



HEPTARES
therapeutics

Revolutionising GPCR Drug Discovery
Corporate Presentation



Company Profile

- Heptares is a drug discovery company focused on identifying novel drug candidates targeting validated G-protein-coupled receptors (GPCRs) in several disease areas
 - Integrated GPCR drug discovery capability
 - Unique stabilised receptor (StaR™) technology and strong IP

- Breakthrough StaR™ technology offers potential to deliver more and better quality drug leads, more innovation, lower risk (fewer safety concerns)
 - Addressing deficiencies in GPCR drug pipelines caused by poorly tractable GPCR targets
 - Enables FULL suite of contemporary drug discovery technologies to be applied to GPCRs

- StaR™ technology is being used to progress a pipeline of best or first in class molecules across the GPCR superfamily
 - Small molecule and antibodies – twin-track approach
 - Aim to progress promising drug leads internally and through strategic collaborations

- Well-funded through £21M Series A fund raise completed Feb 2009

GPCR Drug Discovery

Historical Pharma success rate



- GPCRs once considered highly tractable targets due to history of drug discovery on monoamine receptors
- Current targets much lower success rates
 - Low hanging fruit largely picked
 - Lack of Hits
 - Hits have high molecular weight
 - Poor PK/in vivo activity
 - Difficult to optimize
 - Selectivity problems
 - Other GPCRs, hERG, PGP, Cyps

GPCR Drug Discovery at Heptares

- Heptares' proprietary technology is used to make a stabilised version of target GPCR (StaR™) held in a chosen conformation
 - Stable in functionally-relevant, purified form
 - Only universal method for major stabilisation of GPCRs
 - Growing pipeline of StaRs based on human GPCRs
- StaR reagents and structures are central to a variety of powerful discovery approaches not previously applicable to GPCRs
 - Structure-guided design, Biacore kinetics, fragment screening
 - Novel chemotypes, selectivity, intractable targets
- Application of StaR technology for identification of receptor-specific functional antibodies

Heptares and Cambridge LMB

- World leading institute for Structural Biology
- Chris Tate, Gebhard Schertler and Richard Henderson
 - World leaders in GPCR protein structure field
- **Ongoing exclusive consultancies and active collaboration with LMB**
- **Heptares has access to all IP generated from LMB relating to the application of the technology to GPCRs and other transmembrane proteins**



3D structure of Bacteriorhodopsin
Henderson and Unwin, 1975

Scientific Advisory Board

Pat Humphrey

GSK, Theravance

Greg Winter

MRC, CAT, Domantis

Graeme Milligan

*Glasgow University,
Cara Therapeutics*

Jonathan Javitch

Columbia University

Ed Hulme

*MRC National Institute for
Medical Research*

Stefan Knapp

*Oxford Structural Genomics
Consortium*

David Brown

Roche, Cellzome

Management Team and Board

- **Malcolm Weir (CEO)**
 - CEO Inpharmatica
 - GW: VP Molecular Sciences Division, structure-based design, antivirals, proteases
- **Fiona Marshall (CSO)**
 - Expert in GPCR biology and pharmacology (dimerisation, deorphanisation)
 - Senior drug discovery leadership positions in GSK and Millennium
- **Miles Congreve (Head of Chemistry)**
 - GSK, Astex Director of Chemistry
 - Broad medicinal chemistry, structure-based design, fragment screening
- **Barry Kenny (CBO)**
 - Pfizer, Biofocus, Takeda Cambridge VP Drug Discovery
 - CNS and GPCR drug discovery

John Berriman
(Chairman)
Algeta, Alynlam, Celltech
Michael Steinmetz
Clarus Ventures, MPM,
Roche

Anja Koenig
Novartis Option Fund
Martin Murphy
MVM Life Science Partners
Richard Henderson
MRC LMB

Heptares Therapeutics Ltd



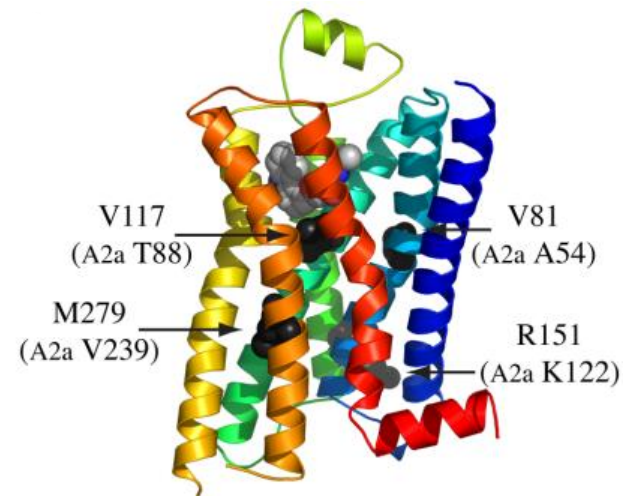
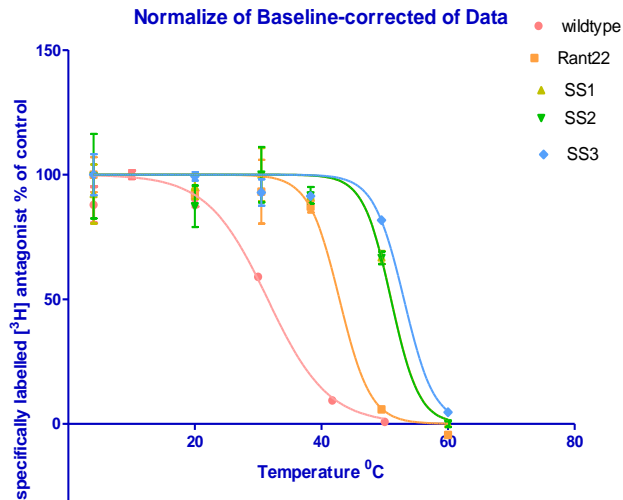
- 40 Employees
- Based at BioPark, Welwyn Garden City, UK
- Scalable pharma standard facilities
 - Molecular biology, protein engineering and scale-up
 - Pharmacology and screening
 - Medicinal and computational chemistry
 - X-ray crystallography
- Significant outsource of non-core support
 - DMPK/tox
 - Synthetic chemistry
 - In vivo pharmacology

Heptares Welwyn BioPark facility



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
 - Completely novel and proprietary
 - Suitable for uHTS, Biacore, crystallisation etc.
 - Transferrable across GPCR families

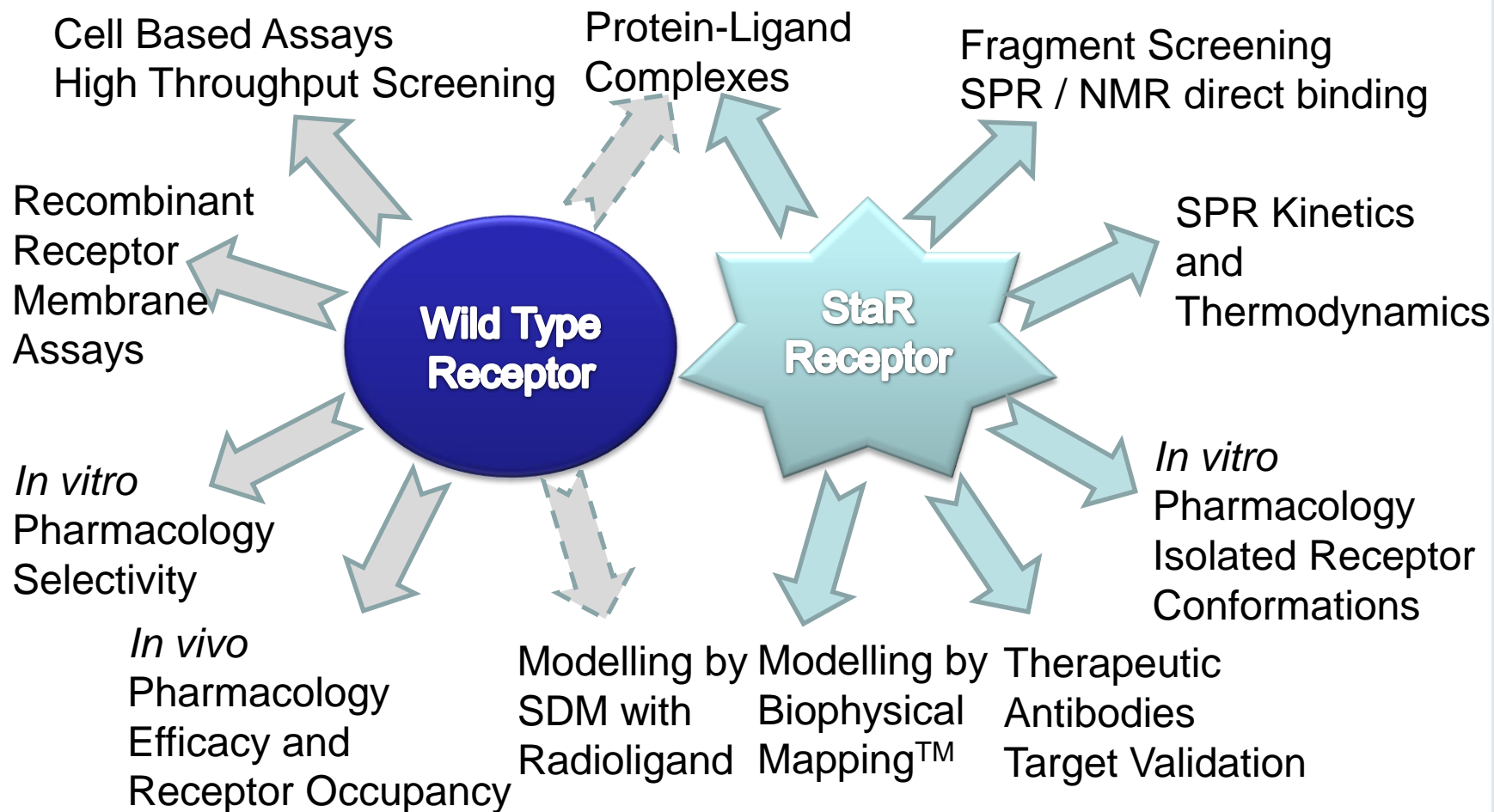


Heptares StaR™ Technology

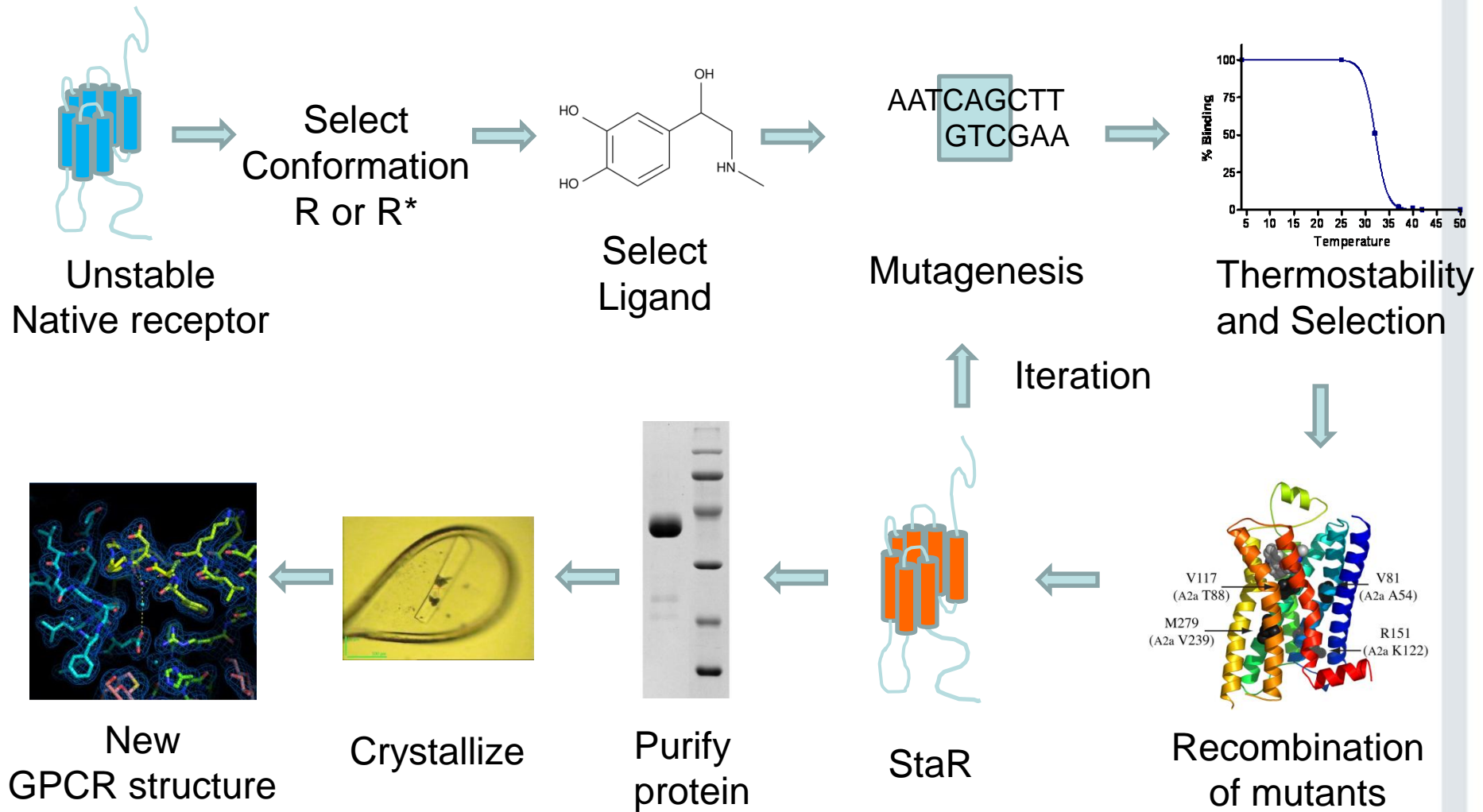


- Receptor embedded in cell membrane, highly unstable when removed
 - Aggregates and loses function when purified in detergent
 - Current drug discovery approaches rely on cell-based assays
- General method required to engineer stabilized, less flexible receptor held in specified functional conformation
- Heptares' proprietary technology is used to make a stabilized version of target GPCR ('StaR™') held in a chosen conformation
 - Stable in functionally-relevant, purified form
 - Dependent on ligand used

Enabling technology for GPCR Drug Discovery



Process for Creating StaRs



Expression

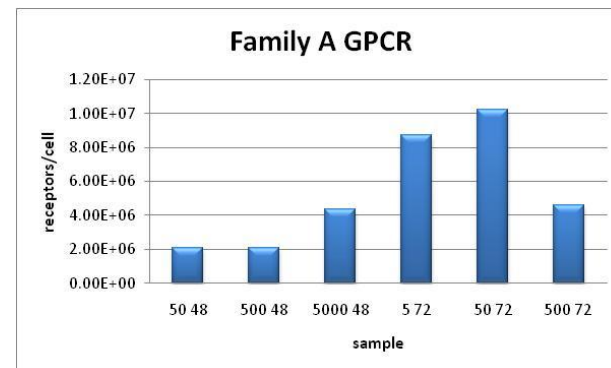
- StaR GPCRs are optimised to give expression levels of $\sim 1 \times 10^7$ receptors/cell (3mg/L)

- Scale 0.1-50L



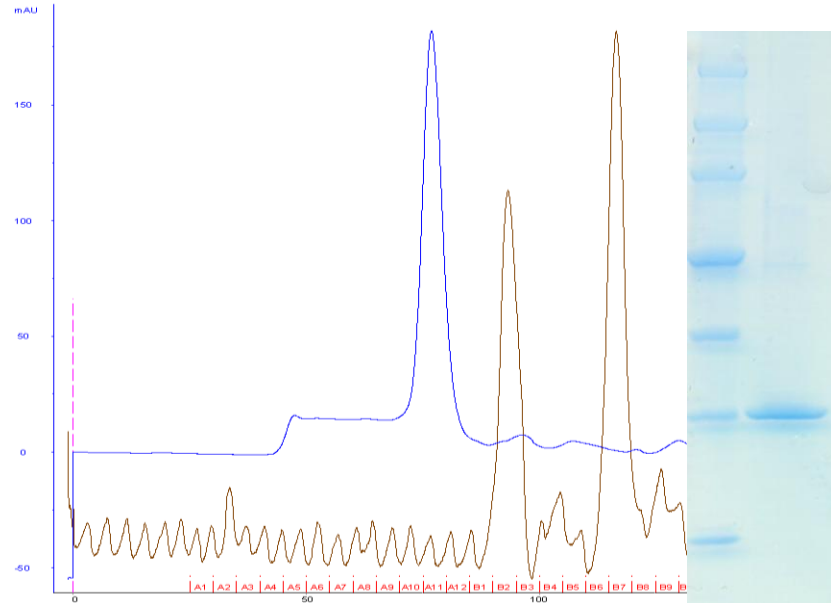
The Baculovirus / insect cell expression optimized:

- Optimized expression construct (receptor truncates)
- Signal peptide
- Cell Line
- Cultivation conditions
- Glycosylation pattern



GPCR Purification

- Range of diverse purification strategies utilised
 - Ligand affinity resins, IMAC, lectin
 - Optimizing protocol (additives, ions, ligands)
- Monitor throughout process
 - purity, yield, activity, specific activity (radioligand assay)
- Protein QC
 - SEC, SDS Page, activity, MS



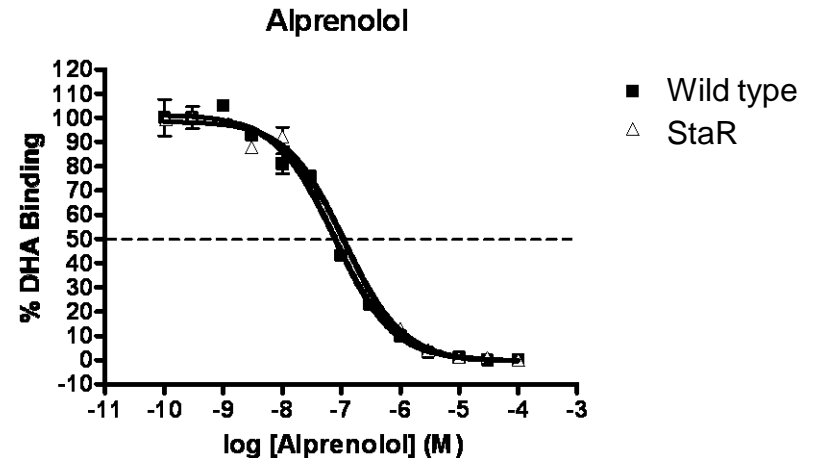
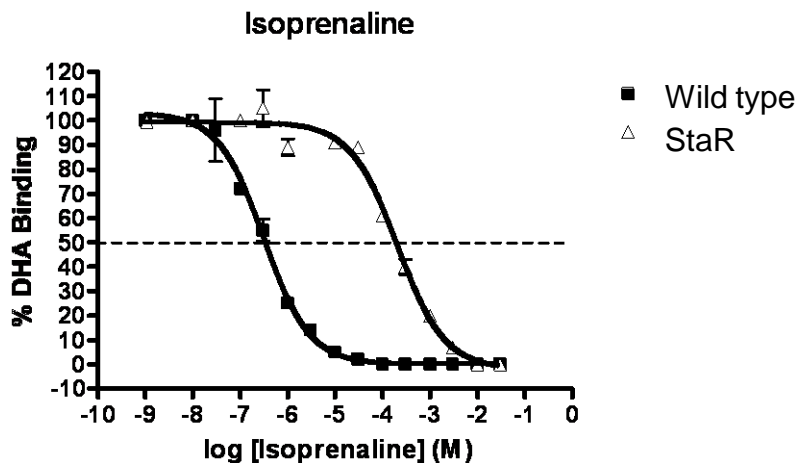
Family A GPCR

- *Purity > 98% by SDS-PAGE*
- *Homogeneous (SEC)*
- *Active (binding assay)*
- *Yield 2 mg/L (66% receptor recovery)*

β 1 Adrenoceptor StaR Stabilized in an Antagonist Conformation

Agonist affinity is reduced

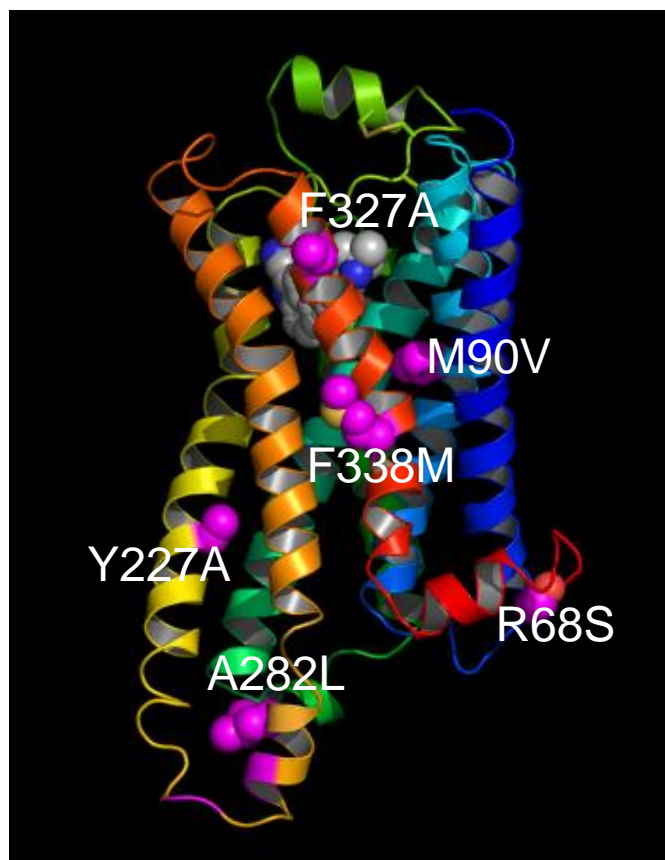
Antagonist affinity unchanged



Mutated receptor fully functional in cells with excess agonist

Zero basal activity (fully antagonist-form resting state)

Beta-1 Adrenoceptor StaR Crystal Structure



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

← Drug binding pocket

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

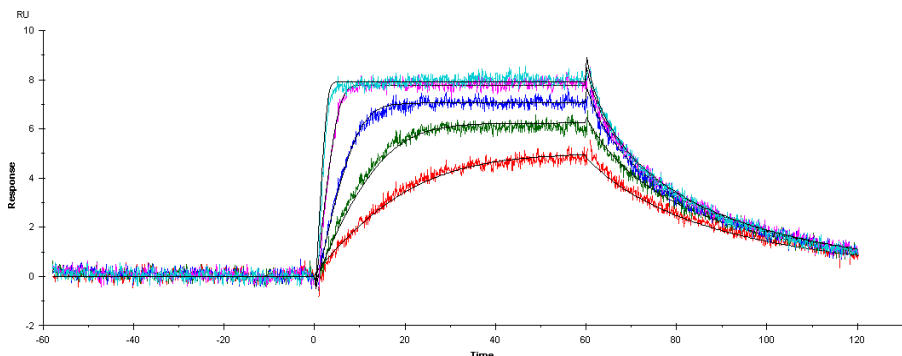
← Activation and G-protein binding region retained
Multiple **loop conformations** resolved cf biased agonism

Biophysical Screening

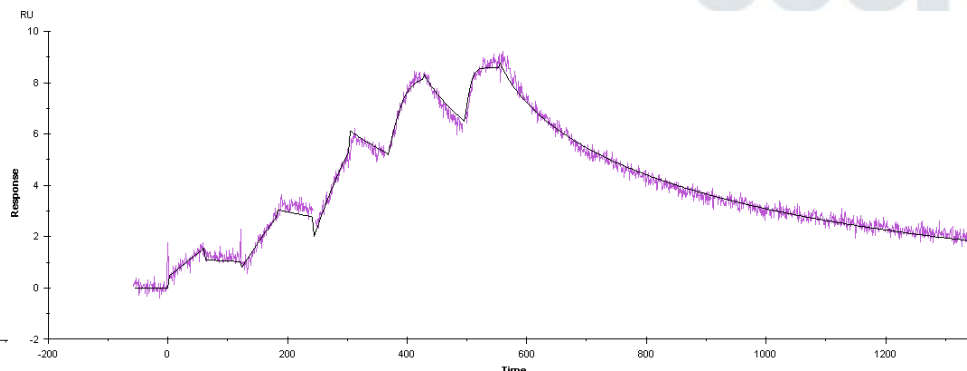


- StaRs broadly applicable to biophysical approaches
 - Stability
 - Protein production
- Heptares will investigate multiple methods
 - SPR
 - NMR screening methods
 - Thermal denaturation approaches
- Heptares is establishing a GPCR targeted fragment library
 - *In silico* for virtual screening
 - *In vitro* for diversity screening

Using StaRs to characterise kinetics



Compound 85 (A2a *in vivo* active) MCK format
KD = 12nM

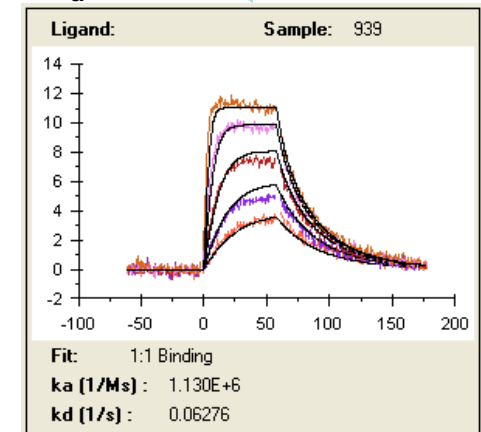
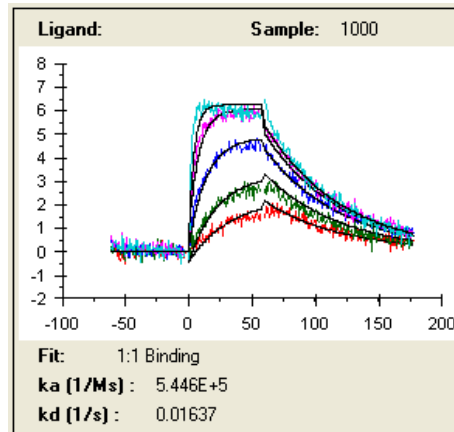
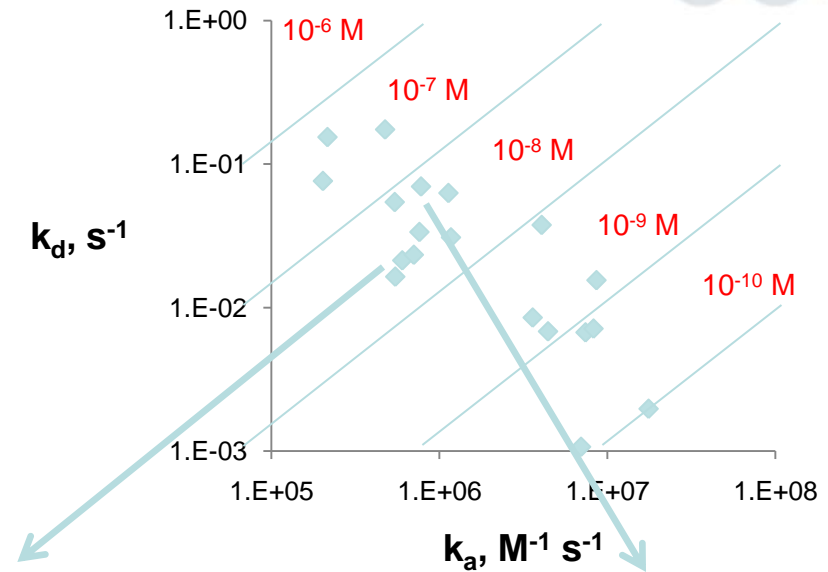


Compound 107 (close analogue) SCK format
KD = 0.9nM
30-fold slower dissociation

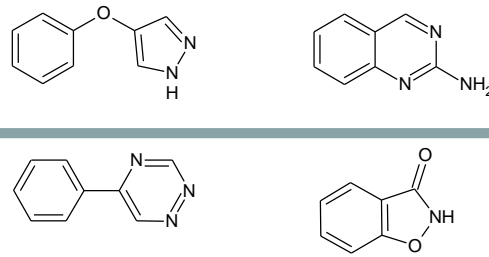
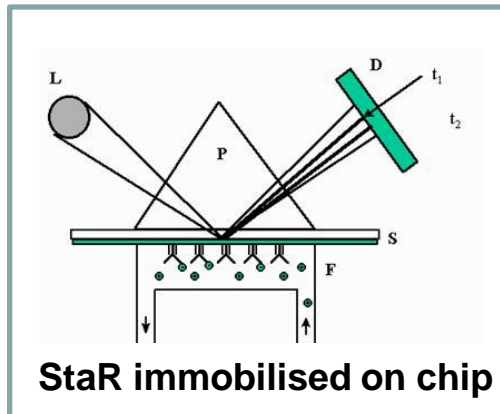
- SPR (Biacore) binding data
- Direct binding of unlabelled compounds eg screen hits, lead series, fragments
- Provides information on kinetics
- Optimising off-rates of drugs key to success eg tiotropium
 - Determines efficacy and safety profile
 - Informs PK/PD modelling
- Most marketed peptide receptor drugs have very slow off rate/insurmountable antagonism

Kinetic screen of A_{2A} receptor antagonists

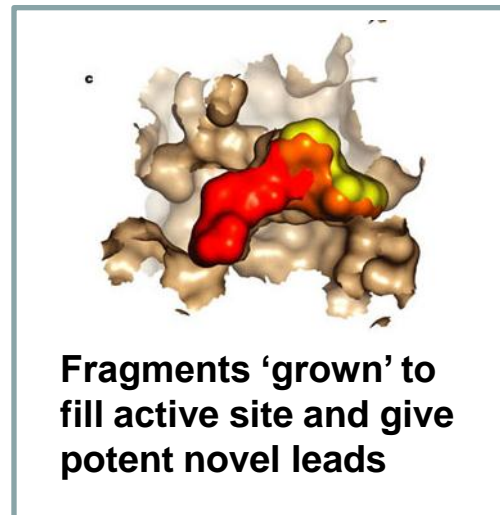
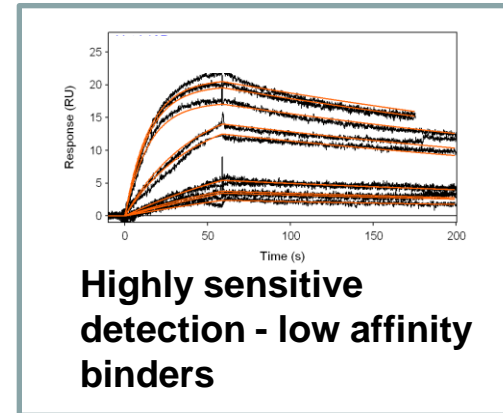
- Biacore kinetics allows compounds with identical affinities to be discriminated with regard to off-rate
- Affinity increases can then be driven by slower off rates in a rational way
- Slow off rate compounds selected for *in vivo* efficacy studies



Fragment Screening using Biacore

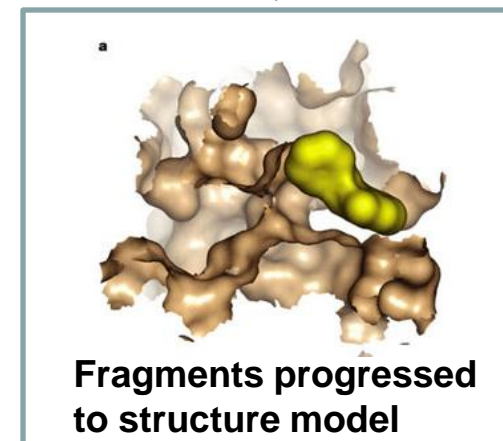


*Challenged with Fragments
(~150-250 Da)*

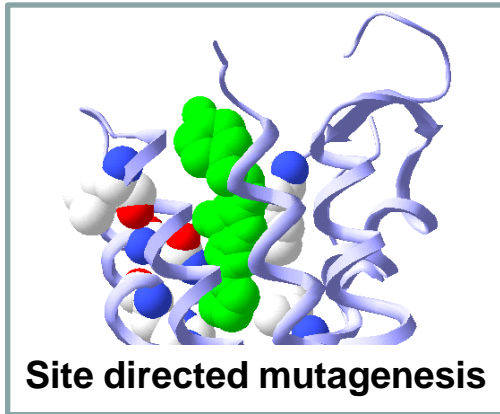


Fragment-based

Drug Discovery



Biophysical Mapping™ using Biacore

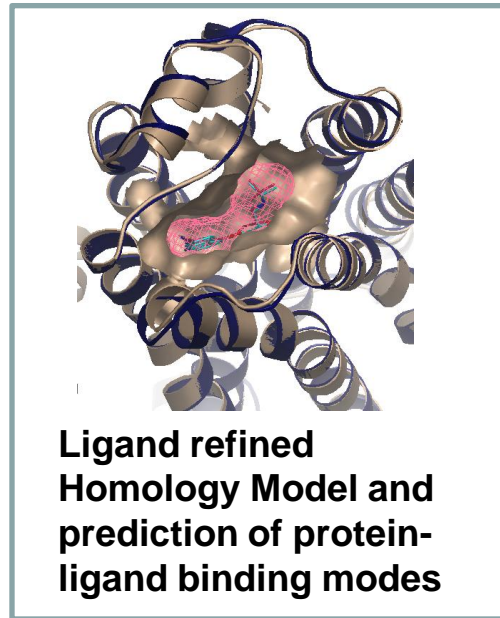
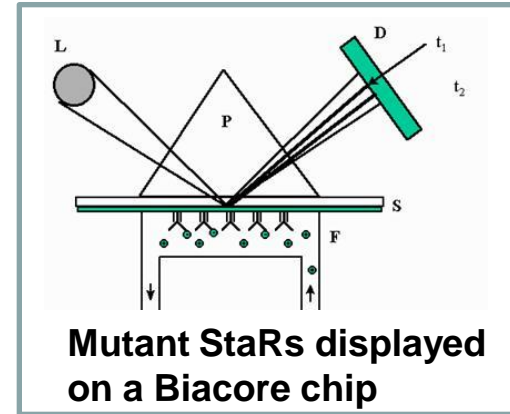


Wild-type: GGGCTCATAGGTA-TTTGTATGCGTGAGTT

Mutants:

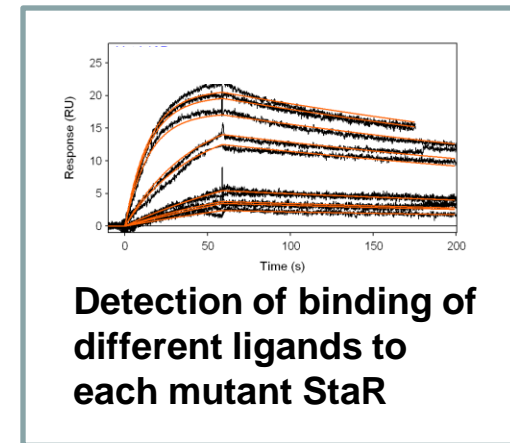
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90-24	GGGCTATAGGTA-TTTGTATGCGTGAGTT
90-40	GGGCTATAGGTA-TTTGTATGCGTGAGTT
120-1	GGGCTATAGGTA-TTTGTATGCGTGAGTT
90-37	GGGCTATAGGTA-TTTGTATGCGTGAGTT
90-21	GGGCTACGGTA-TTTGTATGCGTGAGTT
90-2	GGGCTCAGGTA-TTTGTATGCGTGAGTT
120-22	GGGCTCAGGTA-TTTGTATGCGTGAGTT
90-43	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
90-1	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
90-46	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
120-17	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
90-31	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
90-41	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
90-45	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
90-3	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
120-10	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
120-9	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
90-35	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
120-19	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
120-30	GGGCTCATAGGTA-TTTGTATGCGTGAGTT
90-39	GGGCTCATAGGTA-TTTGTATGCGTGAGTT

10-30 Mutations to the Binding site region



Biophysical Map of binding site

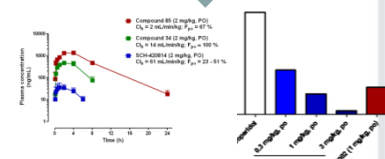
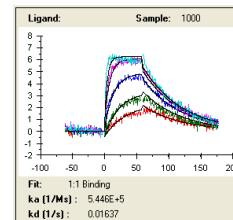
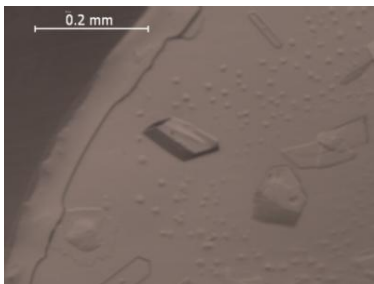
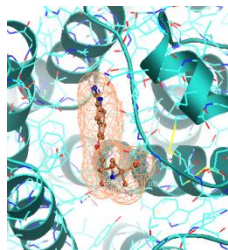
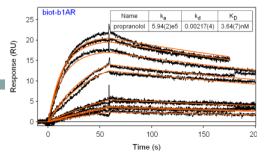
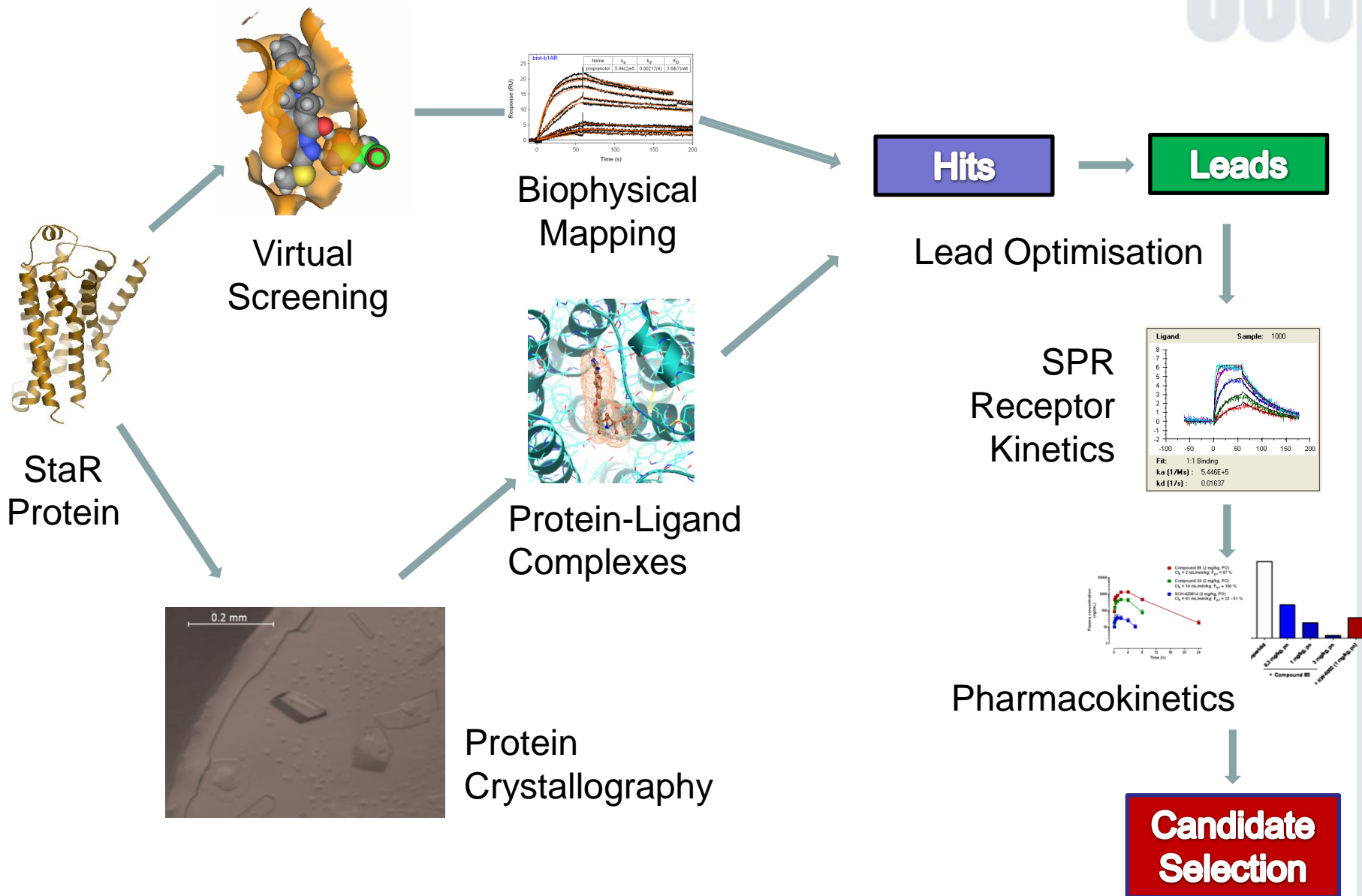
Correlating binding data from Multiple ligands with multiple Mutant StaR proteins



Biophysical Mapping: Key Advantages Compared to Conventional SDM/Modelling Studies

- Wide range of unlabelled compounds with varying potencies can be studied within med chem cycle time
- Direct binding – no requirement for competitive displacement of labelled ligand
- Large body of data (compound/mutant matrix) enables accurate assignment of ligand binding modes
- Decide between alternative docked poses
- Evaluate relative contribution of side-chain interactions
- Binding to immobilised purified protein avoids artefacts due to effects of mutations on protein expression

Heptares A_{2A} Drug Discovery



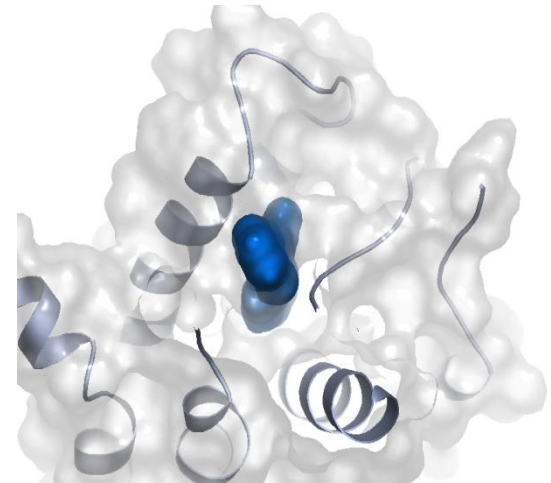
Heptares A_{2A} Antagonists

July - Sept 2008: Virtual screen of 550K compounds

- 230 compounds screened
- 12 novel chemotypes
- 4 series progressed through hits to leads
- IP filed on 2 series

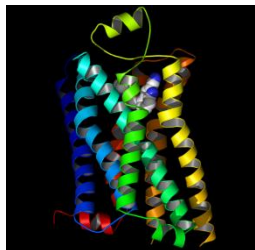
Jan 2010: Candidate selection phase

- Novel non-furan, non-xanthine, low MW (< 300 Da)
- Nanomolar affinity and selectivity
- Understanding of receptor kinetics and relationship with *in vivo* pharmacodynamics
- Excellent oral bioavailability (70-100%), low clearance, low plasma binding (~90%), high solubility
- Oral efficacy *in vivo* ED₅₀ of <1 mg/kg across multiple compounds



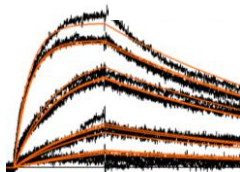
StaR Drug Discovery Applications

X-ray structure



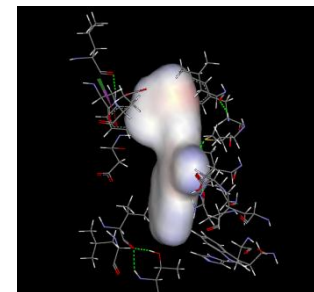
Docking
Virtual screening

Biacore

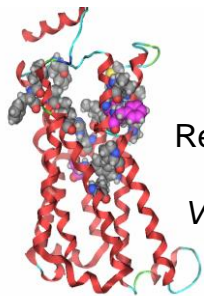


Fragment screening
Kinetics

Ultra HTS



Residues involved in
Ligand binding



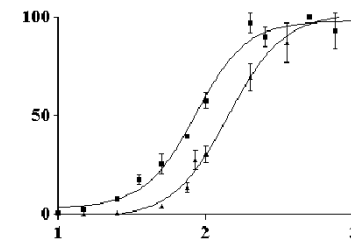
Refined homology
Model
Virtual screening

**StaRs
Ag/Antag
Conformations**

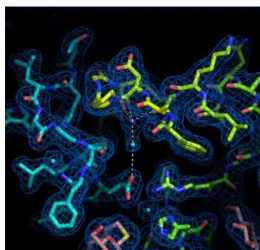
Wild-type:	GGGCTCATAGGGTA-TTTGTATGCGTGAAGTT
Mutants:	
90-9	GGGCTCATAGGGTA-TTTGTATGCGTGAAGTT
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90-3	GGGCTCATAGGGTA-TTTGTATGCGTGAAGTT
120-10	GGGCTCATAGGGTA-TTTGTATGCGTGAAGTT
120-9	GGGCTCATAGGGTA-TTTGTATGCGTGAAGTT
90-35	GGGCTCATAGGGTA-TTTGTATGCGTGAAGTT
120-19	GGGCTCATAGGGTA-TTTGTATGCGTGAAGTT
120-30	GGGCTCATAGGGTA-TTTGTATGCGTGAAGTT
90-39	GGGCTCATAGGGTA-TTTGTATGCGTGAAGTT

**Mutagenesis data
Binding site mutants**

Pharmacology vs
Binding modes

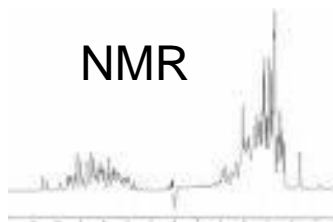


Compound mechanism
of action



Ligand co-structures
Binding site definition

NMR



Fragment screening
Binding site definition