Future Directions in Drug Design & Discovery

Jonathan S. Mason
Head of Computational Chemistry
Heptares Therapeutics, Welwyn Garden City, UK &
Chief Scientist (Predictive Technologies & Drug Design) Medicinal Chemistry
Lundbeck Research, Valby, Copenhagen, Denmark

jonm@lundbeck.com
jonathan.mason@heptares.com
drugdesign@email.com

The menu...

▶ The drug discovery problems
▶ The drug design problems
▶ The opportunities
The Drug Discovery Problem

Attrition / Productivity

- Need to
  - choose the right targets
  - identify suitable hits
  - progress rapidly to a molecule with the desired activity and properties
  - that does not fail (attrit) during development

Attrition / Productivity – Issues → Opportunities

Toxicity – Safety Issues

- large 5 year EU funded project (IMI eTox) to develop models based on pooled data from all major pharma companies (4 week rat)
Design, make & screen compounds that are “drug-like” & “lead-like” - and have biological activity!

**Tackle attrition & productivity at an early stage**

- **100 Ideas** → 1 NCE
- **80 Ideas** → 2 NCE

**The drug discovery problems**

**The drug design problems**

**The opportunities**
The drug discovery problems
The drug design problems
The opportunities

Future trends: The opportunities...

Number 1:
- Drive design with experimental data!
- Synergise, not compete..

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Future trends: The opportunities...

Number 2:

- Continue to think as much about properties as potency (physicochemical, LE, LLE etc)
- Multi-parametric optimisation
- But now with kinetics (off-rates etc) from an early stage - from biophysical screening (Surface Plasmon Resonance (SPR)/Biacore etc)

Biophysical Screening + Site-directed Mutagenesis

De novo SBDD

Future trends: The opportunities...

Number 3:

- Apply “rational design”, SBDD to the major drug target class GPCRs
- Stabilise the GPCR to the desired conformation (agonist, antagonist etc)
- Finally gain understanding at the atomic level of antagonism-agonism & use that in design
- Sub-type selectivity → Sub-state selectivity

Biophysical mapping to determine binding modes, optimise models
Biophysical screening for kinetics - off rates
X-ray structures with ligands
Future trends: The opportunities...

Number 4:

- Drive data-driven design in cerebro
- Aid intuition, experience, knowledge with target derived information (e.g., hotspots for functional groups, unfavourable waters etc.)

GRID energetic survey of binding site  
Binding-site water energetics (GRID, Watermap...)

Using 3D stereo! Now possible with NO loss of quality or interference (PLANAR)

Future trends: The opportunities...

Number 5:

- New energy and datasets to model toxicity
- IMI eTox project with input from many key players and data pooled from all major Pharma with European site.

Hopefully models based on more than chemical substructures...
Future trends: The opportunities...

- **Number 6:**
  - Think about structures based on how proteins see them, not how we synthesise them!
  - 2D → 3D → Biological profile/fingerprint

\[
\text{Off-target screening} \\
\text{In silico} \\
\text{Prediction of off-target profiles}
\]

Future trends: The opportunities...

- **Number 7:**
  - Simulation
  - Get back to using molecular dynamics etc, - both the lock and the key are flexible & solvated!

Model Refine Predict free-energy of binding
Future trends: The opportunities...

Number 8:
- Integration of data - now plenty available!
- Integration of methods, use to solve what the real problem/bottleneck is, not restricted to "traditional" software & methods

"Computational chemistry"
→ "Computational Medicinal Chemistry"
→ Computer-Aided Drug Design/Discovery (CADD)

Future trends: Still Good...

Number 9:
- Pharmacophore-based concepts:
  - now for both proteins & ligands
  - GRID energetic analysis of binding sites with functional groups present in drug molecules
- Virtual Screening
  - more structure-based but ligand-based with relevant descriptors still useful for hit/tool finding
- Physicochemical properties (logP...)
Pharmacophores from Ligands & Proteins

**FLAP** *(new method from Mol. Discovery / U. Perugia)*

a) Molecular Interaction Fields (MIF) calculated in the active site of a protein structure using GRID

b) MIF is condensed into a few Target-based pharmacophoric points.

c) All possible arrangements of 4 pharmacophoric points are generated

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Steric hindrance as a filter for the many solutions found for each ligand when "docked" in the protein active site.

⇒ A molecule/fragment will be posed/fitted using meaningful contacts ...

(pharmacophoric) b
An early example of fragment screening → better leads

- Factor Xa serine protease - the transformation binding mode “flip”

\[
\text{NH}_2 \quad \text{NH} \quad \text{N} \quad \text{O} \quad \text{S} \quad \text{Cl} \quad \text{O} \quad \text{S} \quad \text{Cl} \\
\]

Hydrophobic / lipophilic interactions

Cation-anion interaction with Catalytic aspartate

\[ \pi \text{-cation interaction} \]

Displaces an energetically “unhappy” water in a lipophilic hotspot

A great fragment, that could have been found....

GRID maps show lipophilic hotspot in S1 - needed to ignore dogma, force-fitting

- Through dogma of S1 needing a basic group did not screen widely or follow up on \textit{in silico} reversed binding and hotspots

“Expected” mode

Broke dogma of basic group needed in S1
Polypharmacology / promiscuous behaviour is one of the many issues that is more probable with clogP > 3.

Another big challenge: Increasing Potency by Adding Grease correlates with Increased Clearance.

...& promiscuity, hERG, phospholipidosis, PPB, P450, insolubility, metabolic, exposure (Vd) ... etc
Beyond Structural Alerts: The Rule of 3/75

**In Vivo Toleration Database**
- 349 IVT studies
- 315 compounds
- 985 doses
- 10,785 organ evaluations

1. Basic compounds 3.6x more likely to be toxic in IVT than neutrals
2. Basics with clogP >3 and TPSA <75 have 24x higher odds for being toxic vs <3 and >75

ClogP and TPSA are key compound design physical property descriptors

Source: Martin Edwards, Pfizer Inc

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Future trends: The opportunities...

- **Number 1:**
  - Drive design with experimental data!
  - Synergise, not compete..

Fragment Biophysical Screening + De novo SBDD
Biophysical Screening + Site-directed Mutagenesis BPM
Off-target screening + In silico Prediction of off-target profiles
“Fragments” or “Components” of a drug molecule are reduced complexity starting points
- Enable a much larger proportion / diversity of relevant drug space to be explored:
  - new targets, new indications (e.g., CNS) without screening file bias

Fragments: Reduced complexity screening

Lower hit-rate for HTS-sized cpds, high-LE fragments rarely show up in hits.

Reduced ligand complexity increases the chances of a match but binding will be low.
Fragment screening delivers high effective chemical diversity from small libraries.

- Good high-LE starting points, even though existing within compounds in the screening file are often missed “dressed up” wrongly (➔ greater chances for H-bond mismatch or steric clash etc.)
Some of the questions to consider...

- Can we find “better” leads?
  - Optimisable (non-flat SAR)
  - Better properties, high ligand efficiency (LE), & ligand lipophilic efficiency (LLE: -logKi - clogP or clogD)
  - Allosteric binding sites...

- Can we finding a hit/lead when HTS fails?
  - Sampling of drug relevant chemical space
  - Sensitive screening method (label-free: biophysical)

- Can we use on the major drug class of GPCRs?
  - Better leads (as above)
  - Control polypharmacology

- What do we need to be successful?
  - Learnings from the past
  - New methods for membrane proteins - stabilization of conformation(s) of interest → biophysical screening, kinetics & X-ray structures

A recent example of fragment screening → better leads: BACE aspartate protease

- Poor/no HTS hits (many companies...)
- Hydroxyethylamine (HEA) compounds difficult to optimise to clean brain penetrant compounds (many companies)
- Fragment screening by many different biophysical methods able to find diverse new different / reduced basicity “warheads”

Biophysical screening platforms (e.g. X-ray, SPR, TINS) used to identify & facilitate evolution of weak mM fragments to nM leads
Fragments: Are all the screening libraries similar?

- Analysis of a partially built in-house “hand-picked” fragment library and the fragments from 3 companies offering fragment screening gave surprising results:
  
  - Practically no overlap at the compound level between any of the commercial vendor libraries

  - Different hits will be obtained by fragment screening at multiple vendors (as well as any differences from different methods)

  - Overlap at the compound level between in-house library and all vendor libraries

  - All 3 vendor libraries contained significant numbers of medchem desirable/acceptable hits

Future trends: The opportunities...

- Number 2:
  
  - Continue to think as much about properties as potency (physicochemical, LE, LLE etc)

  - But now with kinetics (off-rates etc) from an early stage - from biophysical screening (Surface Plasmon Resonance (SPR)/Biacore etc)
Better Leads (from Fragments)?

- Kinetics of binding is an important factor for the in vivo efficacy of many drugs
  - Methods already set up for fragment screening & optimization, such as immobilised protein with SPR screening (BiaCore) can be directly used to measure on and off rates during optimization

Better Leads from Fragments?

- Build up a molecule that has multiple properties optimised for the desired target(s):
  - Ligand efficiency, properties [eg for CNS for “difficult” targets]
  - Kinetics [require method & suitable protein, eg SPR/Biacore]
  - Selectivity / Polypharmacology

- To identify, characterize and optimize the fragment binding a purified protein sufficiently stable for immobilization / screening is needed
  - Ok in general for enzymes
  - Previously very difficult for membrane bound proteins such as GPCRs: now possible (StaR™: Stabilized Receptors)
Future trends: The opportunities...

- **Number 3:**
  - Apply “rational design”, SBDD to the major drug target class GPCRs
  - Stabilise the GPCR to the desired conformation (agonist, antagonist etc)
  - Finally gain understanding at the atomic level of antagonism-agonism & use that in design

Fragment screening for mono or poly-pharmacology

Biophysical mapping to determine binding modes, optimise models

Biophysical screening for kinetics, off rates

X-ray structures with ligands

Future trends: The opportunities...

- Use of new stabilized receptors (StaRs) technology with GPCRs for biophysical mapping & X-ray structures with ligands
  - full SBDD (Structure-Based Drug Design)
- **Heptares Therapeutics**
  - GPCR Drug Discovery Company
    - Integrated GPCR drug discovery capability
    - Unique stabilised receptor (StaR™) technology and strong IP
    - Cross Therapeutic
  - Breakthrough stabilised receptor StaR™ technology
    - 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
  - 40 employees located in Biopark, Hertfordshire UK

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**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 ('StaR™') held in a specific chosen conformation
  - Stable in functionally-relevant, purified form
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

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 (kinetics), crystallisation etc.
  - Transferrable across GPCR families
Types of StaRs

- Antagonist StaR
- Inverse Agonist StaR
- Agonist state 1 StaR
- Agonist state 2 StaR
- PAM StaR
- NAM StaR

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

Proprietary Process for Creating StaRs to stabilise GPCRs → Fragment screening, Kinetics, Biophysical mapping, X-ray structures etc

1. Unstable Native receptor
2. Select Conformation
3. Select Ligand
4. Mutagenesis
5. Thermostability and Selection
6. Iteration
7. Crystallise
8. New GPCR structure
9. Fragment screening Kinetics
10. Purify protein
11. Recombination of mutants
12. SPR/Biacore TINS/Zobio
Enabling technology for GPCR Drug Discovery

Cell Based Assays
- High Throughput Screening

Protein-Ligand Complexes

Recombinant Receptor Membrane Assays

In vitro Pharmacology
- Selectivity

Wild Type Receptor

StaR Receptor

Fragment Screening
- SPR / NMR direct binding

SPR Kinetics and Thermodynamics

In vitro Pharmacology
- Isolated Receptor Conformations

Modelling by SDM with Biophysical Mapping™

Modelling by Therapeutic Antibodies

Target Validation

In vivo Pharmacology
- Efficacy and Receptor Occupancy

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

Warne et al, Nature July 24th 2008
Biophysical Screening on GPCRs

- StaRs broadly applicable to biophysical approaches
  - Stability
  - Protein production

- Investigating multiple methods
  - SPR (Biacore)
  - NMR screening methods (TINS)
  - Thermal denaturation approaches

- GPCR targeted fragment library
  - *In silico* for virtual screening
  - *In vitro* for diversity screening

Common Drug Binding site in all GPCRs

**Family A**
- amine, nucleotide, Lipid, eicosanoid

**Family A/B**
- Peptide Receptors

**Family C**
- Metabotropic glutamate

Drug Binding Site located in transmembrane domain

Considered 'allosteric site' for Family B and C

Family A allosteric site overlaps with ligand binding pocket
Future trends: The opportunities...

- **Number 1:**
  - Drive design with experimental data!
  - Synergise, not compete..

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**Biophysical Screening**

- **Fragment Biophysical Screening**
- **De novo SBDD**

**Biophysical Screening + Site-directed Mutagenesis**

**Off-target screening**

- **In silico**
- Prediction of off-target profiles

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**Biophysical Mapping using Biacore**

- **Site directed mutagenesis**
- 10-30 Mutations to the Binding site region
- Mutant StaRs displayed on a Biacore chip
- Biophysical Map of binding site
- Correlating binding data from Multiple ligands with multiple Mutant StaR proteins
- Detection of binding of different ligands to each mutant StaR
- Label free Direct binding method
Biophysical Mapping™: Definition of binding modes

Matrix of ligand-construct binding data → Data interpretation and modelling → Biophysical maps for each chemical series → Decide between alternative docked poses

Fragment-based drug discovery/design (FBDD) for membrane proteins: GPCRs

- Fragment screening has been performed using stabilized GPCRs, giving results comparable to that obtained using soluble (enzyme) targets

The stabilized GPCR can be immobilized and used for fragment screening using:
- Biacore /SPR: Surface Plasma Resonance Biosensor
- TINS (Zobio): Target Immobilized NMR Screening
Fragment Screening: The new possibilities for enzymes & GPCRs

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
TINS (Zobio)

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

TINS method for finding hits: fragment screening

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

TINS = Target Immobilized NMR Screening

Reference

TARGET

Identification of binding position / mode
TINS method for finding hits

The TINS Approach to Ligand Discovery

Fragment Screening: New possibilities for GPCRs (+ enzymes)

Protease
Phosphodiesterase

Enzymes

GPCRs eg:
β1-AR
A2A

TINS screening of Zobio fragment library for 2 enzyme targets + β1-AR GPCR:

→ Sensible looking hits for all targets
80-100 from ~1400 for enzyme targets
~80 from 580 for β1-AR

→ Specific for each target
- Only one fragment hit in common between GPCR & enzyme target hits
StaR™ technology used to generate thermostable mutant of β1AR.
- Stable in purified, detergent solubilised form
- Functional and drug-binding characteristics preserved
- Completely novel and proprietary

**Approach: TINS - Screen immobilized, DDM solubilized β1AR vs OmpA**

» TINS screening using stabilized β1AR was able to identify ~80 hits from 580 fragments screened:
  - reasonable looking structures
  - including compounds where activity could be confirmed by biochemical screening

**Are the GPCR Fragment Screening Hits from TINS (Zobio) generally reasonable / valid?**

» Yes!

» Many reasonable / interesting hits

» Activity confirmed for many by biochemical assay

» Not the same as found for the other targets

» Further work ongoing
GPCR Fragment Screening Examples

- **β₁- Adrenoceptor by TINS NMR**
  - ZoBio library (Gregg Siegal)
  - TINS screening using stabilized 1AR was able to identify ~80 hits from 580 fragments screened
  - Promising chemical structures
  - Including compounds where activity could be confirmed by biochemical screening

- **Adenosine A₂ₐ by SPR/Biacore**
  - University of Utah (David Myszka)
  - Xanthine derivatives identified as hits
  - 200 μM screening concentration

Fragment Screening using Biacore

- StaR immobilised on chip
- Challenged with Fragments (~150-250 Da)
- Fragment-based Drug Discovery
- Fragments 'grown' to fill active site and give potent novel leads
- Highly sensitive detection - low affinity binders
- Fragments progressed to structure model
**SPR fragment screening**

**Analysis of hits**

- Hits are further analysed by
  - Detection of non-specific binding on control surface (e.g., denatured protein)
  - Full concentration response curves
  - Biophysical mapping™
  - Analysis vs receptor with binding site mutations
  - Co-crystal structures

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**3D Structural Information on Industrial Timescale**

**Heptares GPCR Crystallography**

- State of the art facility for GPCR crystallisation set up
- Vapour diffusion crystallography
- Co-crystallisation with multiple ligands
- Nanolitre lipidic cupid phase robotics
- Synchrotron sources at Diamond, ESRF, SLS
StaR Drug Discovery Applications

Need to be careful about correlations...

The Correlation Problem: Spurious Correlations!

San Francisco Statistics:
The number of fire engines at each fire increases as do the damages at each fire.

Conclusion: Fire engines cause the damage!

Courtesy of Arthur Doweyko, BMS
And about biases in how we see data

Future trends: The opportunities...

▶ Number 6:
  ▪ Think about structures based on how proteins see them, not how we synthesise them!
  ▪ 2D → 3D → Biological profile/fingerprint

Off-target screening + In silico Prediction of off-target profiles
Describing molecules: The Protein View

- 2D structure? 
  X

- 3D structure? 
  Better!

- Fingerprint of binding to 150 diverse targets? 
  Better?

- Experimental biophysical characterization (BPM, kinetics, X-ray structure etc.)

Describing molecules: The Protein View

- “Biological fingerprints”? 

  In Vitro Profile  Predictive associations  In Vivo Effect

  120-150 in vitro pharmacological,
  38 ADME(T) & pharmaceutical assays

Cerep BioPrint®
Experiences from BioPrint Broad Pharmacological Profiling

- These fragment cores are in potent hit compounds with undesirable off-target activities that could not be removed.

- This fragment core was in a “clean” potent hit compound without the undesirable off-target activities → candidate.

→ Can be critical to get core (fragment) starting point right.

Experiences from BioPrint Broad Pharmacological Profiling

- Very similar structures can have very different polypharmacology:

\[
\text{IC}_{50} = 4 \text{ nM, ClogP 5} \\
\text{IC}_{50} = 2 \text{ nM, ClogP 2}
\]

BioPrint profiling:

- extremely promiscuous
- 32 Submicromolar binding activities
- 2 Submicromolar binding activities
- + 1 CYP
Experiences from BioPrint
Broad Pharmacological Profiling

Very different polypharmacology, both for related targets and broadly for candidates for a 5HT target:

Correlation of toxic effect with BioPrint profile was found for a CNS target.

The prediction challenge

Potent hits

Cluster using structural fingerprints?
\[ \text{e.g. Daylight} \]

Cluster using a circular structure-based fingerprint?
\[ \text{e.g. Scitegic FCFP6} \]

Cluster using \textit{in vitro} biological activities, diverse targets?
\[ \text{e.g. BioPrint} \]

Relatively selective

Can get individual models for \( \sim 50\% \)
In Silico Prediction of Broad Profile for Polypharmacology

- Warning: “Similar” compounds often do not have similar off-target activities!

- For project compounds not yet viable to replace experimental screening - too many false positives / negatives

Predictions from ChEMBL dataset (7.2K targets, 0.6 million compounds, 2.4 million activities) + IUPAR +

In Silico Prediction of Broad Profile for Polypharmacology

- Experimentally validated examples of predicting new targets for drugs (richer chemical space than for many new project compounds) but “bigger picture” may be missed:

- Paxil (paroxetine):
  - SEA Predicted beta adrenergic blocker - confirmed at 10 μM on β1
  - Experiment (BioPrint):
    - 4 Active at 100nM-1μM
    - 9 Active at 1-5 μM

- Prozac (fluoxetine):
  - SEA Predicted beta adrenergic blocker - confirmed at 4.4μM on β1
  - Experiment (BioPrint):
    - 5 Active at 100nM-1μM
    - 4 Active at 1-5 μM
    - 6 Active at 5-10μM

- Clonazapam
  - Good prediction

Good prediction of BioPrint assays, + some interesting predictions of other activities but false positives

Difficult to predict BioPrint assays, false positives + some interesting predictions of other activities
**In Silico Prediction of Broad Profile for Polypharmacology**

- Use of docking into X-ray structures an exciting future “de novo” approach

- BUT from results seen so far a current NO-GO!

**Summary**

- Fragments & SBDD can provide “better” starting points for both enzyme & GPCR targets
  (where binding is relatively optimal & properties (LE etc) good vs sub-optimal but more potent hits from HTS etc)

- Full “rational design” (SBDD) now possible for major drug class of GPCRs

- Analyse & use all the data to drive better drug discovery decisions:
  - Physicochemical properties etc
  - Protein structure (SBDD) & profiling (BioPrint etc)
  - But don’t forget *in cerebro*! Aid creativity, intuition, knowledge with 3D. Think about relevance & beware of bias/overfitting
Summary: Future trends & opportunities

- **Fragments**
- **Properties: physicochemical, LE, LLE etc - kinetics**
- Biophysical screening / mapping
- **GPCR knowledge antagonists - agonists etc**
- **In cerebro - with 3D**
- Toxicity prediction
- Describe structures in a biologically relevant way
- Simulation (MD etc) → FEB
- Integration of data and approaches - no bounds

Acknowledgements

**TINS**
Gregg Siegal, Francis Figarao, Johan Hollander, Eiso AB (U. Leiden & Zobio)

**SPR**
David Myszka, Rebecca Rich (University of Utah)

**FBDD/SBDD - GPCRs**
All the team at Heptares (Platform-Discovery-Management)
LMB: Richard Henderson, Gebhard Schertler, Chris Tate, Tony Warne

**FBDD / Enzymes**
Lena Tagmose (Lundbeck)

**BioPrint**
BioPrint teams at Cerep and Pfizer