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Use cases of SPR in Membrane Protein Analysis

Protein Production, Binding Assays, MOA Analysis, and Ligand Screening

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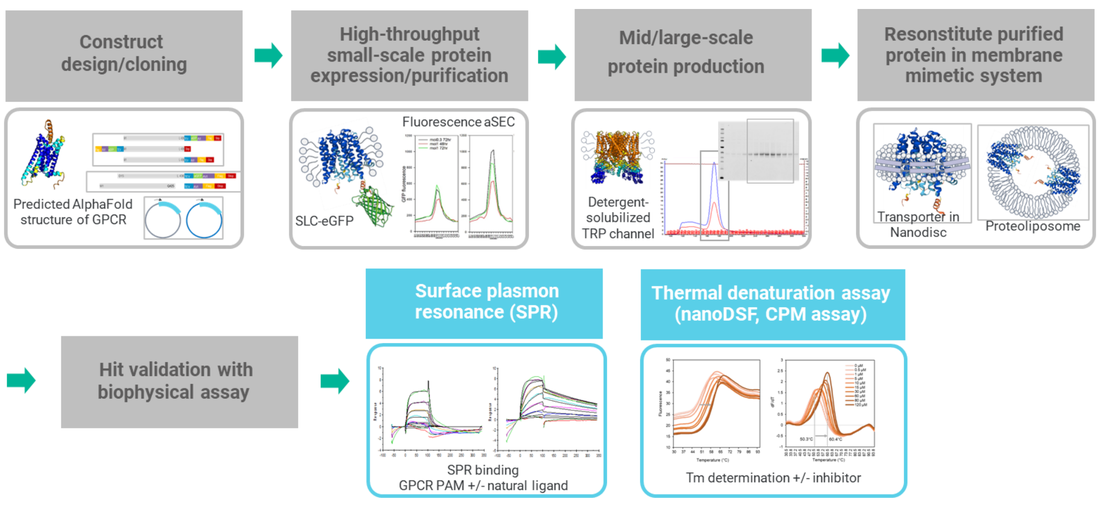
Summary

Our membrane protein production platform is capable of producing membrane proteins, including **ion channels**, enzymes, **GPCRs** and **transporters** for assays and structural biology, which routinely involve incorporation into membrane mimetic systems (e.g., **nanodiscs**). These quality-controlled proteins are then employed in conjunction with diverse technologies like affinity-selection mass spectrometry (AS-MS), thermal shift assays, and surface plasmon resonance (SPR), or for in-depth **structure-affinity studies**, where we utilize cryo-electron microscopy (**cryo-EM**) and lipidic cubic phase (**LCP**) crystallography.

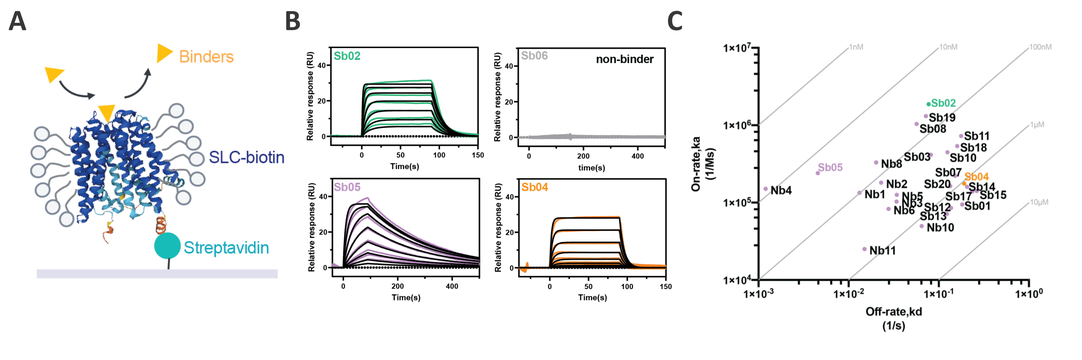
Surface-plasmon resonance (SPR) is used to validate protein folding, protein functionality, **ligand binding,** and protein-protein interactions of the produced membrane proteins.

For screening, **SPR** can be combined with AS-MS and thermal shift assays to facilitate **hit identification** and **validation**.

Membrane-protein production and biophysics



Case study biophysics – high-throughput SPR binder screening to SLC transporter



Case study: High-throughput SPR screening of sybody binding to a solute carrier (SLC) transporter

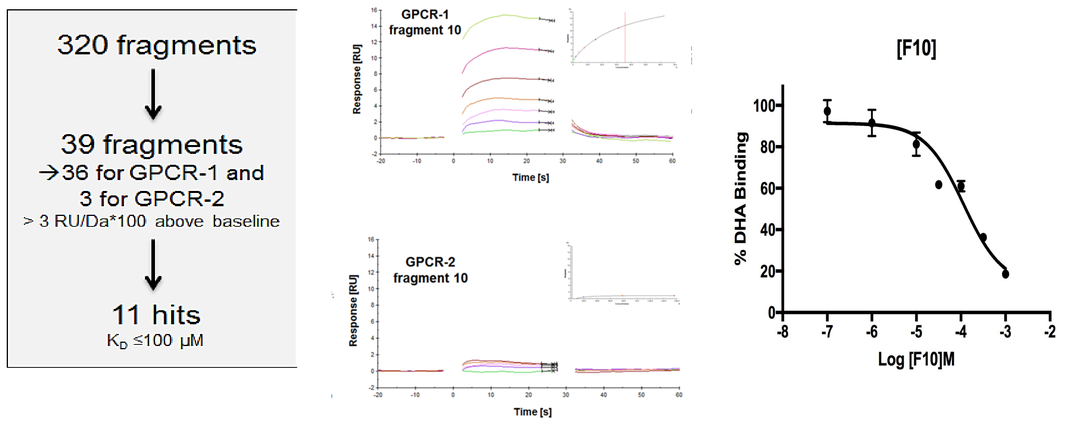
A Sybodies and **nanobodies** were purified from high-throughput small-scale purification pipeline (>90% purity), and subsequently analyzed against immobilized biotinylated SLC transporters on SPR in concentration range of 0 to 3-10 µM.

B Selected representative SPR sensorgrams of binders with different **binding kinetics** and a non-binder.

C Binding kinetics of all binders analyzed with on/off rate plot.

Case study SPR – fragment screen with membrane proteins

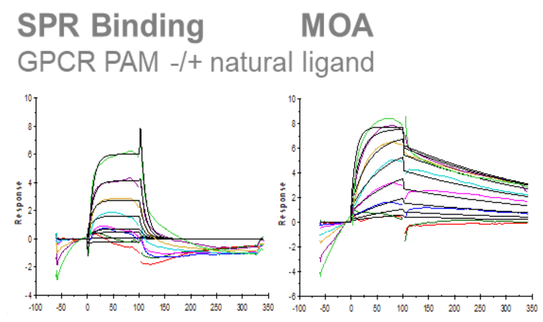
Screening of a 320 compounds from a fragment library against a class A GPCR yielded in hits with a binding affinity below 100µM.



The identified hit fragment F10 also shows activity in the cellular radioligand binding assay.

Case study SPR – for mode of action investigations on GPCRs

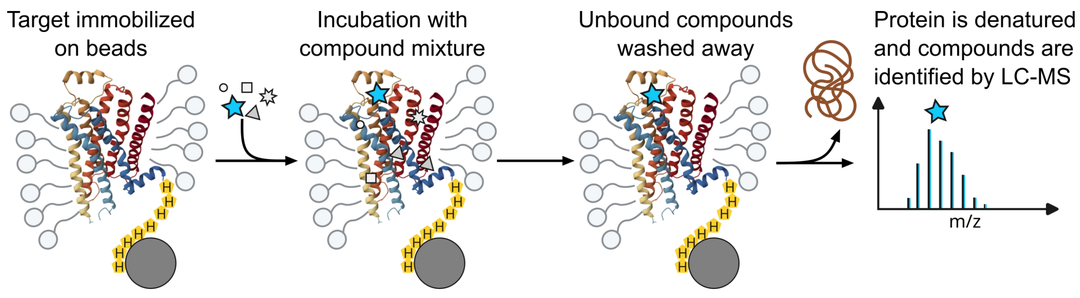
SPR can be used for mode of action (competition, cooperativity) determination of GPCRs.



The figure shows how the presence and absence of the natural ligand influences the binding kinetic of the PAM.

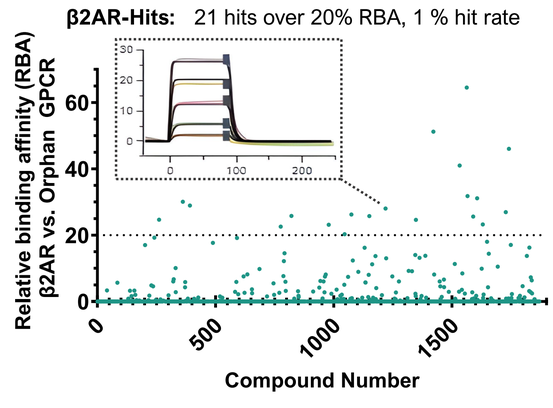
Case study AS-MS – SPR for hit validation of AS-MS fragment screen with GPCRs

AS-MS is a high-throughput screening method to identify or validate binders



Case study fragment screen **orphan GPCR** vs. **β-2AR**:

- Identification of unique hits for both an orphan GPCR and β-2AR.
- Hit validation using SPR
- Hits for both GPCRs are reproducible



References:

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[2] Heyder, Nicolas A., et al. "Structures of active melanocortin-4 receptor–Gs-protein complexes with NDP-α-MSH and setmelanotide." *Cell Research* 31.11 (2021): 1176-1189.
[3] Gelová, Zuzana., et al. "Protein binder toolbox for unlocking solute carrier transporters" (manuscript in preparation)

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