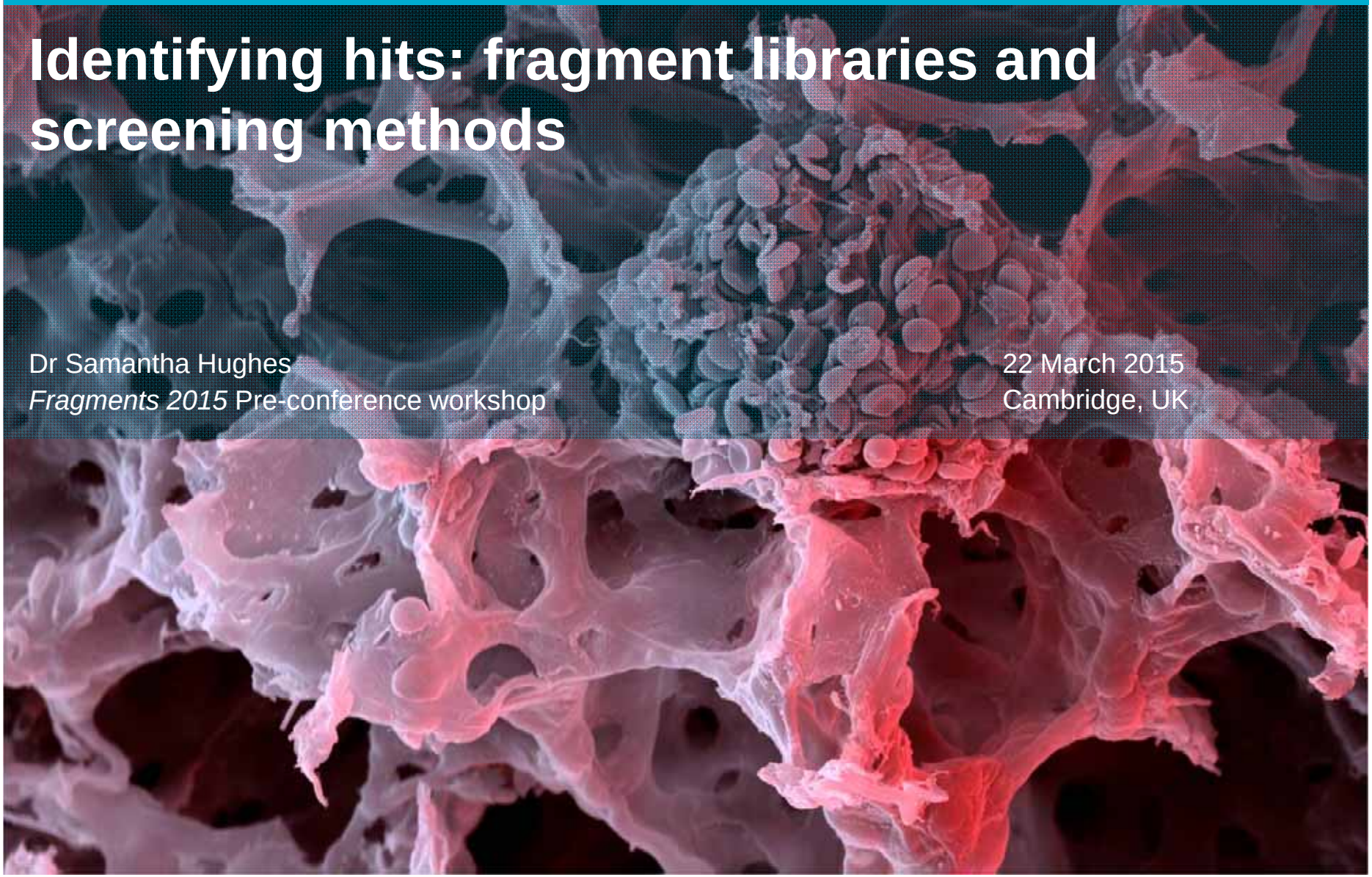
AstraZeneca
IMED Biotech Unit

Identifying hits: fragment libraries and screening methods



Dr Samantha Hughes
Fragments 2015 Pre-conference workshop

22 March 2015
Cambridge, UK

Outline of this workshop session

1. **Fragment library design**
   - What makes a good fragment?
   - 2D vs 3D fragments
   - What makes a good fragment library?
   - Assembling a fragment library

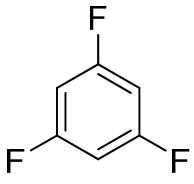
2. **Fragment screening methods**
   - Popular screening methods
   - Orthogonal fragment screening
   - Ligandability assessment by fragment screening

Fragment screening libraries

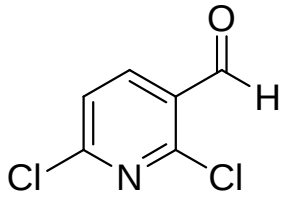
What makes a good fragment ?

- Rule of three physicochemical guideline not sufficient[1]
  - HBA can be relaxed
- Some polarity & functionality but not too complex
- Several vectors for synthetic elaboration
- Novelty
- No structural alerts
- In *general* avoid pan-assay interfering compounds[2]
  - aggregators, redox cyclers
  - be wary of frequent hitters
- *Method-specific* detection “handles”
  - e.g. F (NMR), covalent warheads (tethering)

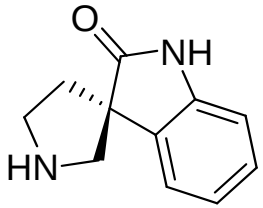
Ro3-compliant compounds



too simple



too reactive

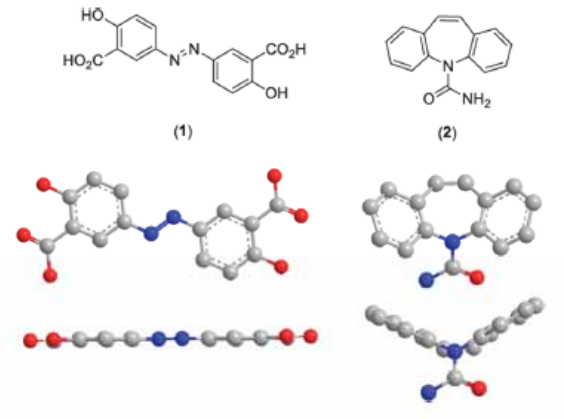


just right?

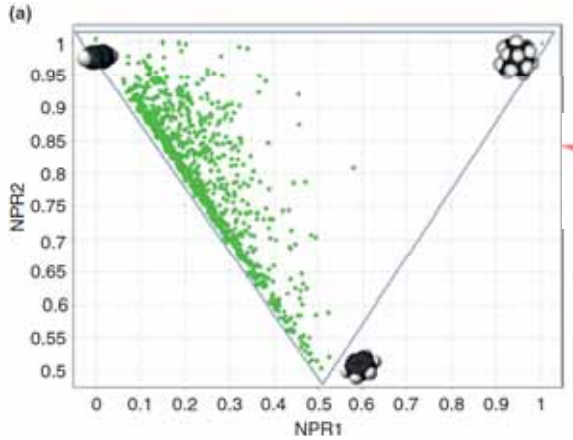
2D vs 3D fragments

An ongoing debate

- Do we need 3D fragments to prosecute novel target classes ?
  - No evidence yet for PPI's[3] but other target classes ?
- Are "3D" fragments synthetically challenging to follow-up ?
  - Not necessarily[4]
- Will 3D fragments give a lower hit rate?
  - Possibly
- How to measure "3-dimensionality" [5,6]



Fsp^3



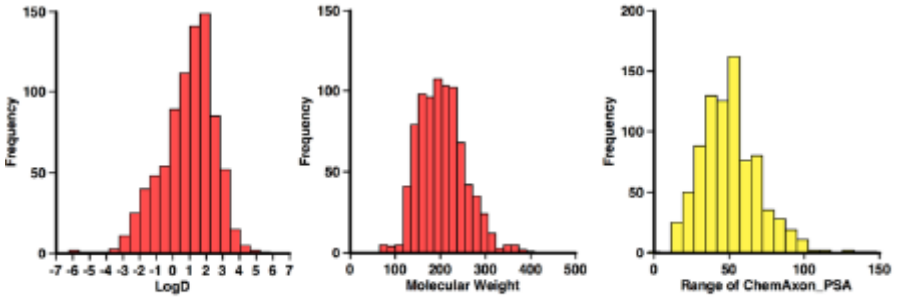
PMI triangle plots [4,5]

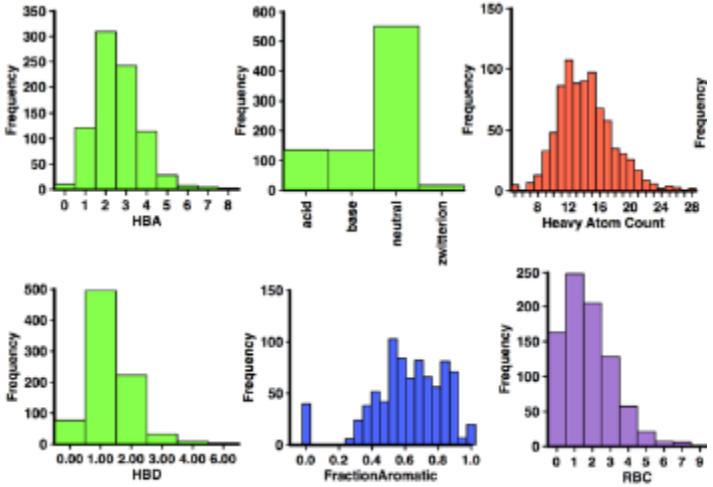
Plane of best fit[6]

Fragment screening libraries

What makes a good fragment library ?

- Appropriate physicochemical space coverage
- Chemical space coverage (diversity)
  - scaffolds, fingerprints, pharmacophore triplets, 3D
  - acids/bases/neutrals/zwitterions
- Commercial or in-house availability of near neighbours for follow-up
- High aqueous solubility (>500 µM, typically ~ 1mM)
- High purity
- Stable in DMSO and screening buffers
- Non-aggregating





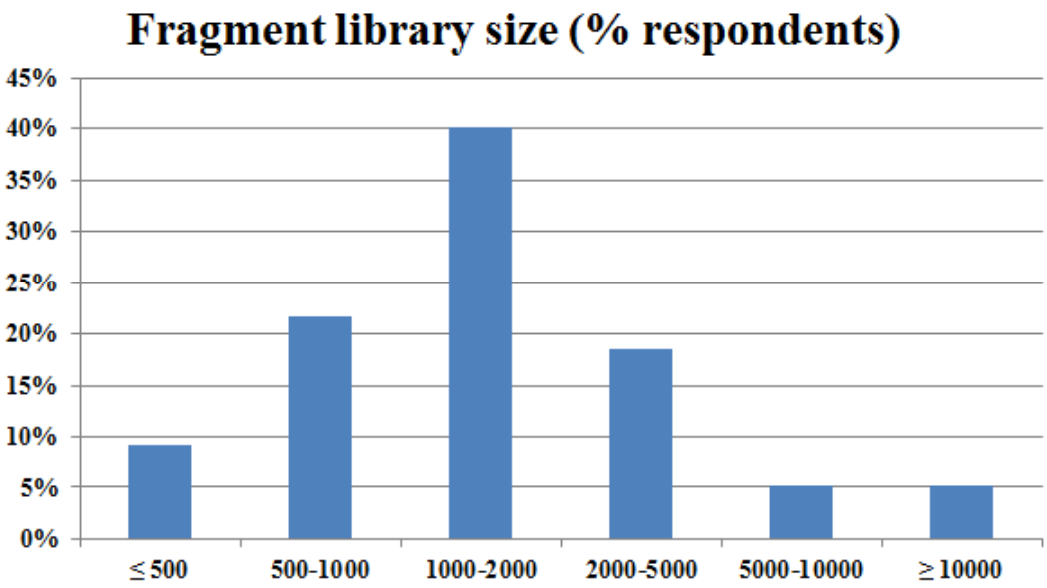
Profile of published fragment hits provided by Chris Swain[7] (www.cambridgemedchemconsulting.com)

What is the ideal library size?

Reported fragment library sizes

1. *Practical Fragments* poll (right)
2. Analysis of 22 published libraries show a median of 1300 fragments[8]

***Practical Fragments* Poll 2013**



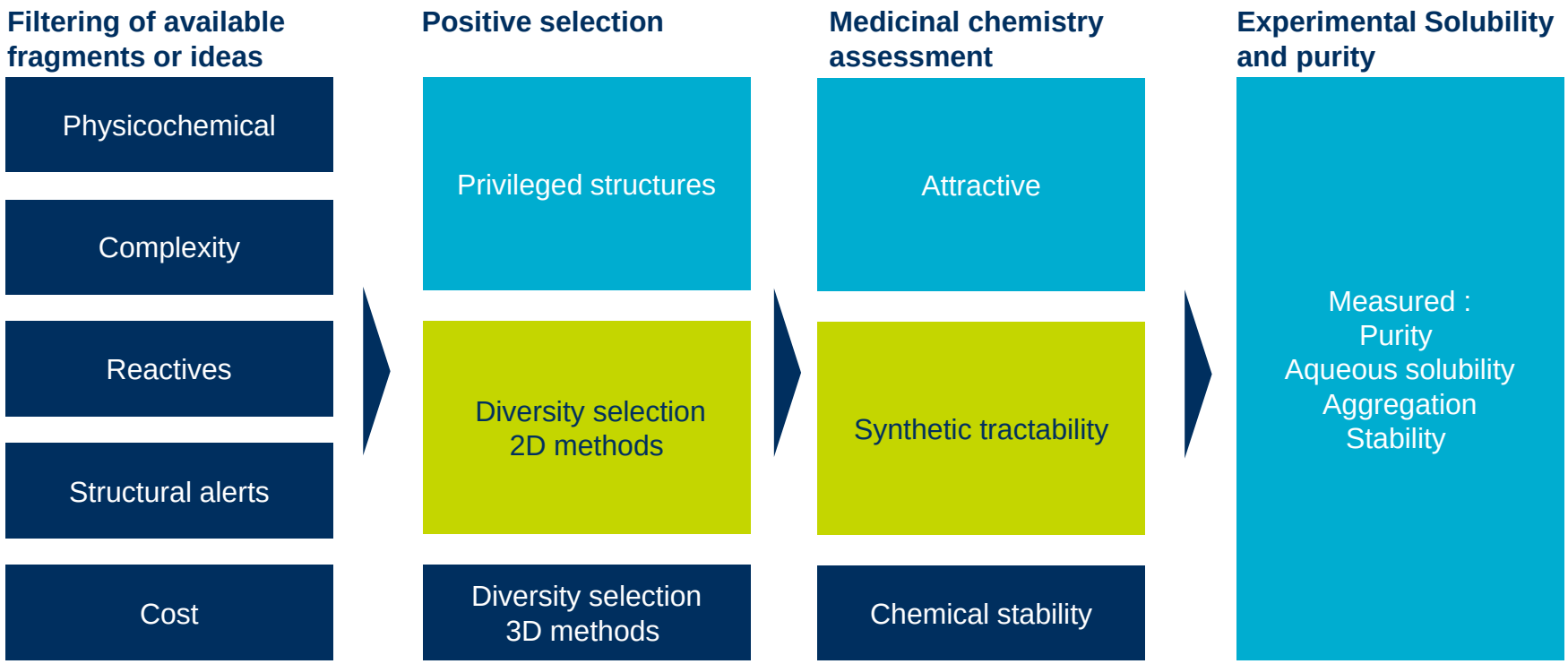
http://practicalfragments.blogspot.nl

The ideal library size depends on your screening methods

Quality of library is more important than size

Fragment library selection/design process

Generic workflow



For example fragment library papers, see references 1 and 9-12

Fragment screening methods

Key points

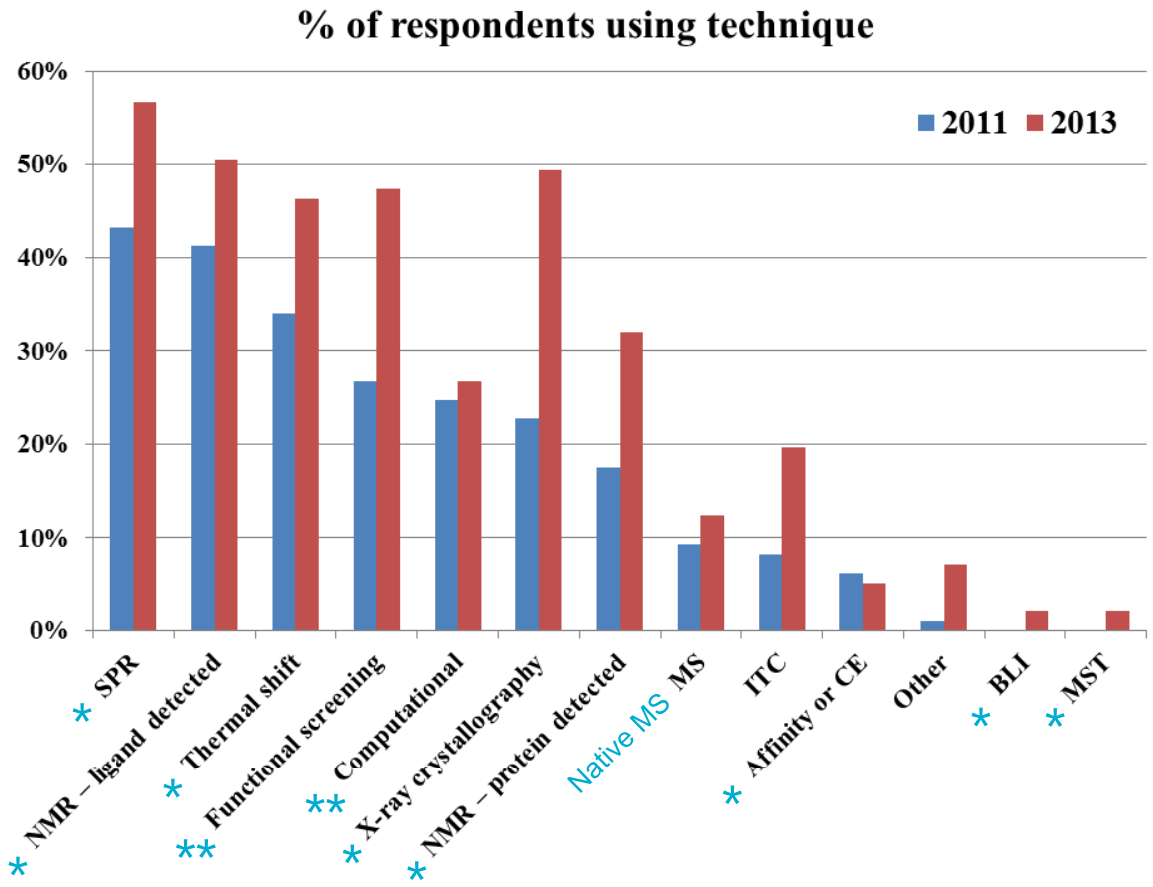
Weak binders require **high sensitivity** detection methods and/or testing at **higher concentrations (around K_D)** in order to detect the event.

Screening in several orthogonal techniques is common

If you do not choose the methods or concentration ranges appropriately **you will potentially miss true hits**

What methods are we using ?

Practical Fragments polls



Moderate (*) to high throughput (**) methods

http://practicalfragments.blogspot.nl

Most use 3 methods or more

The top 4 screening methods

X-ray and NMR screening

X-ray crystallography

Information: ligand protein binding site and interactions; stoichiometry

Pros: No limits on affinity, enables SBDD

Cons: false negatives

Requires: a soakable, robust crystal system, pooling and deconvolution for primary screening

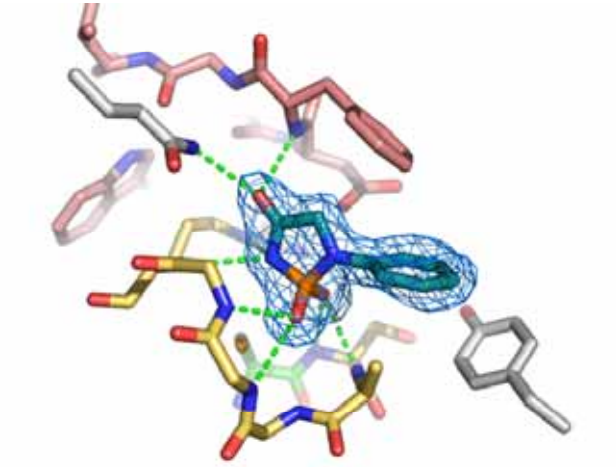
NMR (ligand and protein observed)

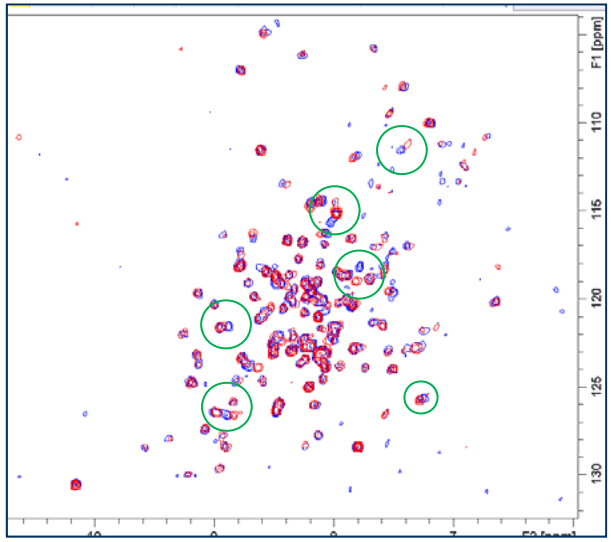
Info: K_D and binding site

Pros: very sensitive, low false positive rate, solution measurement

Cons: blind to high affinity ligands, protein size limitations (protein-detect mode)

Requires: labelled protein (for protein observed NMR), pooling & deconvolution for primary screening





The top 4 screening methods

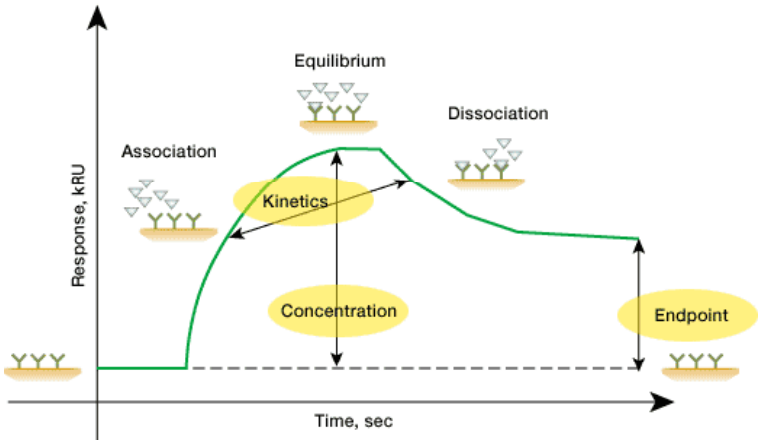
SPR and Functional (biochemical) screening

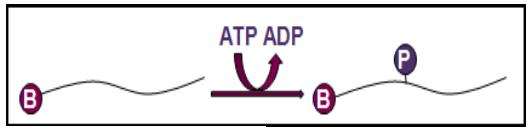
SPR

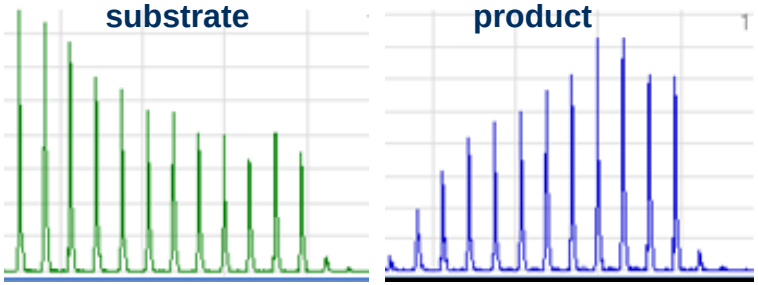
Information: K_D, kinetics, stoichiometry
Pros: low protein requirement
Cons: immobilization can block ligand access, fragment size restrictions for large proteins
Requires: immobilization of protein, competitive ligand for binding site ID

Functional (biochemical) screening

Information: K_i/IC_{50}/EC_{50}
Pros: high throughput, low protein requirement, solution measurement
Cons: potential high false positive/false negative (but these can be reduced if directly detect substrate/product)
Requires: a biochemical reaction, competitive ligand for binding site ID







Orthogonal fragment screening

Methods don't always agree

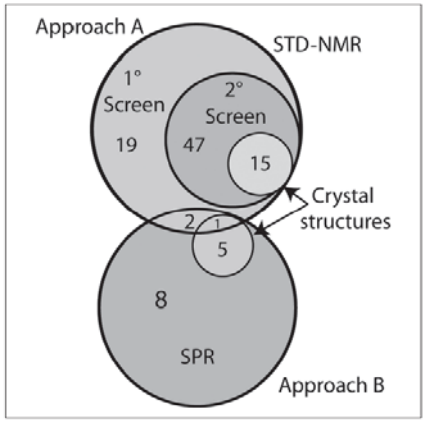
Orthogonal screening benefits:

- Provides complementary information e.g. functional effect and direct binding measure
- Reduces false positives
- Prioritises hits for lower throughput methods

But not all methods agree[13,14] due to:

- Different buffers, T, pH, concentrations
- Sensitivities of methods
- Solution *vs* immobilised protein
- Different protein constructs

HIV-1 IN[2]



Orthogonal screening: HSP90[1]

Multiple methods can provide more starting points for FBDD and provide a degree of consensus

Which methods to use ?

Questions to ask

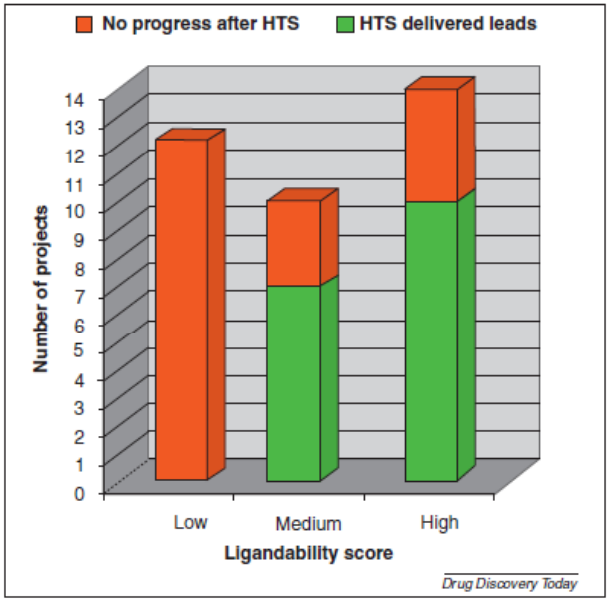
- **What information do you need to start a hit-to-lead program ?**
- Which combination of methods will give this information ?
- Assay throughput *vs* fragment library size ?
- Sensitivity range of assay *vs* likely affinities of fragments?
- Protein requirements:
  - size, stability, purity, amount, labelling, DMSO tolerance
- Availability of a soakable crystal system of sufficient resolution
- Will immobilization impede ligand access?
- Is a competitive ligand required ?

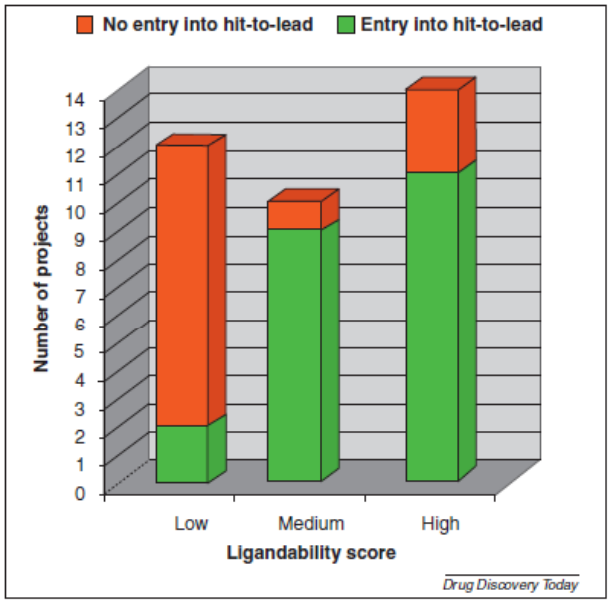
Select methods appropriate for your target and library

Fragment screening and target ligandability

- Fragment screening can give an indication of target ligandability[15,16]
  - Assess fragment screen hit rate, diversity and affinities
- AstraZeneca analysis[16]
  - Low ligandability score correlates with HTS failure
  - Focus efforts on non-HTS approaches for hit ID

AstraZeneca Ligandability Assessment[4]





References

1) Jhoti H. *et al*, *Nat. Rev. Drug. Disc*., 2013,12, 644
2) Baell J.B. & Holloway G.A., *J. Med. Chem*. 53, 2010, 2719-2740; Dahlin, J.L *et al*, *J. Med. Chem.*, 2015, just accepted, DOI 10.1021/jm5019093; Soares, K.M *et al*, *Assay Drug Dev. Technol*., 2010, 8,152–174
3) Turnbull A.P *et a*l, *Research and Reports in Biochem.* , 2014, 4, 13-26
4) Morley A.D *et al*, *Drug Discov. Today*, 2013, 18, 1222-1227
5) Sauer, W.H.B. and Schwarz, M.K., *J. Chem. Inf. Comput. Sci*., 2003, 43, 987- 1003
6) Firth N.C *et al*, *J. Chem. Inf. Model*, 2012, 52, 2512-2525
7) Downloaded from www.cambridgemedchemconsulting.com
8) Doak B.C. *et al, Aust. J. Chem.* 2013, 66, 1465-1472
9) Schuffenhauer A. *et al*, *Curr. Top. Med. Chem.,* 2005, 5, 751-762
10) Chen I-J & Hubbard R.E., *J. Comput. Aided Mol. Des.*, 2009, 23, 603-620
11) Blomberg, N. *et al*, *J. Comput. Aided Mol. Des.,* 2009, 23, 513-525
12) Lau W.F. *et al*, *J. Comput. Aided Mol. Des.,* 2011, 25, 621-636
13) Meiby, E. *et al, Anal.Chem.* 2013, 85, 6756-6766
14) Wielans, J. *et al*, *J. Biomol. Screen.*, 2013, 18, 147-159
15) Hajduk P.J. *et al*., J.*Med. Chem*.,2005,48,2518
16) Edfeldt F.N.B *et al, Drug Discov. Today*, 2011,16,284-287

Acknowledgements

- AstraZeneca
  - Scott Cowen, Loredana Spadola, Heike Schoenherr, Nathan Fuller, Joe Patel, Chris Phillips, Jon Read, Willem Nissink, Elisabetta Chiarparin, Derek Barratt
- The *Fragments 2015* committee
  - Mark Whittaker, Mike Hann, Thorsten Nowak, Gordon Saxty
- Dan Erlanson (*Practical Fragments* Blog)
- Chris Swain (Cambridge MedChem Consulting)