Fragment Screening of Adenosine A2a Receptor using Polymer-Encapsulated Nanodiscs

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Introduction

- As an alternative to the traditional detergent-based extraction methods, the recent development of amphipathic co-polymers offers the possibility for detergent-free purification of integral membrane proteins without stripping away the native lipid bilayer
- Amphipathic polymers, such as SMA, DIBMA, AASTY and related polymers, can insert directly into cell membranes and spontaneously form nanodisc structures of native membrane lipids encapsulated by polymer (polymer-lipid-particles, PoLiPa). This allows membrane proteins to be extracted without removing them from the native lipid environment
- We showcase the extraction and purification of the Adenosine A2a G-protein coupled receptor (GPCR) and the subsequent use of a Spectral Shift binding assay for fragment screening

PoLiPa
Accelerating Membrane Protein Research

Polymer Lipid Particles

Solubilisation of lipid membranes

- Amphipathic polymers insert directly into cell membranes
- The polymer self-assembles into discs of lipid bilayer that allows solubilisation of membrane proteins, maintaining the native lipid environment
- Detergent-free

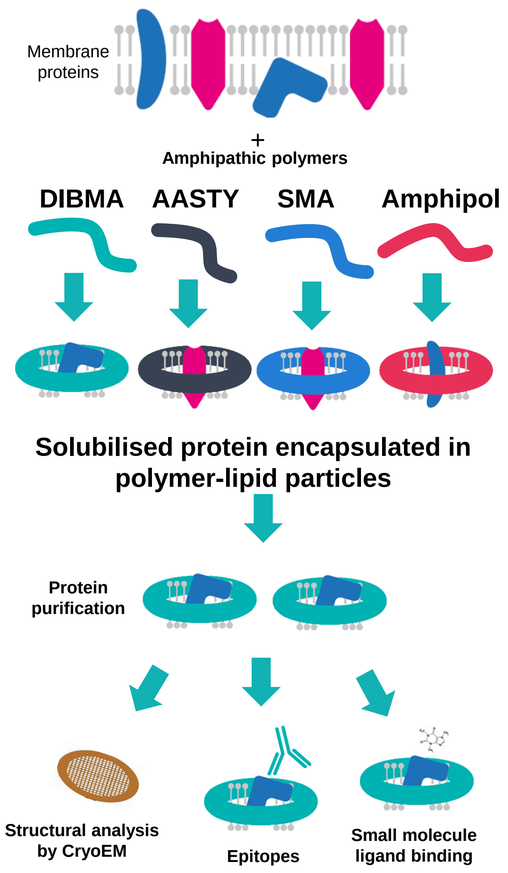
Purification of polymer solubilised membrane proteins

- Compatible with conventional chromatography methods (e.g. affinity, size exclusion)
- Polymer is not required in subsequent purification steps
- Generic solubilisation conditions mean similar purification conditions can be used for any membrane protein

Applications

- Antibody generation, ELISAs, structural studies (CryoEM), and biophysical studies (GCI [Creoptix/Malvern], Spectral Shift, nanoDSF [NanoTemper])

Fig. 1. Description of amphipathic polymer extraction



Adenosine A2a Receptor Purification

- The A2a receptor was expressed in insect cells using the baculoviral expression system
- Extracted membrane fractions were solubilized using either detergent or selected polymer from the solubilization screen and the proteins were purified using Ni-NTA affinity and size exclusion chromatography
- Purification yields (purity > 90%) are comparable for both detergent and Polymer formulations. DLS confirms protein to be monodisperse.

Protein was suitable for downstream applications

Fig. 2. PoLiPa workflow (left) and Western blots of solubilized membrane fractions

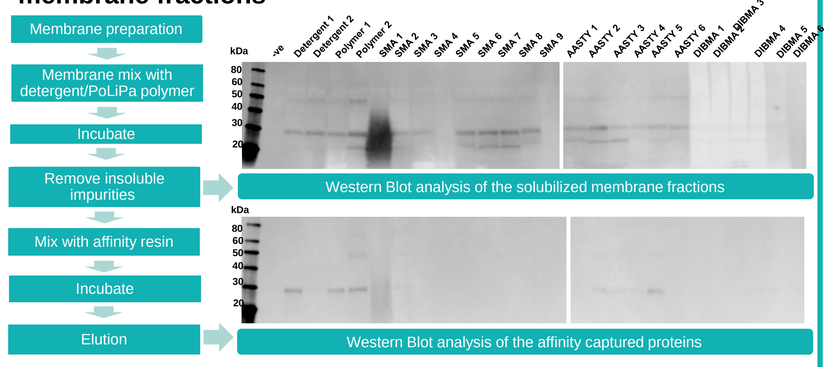
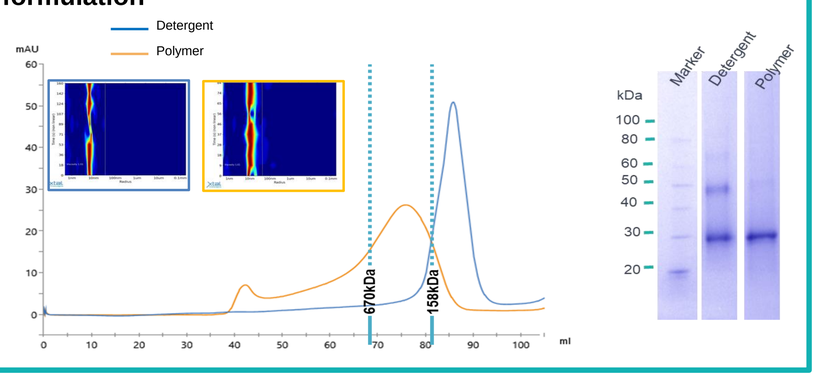


Fig. 3. Size exclusion chromatography (left), DLS (left- inset) and SDS-PAGE (right) of A2a in either detergent or polymer formulation



Adenosine A2a Receptor Spectral Shift Assay

Protein Validation via nanoDSF

- The receptor had a single inflection point, consistent with a single folded species and known antagonists increased the melting temperature by +3 to +13ºC, indicating the receptor is physiologically folded

Spectral Shift Assay (NanoTemper)

- Spectral Shift (NanoTemper) is a biophysical assay that enables the direct measurement of molecular interactions in solution
- Binding is detected when ligands cause very subtle changes in the emission spectra of a fluorescent dye attached to a molecular target
- A Spectral Shift assay was developed using an NTA-labelling kit on the Dianthus instrument (Nanotemper)

Fig. 4. NanoDSF data for A2aR protein samples in the presence and absence of tool compounds

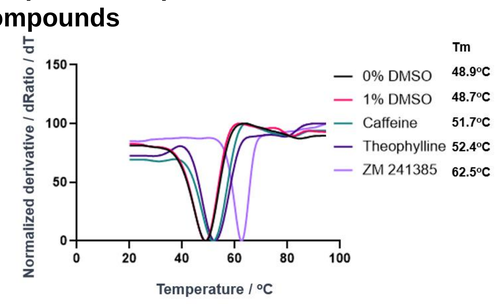
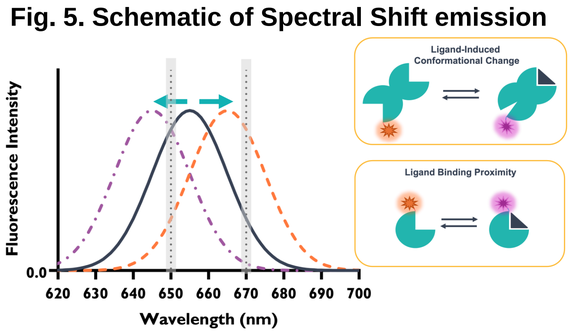
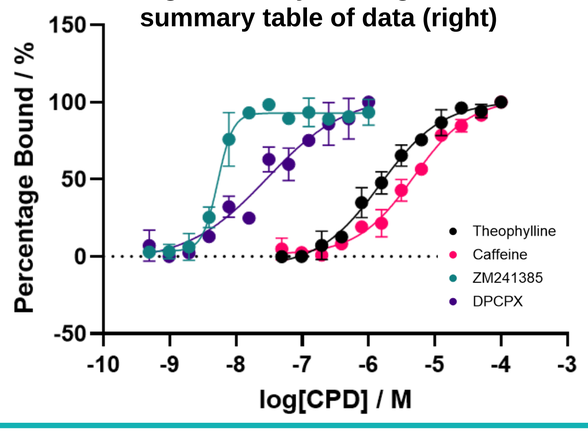


Fig. 5. Schematic of Spectral Shift emission



- Reproducible concentration-response curves for four known antagonists were obtained
- The K_D value for ZM241283 was weaker than expected due it reaching the tight binding limit of the assay

Fig. 6. Affinity binding curves for four known A2aR small molecules (left) and summary table of data (right)



Compound	Hill Slope	Spectral Shift (pK_D)	Literature values^ (pK_i)
Caffeine	1.01	5.3	4.6-5.6
Theophylline	0.92	5.8	5.2-5.8
DPCPX	0.72	7.5	6.6-7.2
ZM241283	3.75*	8.3	8.8-9.1

*at the assay tight binding limit
^IUPHAR/BPS Guide to pharmacology website https://www.guidetopharmacology.org/

Adenosine A2a Receptor Fragment Screen

Fragment Screening

- The Domainex fragment library (960 fragments) was screened at 250 μM against adenosine A2A receptor using spectral shift
- A 9% hit rate was obtained using a user specified threshold

Fig. 7. Data for 160 fragments in duplicate

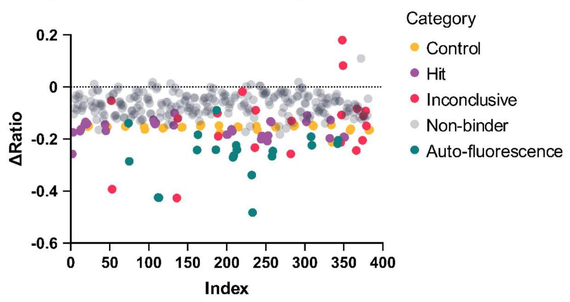
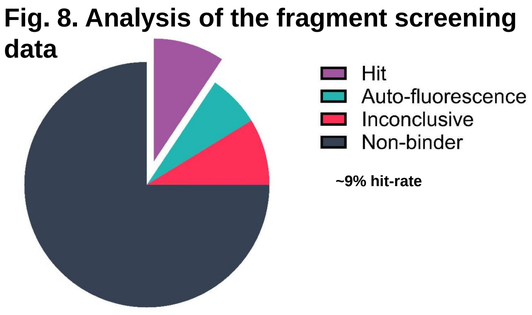


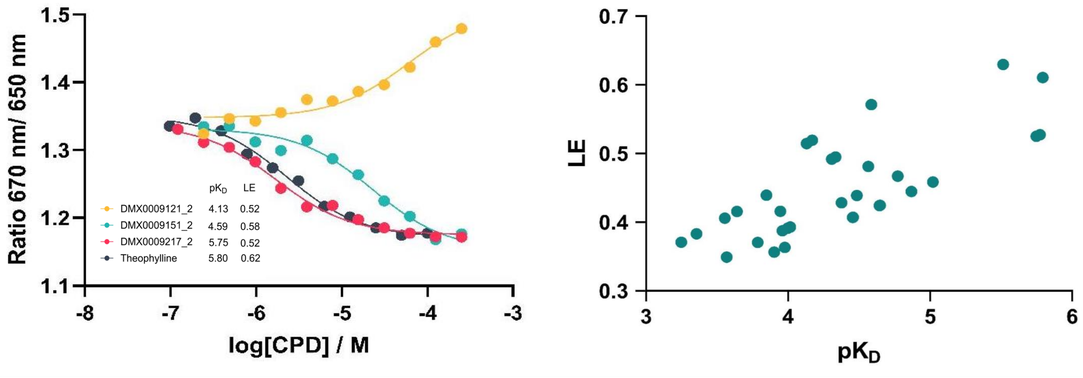
Fig. 8. Analysis of the fragment screening data



Fragment Affinities

- 32 fragment hits were selected from the fragment screen based on their binding signal and heavy atom count (HAC)
- One fragment hit gave a binding curve that increased spectral shift rather than decreased, suggesting the binding mode or site of this hit may be different
- More than 30 fragment hits were identified with ligand efficiencies (LE) greater than 0.3 (LE = 1.37*(pKd/HAC)

Fig. 9. Example binding curves (left) and plot of Fragment Hit Ligand Efficiencies versus pKd (right)



Conclusions

We have prepared a Polymer-Encapsulated Nanodisc (Polymer-lipid-particles - PoLiPa) for the Adenosine A2a GPCR at high purity and yield

We believe this is the first fragment screen with a membrane protein purified in a Polymer-Encapsulated Nanodisc

Ligand efficient fragment hits have been identified and work is ongoing to develop compound SAR via plate-based chemistry (D2B) and to determine crystal structures of the fragment hits

Services/Contact

If you would like to speak with us about A2a or drug discovery services please contact: enquiries@domainex.co.uk

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