Using Direct-to-Biology in a Spectral Shift-PoLiPa Platform to Enable Fast Fragment Follow-Up for Adenosine A2a Antagonists

Vincent Duong[1], Andrea Bombana[1], David Gibson[1], Philip Rawlins[2], Jim Reid[2], William Lee[2], Holly Madden[2], Soumya Dhanavade[2], James Craswell[2], Satya Bhamidimarri[2], Anna Hopkins[1], Joe Mason[1], Marieke Lamers[2], Andrew Ratcliffe[1], Stefanie Reich[2], Trevor Askwith[2].

[1]Domainex Ltd., Churchill Building, Chesterford Research Park, Saffron Walden, CB10 1XL, UK

[2]Domainex Ltd., Sigma Building, Unity Campus, Pampisford, CB22 3FW, UK

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Introduction

- Recent advancements in amphipathic co-polymers enable detergent-free purification of integral membrane proteins while preserving the native lipid bilayer – Polymer Lipid Particle (PoLiPa)
- Spectral Shift assays (NanoTemper) is a fluorescence-based biophysical technique used to determine ligand binding
- Direct-to-Biology (D2B) is the rapid nanoscale synthesis of molecules in plate-based format combined with direct screening of crude reaction mixtures

PoLiPa
Accelerating Membrane Protein Research

Direct to Biology
Galaxy of opportunity

Adenosine A2a Receptor and Polymer Lipid Particles

Adenosine A2a Receptor

- The adenosine A2a receptor (A2aR) was expressed in insect cells using the baculoviral expression system
- Extracted membrane fractions were solubilized using either detergent or selected polymer from the solubilisation screen and the proteins were purified using Ni-NTA affinity and size exclusion chromatography

Polymer Lipid Particles

- 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
- Compatible with conventional chromatography methods

Applications

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

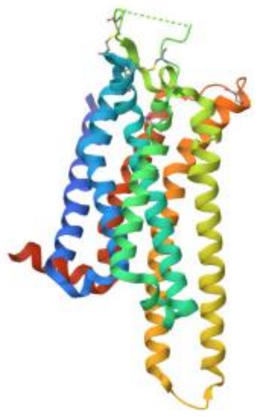

Fig. 1. A2aR protein complexed with caffeine

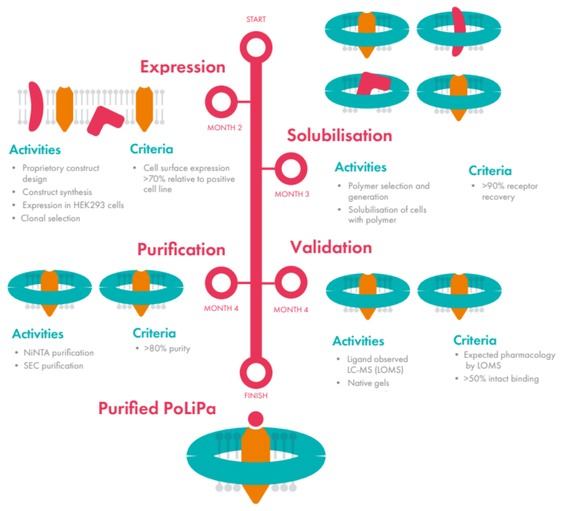

Fig. 2. PoLiPa protein extraction platform

Adenosine A2a Receptor Spectral Shift Assay

Spectral Shift Assay (NanoTemper)

- Binding validation of A2aR protein with known antagonists via nanoDSF (Fig. 3)
- Binding is detected when ligands cause very subtle changes in the emission spectra of a fluorescent dye attached to a molecular target (Fig. 4)
- A Spectral Shift assay was developed using an NTA-labelling kit on the Dianthus instrument (NanoTemper)
- Reproducible concentration-response curves for four known antagonists were obtained (Fig. 5)

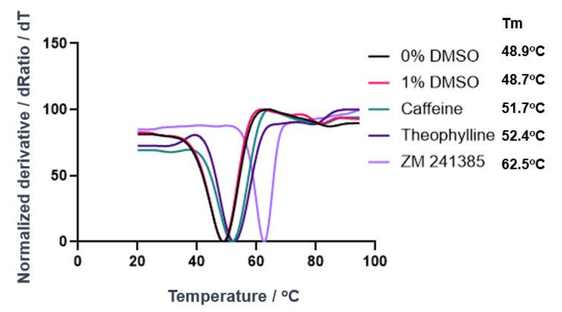

Fig. 3 . NanoDSF data forA2aR protein sample in the presence and absence of tool compounds

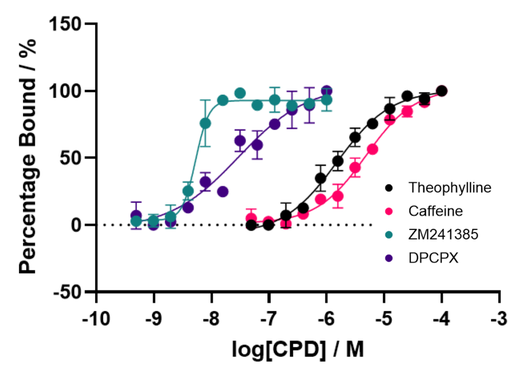

Fig. 5. Affinity binding curves for four known A2aR small molecules (left) and summary table of data (right)

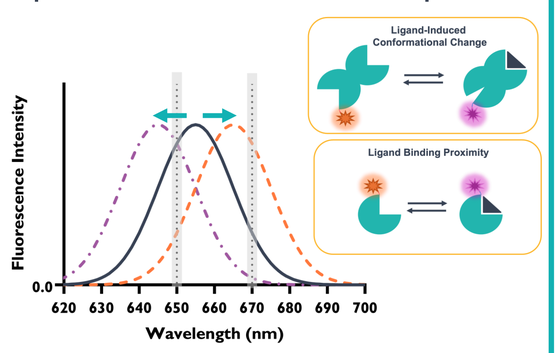

Fig. 4. Schematic of Spectral Shift emission

Compound	Hill Slope	Spectral Shift (K_D, μM)	Literature values^ (pK_i)
Caffeine	1.01	5.01	4.6-5.6
Theophylline	0.92	1.58	5.2-5.8
DPCPX	0.72	0.03	6.6-7.2
ZM241283	3.75*	0.05	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

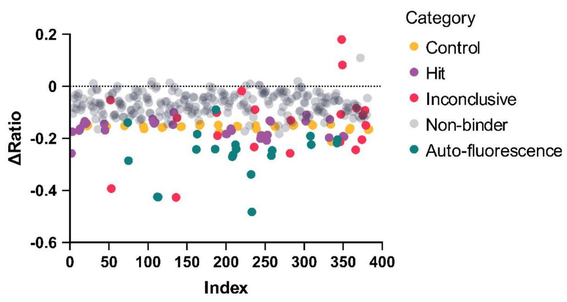

Fig. 6. Data for 160 fragments in duplicate

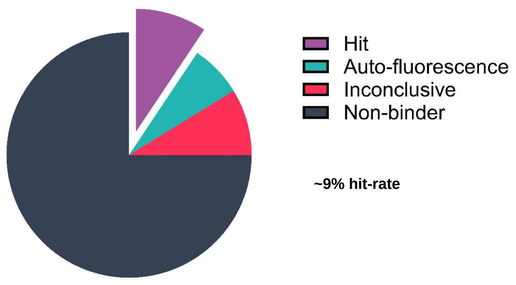

Fig. 7. Analysis of the fragment screening data

Fragment Affinities

- 32 fragment hits were selected from the fragment screen based on their binding signal and heavy atom count (HAC)
- 29 fragments gave >0.3 LE
- 4 diverse fragments with appropriate properties were selected as scaffolds for fragment expansion using D2B synthesis

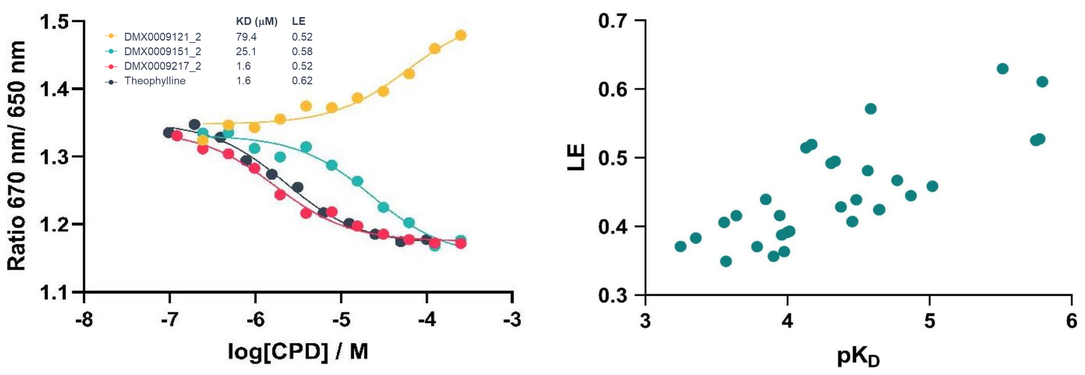

Fig. 8. Example binding curves (left) and plot of Fragment Hit Ligand Efficiencies versus pKd (right)

Direct-to-Biology (D2B)

Compound Selection

- 37 amines were selected from in-house 4710 amine library through property filtering and 'bbSelect' diversification
- 4 acid scaffolds x 37 amines → 148 target compounds

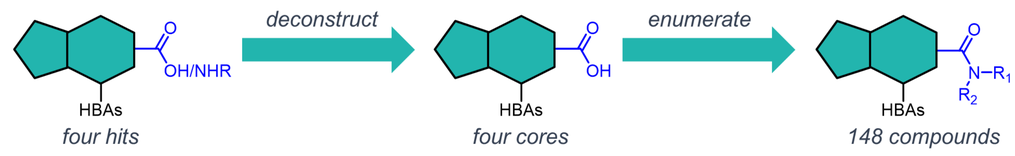

Fig. 9. Fragment expansion design for D2B synthesis

D2B Synthesis and Binding Affinity

- Model amide coupling reactions were compatibility tested to ensure non-interference
- 109/148 compounds were successfully synthesised on-plate (Fig. 10)
- Binding data and a crude "single shot Ligand Efficiency" was measured for all crude reaction products
- 21 D2B hits were selected and resynthesised
- Up to **200-fold** increase in binding with purified compound compared to initial fragment hit (Cpd 1 vs 2)
- D2B synthesis required 1 FTE day compared to 15 weeks using conventional chemistry and purification of final compounds

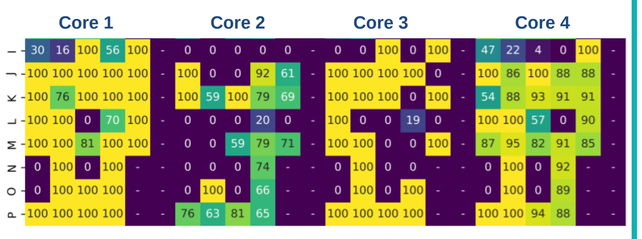

Fig. 10. Heatmap for D2B synthesis

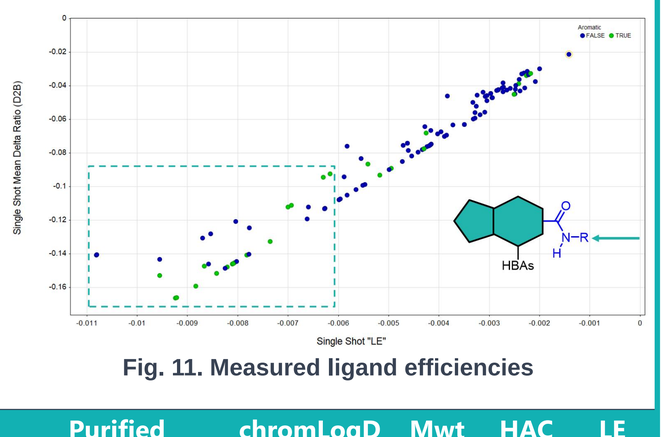

Fig. 11. Measured ligand efficiencies

Cpd	Crude sample K_D (μM)	Estimated Conversion (%)	Purified Sample K_D (μM)	chromLogD	Mwt	HAC	LE
1 (Initial hit)	16.5	100	**48**	1.87	206	15	0.39
2	2.3	56	**0.21**	2.70	249	17	0.54
3	2.2	100	2.1	3.65	248	18	0.43
4	3.9	100	3.9	2.92	262	18	0.41

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
- Successful D2B synthesis following fragment hit, with significant (>200 fold) increase in binding affinity compared to initial hit

Services/Contact

If you would like to speak with us about D2B, A2a or drug discovery services please contact: enquiries@domainex.co.uk

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