HEPTARES
therapeutics

Enabling fragment-based lead discovery & structure-based design for GPCRs using stabilized receptor (StaR®) technology

Jonathan S Mason

Overview

- GPCRs the largest drug-target gene family
  - 50 well validated but poorly tractable current Pharma targets
  - Instability of isolated GPCRs major obstacle to drug discovery
- Integrated GPCR Drug Discovery Engine based on stabilised receptor (StaR®) technology overcomes this issue
- $33M Series A fund raise completed Feb 2009
- Focus on internal drug discovery pipeline
- $200M deal on single non-pipeline target with Novartis
- Scope for additional, broad-based strategic alliance

GPCR Drug Discovery

Pharma HTS success rate only 1:10

100 GPCRs screened in HTS → 50 GPCR Hits → 25 GPCR leads → 10 GPCR candidates

- 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

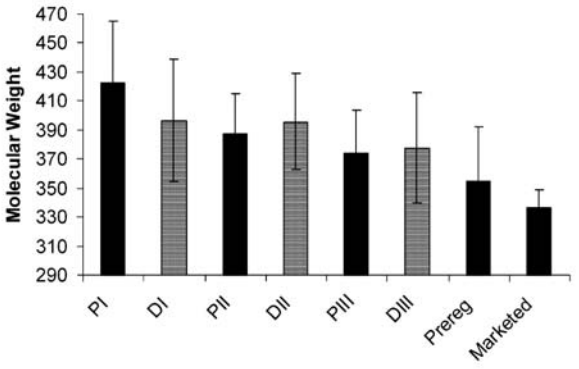


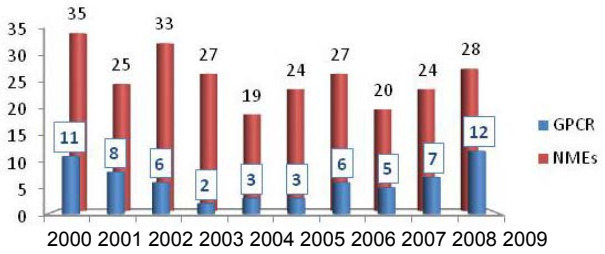
Figure 3. Mean molecular weight for drugs in different phases.

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

GPCR Drug Launches

- 24% of launched drugs in the last decade hit GPCRs
- This is 63 NMEs
- The numbers of launched GPCRs has actually increased in the last few years
- However only about 1 new GPCR is drugged per year
- Many drugs are 'me-too' or have spectrums of activity vs multiple previously drugged receptors
- There have been multiple phase 3 failures in the last 2 years for new MoAs

GPCR Drugs Launched compared with all NMEs

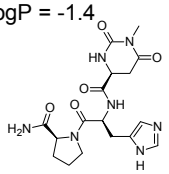


GPCR Drugs Launched in 2009

Nuvigil	armodafinil	α1-adrenoceptor agonist
Saphris	asenapine	poly-pharmacology monoamine receptors
Firmagon	degarelix acetate	GnRH antagonist
Fanapt	lloperidone	D2/D3/α2c/5HT1A/5HT6
Onbrez Breezhaler	indacaterol	β, agonist
Victoza	liraglutide	GLP1 agonist
Remitch	nalfurafine HCl	κ-opioid
Mozobil	plerixafor	CXCR4
Talion	bepotastine	H1 antagonist
Effient	prasugrel	P2Y12 antagonist
Nucynta	tapentadol	MOR agonist (and noradrenaline reuptake inh)
Samsca	tolvaptan	vasopressin V2 antagonist

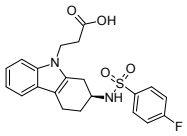
Taltirelin (2000)

TRH receptor agonist / oral
MWT = 405
cLogP = -1.4



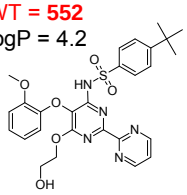
Ramatroban (2000)

Thromboxane receptor antagonist
DP_2 antagonist / oral
MWT = 416
cLogP = 4.0



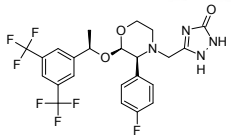
Bosentan (2001)

Endothelin receptor antagonist
(ET_A / ET_B) / oral
MWT = **552**
cLogP = 4.2



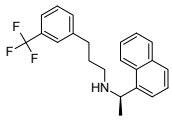
Aprepitant (2003)

NK_1 antagonist / oral
MWT = **534**
cLogP = 4.8



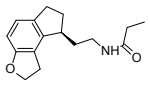
Cinacalcet (2004)

Calcium-sensing receptor allosteric modulator / oral
MWT = 357
cLogP = **6.4**



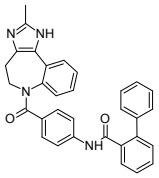
Ramelteon (2005)

$MT_{1/2}$ agonist /oral
MWT = 259
cLogP = 2.5



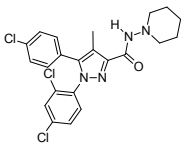
Conivaptan (2005)

V_{1A}/V_2 antagonist / IV
MWT = 499
cLogP = **5.0**



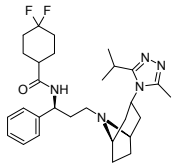
Rimonabant (2006)

CB_1 inverse agonist / oral
Now withdrawn
MWT = 464
cLogP = **6.5**



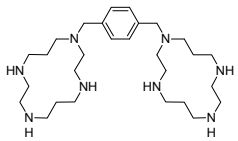
Maraviroc (2007)

CCR5 antagonist / oral
MWT = **514**
cLogP = **3.3**



Plerixafor (2009)

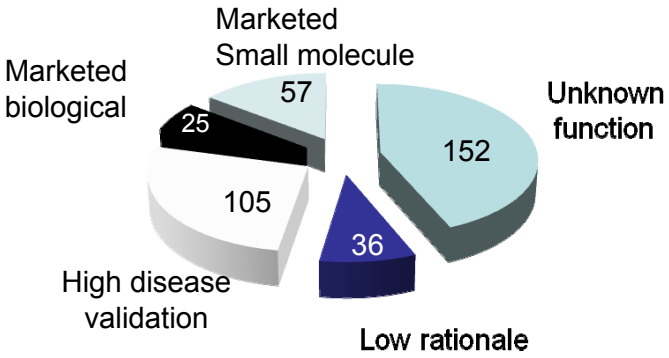
CXCR4 antagonist / SC
MWT = **502**
cLogP = -0.2



Biological Agents

Atosiban	V_{1A}/oxytocin	(2000)
Ganirelix	GnRH	(2000)
Exenatide	GLP1	(2005)
Icatibant	B_2	(2009)

Intractable GPCR Targets



Lack of Selectivity

Muscarinic M1. M4

Serotonin receptors

Dopamine D1

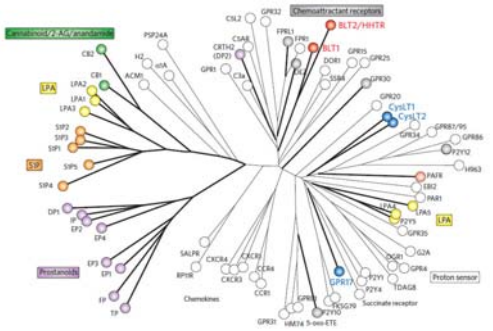
Poor/limited chemistry

Many Chemokines
CRF1
MC4
Lipid receptors/fatty acid
TGR5
Orexin
Neuropeptides
Complement C3a,C5a
CGRP
GnRH
mGluRs

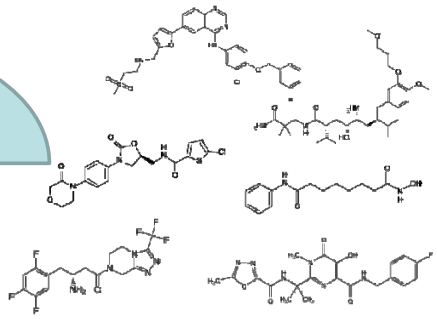
No drug-like molecules

GLP1
PTH
Ghrelin
VIP
Glucagon
PARs
FSH
Bradykinin

StaRs® are a bridge between established discovery paradigms



StaR® Technology



GPCRs

Proven Target Superfamily

Structure-based Drug Design

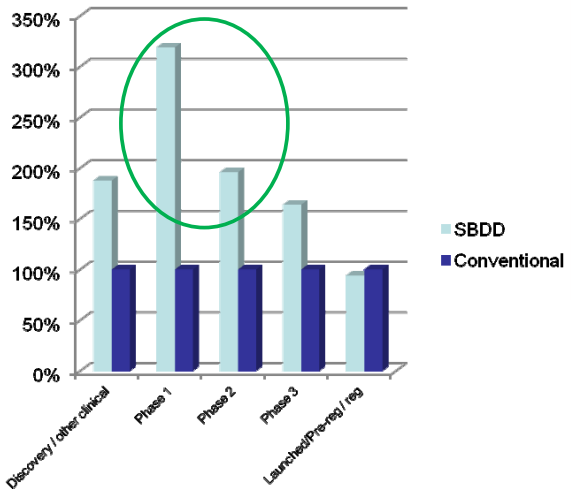
Proven Technology

Advantages of SBDD over Empirical Lead Optimisation

SBDD targets out perform

GPCR targets in terms of numbers of clinical compounds and smaller numbers of discontinued projects

- 3 times the success rate of agents in Phase 1 for SBDD vs GPCR
- Higher numbers of agents in P3 and pre-registration (28 vs 12)
- 70% GPCR projects discontinued vs 43% SBDD



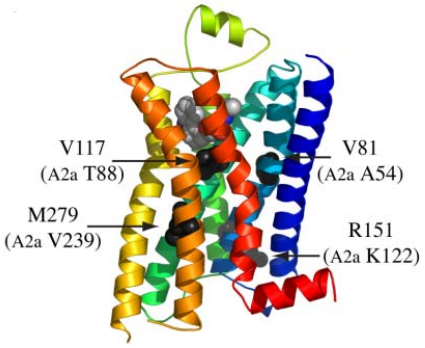
2 carefully matched sets of 10 targets, SBDD vs GPCRs

- **+/- Same number of launched drugs for both**
- Clinically validated MOA
- Industry 'hot' targets
- Large data set (Thomson Pharma

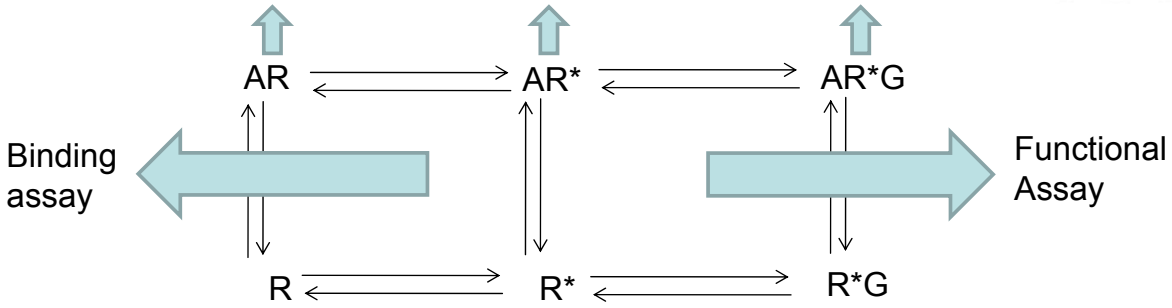
n=1095)

What is a StaR®?

- A GPCR containing a small number of point mutations that greatly improve its thermostability
  - Stable in purified, detergent solubilised form
  - Functional and drug-binding characteristics preserved
  - Trapped in relevant conformation that matches drug Product Profile
  - Patent protected technology
  - Suitable for uHTS, Biacore (kinetics), crystallisation etc.
  - Transferrable across GPCR superfamily

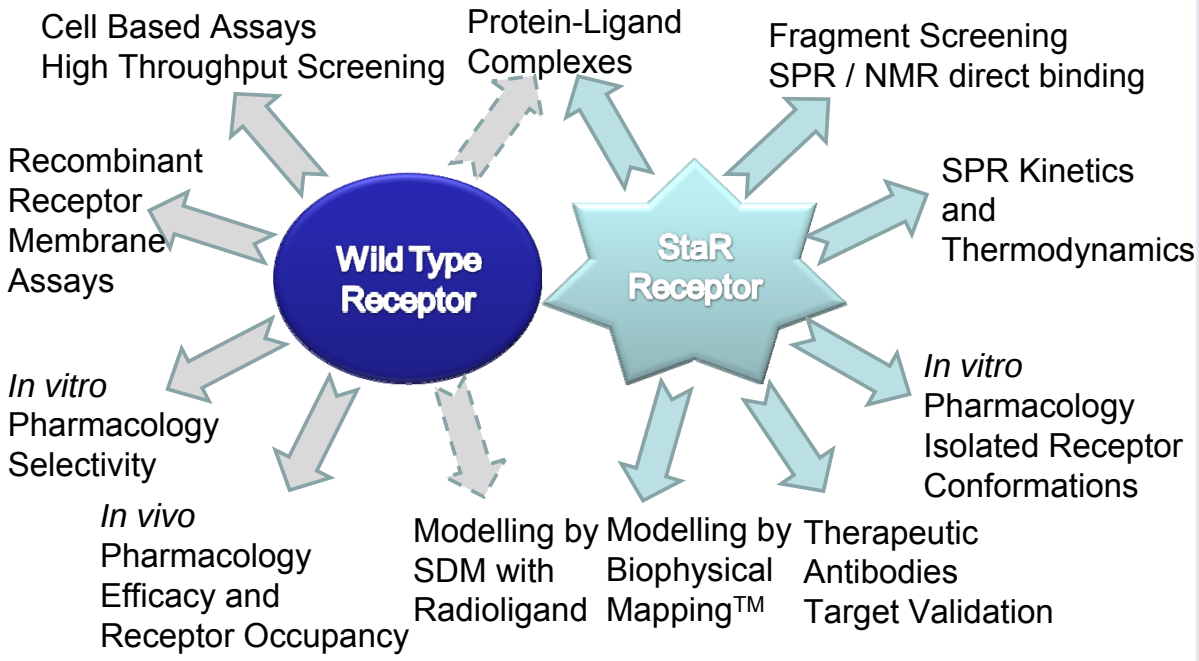


Heptares StaR® Technology



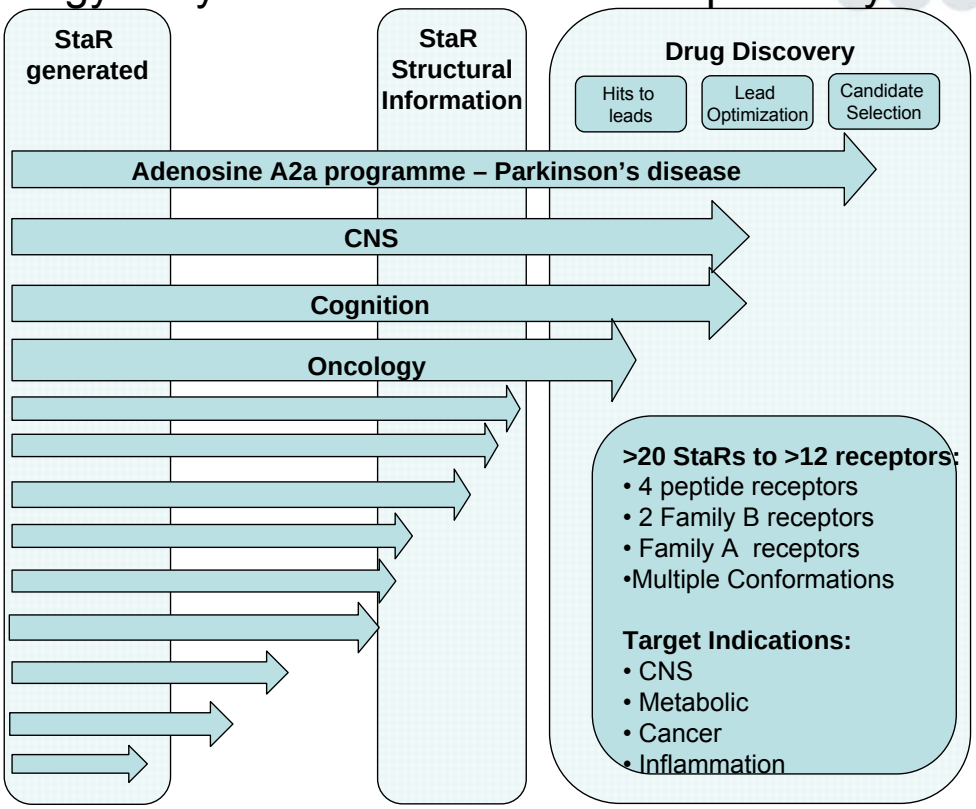
- Receptors embedded in cell membrane exist in multiple conformations
  - Highly unstable when removed
  - Not suitable for structure based drug discovery methods
- Heptares' technology is used to make a stabilized versions of target GPCRs (StaRs) held in a specific chosen conformation
  - Stable in 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

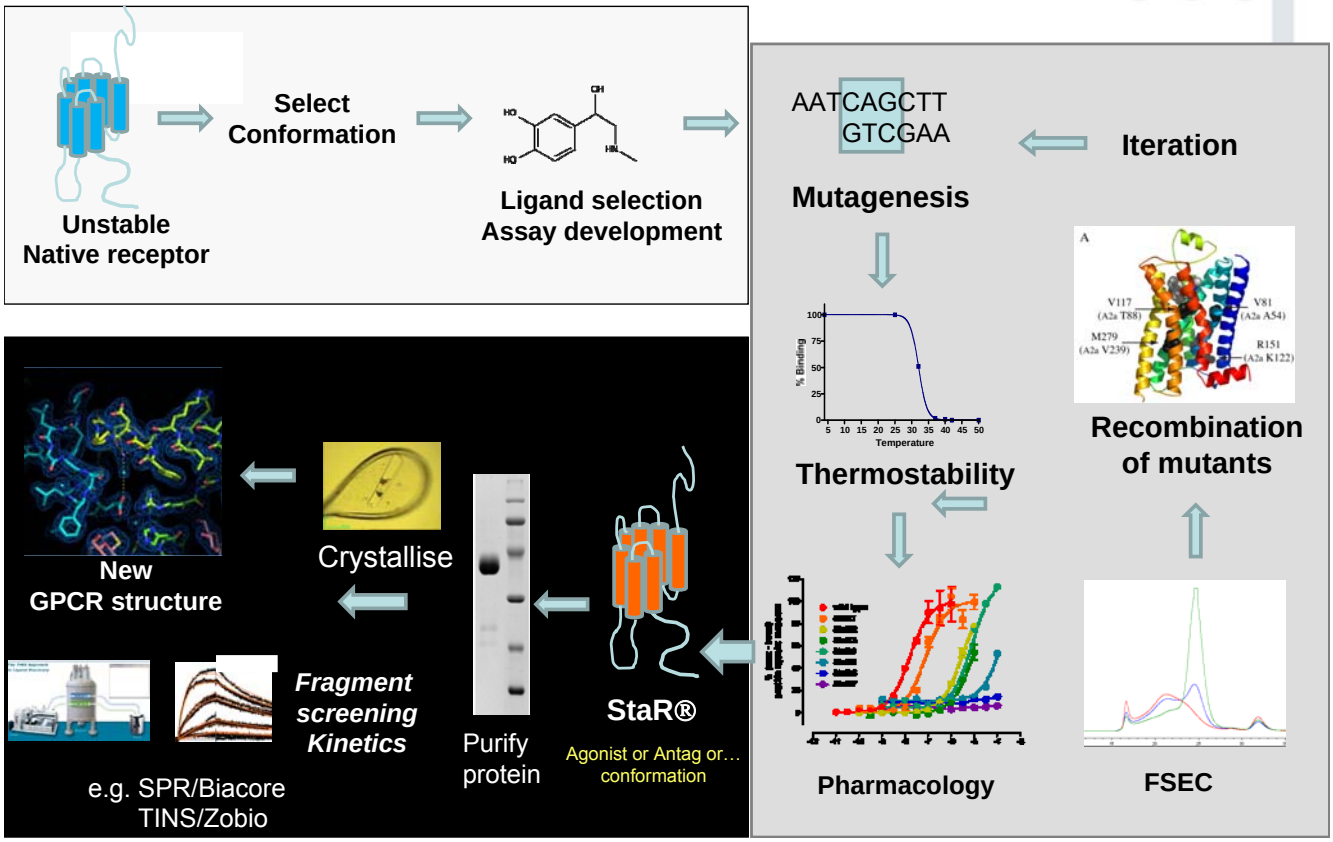


Rapid Pipeline Progress Since Series A

Technology Fully Transferrable Across Superfamily



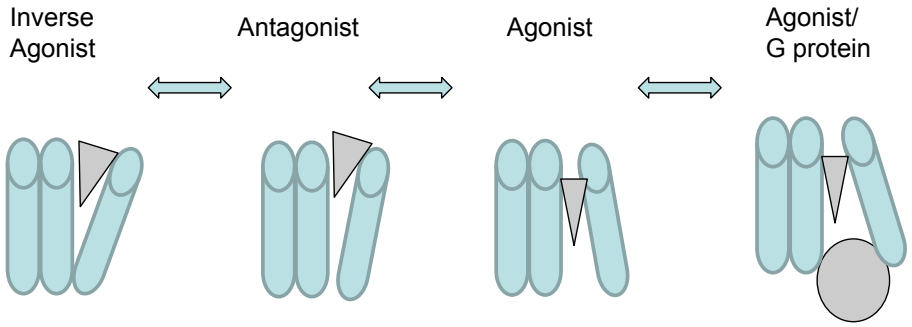
Proprietary Process for Creating StaRs



Types of StaRs

StaR proteins are locked in the conformation derived from the pharmacology of the ligand used in their creation

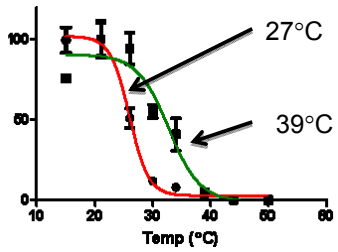
Types of StaRs



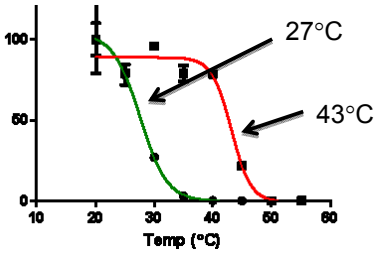
	Inverse Agonist	Antagonist	Agonist	Agonist/ G protein
Agonist affinity	Low	Low	High	High
Antagonist affinity	High	High	Low	Low
Signalling	N/Y (high agonist)	Y (high agonist)	Partial or N	Y
Ionic lock	Yes	Partial	Broken	Broken

StaRs give a general approach to thermostabilisation

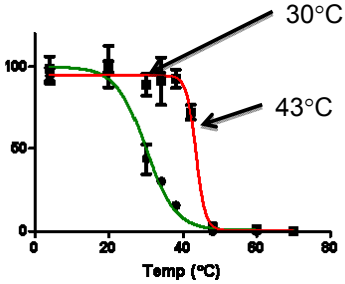
Family B



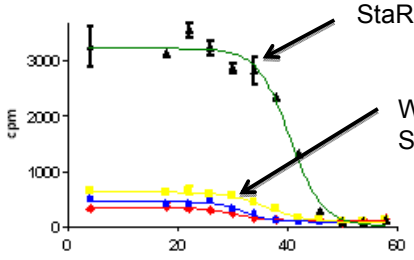
Family A – solubilised (normalised data)



Chemokine receptor

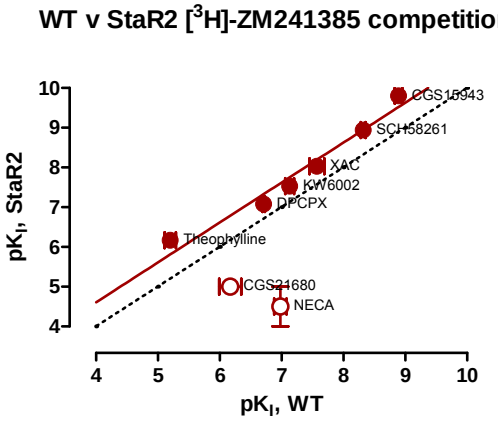


Family A peptide - purified (raw data shows higher yield of functional protein)



Pharmacology correlates with the isolated conformation

- Inverse agonist StaR shows excellent correlation to wild-type for binding of antagonists / inverse agonists from a range of chemical classes
- **Indicates antagonist binding site is unaltered**
- Improved affinity for StaR due to inverse agonist conformational trapping
- **Conformation specific to pharmacological class not chemotype**

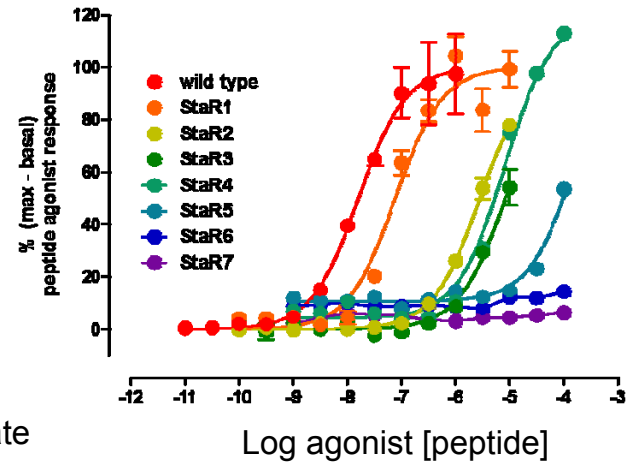


StaRs show increasing conformational selection during optimisation

Antagonist StaR Generation Process

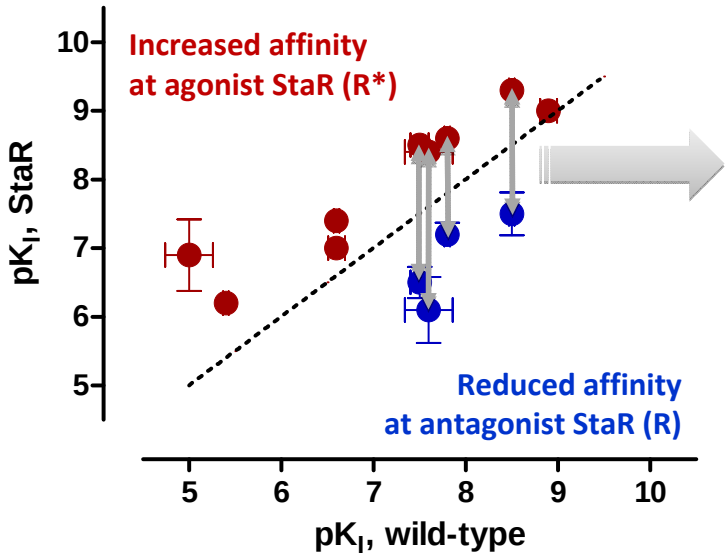
- Thermostability
- Number of mutations
- Conformational selection
- Shift in equilibrium to ground state
- Agonist potency

Family A peptide receptor StaR signalling



Isolating R and R* conformations of GPCRs

- StaRs of different conformations of the *same receptor* highlight ability to screen for desired pharmacology using a binding assay
- Agonist affinities at Family A **agonist** and **antagonist** StaRs
- Useful for screening for specific pharmacologies



Does the difference in affinity at **agonist** and **antagonist** StaR represent efficacy ?

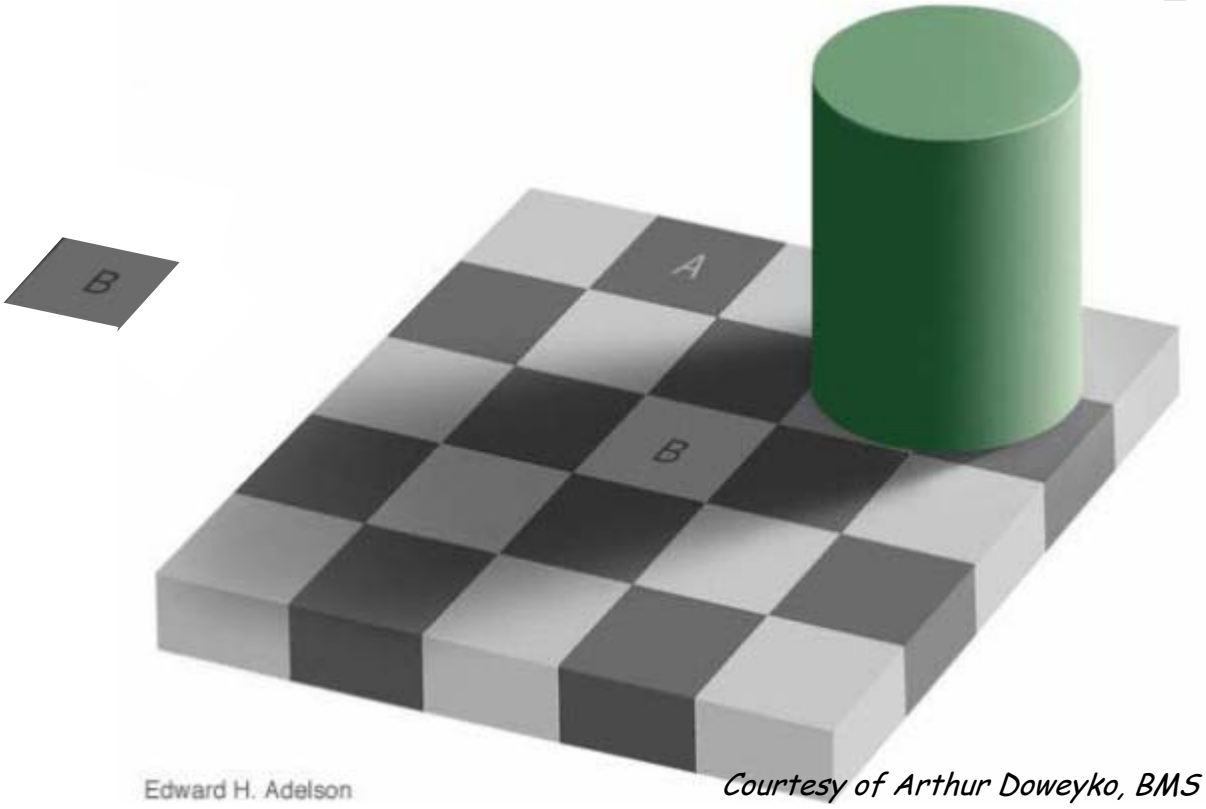
Understanding GPCR Pharmacology

➔ First remove the immense bias and potential "force fitting" we have when only ligand structures are known

➔ A real issue for the key drug class of GPCRs

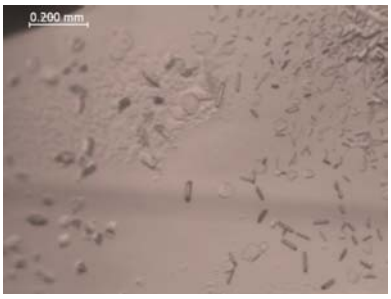
… Until we could stabilize them in antagonist/agonist/… conformations and do
X-ray structures with ligands / fragments
& biophysical (fragment) screening
& binding site mapping (using stabilized mutant structures)

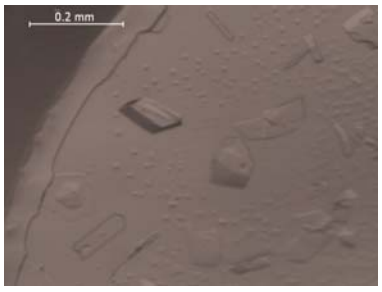
Need to be careful about biases in how we see data – based only on ligands?



Courtesy of Arthur Doweyko, BMS

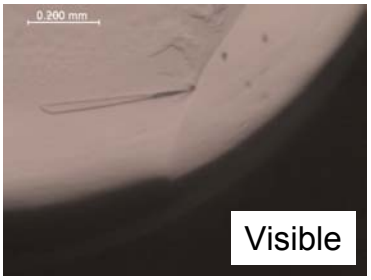
A_{2A} StaR Crystallography
Conventional Detergents/Vapour Diffusion

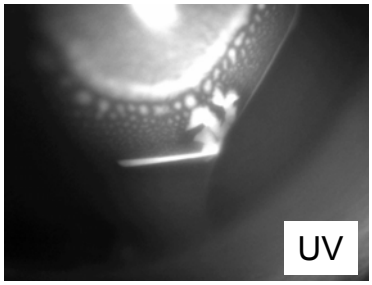




Wide range of crystals in 5 different detergents

Crystals up to 0.5mm in size



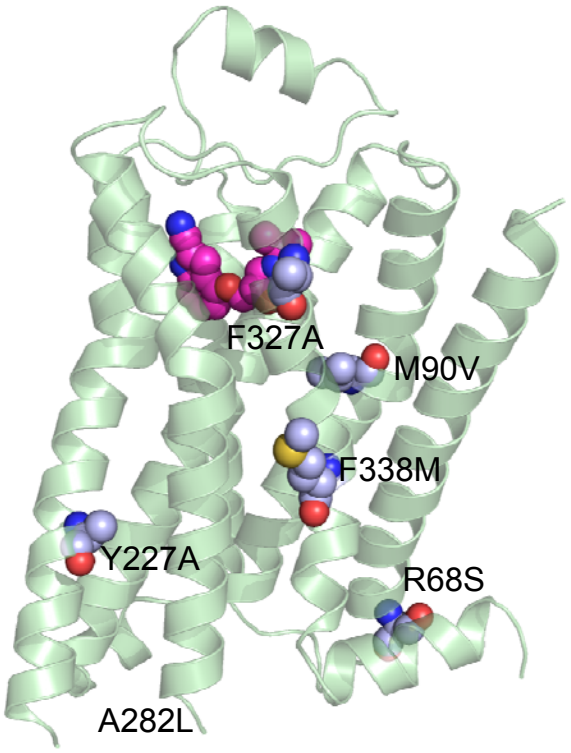


Heptares A_{2A} crystal structure solved from single crystal

8 co-structures, wide range of potency and size (10nM to 20uM, 2-600 Da)

Greater stability => better quality protein, reduced flexibility => better crystals

Beta-1 Adrenoceptor (β_1 AR) StaR X-ray Structure
Collaboration with LMB



Entrance to ligand binding site well defined – **high resolution**

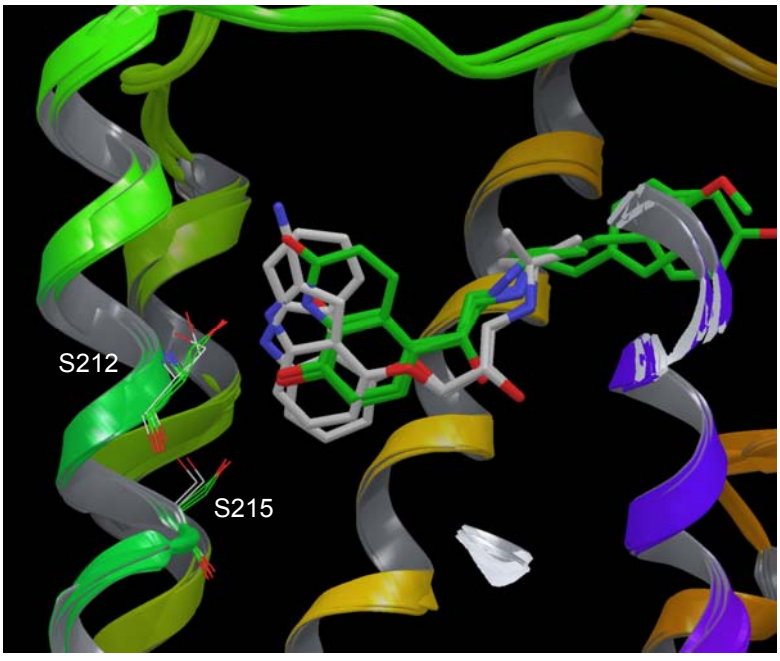
9 drug **co-crystal** structures now solved in detergent
Agonists and **Antagonists**
Low and **High** Affinity

Activation and G-protein binding region retained
Multiple **loop conformations** resolved cf biased agonism

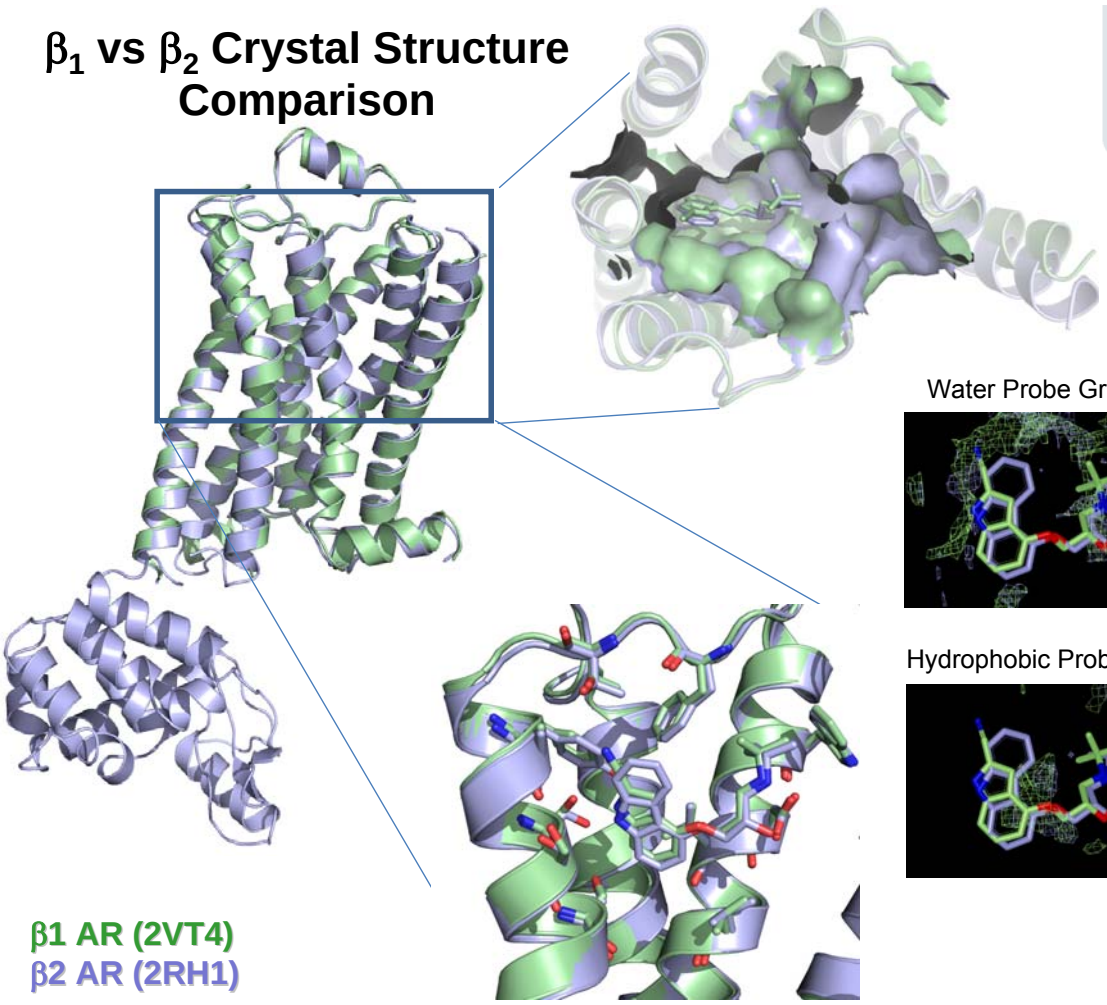
β_1 AR agonists & antagonists cluster into different binding modes

- Agonist ligands
  - green carbons
- Antagonists ligands
  - light carbons

Significant changes in ligand position, hydrogen bonding, backbone and side-chains observed



β_1 vs β_2 Crystal Structure Comparison



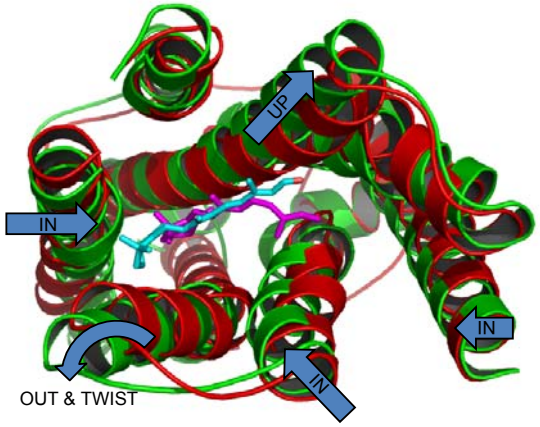
β1 AR (2VT4)
β2 AR (2RH1)

Water Probe Grid Map

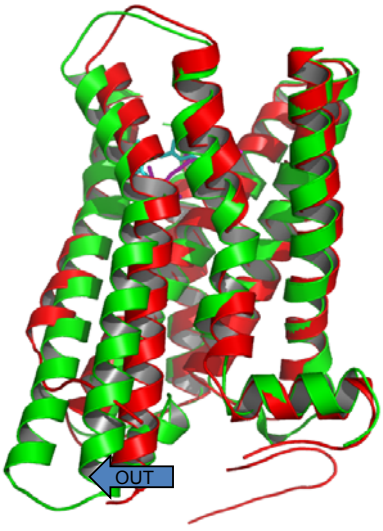
Hydrophobic Probe Grid Map

rhodopsin agonist v's antagonist 2X72 c.f. 1HZX

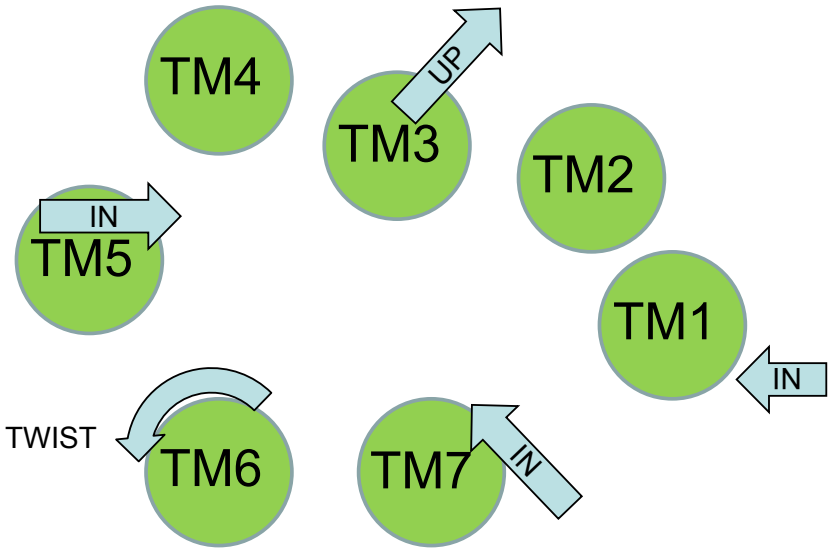
top



side

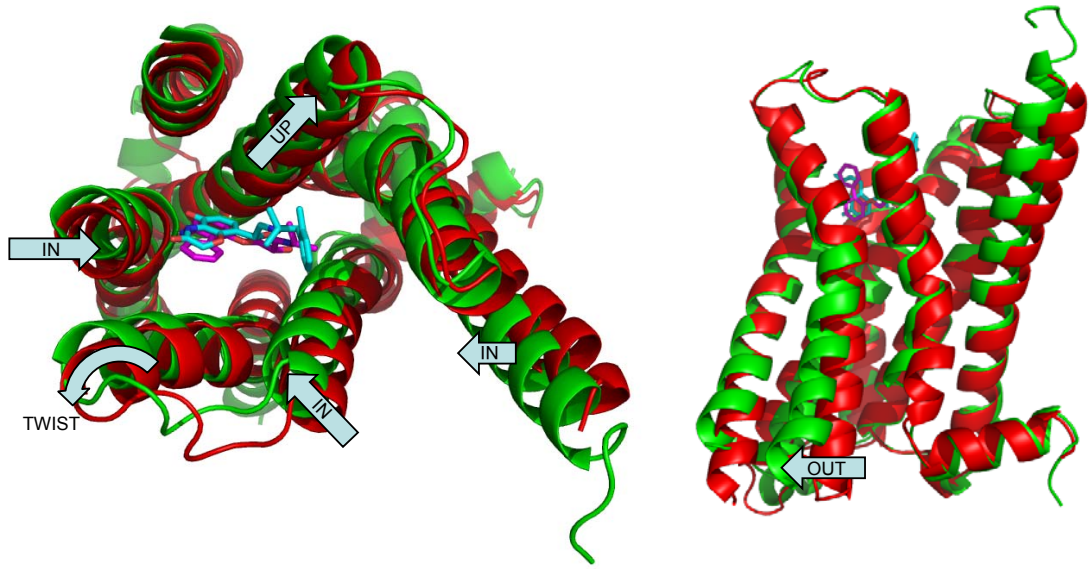


What causes activation?

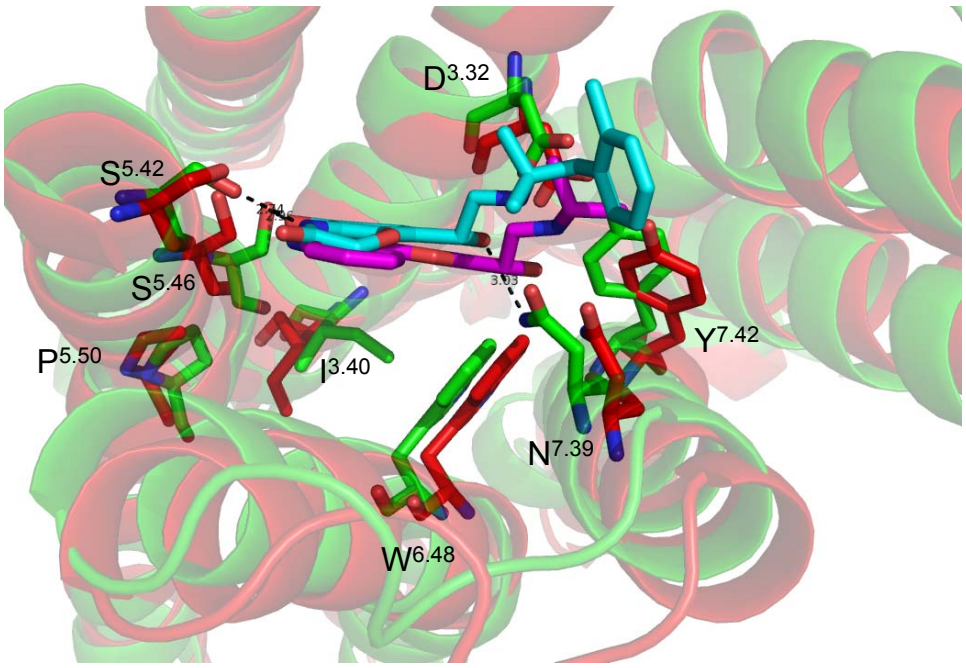


β2 agonist v's antagonist
3POG c.f. 2RH1

top **side**

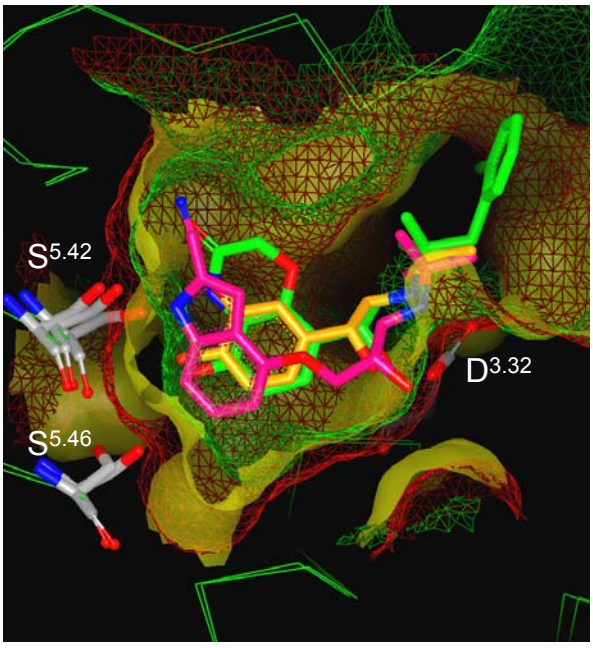


β2 agonist v's antagonist
3POG c.f. 2RH1

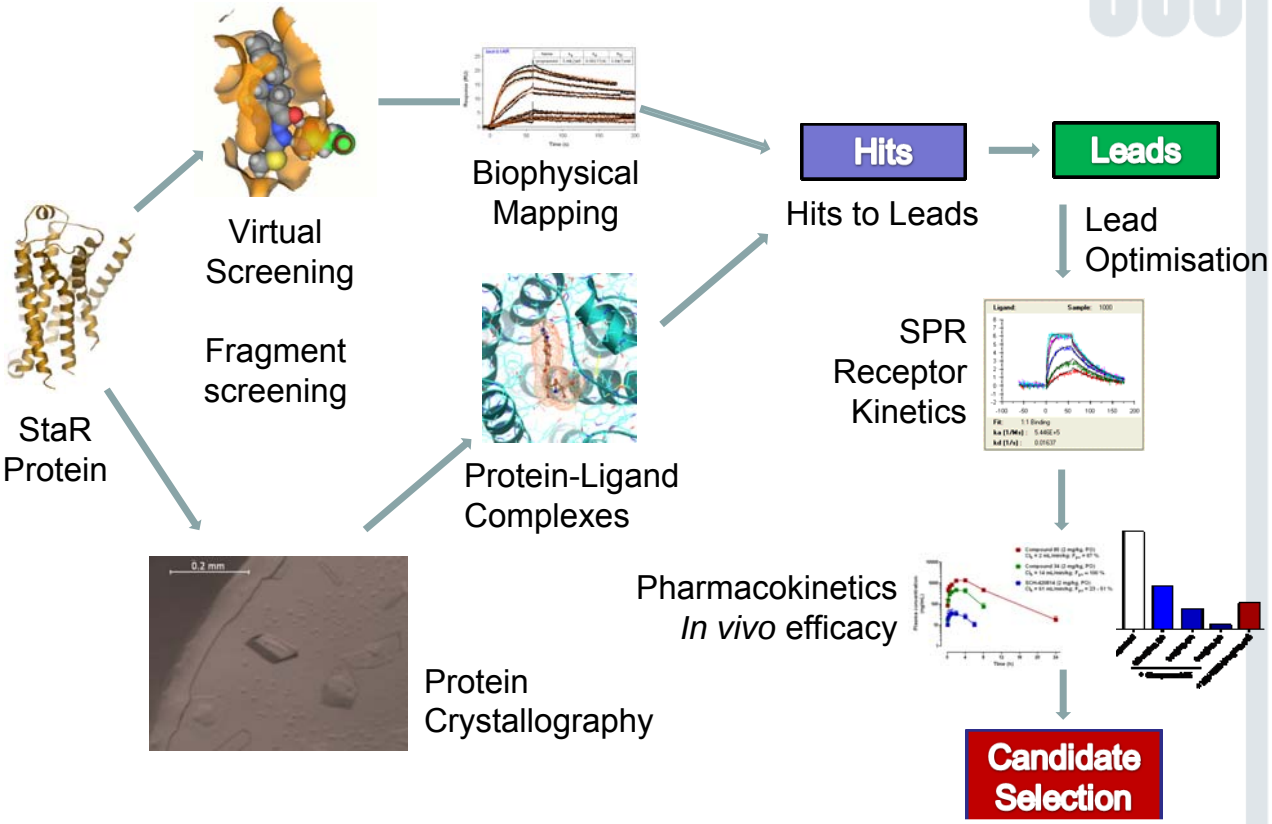


Agonists, "agonists" & antagonists

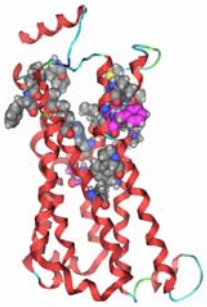
- Binding site surfaces of β2 agonist in β2 agonist structure (3POG), β1 agonist in β1 antagonist StaR structure (2Y03) and β1 antagonist in β1 antagonist StaR structure (2VT4) showing contraction of the site due to agonist binding and receptor activation

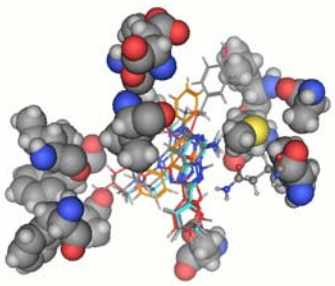


Heptares Drug Discovery



Adenosine A_{2A} Antagonist Virtual Screen





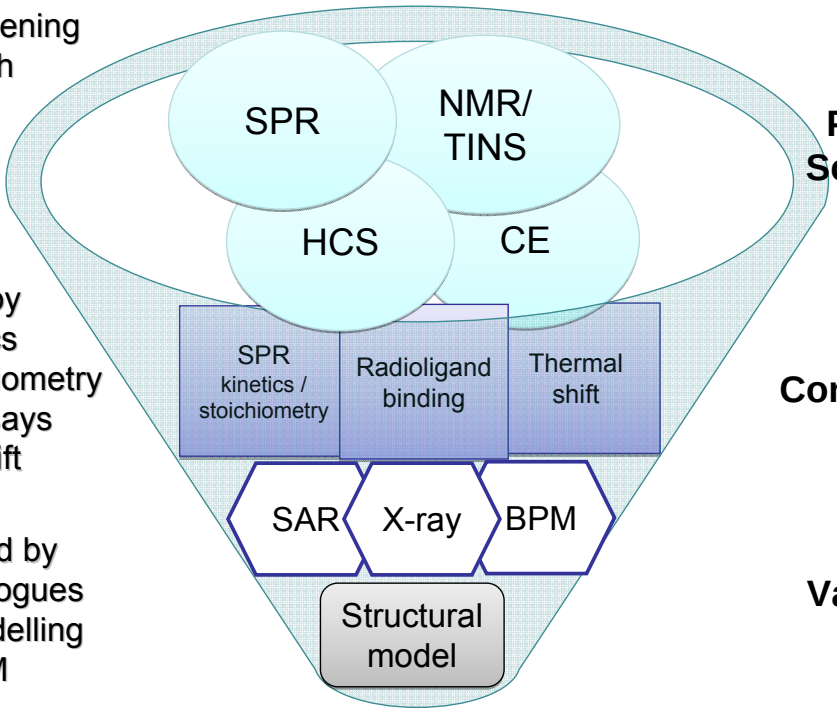
- Homology models based on β1 structure built and refined by extensive mutagenesis data. Point mutants that affect ligand binding cluster around active site.
  - Model adjusted significantly to fit with mutation/ligand-binding data (Modeller, MOE)
- Library of 540K compounds (CNS property-filtered etc) screened *in silico* by docking using Glide/SP. Bias towards compounds which docked into the most buried part of the site, remote form the low confidence region bordered by the ECL2 loop.
- 372 compounds were prioritized following post-processing and visualization in the models. 231 compounds were purchased
  - 20 exhibited activity in binding assay (IC50<55μM) covering 12 chemotypes
  - Hit rate of 9%
- The most potent and ligand efficient molecules were selected
  - Resulted in 4 hit series
- Subsequent comparison with X-ray structure showed good agreement in particular around the binding mode of ZM-241385.

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



Primary Screening

Hit Confirmation

Hit Validation

HIT SERIES

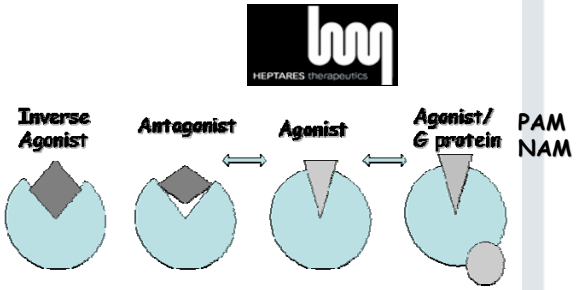
Fragment Screening: The new possibilities for GPCRs as well as enzymes

Enzymes

GPCRs

StaR™

Select conformation Stabilize



StaR proteins are locked in the conformation derived from the pharmacology of the ligand used in their creation

Immobilized protein fragment screening
SPR (Biacore)
TINS (Zobio)

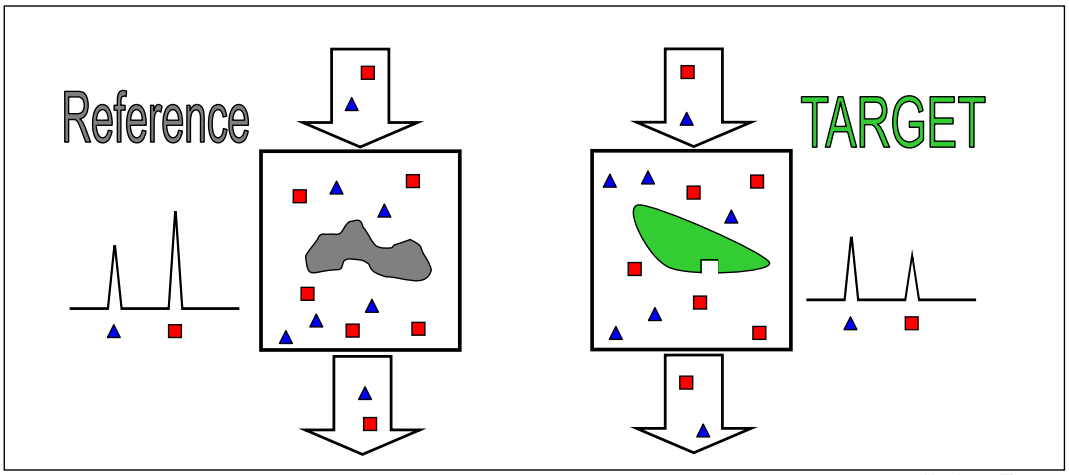
Identify binding position / mode

Biophysical mapping (SPR), Competition studies (SPR & TINS) & X-ray structures

NMR/TINS method for finding hits: fragment screening

- Immobilized protein – only small amounts needed (~1mg)
- Very sensitive: higher mM hits identified (not found by SPR)

TINS = Target Immobilized NMR Screening:

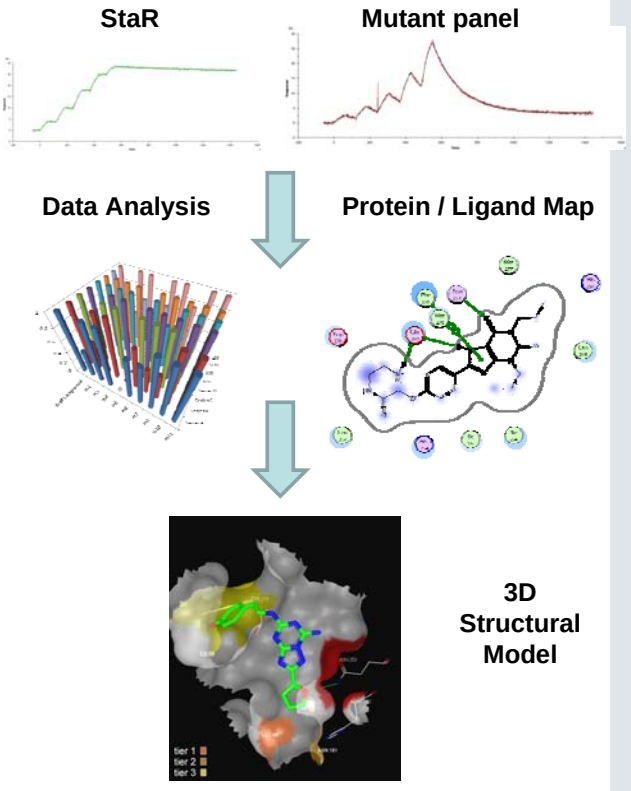


Heptares Therapeutics Ltd

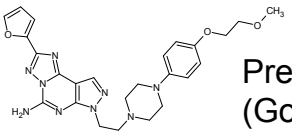
Adenosine A_{2A} Binding Modes:
Biophysical Mapping comparison with Crystal Structures

Biophysical Mapping

- Not possible with native receptors due to instability and lack of sensitivity
- Provides detailed 3D structural data in the absence of X-ray structure
- Structure based approach drives efficient lead optimisation
  - Maintain ligand efficiency whilst improving potency/selectivity
  - Greatly reduced timelines during LO.
  - Knowledge driven rather than empirical chemistry
  - Greatly improved drug like properties leading to reduced risk and attrition

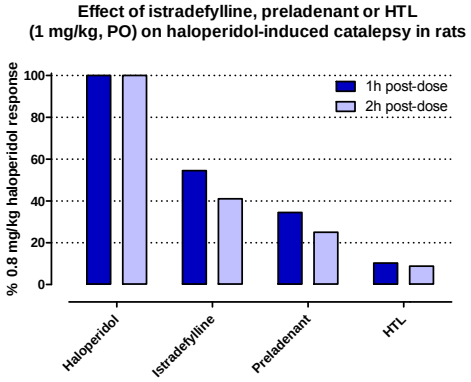


Structure based discovery of A_{2A} Antagonists for Parkinson's Disease



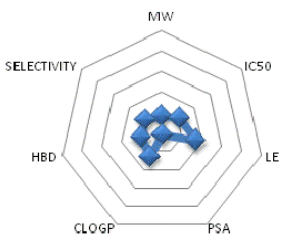
Preladenant in Ph III
(Gold Standard)

- Range of Structure based approaches used to discover novel series of A_{2A} antagonists
- Lead generation from virtual screening and fragment screening
- Very rapid lead optimisation phase
  - 18months to candidate selection phase
- Lead optimisation informed by:
  - Biophysical mapping using SPR
  - Rapid co-crystallization of lead compounds
- Kinetic profiling by SPR on all compounds
  - Selection of slow off rate compounds
- Heptares candidate
  - Greatly improved properties compared to other A_{2A} antagonists (eg molecular weight, pharmacokinetics)
  - Nanomolar affinity and selectivity
  - Very high oral bioavailability (80-100%), low clearance, low plasma binding (~90%), high solubility
  - Oral efficacy in vivo ED50 of <1 mg/kg across multiple compounds

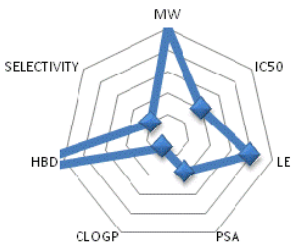


Family A Chemokine Receptor Antagonist Breakthrough to a Highly Intractable Target

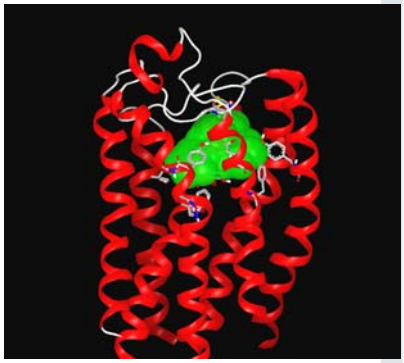
- SBDD and fragment screening
- 5% hit rate from Heptares' 800-member Fragment library
- Clinical gold standard is not Rule of 5 compliant
- Potent and low molecular weight start-point
- Promising low-nanomolar atom efficient lead series



Heptares' Lead



Clinical Gold Standard

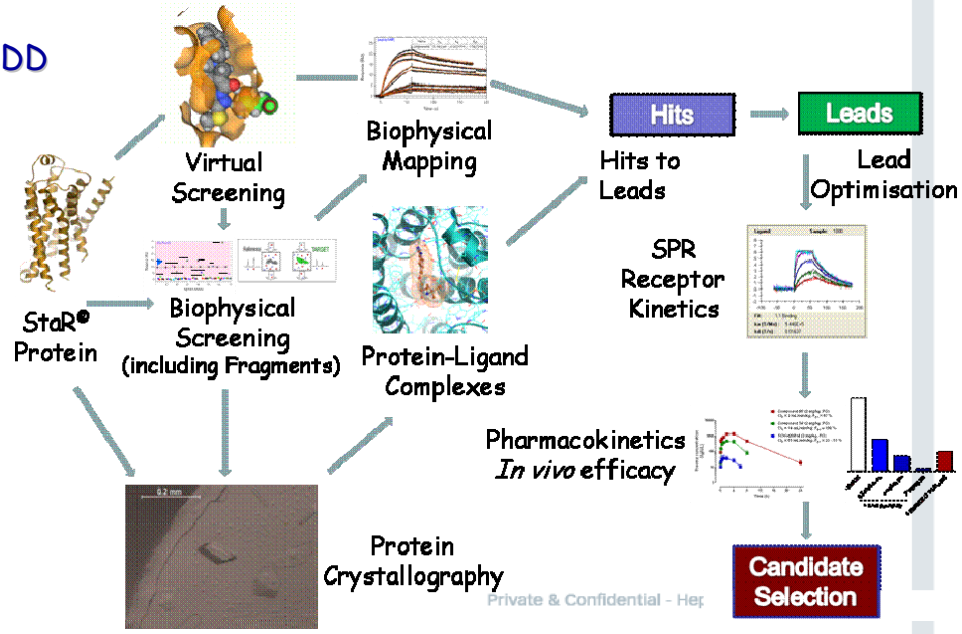


Surface of hit compound bound to chemokine homology model

Summary - Heptares

- Transformational technology for GPCR drug discovery
  - Structures at last for agonist and antagonist ligands etc + early biophysical screening to identify fragments etc + binding modes etc

- Validated StaR® SBDD platform



Summary

- *Transformational technology for GPCR drug discovery*
- *Validated StaR technology platform*
- Experienced management
- Established drug discovery capability
  - Adenosine Receptor programme (A2a antagonist in PD) in candidate selection
- Balanced business model:
  - 'Platform & Product'
  - Pipeline focussed on difficult/intractable but validated targets
  - First major deal ($200M) done with Novartis Oct 2009 on a single target
  - Discovery Alliance – new drug leads to designated set of targets
- Strong cash position to invest in future growth and development
  - $30M Series A February 2009 – Clarus, MVM, NOF

Heptares

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