



Comparisons between SMALP'ed and detergent solubilised GPCRs

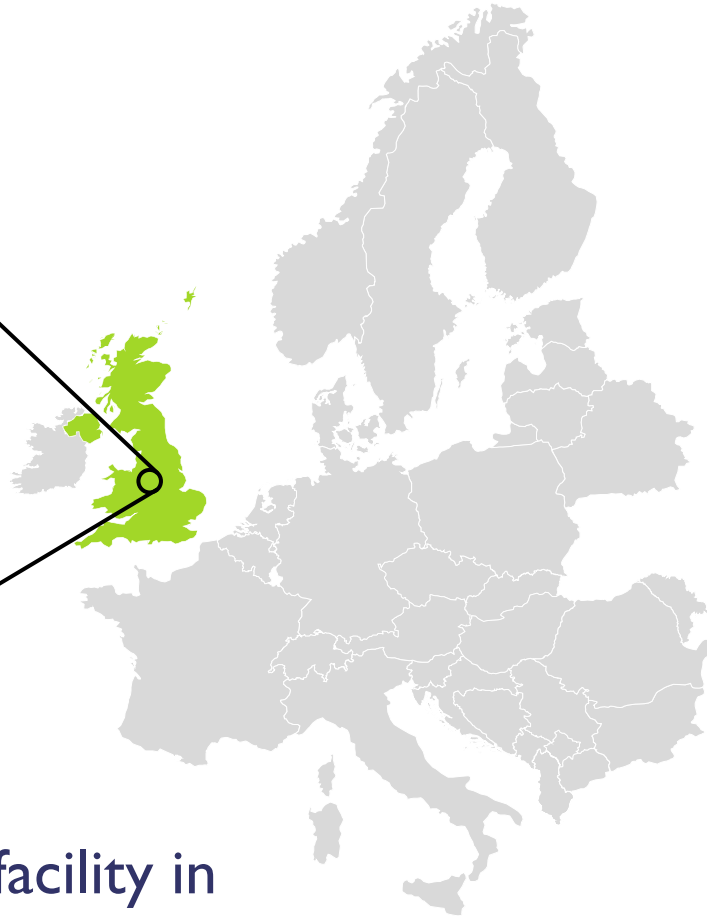


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Peak Proteins

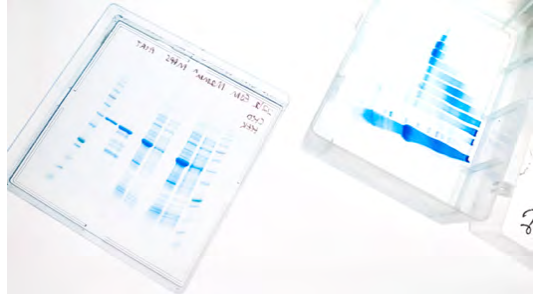
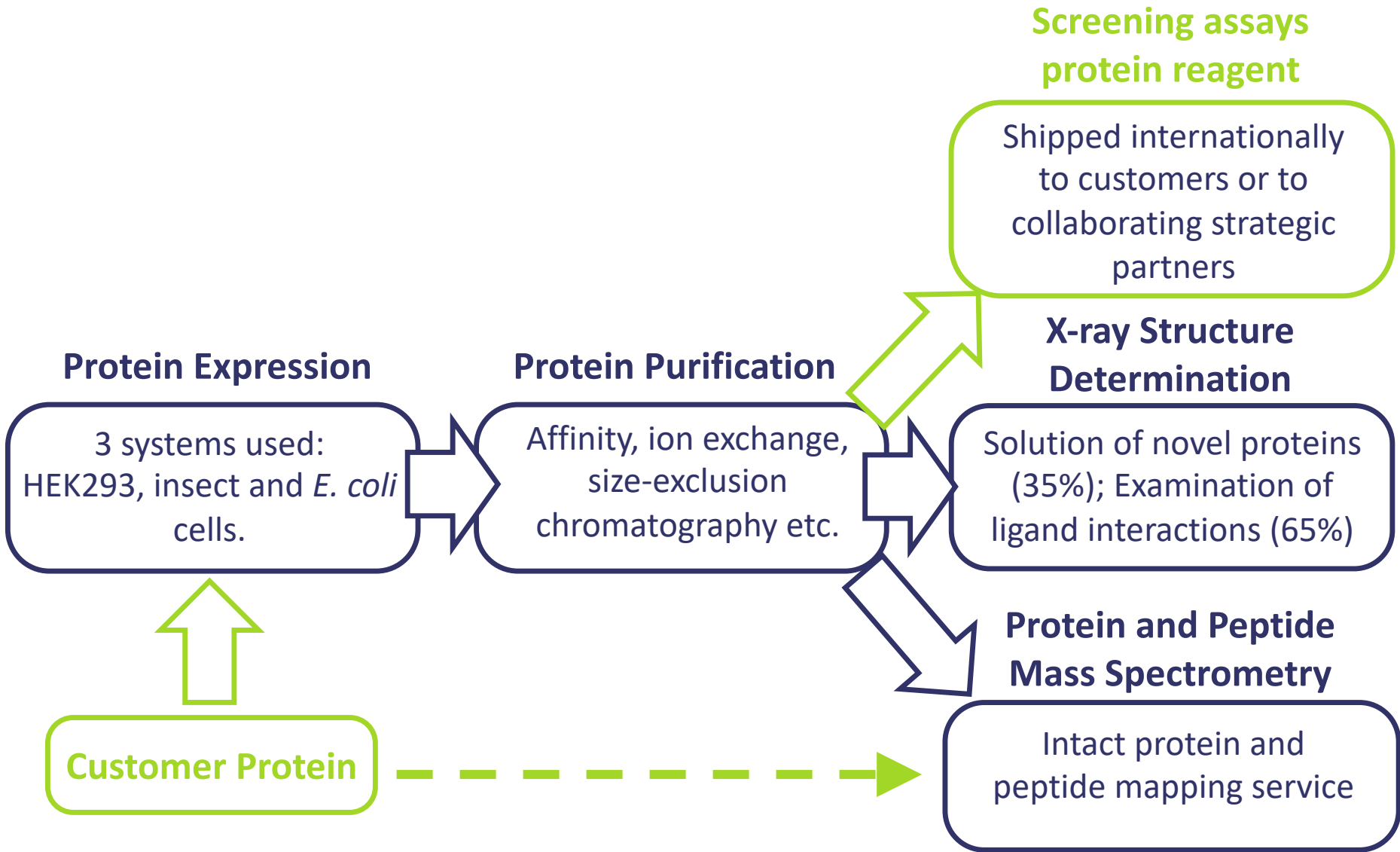
Contract research organisation (CRO) established in October 2014



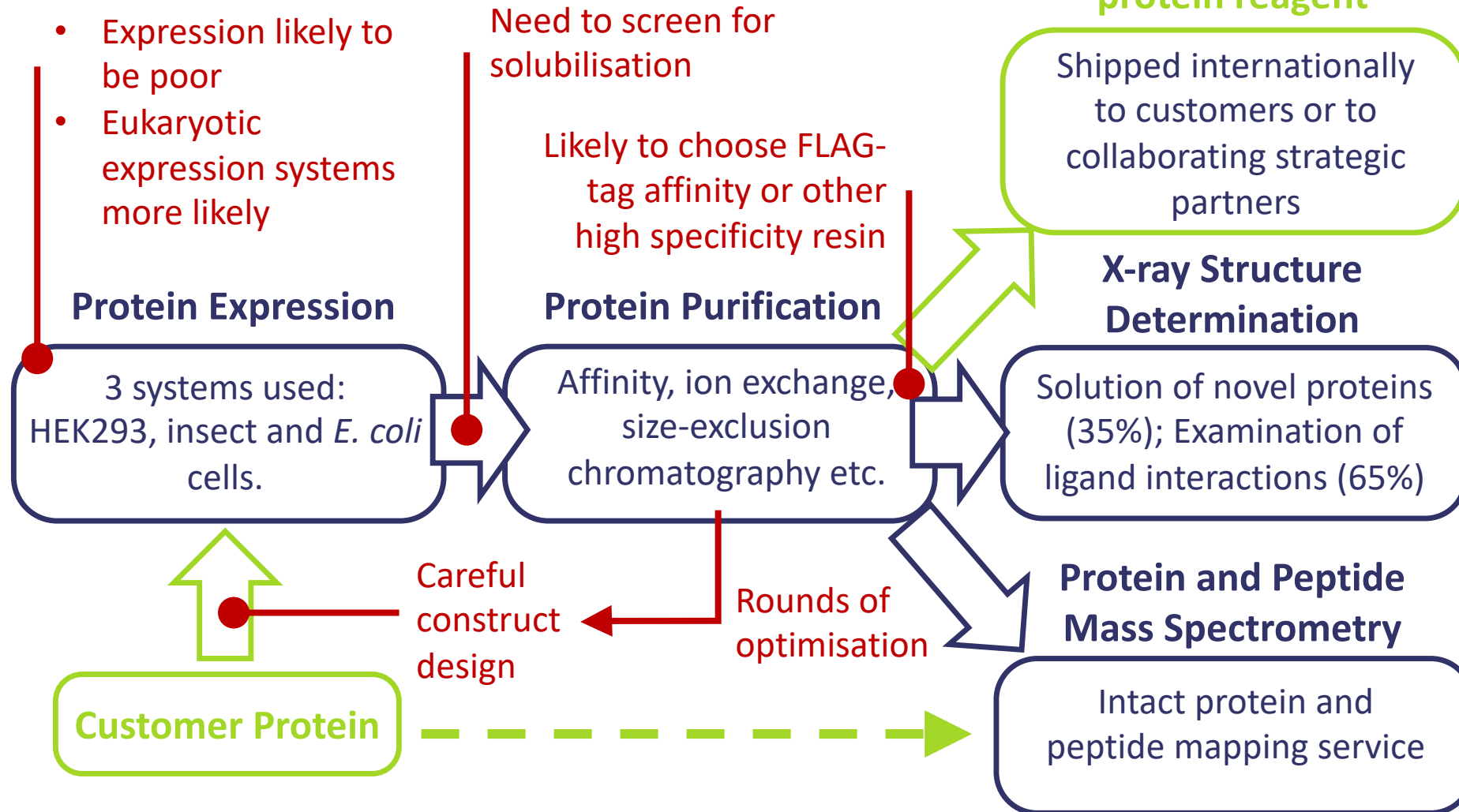
Mark Abbott (CEO)

Based at Alderley Park research facility in
Cheshire, UK

Peak Proteins Services



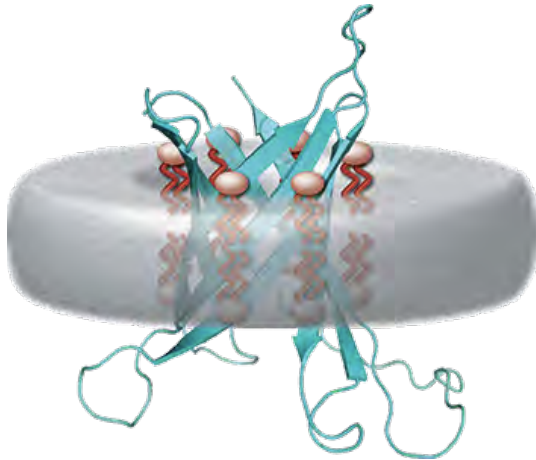
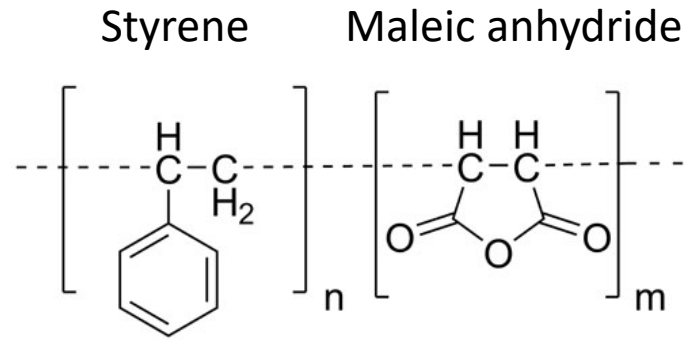
Production of membrane proteins



Screening assays protein reagent



Can SMALPs help?

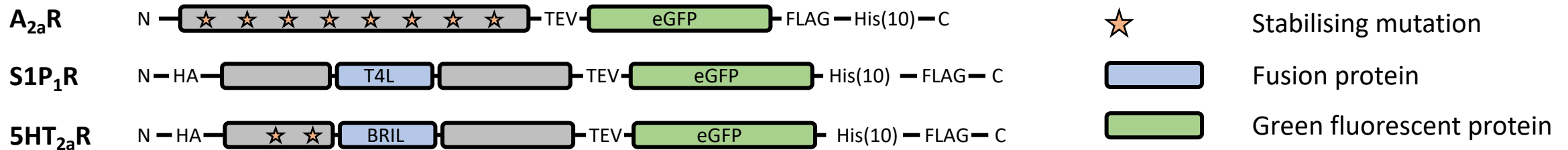


	Detergents	SMALPs
Cost	✗ (academic) ✗ (industry)	✓✓ (academic) ✗ (industry)
Ease of use	✓	✓✓
Diversity	✓✓	✓
Solubility	Needs optimising	✓
Stability	Needs optimising	✓
Purity	✓	✓✓

GPCR test cases

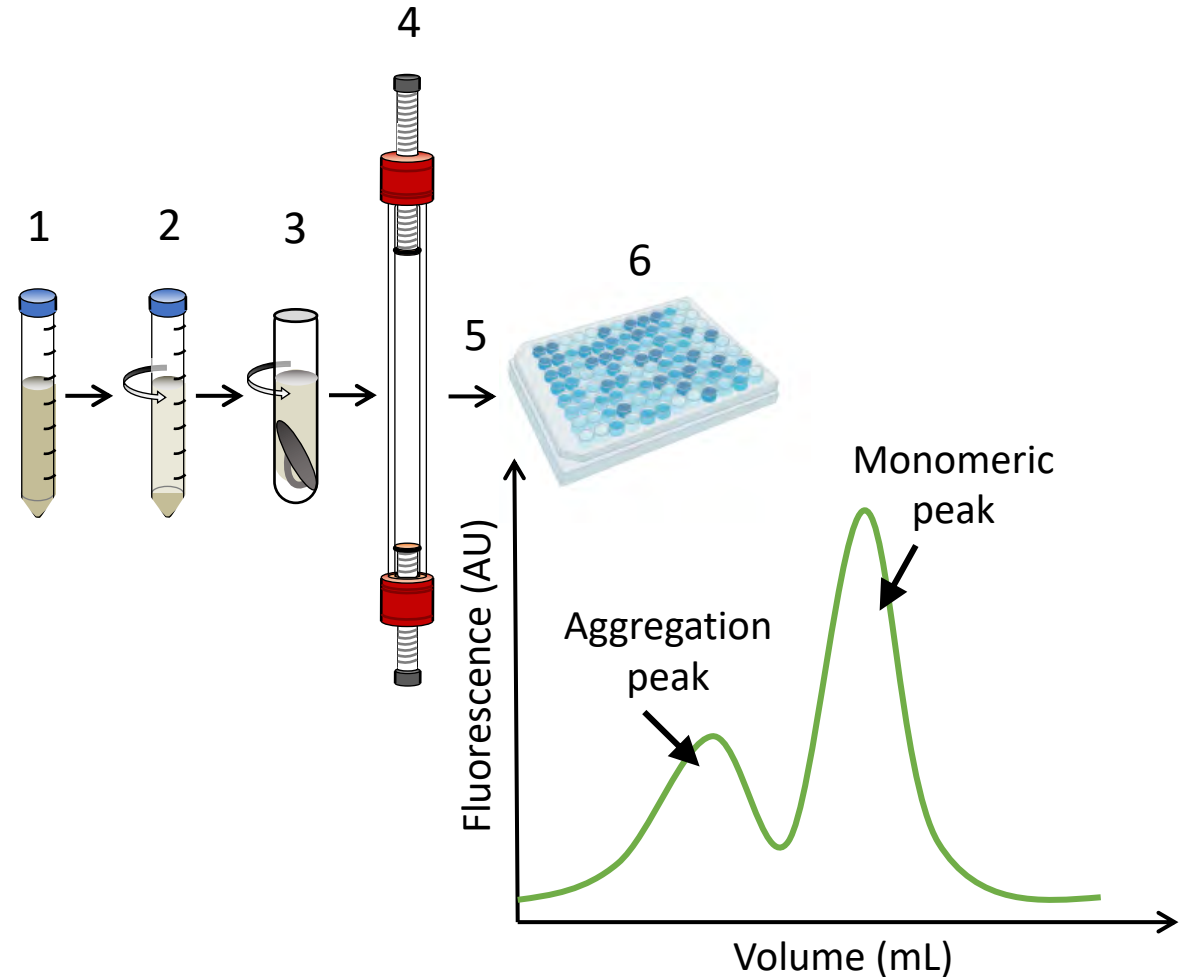
- Use three well characterised GPCRs as test cases:
 - Adenosine receptor ($A_{2a}R$).
 - Sphingosine-1-phosphate receptor (SIP_1R).
 - Serotonin receptor ($5HT_{2a}R$).
- Structures of all have previously been solved by X-ray crystallography
- Used the crystallography constructs as a basis
- Added a C-terminal fluorescent protein tag in each case

Construct design:



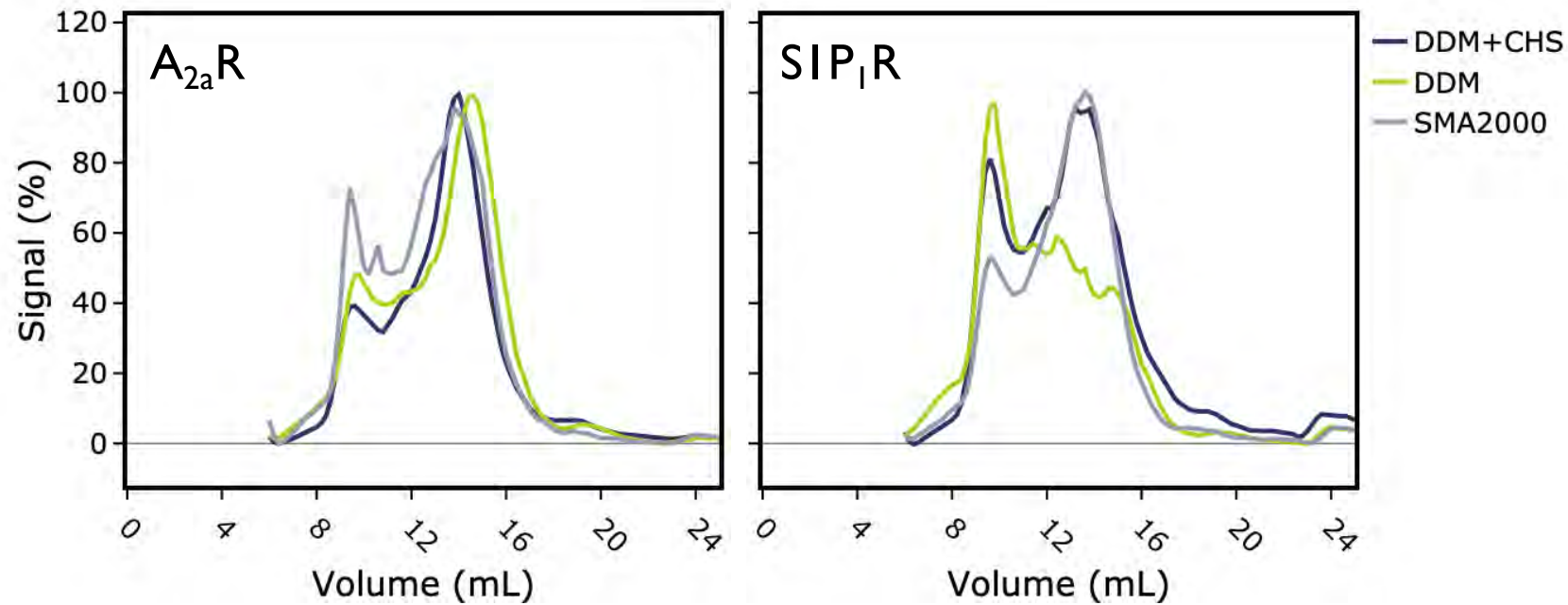
Fluorescent size exclusion chromatography (FSEC)

1. Solubilise insect cells for 30 mins at 4°C with DDM (with and without CHS), or at room temperature with SMA
2. Low-speed spin at 2,000 x g for 10 min
3. High-speed spin at 100,000 x g for 30 min
4. 500 µL of supernatant loaded onto a 24 mL Superdex S200 column
5. 200 µL fractions collected
6. GFP-fluorescence detected using plate reader



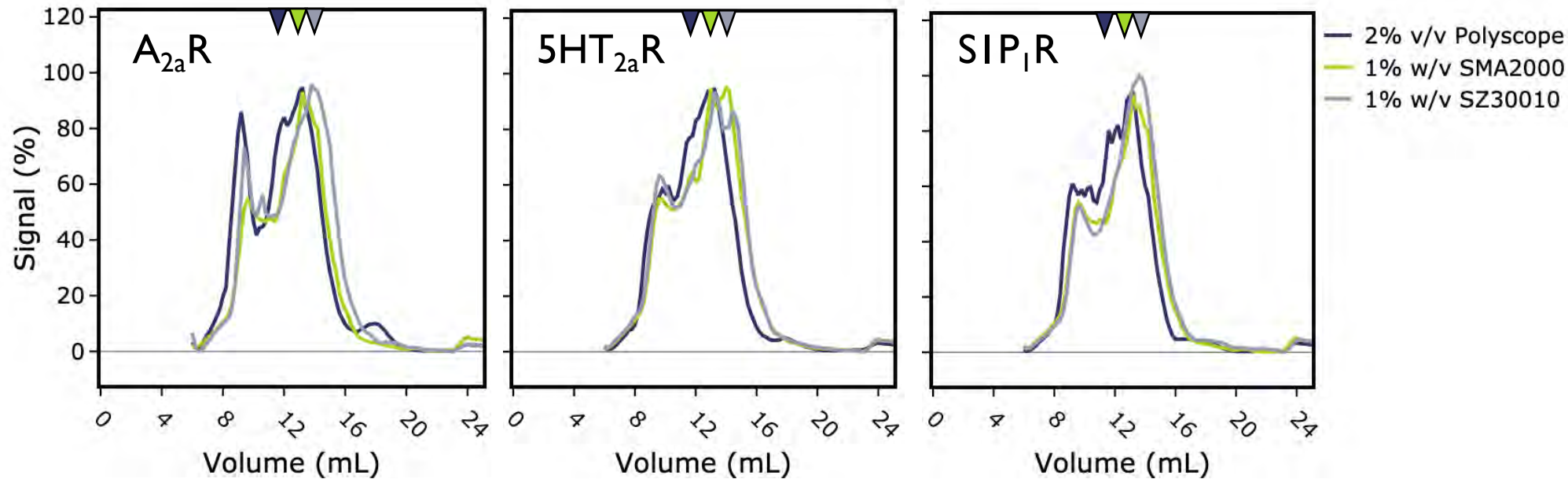
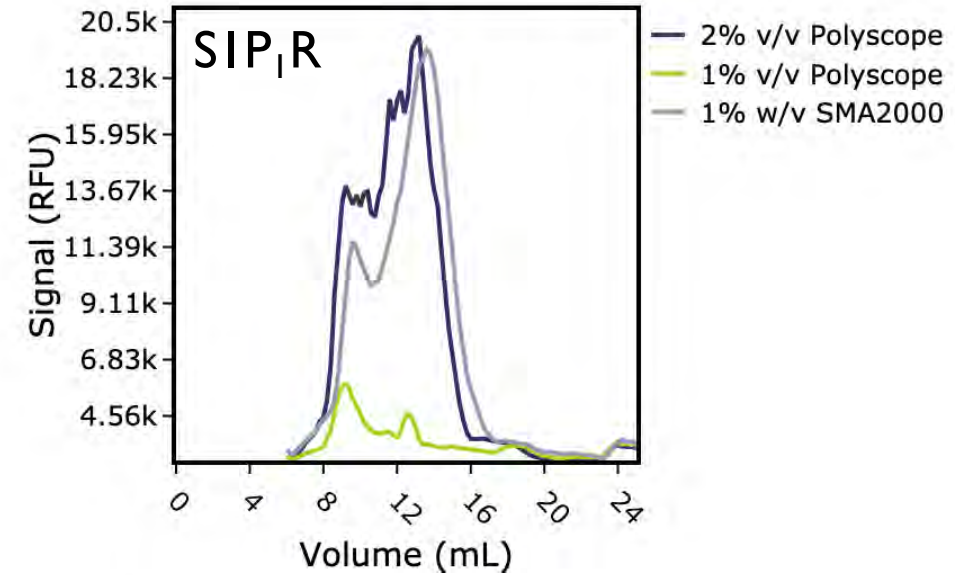
FSEC results (I)

- For $A_{2a}R$:
 - Solubilisation and monodispersity similar between DDM+CHS solubilisation and SMA solubilisation
- For SIP_1R :
 - Solubilisation and monodispersity better with addition of CHS compared to DDM alone
 - SMA solubilisation is the best

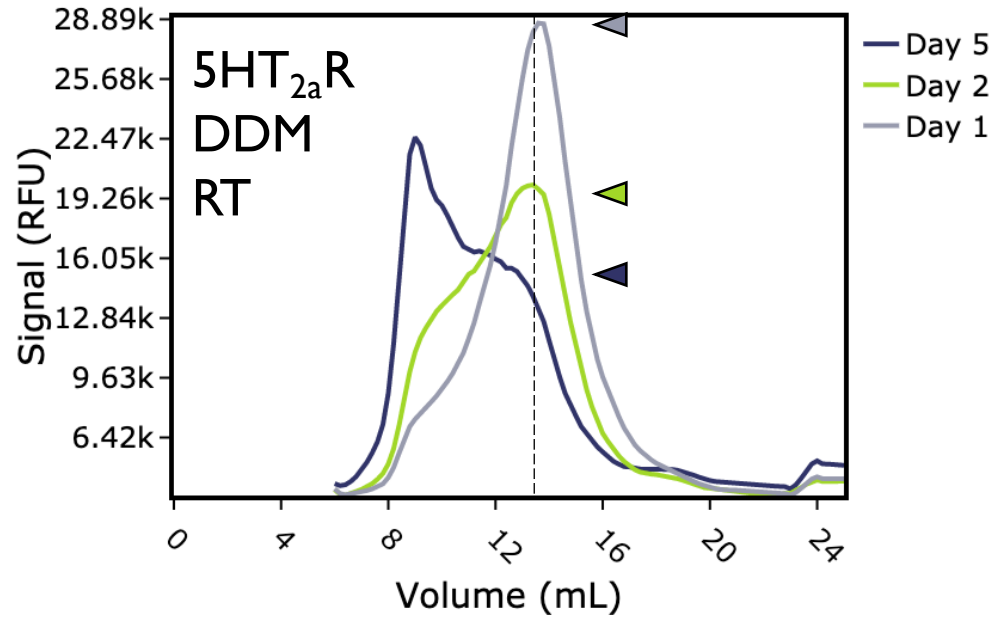


FSEC results (II)

- Comparison of 'home-made' SMALPs with 300010P from Polyscope
- Technical difference of adding liquid versus solid: need 2% (v/v) liquid SMALP compared to 1% (w/v) solid SMALP
- Size difference between the different polymers:
 - shoulder indicating a higher molecular weight species
 - consistent between receptors

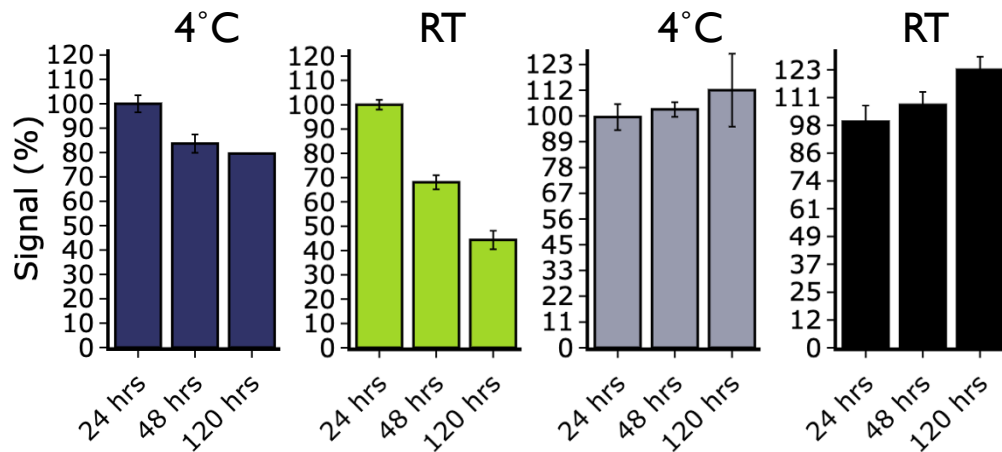


Stability over time, DDM versus SMALPs



DDM

SMALPs



- Used 5HT_{2a}R – solubilised in SMALP or DDM
- Monitored the FSEC trace of sample on day 1, day 2 and day 5 at two different temperatures (4°C or room temperature)
- Clear drop in monomeric peak height and increase in aggregate peak height over time for the DDM solubilised receptor – exacerbated by increased temperature
- No drop in monomeric peak height of SMALP solubilised receptor at either temperature

Can SMALPs help?

YES!

Take home messages:

- SMALPs can provide a valuable alternative to detergent solubilisation
 - Don't need to add CHS to GPCR solubilisations
 - Receptor specific preference for SMALP solubilisation (SIP₁R case)
- Increased protein stability over time for proteins in SMALPs!
- However, SMALPs are not 'magic bullets'
 - Badly folded proteins can't be saved!

Acknowledgments

Peak Proteins Team



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Insect cell culture



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Protein science

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Alan Goddard
Roslyn Bill

THANKS!

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