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Accelerating Drug Discovery

Introduction to FBDD

Fragment screening methods and library design

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Why “fragment” screening methods?

Guess the potency for LE=0.3

MW	Heavy Atoms	Predicted K_i for LE=0.3
150	11	?
200	15	?
250	19	?
300	23	?

- Need to screen at higher ligand concentrations than HTS
- Require robust assays capable of quantifying weak binding

LE: Hopkins *et al*, Drug Discov Today (2004) 9, 430-431

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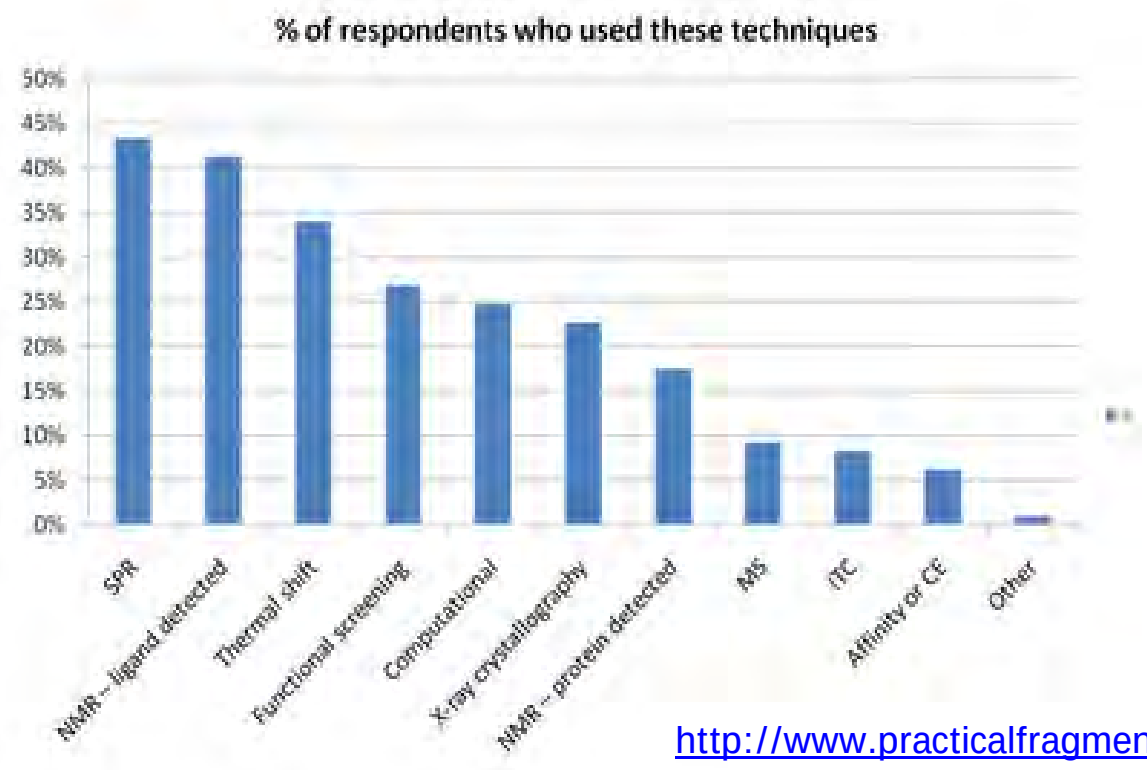
What methods are we using ?
Practical fragments Poll

- A range of methods are used for fragment screening
  - poll on Practical fragments Blog October 2011



Snapshot of the community usage (limited sample!)

Changing landscape ?

http://www.practicalfragments.blogspot.co.uk/

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Principles of screening methods

High concentration functional screening (biochemical assay)	measure substrate or product levels (MS, fluorescence, abs, CE...)
NMR ligand detected	observe effects on 1H or 19F NMR spectra of fragment
Surface Plasmon Resonance (biosensor methods)	detect changes in optical properties of surface containing protein/ligand
Thermal shift	measure thermal stability shift of protein
X-ray crystallography	observe electron density of bound fragment
Computational	docking and scoring of fragments

See Siegal *et al*, Drug Discov Today (2007) 12, 1032-1039 for an overview of methods

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Principles of screening methods

NMR protein detected	observe effects on NMR spectra of labelled protein
Tethering	fragments form disulfide with Cys detect mass of covalent complex
Native MS	detect mass of bound noncovalent complex
Isothermal calorimetry	measure solution temperature changes upon ligand binding
Capillary electrophoresis	measure change in mobility under electrophoretic gradient
Microscale thermophoresis	detect change in hydration shell of protein
Affinity chromatography	immobilise protein on a column, measure retention time of fragment

See Siegal *et al*, Drug Discov Today (2007) 12, 1032-1039 for an overview of methods

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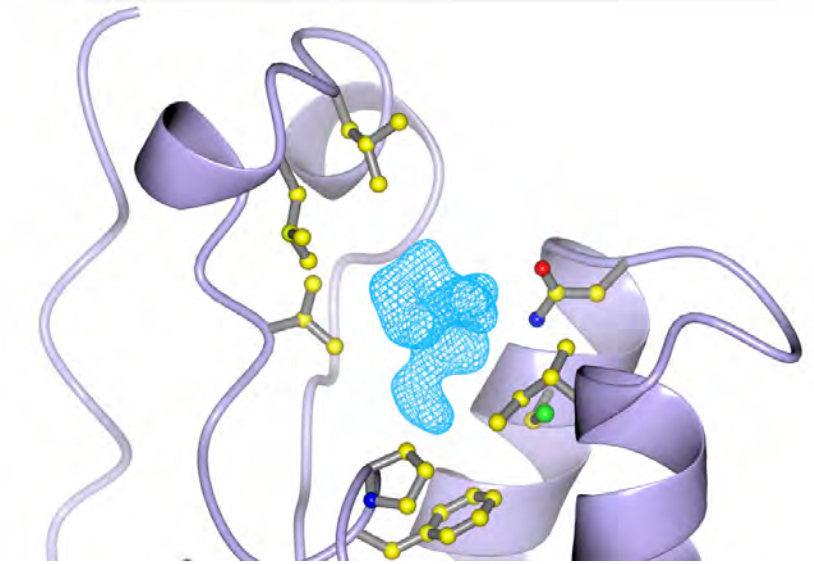
Information that can be obtained

From one or more fragment screening methods

- Fragment binding (yes/no) or inhibition (yes/no)
- Dissociation/inhibition constants K_d, K_i
- Binding mode or binding site
- Stoichiometry
- Kinetics: on-rate, off-rates
- Thermodynamics ($\Delta G, \Delta H, \Delta S$)



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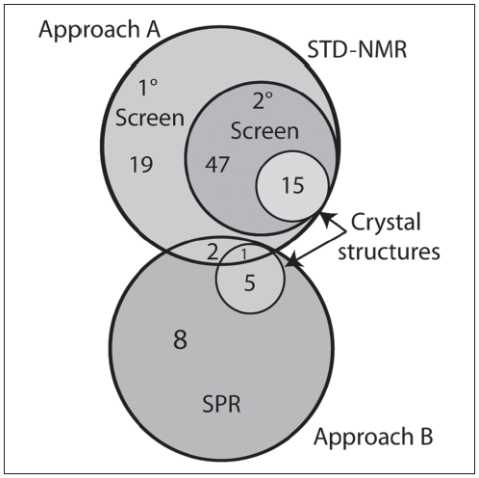
Practical considerations

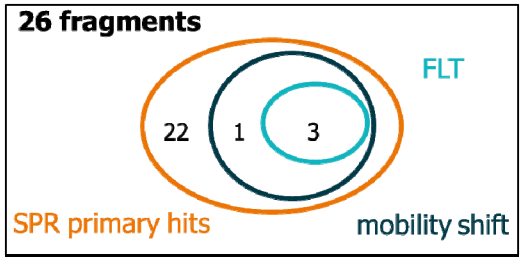
- Assay throughput and concentration range
- Protein size, stability, purity & amount required
- Labels (isotope labels, fluorescence tags)
- Reducing false positives and false negatives
- Orthosteric/allosteric binding site; competitive ligand available?

Orthogonal screening
Considerations

- Sequential orthogonal screening used to confirm initial fragment hits
  - gain more information on hits
  - ideally show a functional effect and confirmed binding to target
- However, true hits often do not show up in all methods

Wielans *et al*, J Biomol Screen (2013) 18, 147-159





Pollack *et al*, J Comput Aided Mol Des (2011) 25, 677-687

- Parallel orthogonal screening can identify more starting points for FBDD

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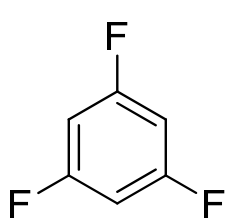
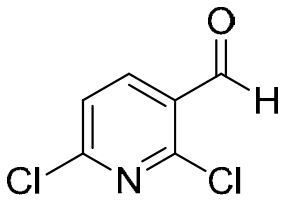
Fragment libraries
Attractiveness

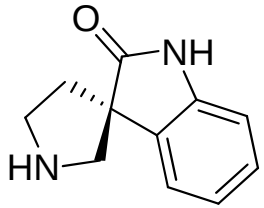
- The purpose of a general fragment screening library is to provide diverse, attractive starting points for medicinal chemistry
  - **a good quality fragment library is key**
- What makes an attractive fragment ?
  - Astex “Rule of 3” is a useful guideline for physicochemical properties
  - further aspects to consider in library design/selection



too simple



too reactive



just right?

Some example Ro3-compliant compounds

Rule of 3: Congreve et al, Drug Discov Today (2003) 8, 876-877

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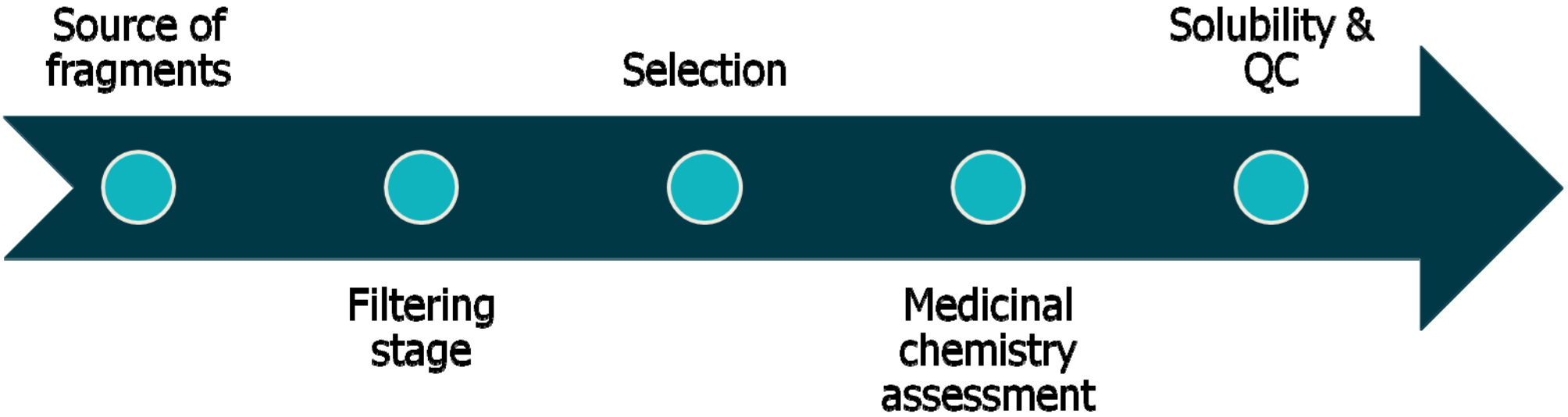
Generic library generation process



Schuffenhauer *et al*, Curr. Top. Med. Chem (2005), 5, 751-762
Chen and Hubbard, J Comput Aided Mol Des (2009) 23, 603-620
Blomberg *et al*, J Comput Aided Mol Des (2009) 23, 513-525
Lau *et al*, J Comput Aided Mol Des (2011), 25, 621-636

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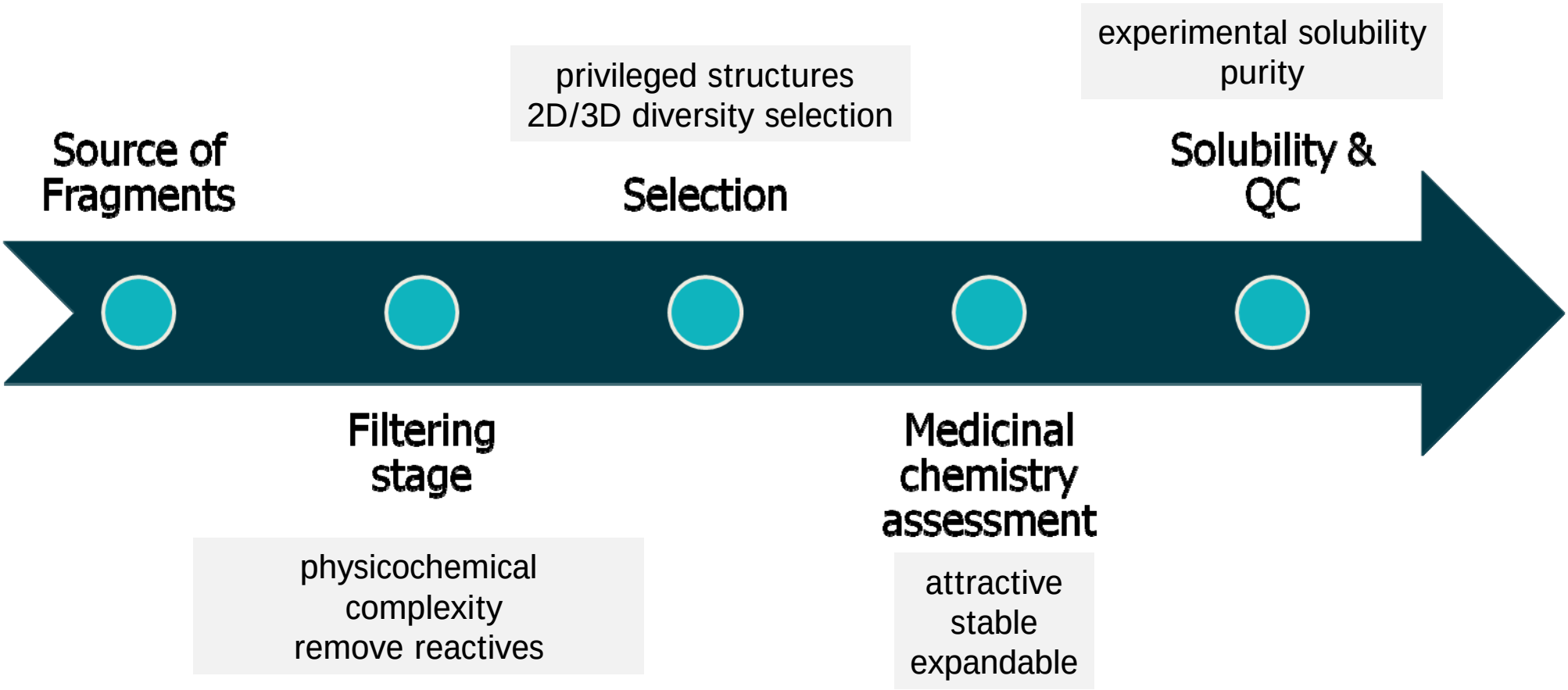
Generic library generation process



A substantial investment of time and resource

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