Fragment screening against detergent-free purified GPCRs by mass spectrometry

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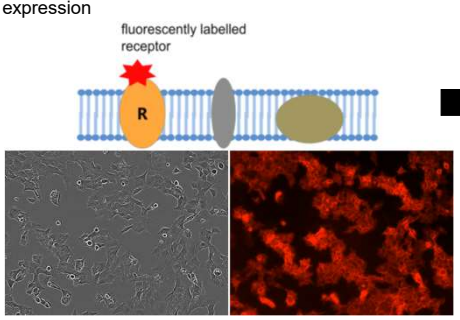
Introduction

- Fragment-based drug design (FBDD) against GPCR targets has been limited by access to high quality purified receptor protein
- To avoid many of the challenges associated with purifying membrane proteins, styrene maleic acid (SMA) polymers have been used to solubilise these targets in their native membrane environment without using detergents by forming SMA lipid particles (SMALPs)
- We have developed a novel liquid chromatography-mass spectrometry (LC-MS) based ligand binding assay against purified SMALP-GPCRs that will enable FBDD at GPCRs
- We aim to offer a platform-based approach for GPCR purification and screening for much faster and more cost-effective GPCR FBDD with SMALP technology, using the neurotensin1 (NTSR1) and β2-adrenergic (β2AR) receptors as test cases.

Purification of SMALP-GPCR

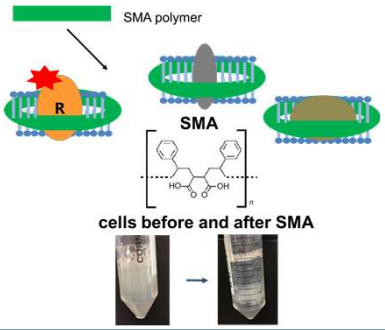
mammalian expression of tagged GPCR

- Clonal isolates of GPCR expressing HEK293 cells generated within 2 months
- Receptor is fluorescently labelled and shows cell surface expression



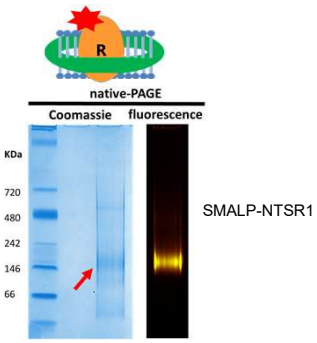
SMA solubilisation of lipid membranes

- SMA polymer inserts into cell membrane
- Crates solution of SMALPs; particles of membrane proteins in SMA-encapsulated lipid bi-layer



purification of SMALP-GPCR

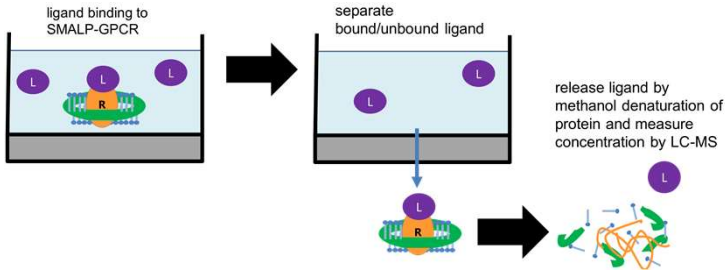
- NiNTA purification of his-tagged GPCR
- Runs as a single homogenous population of fluorescently-labelled protein, with partial purity



LC-MS binding validation

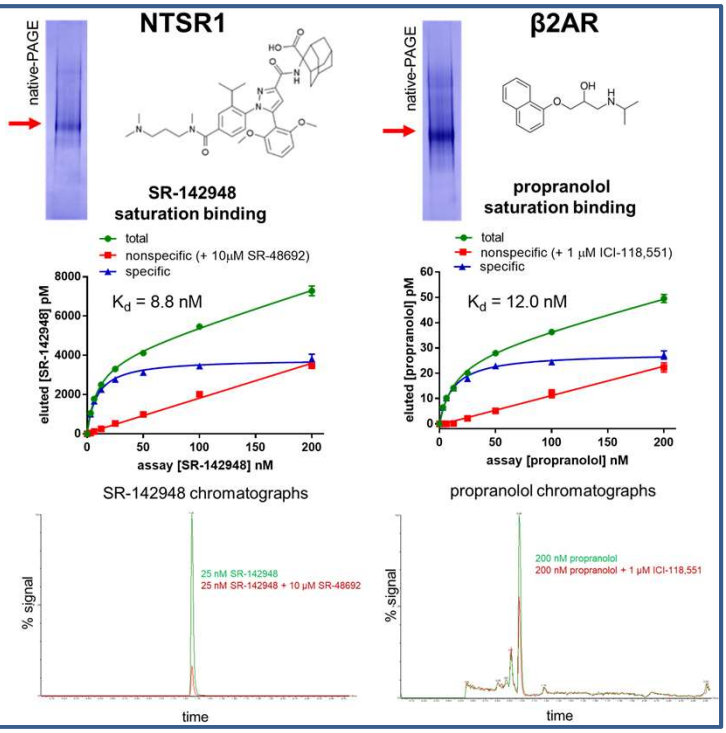
assay principle

- Tracer ligand of choice is measured by LC-MS (therefore does not require ligand labelling)
- Purified SMALP-GPCR incubated with tracer ligand (L)
- Bound and unbound ligand separated by MW cut-off filter in a 96-well format, concentration of bound ligand is quantified against a standard curve



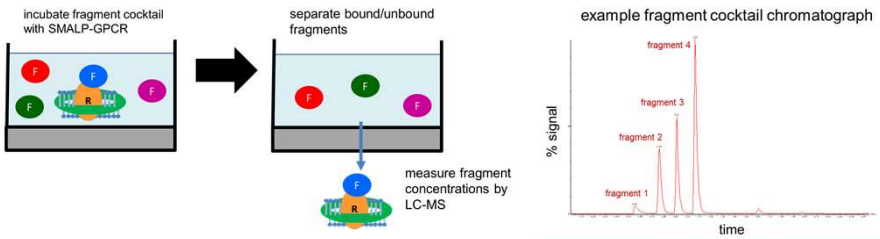
saturation binding curves

- Small molecule antagonists used as tracer ligands for NTS1R and β2AR
- Specific binding sites quantified using competition with an excess of structurally distinct receptor antagonist
- Affinity K_d values for both ligands agrees with data from intact membranes
- Demonstrates that the SMALP-purified GPCRs are folded and pharmacologically intact
- Highlights the application of LC-MS as a robust ligand binding assay



LC-MS fragment screening

- We have developed processes to detect fragments (F) incubated with the SMALP-GPCR in 'cocktails' (mixtures of fragments) by LC-MS
- Measure direct binding of fragments to target protein
- Does not rely on competition with an orthosteric ligand and is sensitive to detection of allosteric binders
- In-house screens at case-study receptors are in process.



Services / Contact

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to enquire about how our drug discovery platform could be applied to targets of interest to you, please contact: tom.mander@domainex.co.uk
www.domainex.co.uk

Conclusions

- Domainex is developing a generic platform approach to solubilise GPCRs in the complete absence of detergents
- By combining this with LC-MS detection of ligand binding our aim is to greatly facilitate FBDD at GPCR targets for our clients