



HEPTARES
therapeutics

Fragment Based Approaches to GPCRs
Miles Congreve
March 2011

GPCR Drug Discovery

Pharma HTS success rate only 1:10



- GPCRs once considered highly tractable targets but very slow progress over last decade
- Yet GPCRs still form 30% of current Pharma targets due to compelling biology
- Most recent pipeline compounds large and lipophilic - high-attrition chemotypes
- Need Structure-Based Design approaches to produce atom-efficient NCEs
- But GPCR discovery previously limited to testing in cells - StaR[®] s are the solution

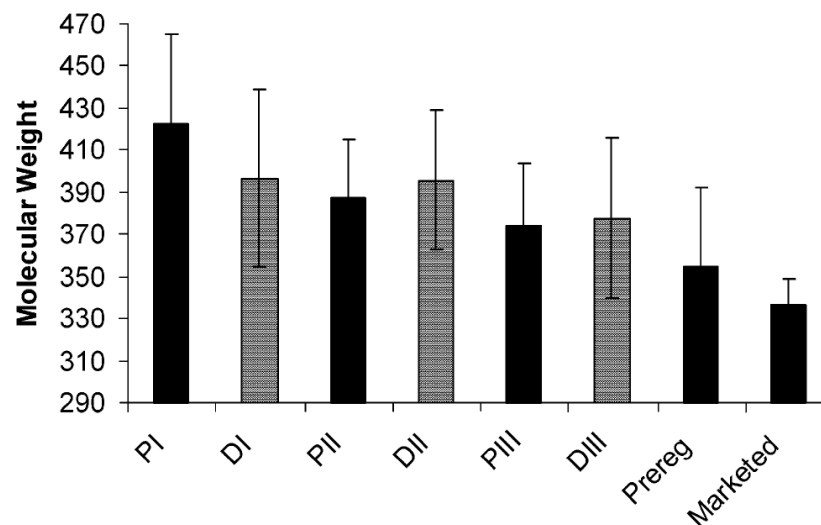
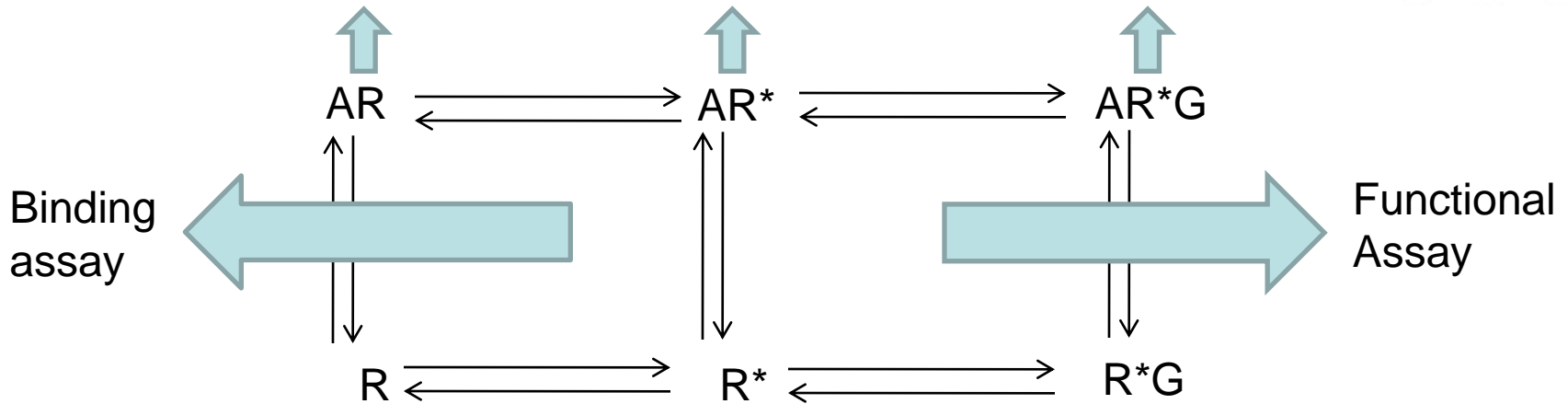


Figure 3. Mean molecular weight for drugs in different phases.

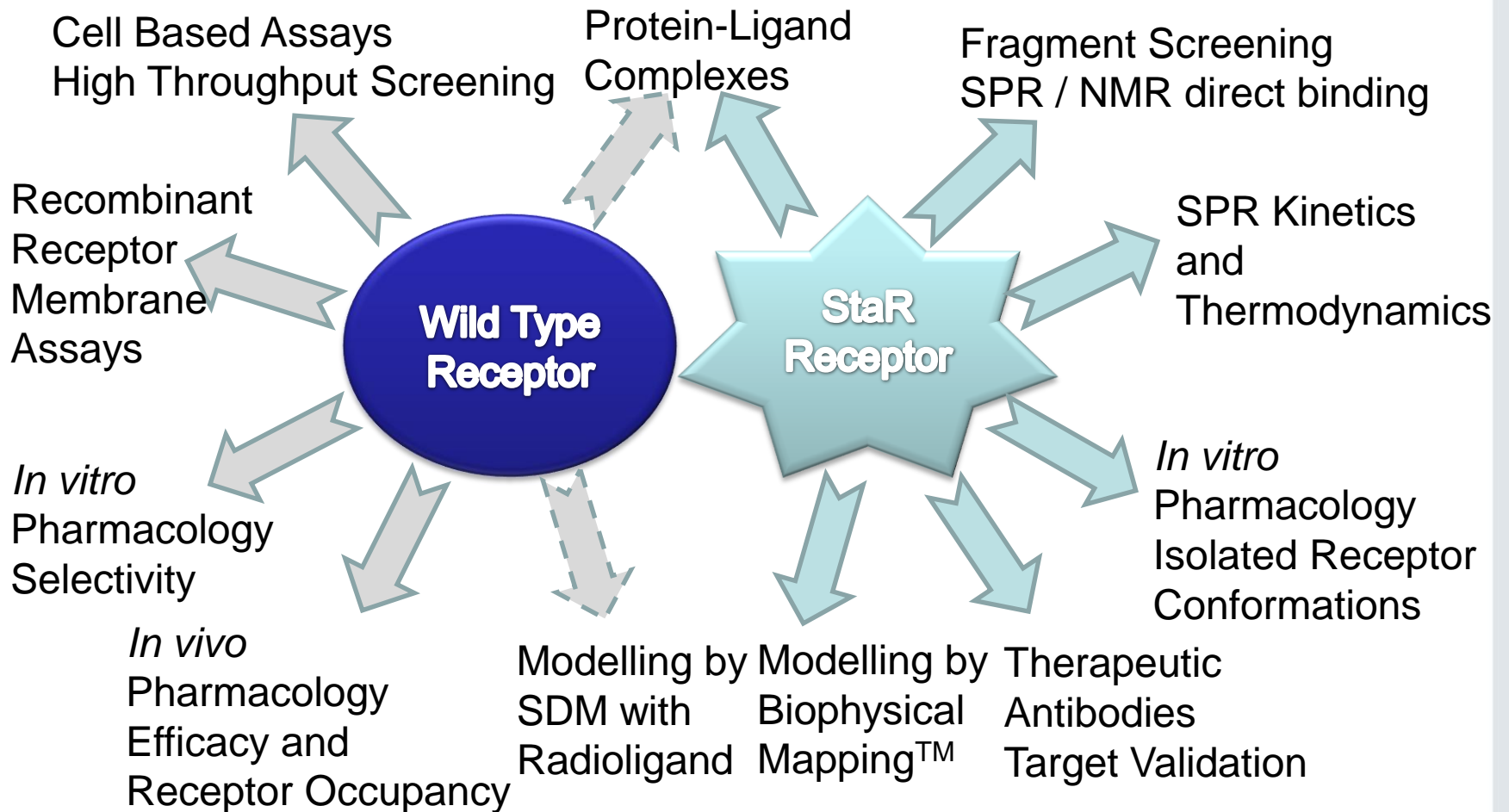
Wenlock, Austin, Barton, Davis and Leeson,
J. Med. Chem. 2003, 1250

Heptares StaR[®] Technology

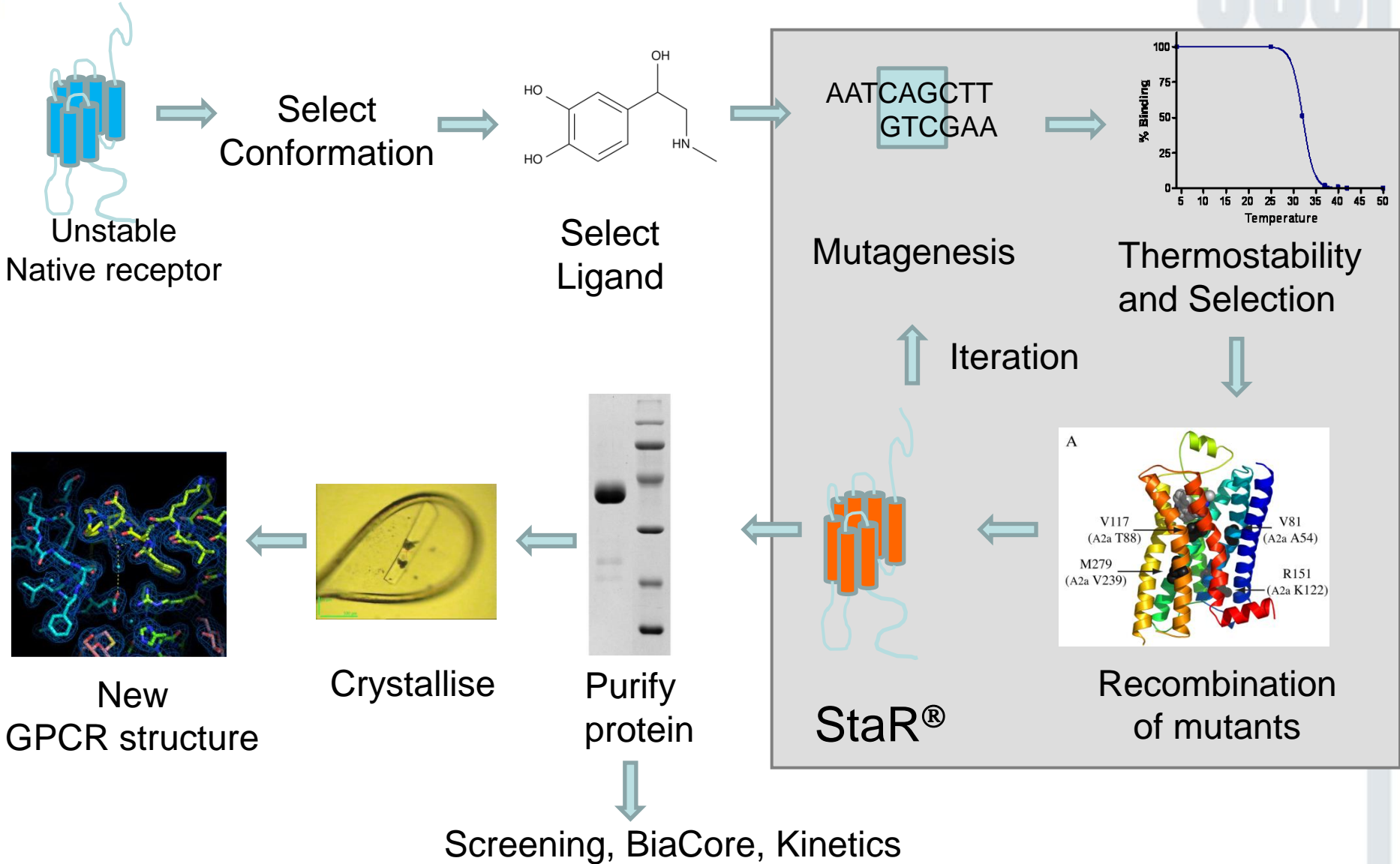


- Receptors embedded in cell membrane exist in multiple conformations
 - Highly unstable when removed
 - Not suitable for structure based drug discovery methods
- Heptares' technology is used to make a stabilized versions of target GPCRs (StaRs) held in a specific chosen conformation
 - Stable in functionally-relevant, purified form
- Discover Leads using the conformation that fits pharmacology of Target Product Profile
 - N.B. always follow up with wild type screens

StaR[®]-Based GPCR Drug Discovery



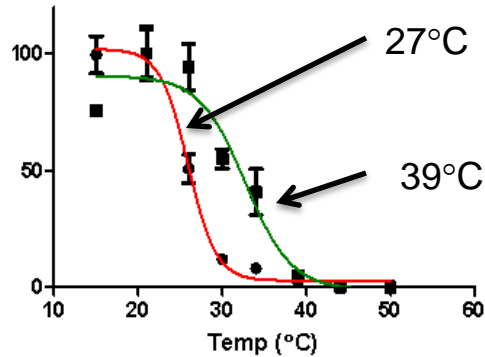
Proprietary Process for Creating StaRs



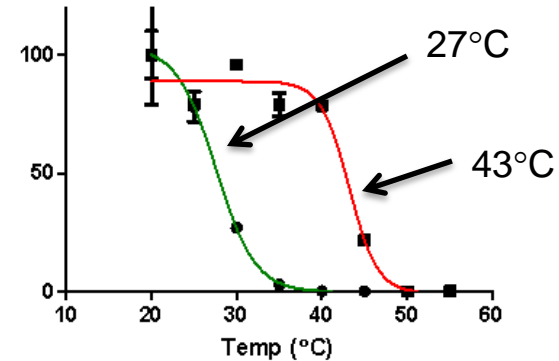
StaRs give a general approach to thermostabilisation



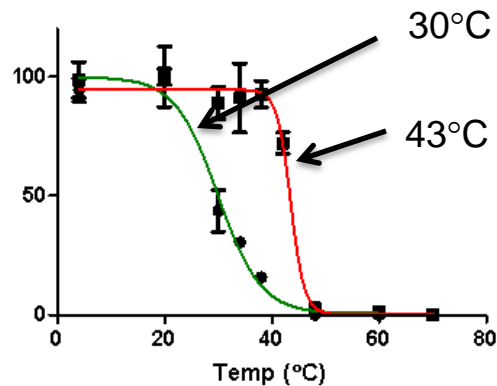
Family B



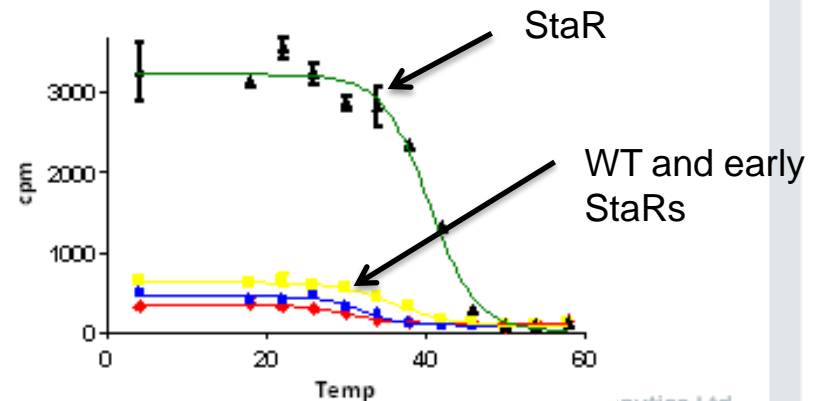
Family A – solubilised
(normalised data)



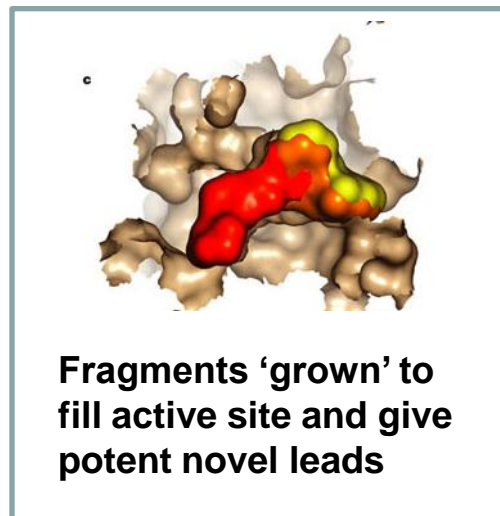
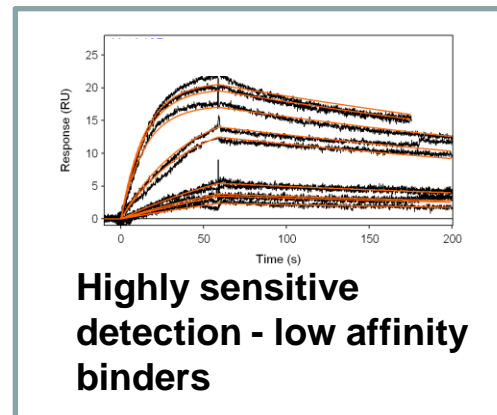
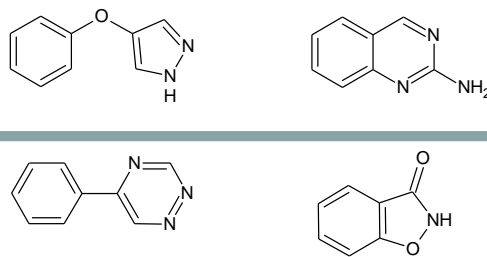
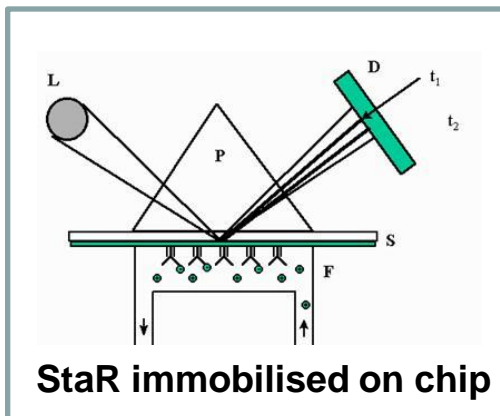
Chemokine receptor



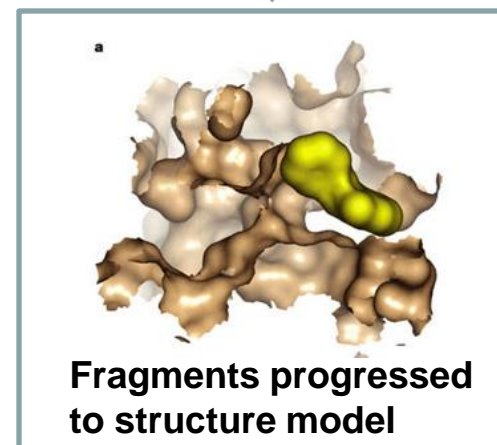
Family A peptide - purified
(raw data shows
higher yield of functional protein)



Fragment Screening



Fragment-based
Drug Discovery



Fragments are very small compounds, expected to bind weakly, but that can be rationally optimised

Fragment Screening Cascade

Primary screening
validated with

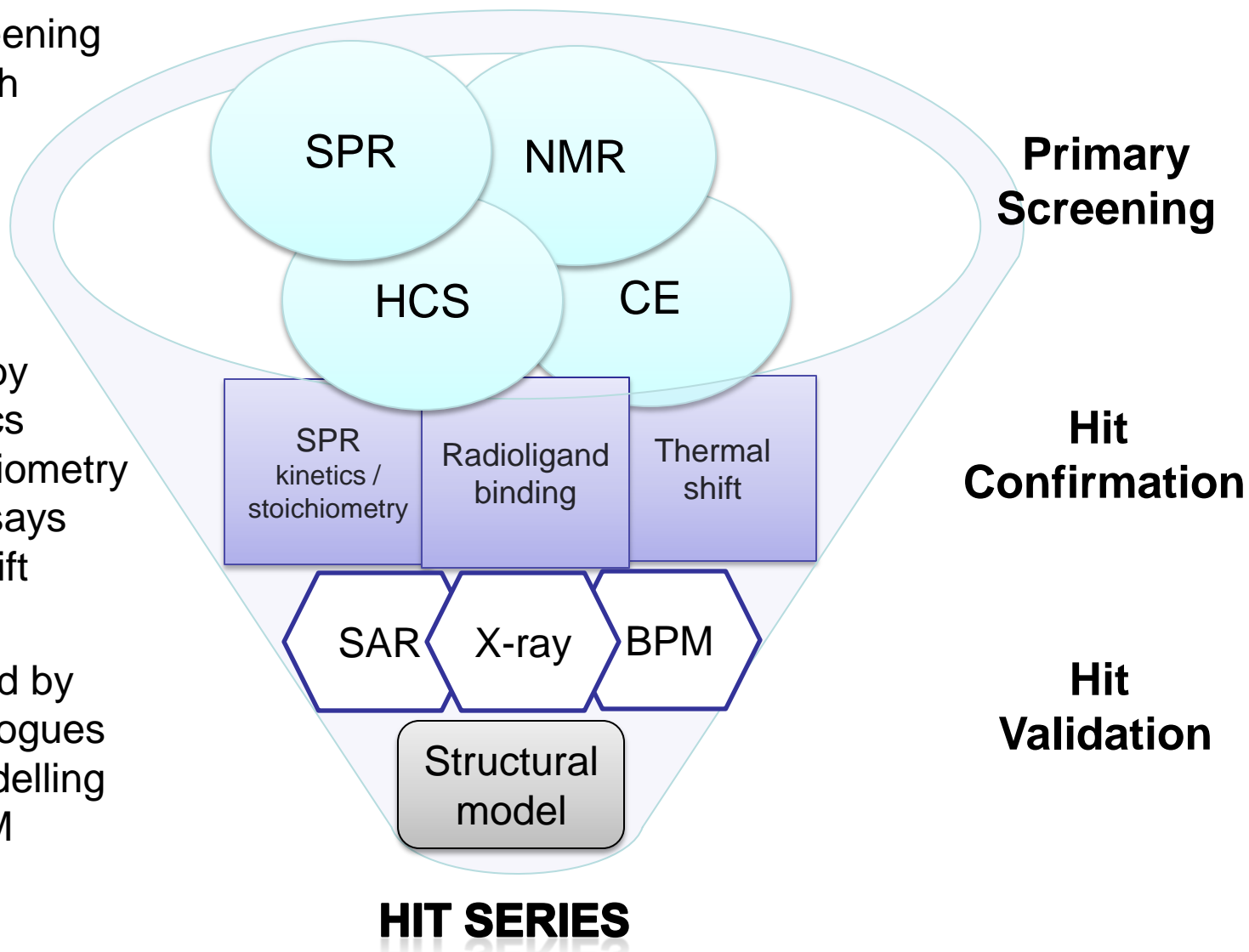
- SPR
- NMR
- HCS
- CE

Hits triaged by

- SPR kinetics
- SPR stoichiometry
- Binding assays
- Thermal shift

Hits validated by

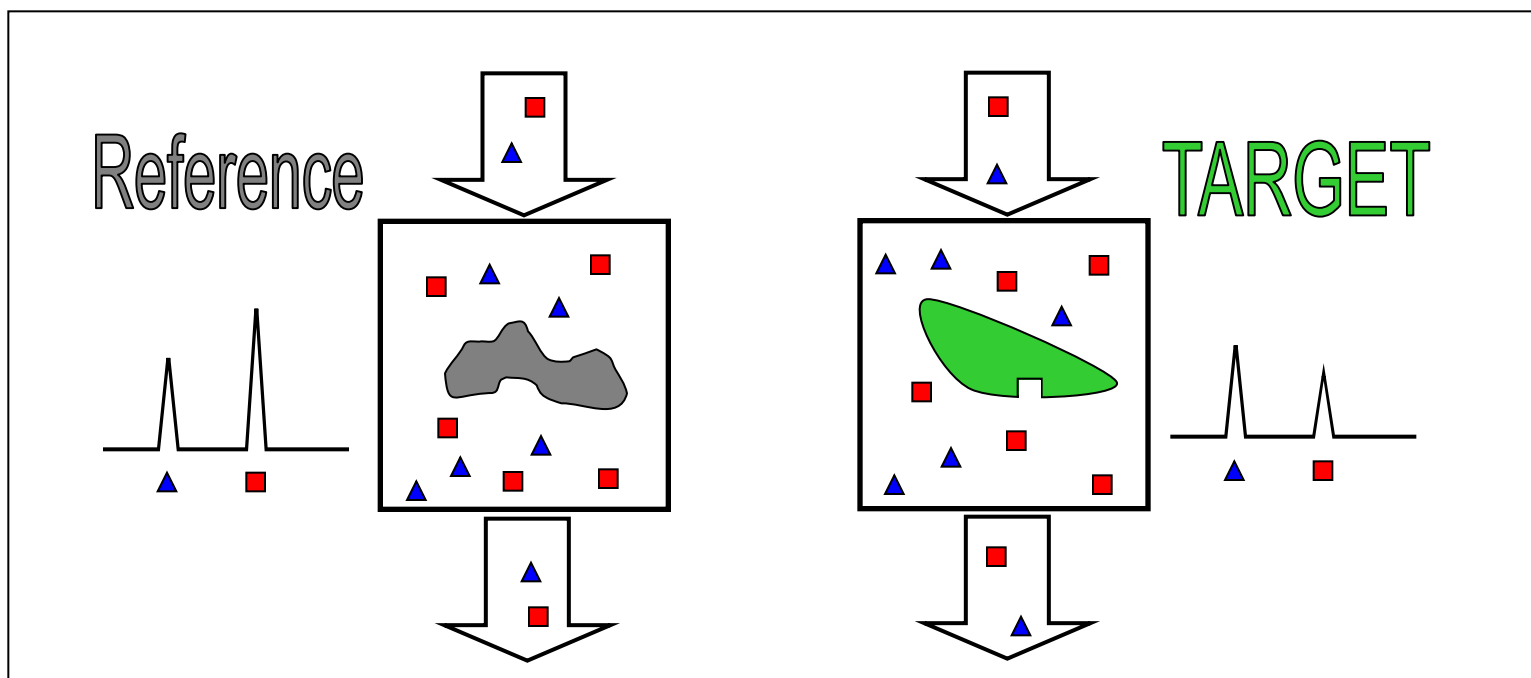
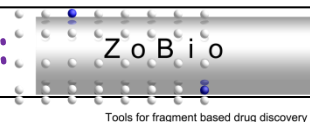
- SAR / analogues
- X-ray / modelling
- BPM / SDM



NMR Fragment Screening (with ZoBio)

- ➔ Immobilized protein – only small amounts needed (~1mg)
- ➔ Very sensitive method can detect very weak binding events

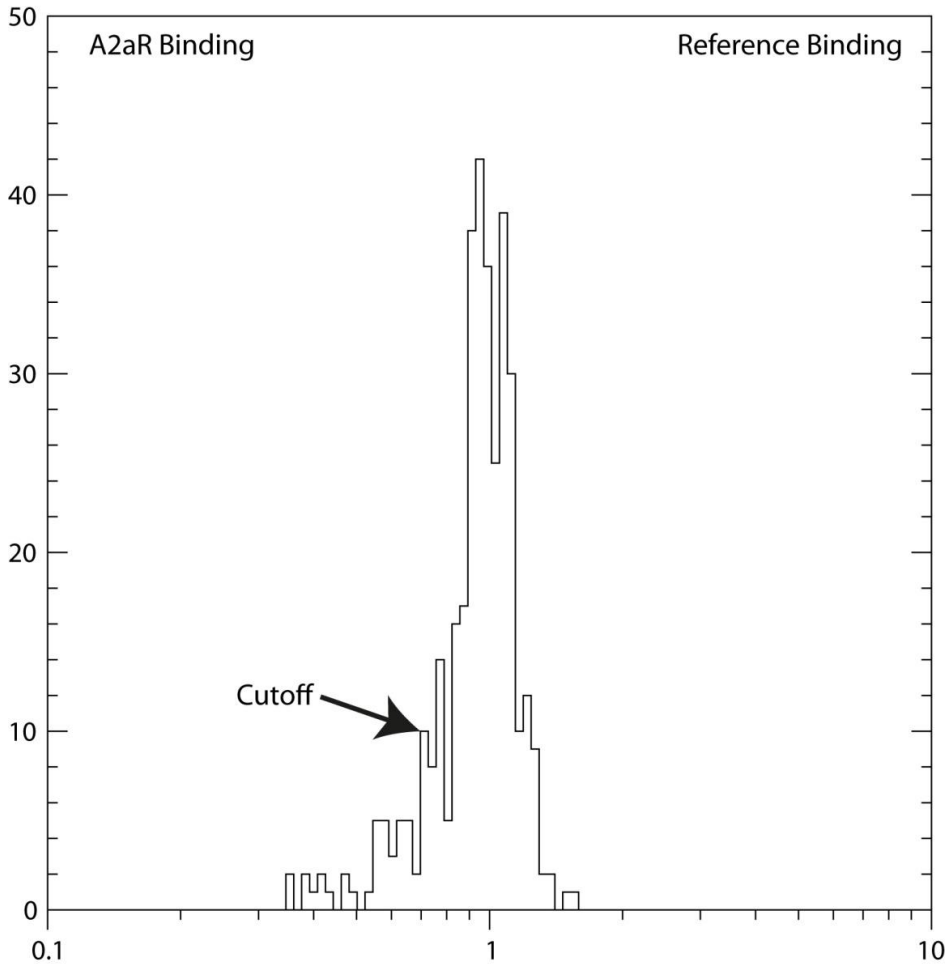
TINS = Target Immobilized NMR Screening



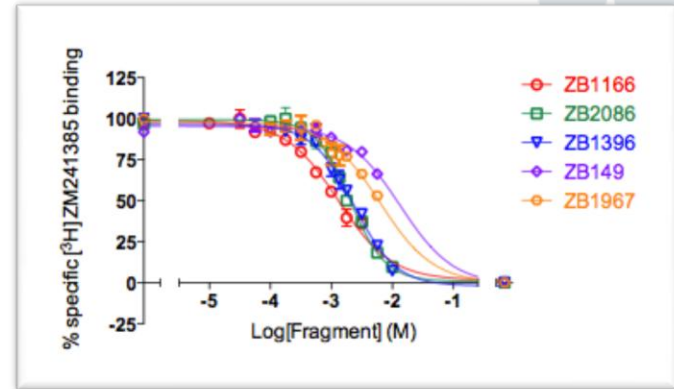
NMR Screening A_{2A}



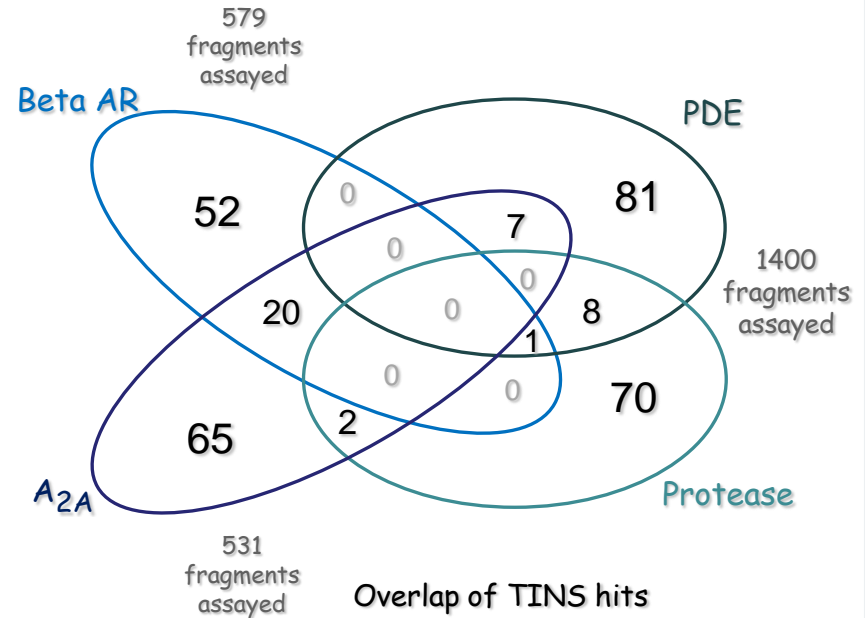
T/R distribution by compound



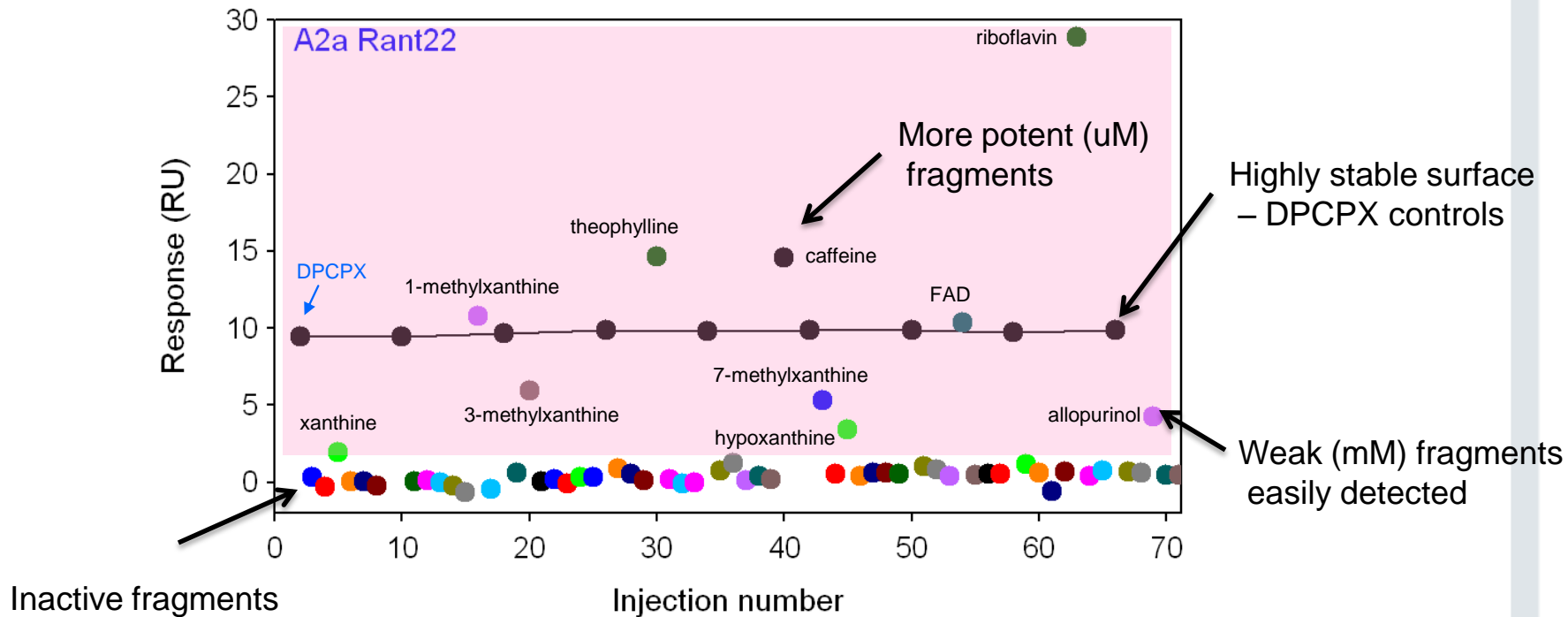
531 fragments assayed against A_{2A}
94 hits



Each hit assayed at 500 μM for ³H- ZM241385 displacement using wild type A_{2A}



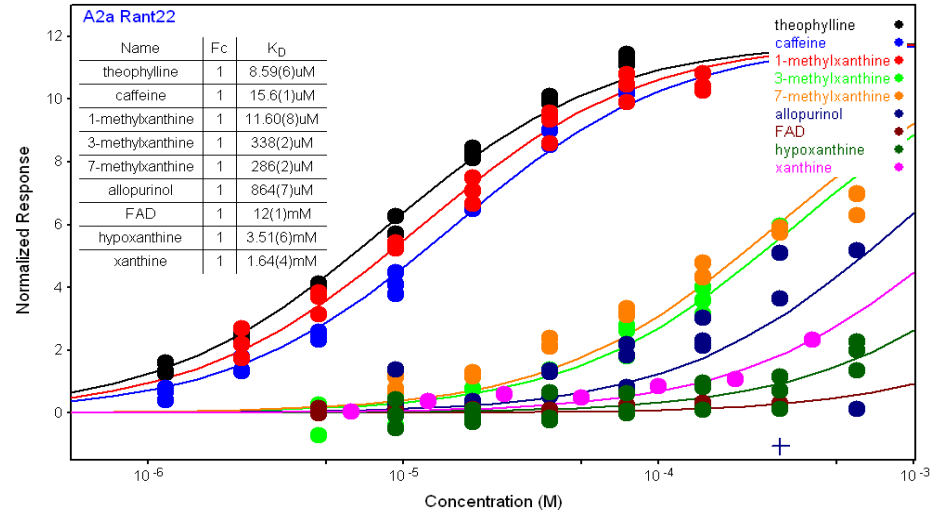
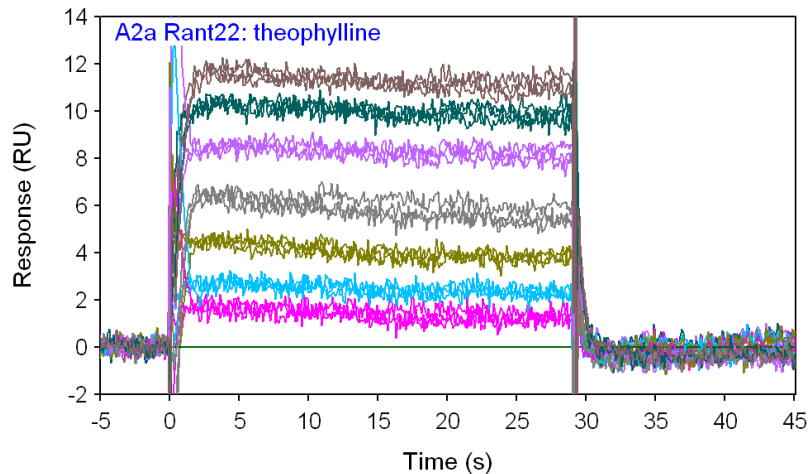
SPR Fragment Screening A_{2A} StaR (with David Myszka)



- Weakly binding fragments hits easily discriminated from inactives
- Xanthines added to library as likely binders
- Chip stable for days

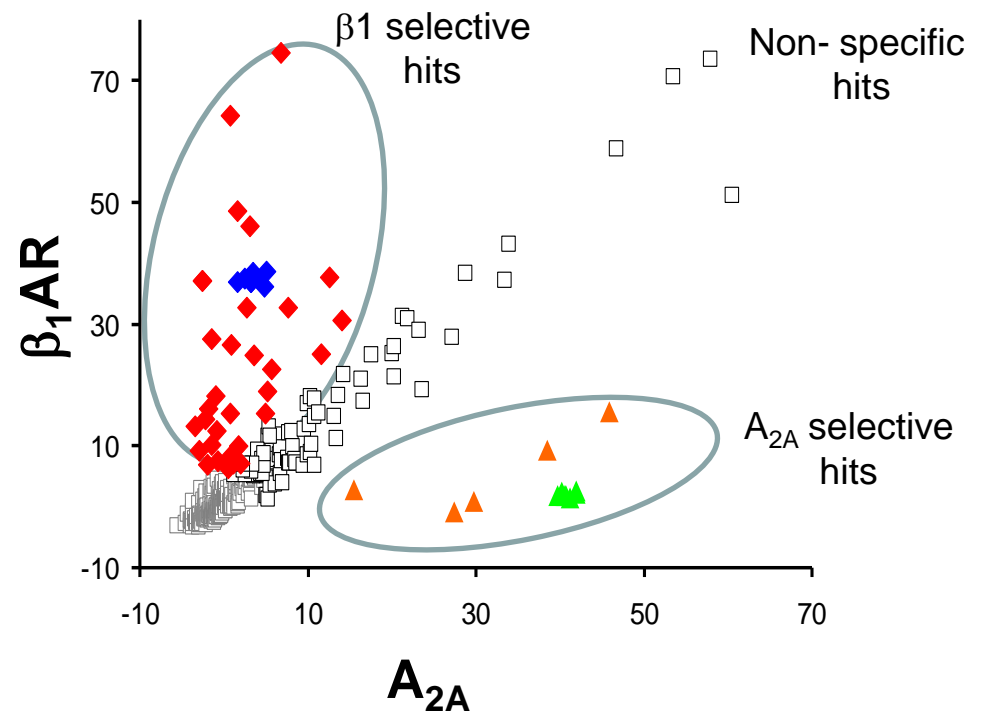
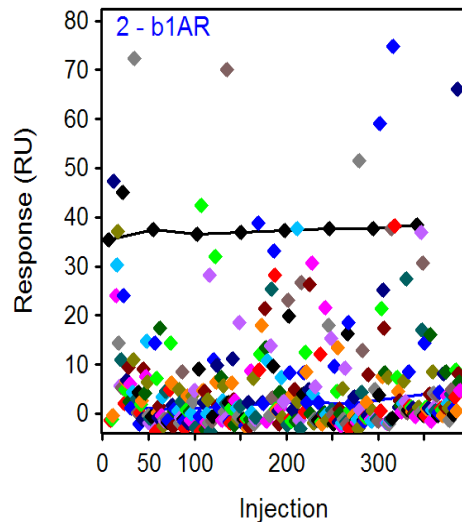
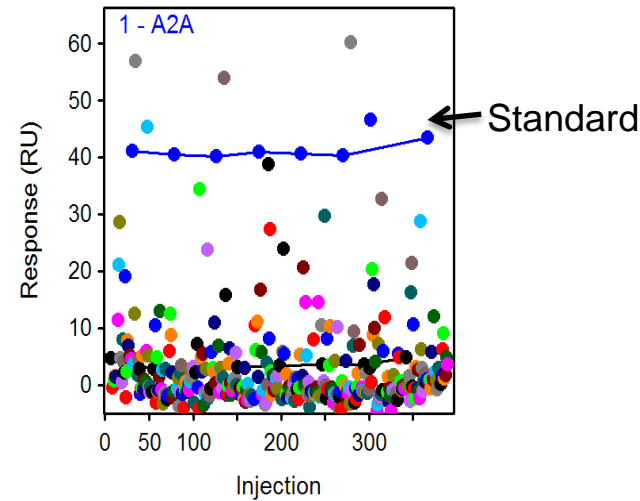
SPR fragment screening

Analysis of hits

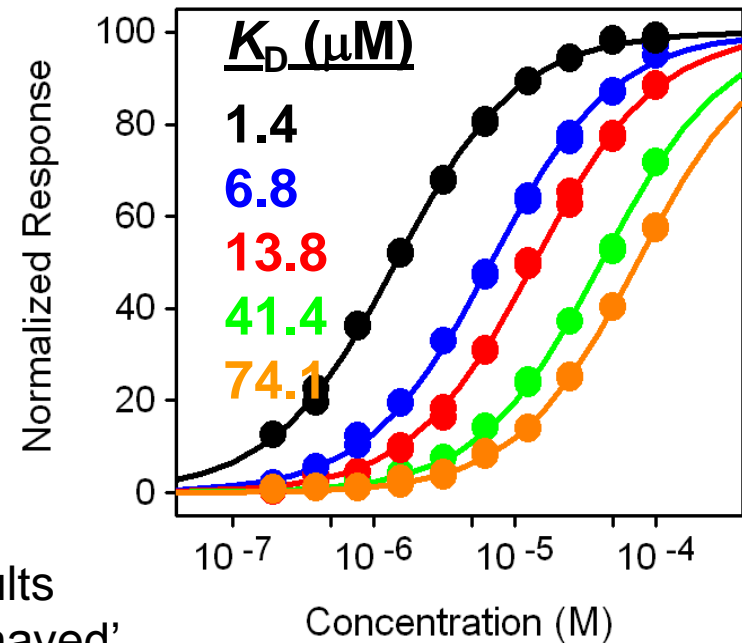
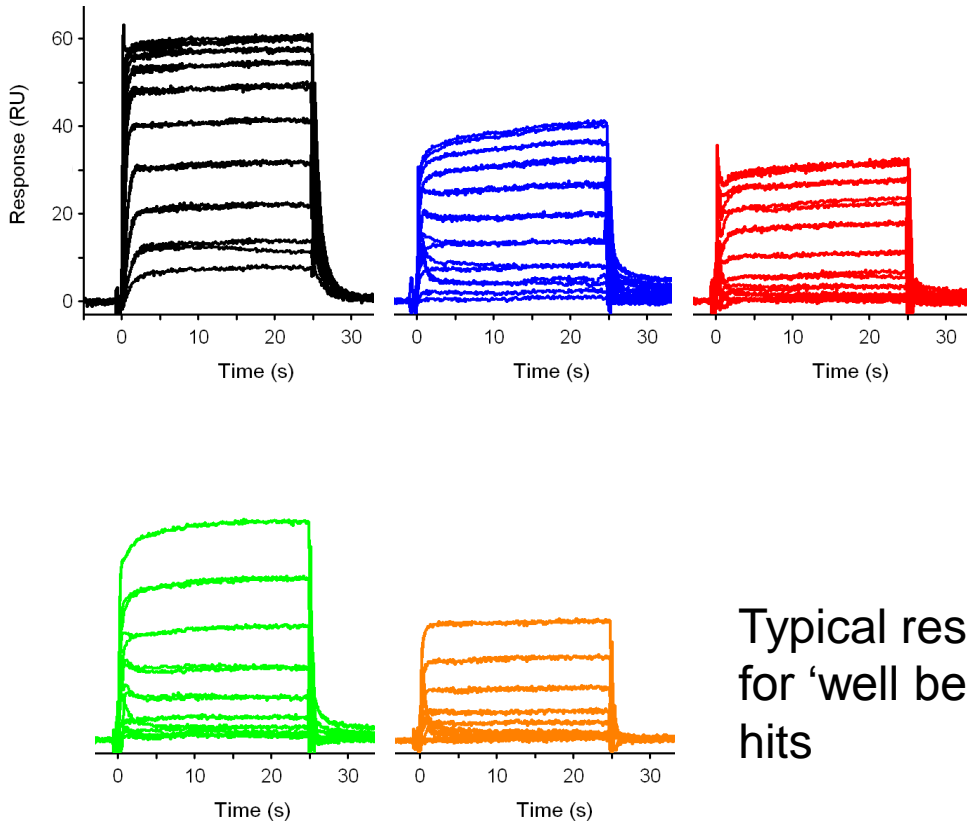


- Hits are further analysed by
 - Detection of non-specific binding on control surface (eg denatured protein)
 - Full concentration response curves
 - Assessment of kinetics – fragments should be fast off
 - Stoichiometry – fragments should ideally bind 1:1 with target

A_{2A} and β_1 AR SPR screen of Maybridge Fragment Library (500 compounds)

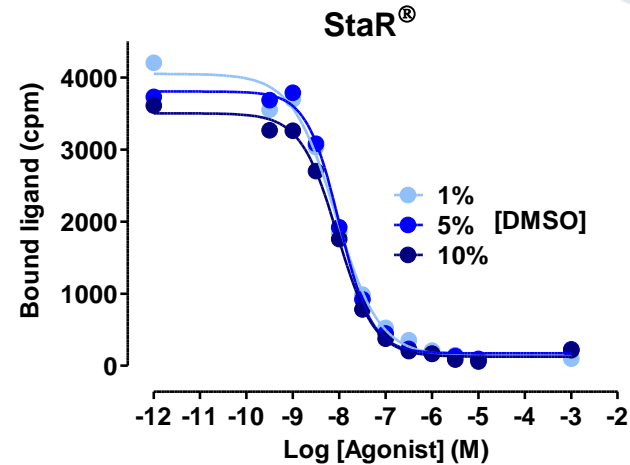
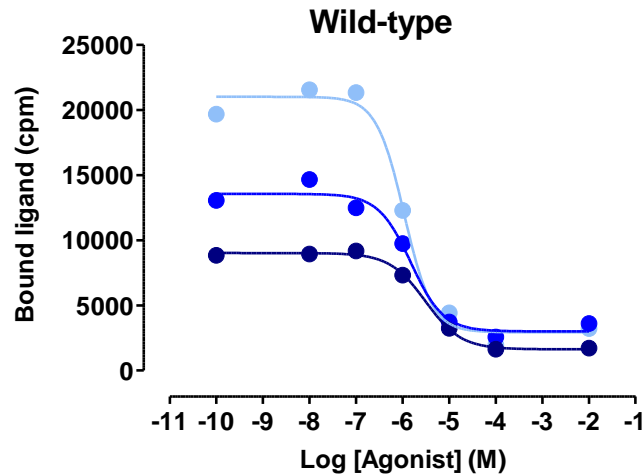


Example Hits from Maybridge Library vs β_1 AR

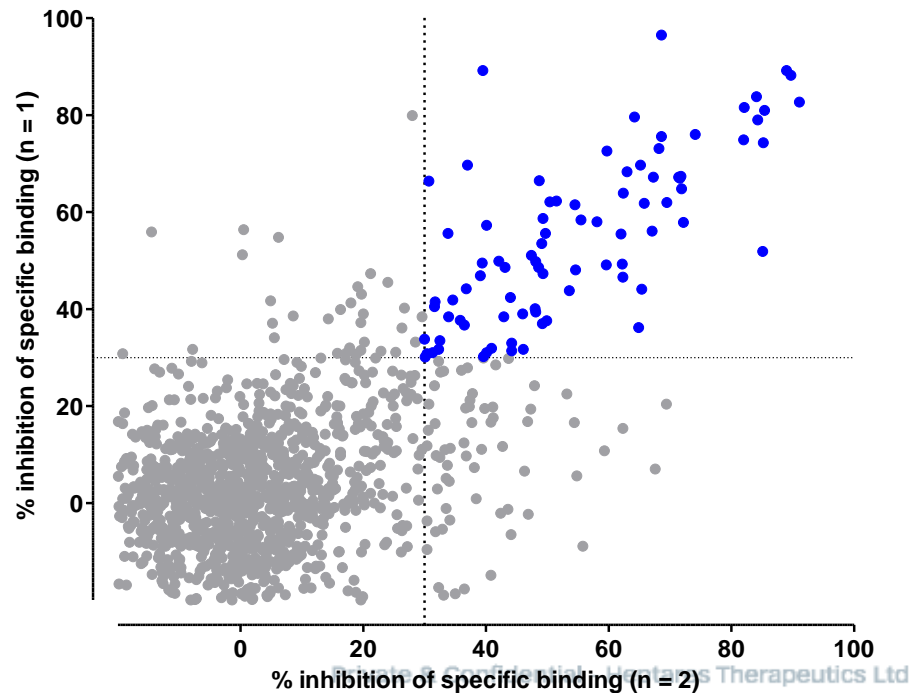


Typical results for 'well behaved' hits

High Concentration Screening Lipid Receptor Agonist StaR



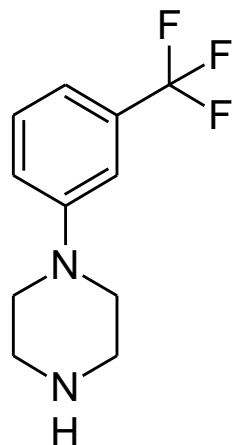
- Agonist StaR binds known agonists with higher affinity compared to wild-type receptor
- 10% DMSO has no effect on ligand-binding to StaR in membranes, unlike wild-type receptor
- Enables screening of fragment library at high concentration (100 μ M) which would be impossible with wild-type receptor
- Approx 6% hit rate (84 / 1419 fragments inhibit > 30% binding n = 2)



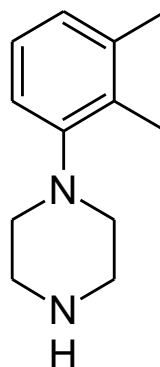
Family A Aminergic Receptor Antagonist Hit 2 Lead

- Identification of structurally related ligand efficient fragments
- Follow up gives clear SAR
- Rapid identification of potent hit series

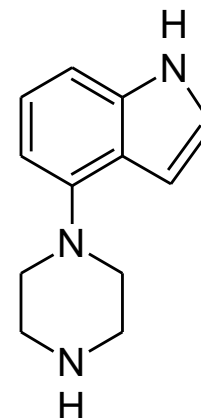
Docking and design in a protein-ligand binding model led to suggestion to introduce donor atom



Example fragment
 $IC_{50} = 16 \mu\text{M}$
(LE = 0.41; SPR)



Follow up 1st iteration
 $IC_{50} = 7 \mu\text{M}$
(LE = 0.5; binding assay)

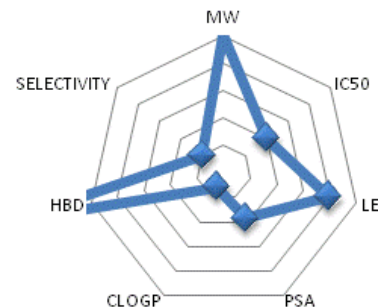
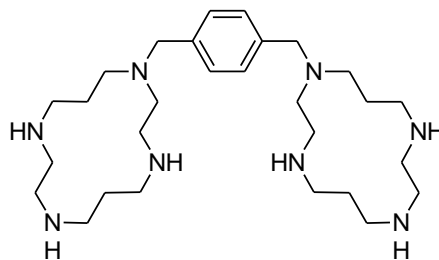


2nd iteration
 $IC_{50} = 58 \text{ nM}$
(LE = 0.66; binding assay)

CXCR4 Chemokine Receptor Antagonist

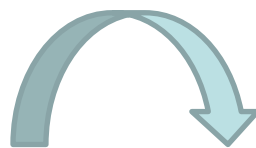
Hit 2 Lead

- 39/808 hits from diversity screening (4.8%)
- Follow up gives clear SAR
- Rapid identification of potent hit series



Standard compound

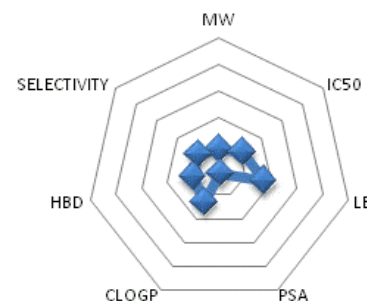
Example fragment hit



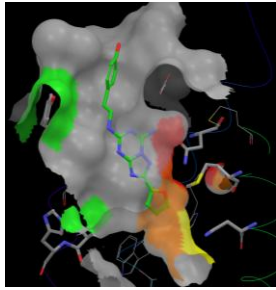
- $IC_{50} = 150 \mu M$ (LE = 0.47)
- Good solubility
- MWT 144, cLogP 1.3, PSA ~39

Hit Series

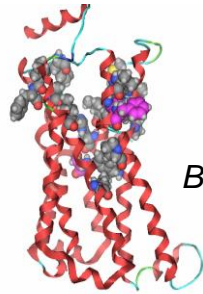
- $IC_{50} = 10 nM$ (LE = 0.34)
- Good solubility
- MWT ~300, cLogP 1.3, PSA ~76



StaR™ Based Drug Discovery

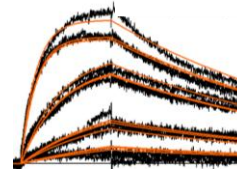


Biophysical Mapping™
Residues involved in
Ligand binding



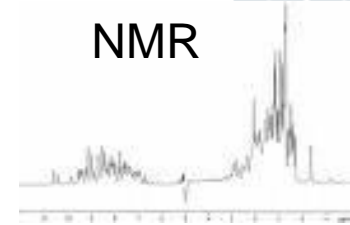
Refined homology
Model
Biophysical Mapping™
Virtual screening

Biacore



Fragment Screening
Kinetics

NMR

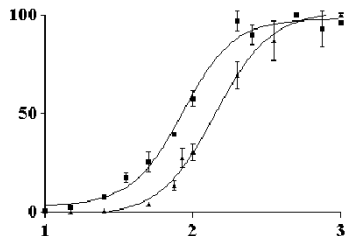


Fragment screening
Binding site definition

Ultra HTS



Pharmacology vs
Binding modes



Compound mechanism
of action

StaRs
Ag/Antag
Conformations

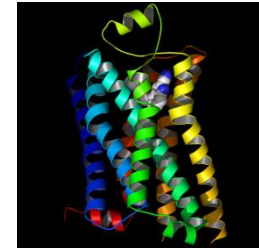
Wild-type: GGGCTCATAGGGTA-TTTCATGCGTGAGTT

Mutants:

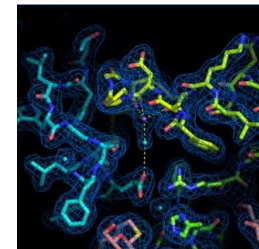
90-9	GGGCACATAGGGTA-TTTCATGCGTGAGTT
90-24	GGGCTTATAGGGTA-TTTCATGCGTGAGTT
90-40	GGGCTGATAGGGTA-TTTCATGCGTGAGTT
120-1	GGGCTGTAGGGTA-TTTCATGCGTGAGTT
90-37	GGGCTCTAGGGTA-TTTCATGCGTGAGTT
90-21	GGGCTACGGTA-TTTCATGCGTGAGTT
90-2	GGGCTCAGGGTA-TTTCATGCGTGAGTT
120-22	GGGCTCAAAGGGTA-TTTCATGCGTGAGTT
90-43	GGGCTATGGGTA-TTTCATGCGTGAGTT
90-1	GGGCTATAGGTA-TTTCATGCGTGAGTT
90-46	GGGCTATAGG-TA-TTTCATGCGTGAGTT
120-17	GGGCTATAGGGATTTTTCATGCGTGAGTT
90-31	GGGCTCATGGGT-TTTCATGCGTGAGTT
90-41	GGGCTCATAGGGTA-TTTCATGCGTGAGTT
90-45	GGGCTATAGGGTA-TTTCATGCGTGAGTT
90-3	GGGCTCATAGGGTA-TTTCATGCGTGAGTT
120-10	GGGCTATAGGGTA-TTTC-ATGCGTGAGTT
120-9	GGGCTCATAGGGTA-TTTCATGCGTGAGTT
90-35	GGGCTCATAGGGTA-TTTCATGCGTGAGTT
120-19	GGGCTCATAGGGTA-TTTCATGCGTGAGTT
120-30	GGGCTCATAGGGTA-TTTCATGCGTGAGTT
90-39	GGGCTCATAGGGTA-TTTCATGCGTGAGTT

Mutagenesis data
Binding site mutants

X-ray structure



Docking
Virtual screening



Ligand co-structures
Binding site definition

Heptares

Molecular Biology
Protein Sciences
Structure Group
Pharmacology
Chemistry
Modelling
Management

LMB

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Gebhard Schertler
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ZoBio

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