput protein crystallography platform

Using industry standard high-throughput instrumentation extended through in-house developments/customizations, we develop crystallography systems for unprecedented and previously described targets. Once established, the platform can support a **throughput of 40-70 compounds/month/project**, by soaking or co-crystallization. Synchrotron diffraction data is processed and analyzed using Pan Dataset Density Analysis (PanDDA) for the identification of ligand binding events.[2] **Our entire pipeline is highly customizable** allowing us to easily meet our client's needs and target's demands.

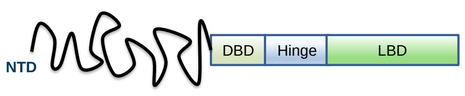
NMR and IDPRs

Work executed for Essa Pharma

NMR uncovers protein-ligand interactions in Intrinsically Disordered Protein Regions (IDPRs) at atomic resolution

Target: Androgen Receptor N-terminal domain (AR-NTD)

Background: Essa Pharma developed an AR inhibitor (**EPI-7386**, clinical candidate) with *in vivo* activity against LBD-deleted AR variants. **EPI-7386** was expected to bind to the NTD, but direct binding evidence was lacking.



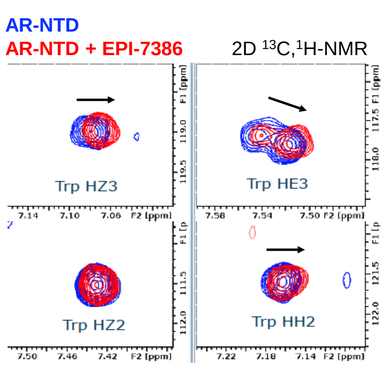
Androgen Receptor (AR)

Goals: Demonstrate direct interaction of **EPI-7386** with the NTD of the AR.

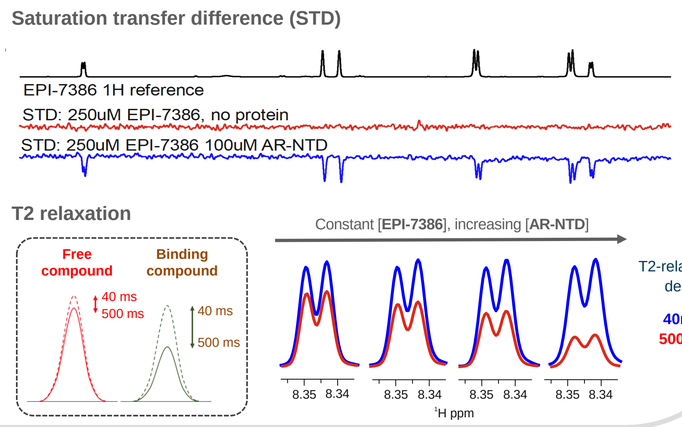
Methodology: Ligand-Observed NMR (LO-NMR) and Protein-Observed NMR (PO-NMR).

Results: Interaction of **EPI-7386** with NTD-AR demonstrated by both ligand-observed experiments (T2-CPMG and STD-NMR) and protein-observed experiments. The latter further shows interaction of **EPI-7386** with two Tryptophan residues in the AR-NTD.

PO-NMR



LO-NMR



NMR and Covalents

Work executed for Sanofi

NMR is an information-rich technique that can deliver insights into covalent complexes at various levels of detail and timescales compatible with the drug development cycle.

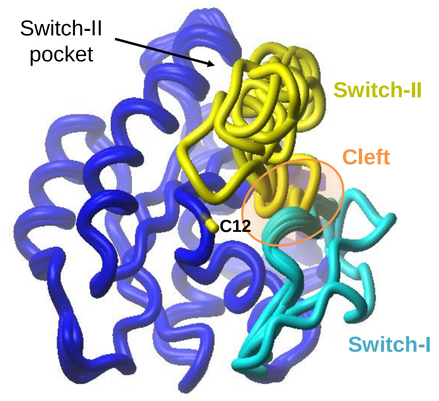
Target: KRAS G12C

Background: Sanofi published crystal structures for 2 compounds (**cdp2** and **cdp3**) that bind to a new site, a cleft between switch-II and switch-I.[3] The dynamic Switch-II and Switch-I regions are sensitive to both compound binding and crystal packing.

Goals: Characterize ligand pose and switch-I/II conformation in solution and compare to the ones observed in crystal structures.

Methodology: Structure determination by NMR; comparison of solution and crystal structures based on NOE violations and observed vs predicted chemical shifts.

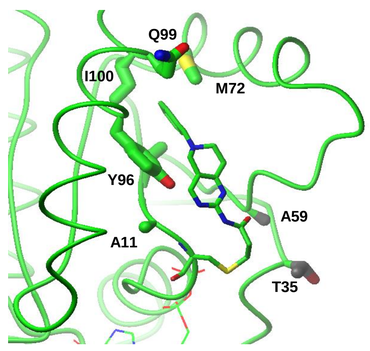
Results: **Structures solved by NMR** showing both compounds have multiple binding modes in fast exchange, some of which is consistent with the crystallography structures.



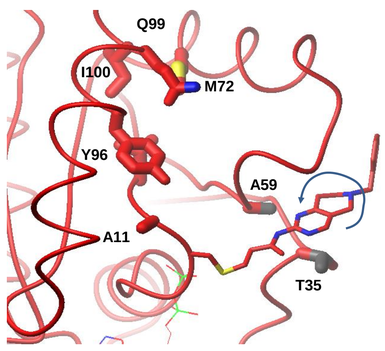
KRAS G12C, Switch-I and Switch-II regions

Most C12-conjugated compounds bind in the Switch-II pocket

NMR structure



Crystal structure



Structures of KRAS (G12C) – cpd3, NMR vs X-ray Crystallography

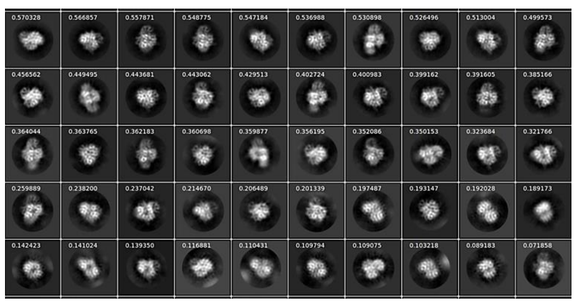
NMR studies showed that in solution **Cpd3** is mainly bound in the switch-II pocket but has a minor binding mode in the cleft (NOEs for A59 and T35 consistent with the crystal structure). We were also able to determine that **Cpd2** is bound in the cleft, but exchanges between 2 orientations, one similar to the orientation in the crystal structure (not shown).

Cryo-EM for Large Protein Assemblies

Cryo-EM supplements our structural biology capabilities for protein assemblies too large for NMR and not amenable to X-ray Crystallography.

ZoBio has rapid access to state-of-the-art Cryo-EM facilities via a strategic relationship with the Netherlands Centre for Electron Nanoscopy (NeCEN).[4]

Our Protein Sciences team readily develops customized workflows to express and purify large protein complexes for Cryo-EM. For example, we recently determined the 3D structure of a new E3-ligase complex to a resolution of ~3.5Å to assist our client's drug discovery goals.



Particle extraction and 2D classification

References

[1] Siegal *et al*, Drug Disc.Today, 2007 (12), 1032; 2

[2] Pearce *et al*. Nat Commun, 2017 (8), 15123

[3] Mathieu *et al*, Small GTPases 2022 Jan;13(1):225-238

[4] *https://www.necen.nl/*

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