GPCR fragment-based design and a novel structure-based perspective on druggability

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*4th RSC/SCI Symposium on GPCRs in Medicinal Chemistry.*
*Windlesham September 2012*
Heptares Therapeutics

- Breakthrough medicines targeting previously undruggable GPCRs
- $40M from leading venture investors since 2009
- Major R&D partnerships with multiple large pharma companies
- Structure-Based Drug Design for GPCRs, enabled for the first time by StaRs® (Stabilised Receptors)
- Leading GPCR capability in industry, integrating chemistry & structural biology
- Engine creating both small molecule NCEs and antibody therapeutics
- Exceptional pipeline of investigational medicines for serious diseases
## Heptares Product Pipeline

<table>
<thead>
<tr>
<th>GPCR</th>
<th>Discovery</th>
<th>Preclinical</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Indication(s)</th>
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<tbody>
<tr>
<td>Muscarinic M1</td>
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<td>Binge Eating, Nicotine Addiction</td>
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<td>Type 2 Diabetes (First Oral NCE)</td>
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<td>Type 2 Diabetes (Disease Modifying)</td>
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<td>Autism, Dyskinesia, Depression</td>
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<td>CGRP</td>
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<td>Migraine Treatment &amp; Prophylaxis</td>
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<td>Adenosine A&lt;sub&gt;2A&lt;/sub&gt;</td>
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<td></td>
<td></td>
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<td>CNS Disorders</td>
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<td>Takeda</td>
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<td>CNS Disorders</td>
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<td>Antibody Therapeutics</td>
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<td>Novartis</td>
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</table>

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Outline of Presentation

- Fragment Screening Methods
- Fragment H2L examples
- A Water perspective...
  - Druggability
  - SAR & Kinetics
StaRs enable Fragment Screening

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

HIT SERIES

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SPR Fragment Screening POC

A$_2$A StaR

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

Adenosine $A_{2A}$ and $\beta_1$ Adrenergic Receptor ($\beta_1$ AR) SPR screen

Similar results for screening Heptares fragment library against $A_{2A}$ and $\beta_1$AR
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NMR Screening: $\beta_1$AR (with ZoBio)

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

TINS = Target Immobilized NMR Screening

High Concentration Screening Lipid Receptor Agonist StaR

- Agonist StaR binds known agonists with higher affinity compared to wild-type receptor (binding assay radioligand usage significantly reduced).
- 10% DMSO has a minimal effect on ligand-binding to the StaR in membranes, unlike wild-type.
- Enables high concentration (100 μM) fragment screening which is not feasible with wild-type.
- Approx 6% hit rate (84 / 1419 fragments inhibit binding > 30%, n = 2).
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Hit to lead example 1: CXCR4 Chemokine Receptor Antagonist

- 39 hits (4.8%) from 808 molecules (fragments + targeted screening set)
- Follow up gives clear SAR
- Rapid identification of potent hit series with considerably better profile than gold standard

Example fragment hit
- $K_i = 150 \, \mu M$ (LE = 0.47)
- Good solubility
- MWT 144, cLogP 1.3, PSA 39

Hit Series Exemplar
- $K_i = 10 \, nM$ (LE = 0.34)
- Good solubility
- MWT 300, cLogP 1.3, PSA 76
Hit to lead example 2: \( \beta_1 \text{AR antagonists} \)

- SPR screening with A\(_{2A}\) as counter screen
- Several related hits

Typical results for ‘well behaved’ hits

K\(_D\) = 16 \(\mu\)M
**β₁AR: Structure-guided hit to lead progression**


### SPR hit
- \( K_D = 16 \ \mu M \)
- \( LE = 0.41 \)

### Analogue purchasing
- \( IC_{50} = 7 \ \mu M \)
- Binding Assay
- \( LE = 0.50 \)

### Analogue purchasing
- \( IC_{50} = 58 \ nM \)
- Binding Assay
- \( LE = 0.66 \)
- MW <250, Highly soluble
β1AR: Fragment X-ray crystallography

- Protein-Fragment crystal co-crystal complexes solved to high resolution.
- Novel chemical matter, breaking the mould of existing chemistry.
  - Lack aminoalcohol motif
Comparison of Heptares GPCR Fragment Hits with Enzyme Targets

<table>
<thead>
<tr>
<th>Target</th>
<th>Method</th>
<th>Hit LE</th>
<th>Target</th>
<th>Method</th>
<th>Hit LE</th>
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</thead>
<tbody>
<tr>
<td>Adenosine A$_{2A}$</td>
<td>TINS NMR</td>
<td>0.56</td>
<td>Protein kinase B</td>
<td>X-ray soak</td>
<td>0.47</td>
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<tr>
<td>Adenosine A$_{2A}$</td>
<td>SPR</td>
<td>0.53</td>
<td>DPPIV</td>
<td>HCS</td>
<td>0.46</td>
</tr>
<tr>
<td>Family A aminergic</td>
<td>SPR</td>
<td>0.41</td>
<td>Thrombin</td>
<td>X-ray soak</td>
<td>0.40</td>
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<tr>
<td>Family A peptidergic</td>
<td>SPR</td>
<td>0.31</td>
<td>BACE</td>
<td>SPR</td>
<td>~0.30</td>
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<tr>
<td>Family A lipid</td>
<td>HCS</td>
<td>0.55*</td>
<td>HSP90</td>
<td>NMR</td>
<td>0.53</td>
</tr>
<tr>
<td>CXCR4</td>
<td>HCS</td>
<td>0.47</td>
<td>PDE4</td>
<td>HCS</td>
<td>0.46</td>
</tr>
</tbody>
</table>

11 GPCRs (Family A, B & C) screened in 14 assays

GPCRs are highly comparable to enzyme targets in terms of quality of hits, when StaR proteins are used for screening.

* StaR assay
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- A Water perspective...
  - Druggability
  - SAR & Kinetics
GPCR structural information can be revolutionary – previously we were biased by ligand + analogs data

Beware of biases in how we see and “force-fit” data

Courtesy of Arthur Doweyko,
Why Water?

- Water molecules play an essential role in the structure and function of biological systems - appear to play a key role in the GPCR structures available to date, perhaps due to the deep pockets present in the GPCR transmembrane binding sites?

- Displacement of waters from a binding site is a key component of ligand binding, with significant binding energy, and thus potency, often from the entropic gain of the displacement.

- But all waters are not equal…
  - Burying an "unhappy" water [i.e. entropically and/or enthalpically worse than bulk solvent] may affect both potency and kinetics.
  - Perturbation of the remaining waters will also affect binding ±

- Opportunity to provide new insights into druggability, to drive SAR and find solutions to many SAR issues (prediction of "magic methyl", SAR not explainable by direct ligand-protein interactions...)
Water: The new wave...

- New computational approaches now available that can create water networks (with or without a ligand present)
  + energetically differentiate "happy" and "unhappy" waters

- Favourable binding sites contain multiple "unhappy" waters, with a cluster being particularly favourable (→ higher affinity/LE fragment hits etc...)
  - in an environment compatible with a drug-like molecule (mixture of hydrophobic & hydrophilic hotspots → rule of 5 etc properties)

... e.g. not all serines are the same

All pockets are equal, but some pockets are more equal than others
1. MD simulation and clustering technique to build a map of water occupancy in the factor Xa active site
2. Chemical potentials assigned to the water sites using the inhomogenous solvation theory
A New Structure-Based Perspective on Druggability
– 3D GRID physicochemical + Water energetics

- GRID (Molecular Discovery) used to identify binding site complementary properties – energetic analysis of binding site with ligand functional groups:
  focus on hotspots for lipophilic probe (C1=) + H-bonding (H2O probe)
  - new combined hydrophobic (DRY) and lipophilic (C1=) probe : CRY

- WaterMap (Schrödinger) & SZMAP (Open Eye) used to generate a full network of waters in binding site with free energy estimation (vs bulk solvent – neutral atom) including a breakdown into entropic & enthalpic contributions

- Combined analysis enables a new druggability assessment:
  - fragment/ligand potency, properties, shape requirements…
Waters that are calculated to have a significant positive free energy (e.g. relative to being in bulk solvent) are termed ‘unhappy’.

- There should be a particularly good free energy gain from displacing these waters; they are only in the site as creating a vacuum is even more energetically unfavourable.

- The number and the relative position of the predicted ‘unhappy’ water clusters reveal hotspots for small molecule ligand binding.
Water Networks: Validation

- Can we generate a water network in a protein-ligand complex similar to that experimentally determined in high resolution X-ray structures?
  - e.g. A$_2$A at 1.8Å (4EIY)

  ⇒ Excellent correlation

  + we also know the relative energies of the waters, and can re-evaluate with different ligands
    - a significant advance

  ? Can we now explain previously unexplained SAR (in terms of direct ligand-protein interactions) etc
Binding Site Analysis for Druggability

- **Druggability** – *ligandability with properties of an oral drug*

- Use a combined analysis of ‘unhappy’ waters & binding site preference (lipophilic/hydrophobic)
  - Analyse numbers, connectivity & distribution of ‘unhappy’ waters
  - ‘Unhappy’ water = >2.5 kcal vs bulk solvent (*vacuum usually worse*)

- The link with protein active site druggability is not only to the total number of ‘unhappy’ waters in each binding pocket but also to their arrangement and proximity (connectivity) to other ‘unhappy’ waters.

- In GPCRs such as A<sub>2A</sub> the predicted ‘unhappy’ waters have a favourable more globular arrangement with an average 2-3 other adjacent ‘unhappy’ waters at < 3 Å

- Two representative protease enzyme targets factor Xa and BACE1 have key ‘unhappy’ waters displaced by the ligand that are not connected
  - extreme case in factor Xa where they are in subpockets almost 10 Å apart, giving a lower value of 0–1 connections;
β₁- (from StaR) & β₂- (from T4L insertion) Adrenergic Receptors

Inactive

Active

β₁ antag

β₁ agonist

β₂ antag

β₂ agonist
Druggability: Dopamine, Histamine & Muscarinic GPCRs

Druggable regions with clusters of ‘unhappy’ waters (WaterMap) with GRID showing concurrent lipophilic & H-bond hotspots

What about more recent structures?

S1P1 Lipid GPCR

Opioid kappa Receptor
Kinases are more like GPCRs for druggability in terms of regions of connected ‘unhappy’ waters.

[Diagram of c-Abl DFG-in and c-Abl DFG-out structures]

Less druggable enzyme targets do not have large clusters of ‘unhappy’ waters

The myth: Basic S1 for serine proteases (factor Xa)

The structure that broke the myth

The clinical candidate

These sparsely distributed ‘unhappy’ waters are distributed in different subpockets, meaning that fairly ‘angular’ ligands are needed, with a requirement for a precise shape and conformation; this could explain why random screening (HTS) often fails. → ranked lower in overall druggability
Less Druggable Sites Still Tractable to SBDD
GPCR Compared to Protease

CXCR4 has a more challenging site:
Structure shows the “hot-spot” for binding to be less deep / druggable (lipophilic, less buried ionic interaction)

BACE has delocalised druggable site – hot-spots linked by SBDD

Conserved mechanism suggests orthostERIC agonists should be attainable for any GPCR

Inactive

Druggable $A_{2A}$ Antagonist site

Active

Druggable $A_{2A}$ Agonist site
First GPCR Candidate Wholly Derived from SBDD

- Superior adenosine A$_{2A}$ antagonists
- Entirely novel chemotypes
- Candidate licensed to Shire
- Treatment of multiple neuro disorders
- Radical binding mode with highly optimised receptor interactions

**Features**
- Non-furan, non-xanthine
- Very low molecular weight
- Relatively polar

**Benefits**
- Attractive safety profile
- Improved oral bioavailability and PK
- Excellent *in vivo* efficacy

Using GRID hotspots enabled the first full GPCR SBDD to give a clinical candidate for the Adenosine A2a

ZM24138

A$_2$A antag

▶ The highly ligand efficient designed structures displaces a cluster of unhappy waters deep in the pocket, missed by previous ZM-like ligands (from HTS etc)
Biophysical Mapping™ uses StaRs to map the binding sites of GPCRs & conf

Site directed mutagenesis on StaR®

10-30 Mutations to the Binding site region

Mutant StaRs screened on Biacore chips

Biophysical Map of binding site

Ligand refined homology model and prediction of protein-ligand binding modes

Correlating binding data from multiple ligands with multiple StaR proteins

Detection of binding of different ligands to each mutant StaR

Experimentally Enhanced Homology Models

- Existing structures
- Knowledge of conformations
- Reported variants

First homology model

StaR mutagenesis

Further X-ray structures

Biophysical Mapping

Multiple generations of Experimentally Enhanced Homology Models

Fragment screening

Virtual screening

Virtual screening

Virtual screening
Structure-Based Lead Optimisation

- Models of A$_{2A}$, refined using the biophysical mapping, & of A$_1$ used -
dockings prioritized from large virtual libraries (1,2,4-triazines)
  - Used GRID hotspots to design compounds that efficiently displaced the
    “unhappy“ waters deep in the site
  - Used GRID surfaces of A$_2$ vs A$_1$ to probe for subtle differences to efficiently
design improved selectivity

- Structure can enable small ligand efficient
changes to modulate selectivity & potency
Waters & GPCR SBDD / Druggability

• New perspective on druggability possible by analysis of binding site using water free energies & 3D physicochemical properties (GRID)

• Looking at pertubation of all waters, including those remaining, is looking promising

• Multiple approaches possible, with combined approaches often useful
  - for apo structure generate water network with WaterMap or GRID
  - for complexes generate initial complete water network with SZMAP & optimize with WaterMap
  - binding site preferences from GRID analysis with functional groups
  - scoring (displacement and perturbation) by all 3 methods: (WaterMap, SZMAP, GRID/CRY probe)
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