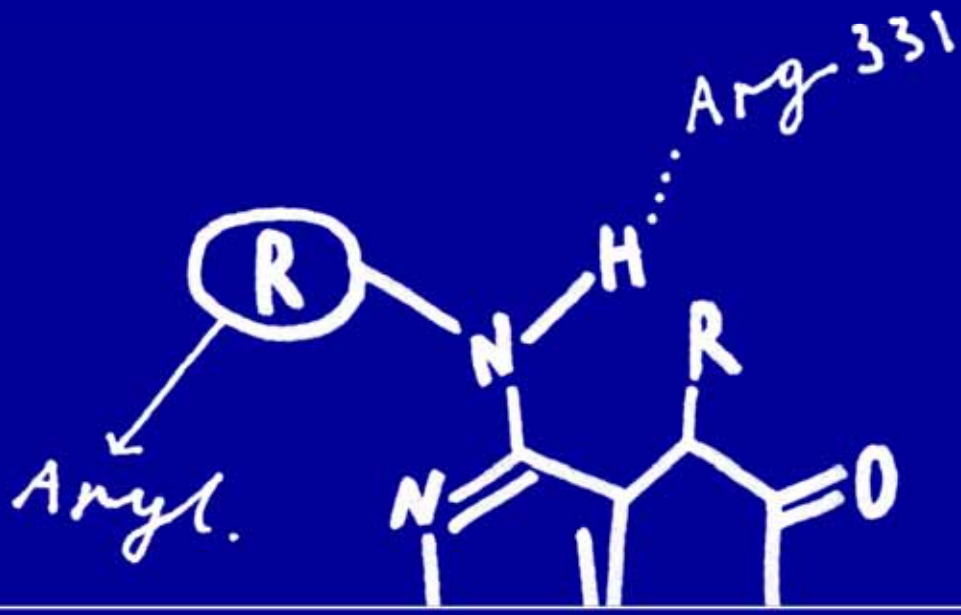
evotec

'RESEARCH NEVER STOPS'
Building innovative drug discovery alliances

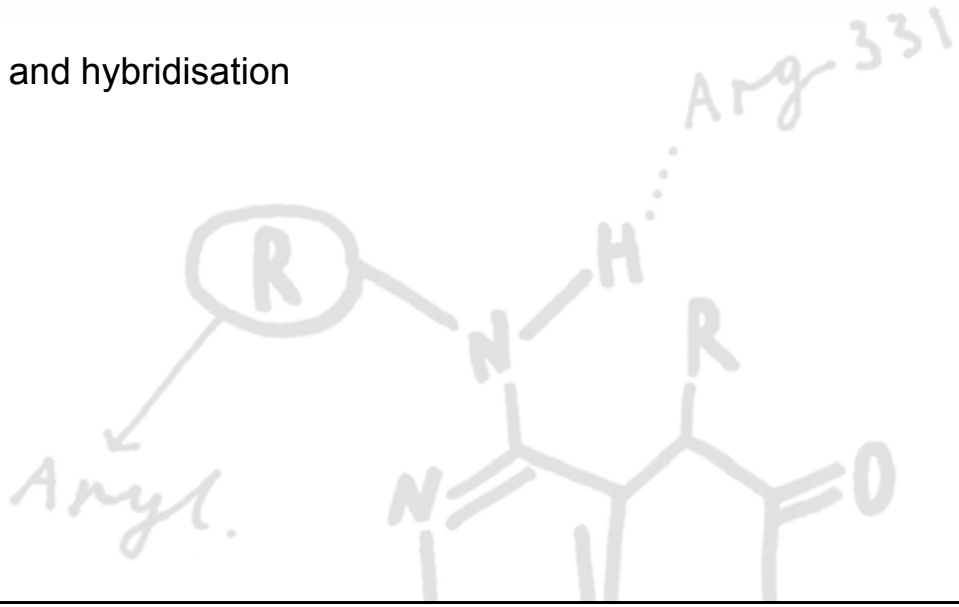
Hit optimisation using fragments

Mark Whittaker



Agenda

- **Fragment optimisation in an ideal world**
- Fragment optimisation in reality
  - Metrics for fragment hit assessment and optimisation
  - Selecting the best fragment hits to work with
  - Fragment expansion
  - Growing, linking, merging and hybridisation
- Summary



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Fragment optimisation in an ideal world…..

but unfortunately in the real world these attributes are not always met?

- Fragment starting points have no obvious structural liabilities and exhibit high ligand efficiency (>0.35) with good affinity/activity confirmed by orthogonal assay methods
- Robust and efficient crystal system provides high resolution crystal structures (<2.5 Å) in a rapid fashion
- Ligand complexes each exhibit a single well defined fragment binding mode suggestive of enthalpic binding
- Clear vectors are available for fragment growing to improve potency by making additional well defined interactions
- Unlimited access to structural biology to enable iterative structure-based design to check and refine design concepts as optimisation progresses
- Maintain, or even improve, original ligand efficiency during optimisation
- Design process addresses key off-targets, particularly family related proteins, from the outset
- Optimisation to provide development candidate, that satisfies all TTP criteria, is completed in a very short time through the synthesis of less than 100 compounds

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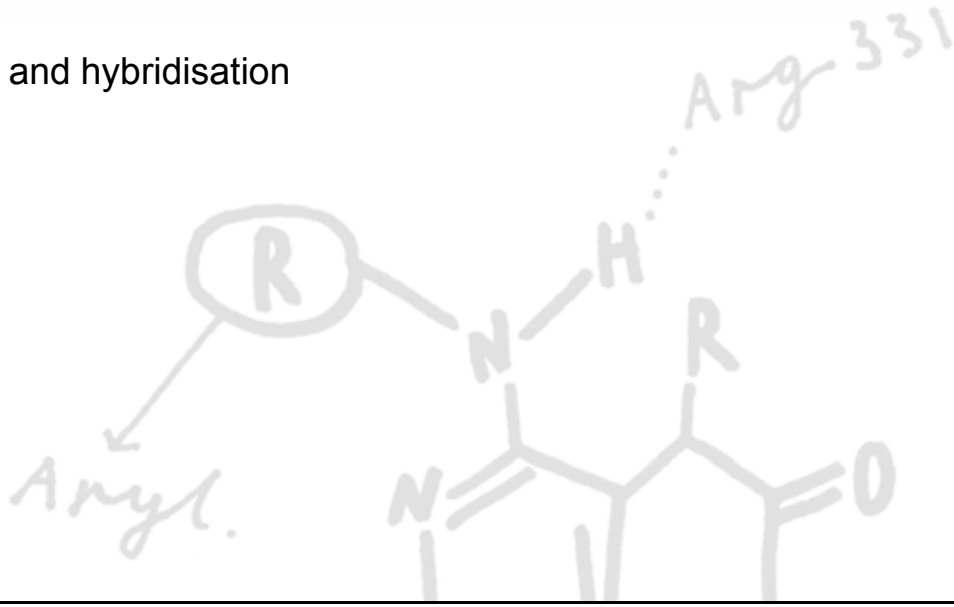
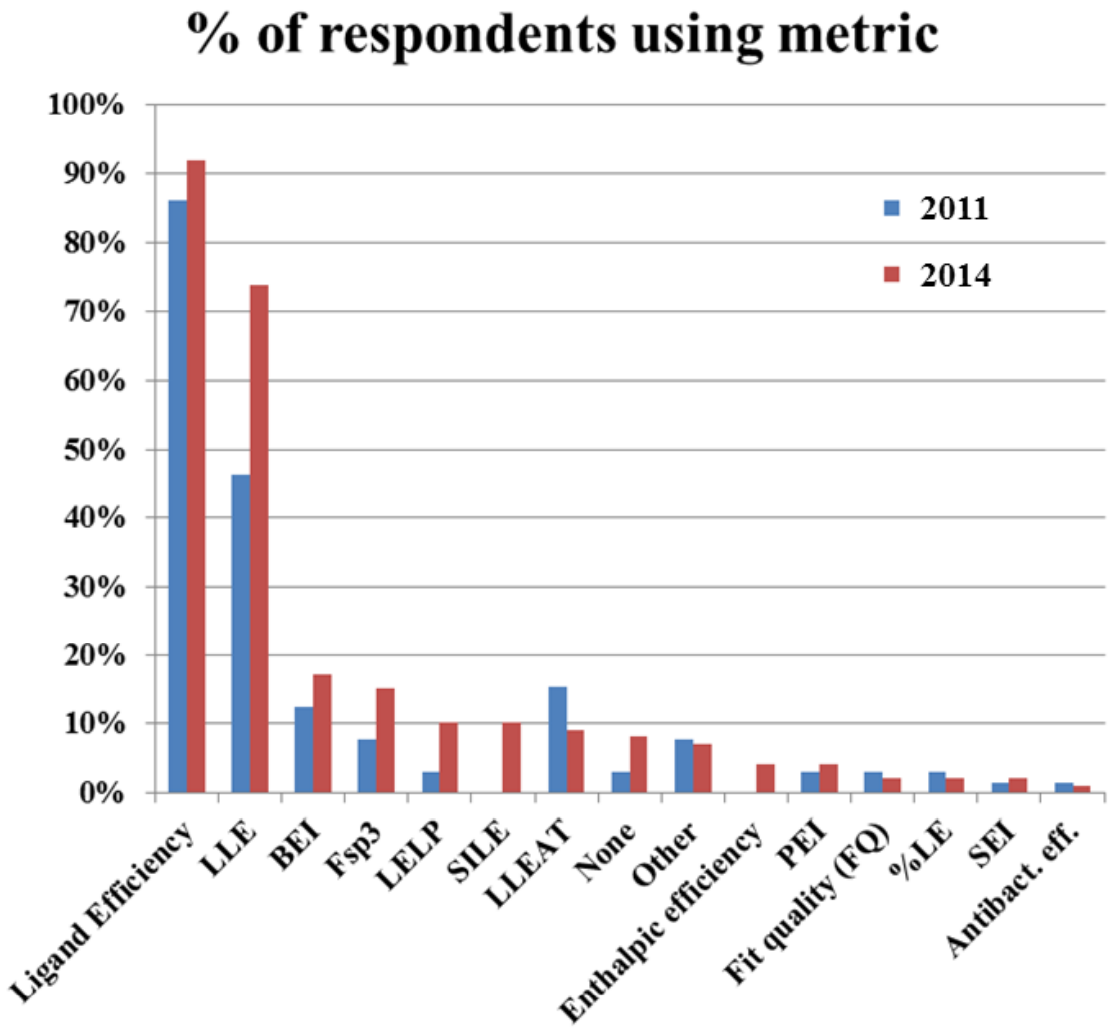


There is a plethora of metrics available to aid fragment hit selection and optimisation

Practical Fragments poll result of metrics used

- Ligand Efficiency and Ligand Lipophilicitiy Efficiency are the preferred metrics
- Additional efficiency metrics include Binding Efficiency Index (BEI), Group Efficiency (GE), Fit Quality (FQ) and Size Independent Ligand Efficiency (SILE)



Source: Practical Fragments 23 June 2014: http://practicalfragments.blogspot.co.uk/2014/06/metric-poll-results-plus-ca-change.html

Ligand efficiency (LE) – the first metric for FBDD

A metric that relates potency to the number of non-hydrogen atoms

- Ligand efficiency LE = $-\Delta G/HAC = -RT \ln(K_d)/HAC$ – usually expressed as kcal mol^{-1}
  - Often simplified as LE = $1.4(-log IC_{50})/HAC$
- Ligand efficiency is used to prioritise fragments for progression
  - Fragments are typically more ligand efficient than HTS derived hits
- LE is also used to monitor the progress of optimisation

HAC = Heavy Atom Count

Hopkins A.L. *et al., Drug Discov. Today*, 2004, **9**, 430-431; Bembenek S.D. *et al., Drug Discov. Today*, 2009, **14**, 278-283; Tsai J. *et al., Proc. Natl. Acad. Sci. U S A*, 2008, **105**, 3041-3046; Murray C.W., et al., *Trends Pharmacol. Sci.*, 2012, **33**, 224-232; Schultes S. *et al., Drug Discoov. Today: Technol.*, 2010, **7**, e-157-e162. Hopkins, A.L. *et al., Nat. Rev. Drug Disc.*, 2014, **13**, 105-121.

Lipophilicity Efficiency

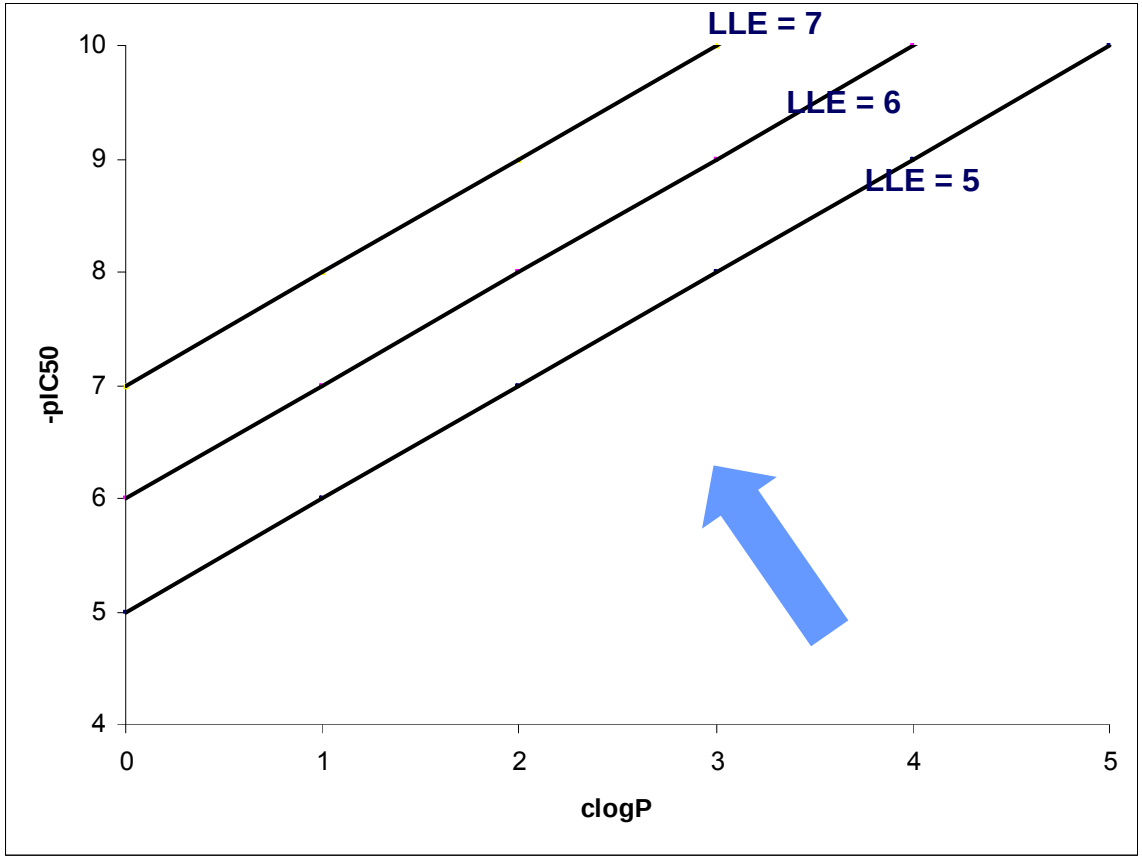
LLE (or LipE) – A simple lipophilicity metric

- Ligand lipophilicity efficiency
  - Ligand lipophilic efficiency (LLE) is a metric used to monitor the lipophilicity with respect to *in vitro* potency of a molecule
  - LLE can be estimated using the equation:

$$LLE = pIC_{50} \text{ (or } pK_i) - cLogP \text{ (or LogD)}$$

  - Ideally target LLE's of ~5-7 or greater
  - Optimisation goal - Improve potency without increasing lipophilicity i.e. optimise in the direction of the arrow

- LLE does not take into account the size of the ligand and so is perhaps better used in the optimisation process than in selecting fragments in the first place
  - This shortcoming is addressed by the LLE_{AT} metric from Astex



1) Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Discov.* 2007, **6**, 881-890.
2) Mortenson P.N.; Murray C.W., *J. Comput. Aided Mol. Des.* 2011, **25**(7), 663-667.

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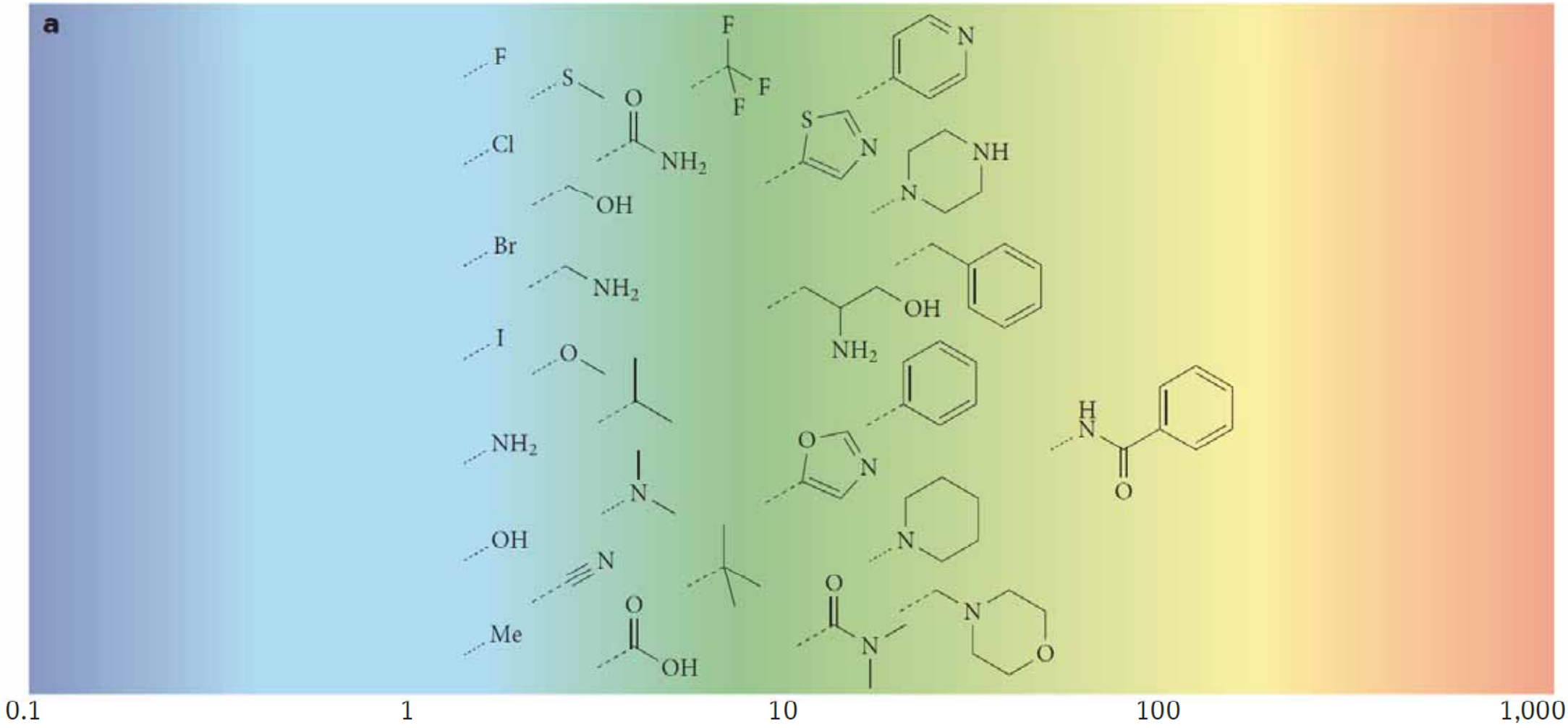
Maintaining acceptable ligand efficiencies during optimization of binding affinity can be challenging

Fold increase in affinity needed to maintain LE of 0.3



Hopkins, A.L. *et al., Nat. Rev. Drug Disc.*, 2014, **13**, 105-121.

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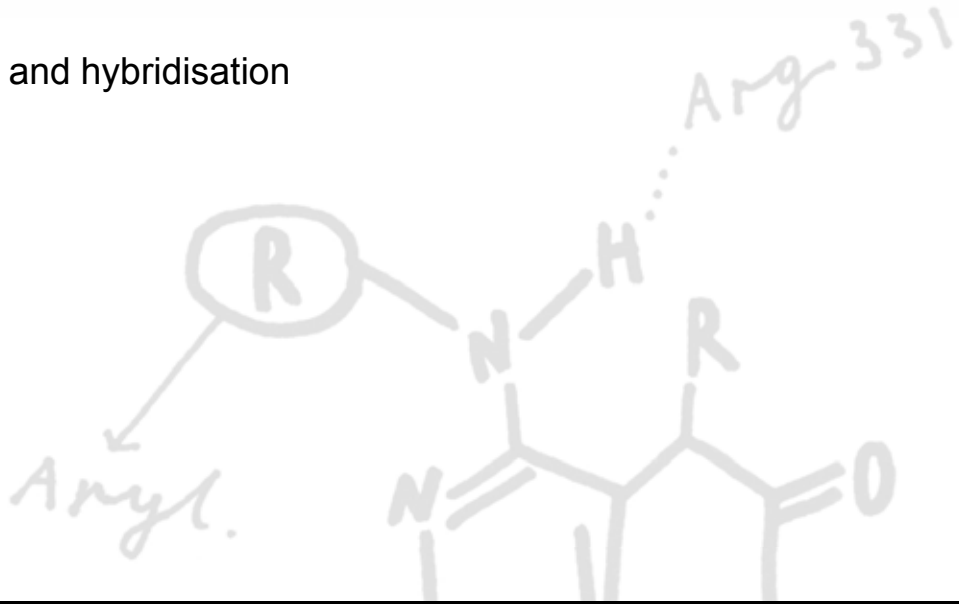
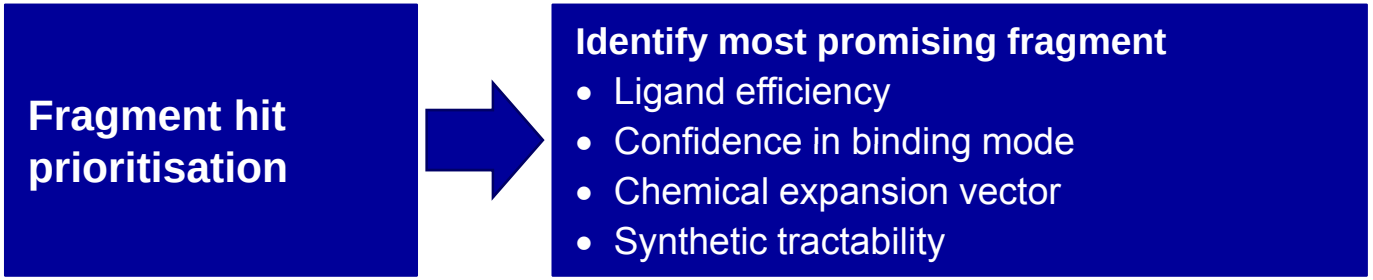


Reviewing fragment hit sets

Starts with biology to select the fragments with best LEs



- The screening hit rate and the level of access to structural biology (as well as the nature of the crystal system) will influence the selection process
- A high hit rate in combination with low throughput crystallography may necessitate preselection of fragments for structural studies
  - Selection based on quality of assay data (e.g. binding curves), LE (ideally >0.35), diversity and medicinal chemistry review
- Access to high throughput crystallography may allow all fragments to be progressed to structural studies
  - Screening directly by crystallography is becoming less of a specialised technique due to greater throughput on modern synchrotron beamlines
- Success in producing high quality protein-ligand structures can vary considerably but in Evotec's experience is rarely greater than 70%
  - Attrition is to be expected

Reviewing fragment X-ray structures

Bring together key disciplines

- Structural biology to assess quality of each structure
  - Ideally resolution should be high (<2.5 Å) with no ambiguities in how the ligand is modelled into the electron density
- Computational chemistry to review the specific interactions that each ligand makes
  - Provide insight into which interactions are key and their potential contribution to binding energy
  - Comment on the available vectors for fragment growing and potential for alternative strategies of fragment merging and/or linking
- Medicinal chemistry to suggest options for optimisation from each fragment consistent with the insights from structural biology and computational chemistry
- Jointly agreed strategy should emerge for fragment optimisation
  - Often starts with analogue by catalogue hit expansion activities

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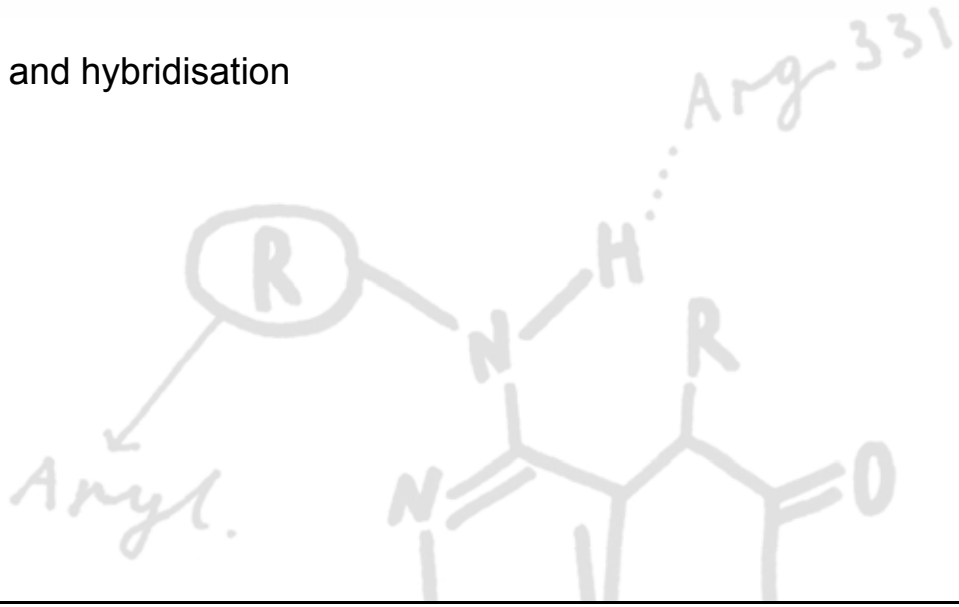
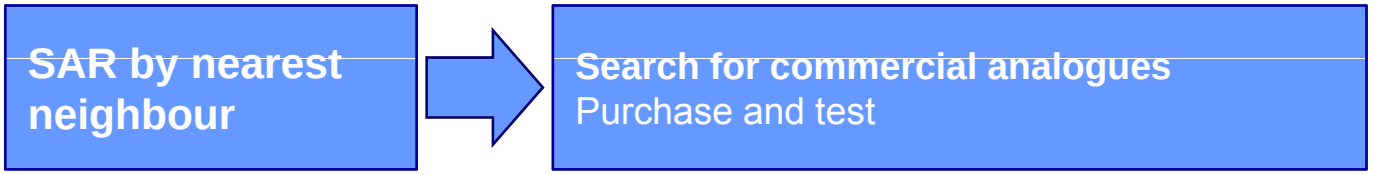


In silico fragment hit expansion

Rapid (and cheap) initial entry into fragment optimisation



- Astex reported that in 39 fragment-to-lead campaigns that on average 80% of the atoms in fragment hits are retained in the derived lead and that the retained atoms exhibit a mean shift of only 0.79Å RSMD between fragment and lead target co-complex structures[3]

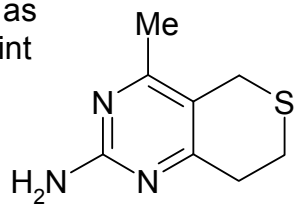
- Dock fragment analogues into the binding site and select those for purchase and testing those compounds in which the part related to the original fragment hit binds in a similar manner to the original fragment

Murray, C.W., *et al.*, *Trends Pharmacol. Sci.*, 2012, **33**, 224-232.

In silico fragment hit expansion example: Hsp90

Example: Hsp90 inhibitors

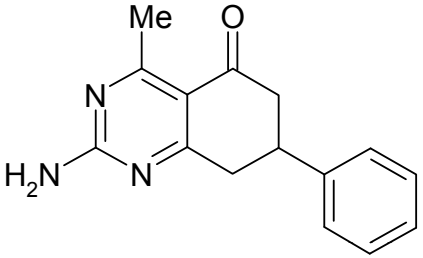
Fragment with highest LE as starting point



Fragment Hit
IC_{50} 15,000 nM LE 0.59

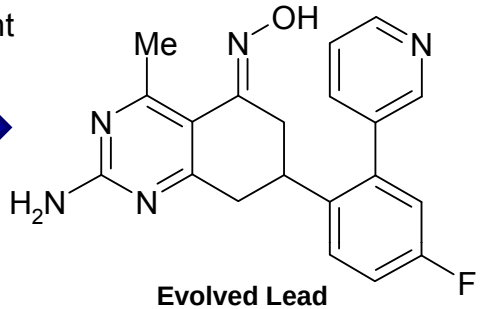
Sub-structure searches performed against 3.8 million available compounds

Followed by constrained docking (GOLD)[1,2]

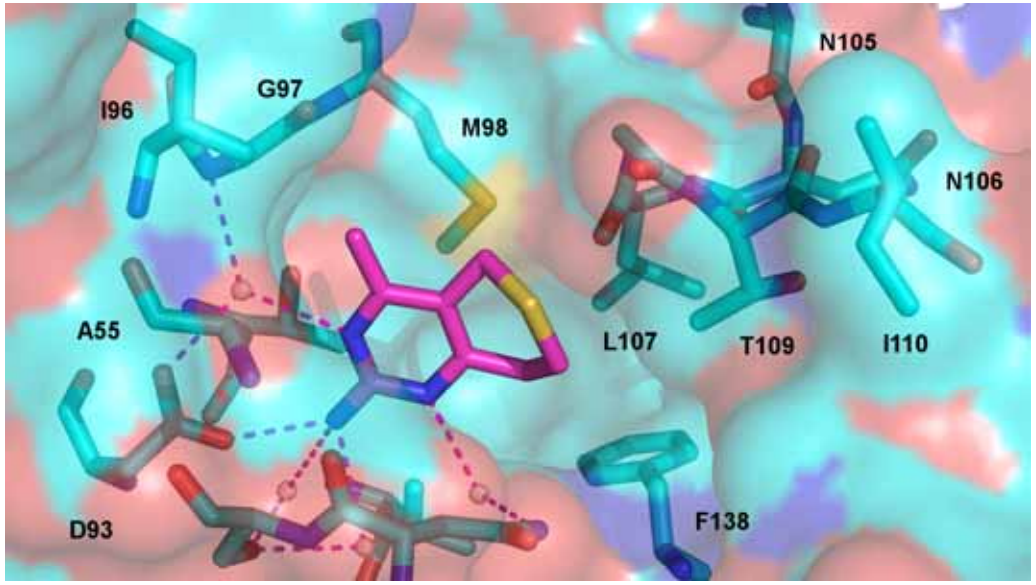


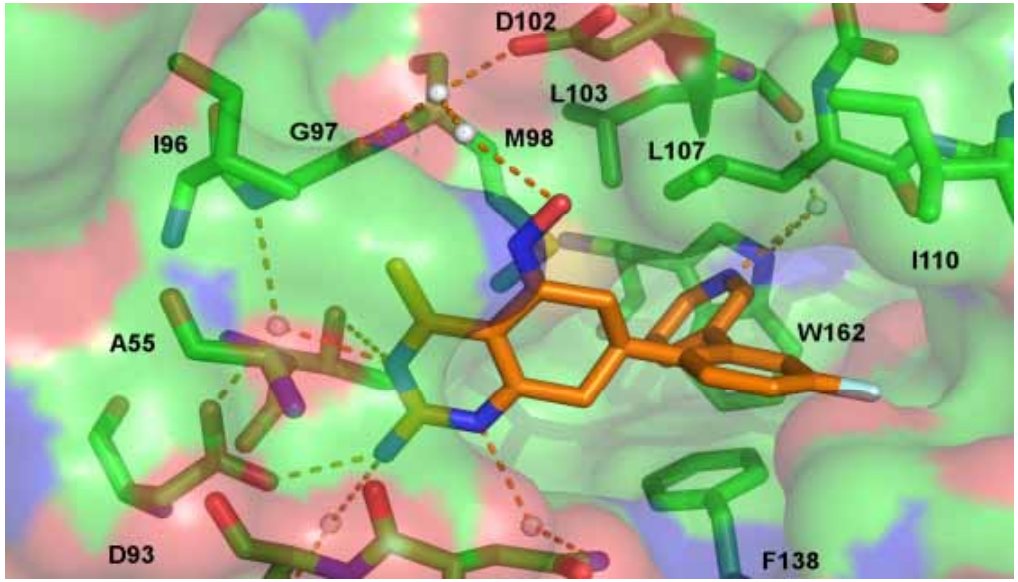
Analogue obtained by *in silico* hit expansion
IC_{50} 800 nM LE 0.46

Further fragment growth



Evolved Lead
IC_{50} 30 nM LE 0.39





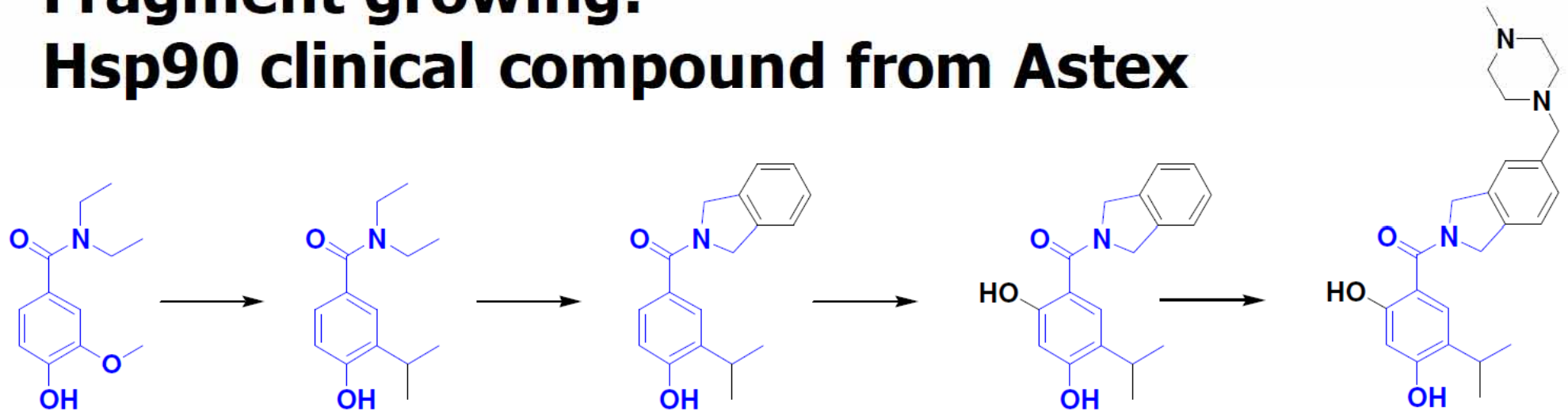
1) Barker J.J., *et al.*, *ChemMedChem*, 2010, **5**, 697-700; Law, R., *et al.*, *J. Comput. Aided Mol. Des.* 2009, **23**, 459-473;

Strategies for Fragment Hit Optimisation

Grow, merge, link or hybridise

Grow	**Grow from fragments** • Start from a ligand efficient fragment • Build in additional interactions
Link	**Connect together fragments in separate binding sites** • Adjacent fragments can be linked • Maintain interactions and poses of each fragment
Merge	**Combine features of overlapping fragments** • Derive a new superior fragment scaffold • Maintain key interactions of 2 or more fragments
Hybridise	**3D Overlay with existing hits and leads** • Design by visual inspection • Apply pharmacophore and scaffold hopping tools

Fragment growing: Hsp90 clinical compound from Astex



Compound 3
K_d = 790 µM
LE = 0.26

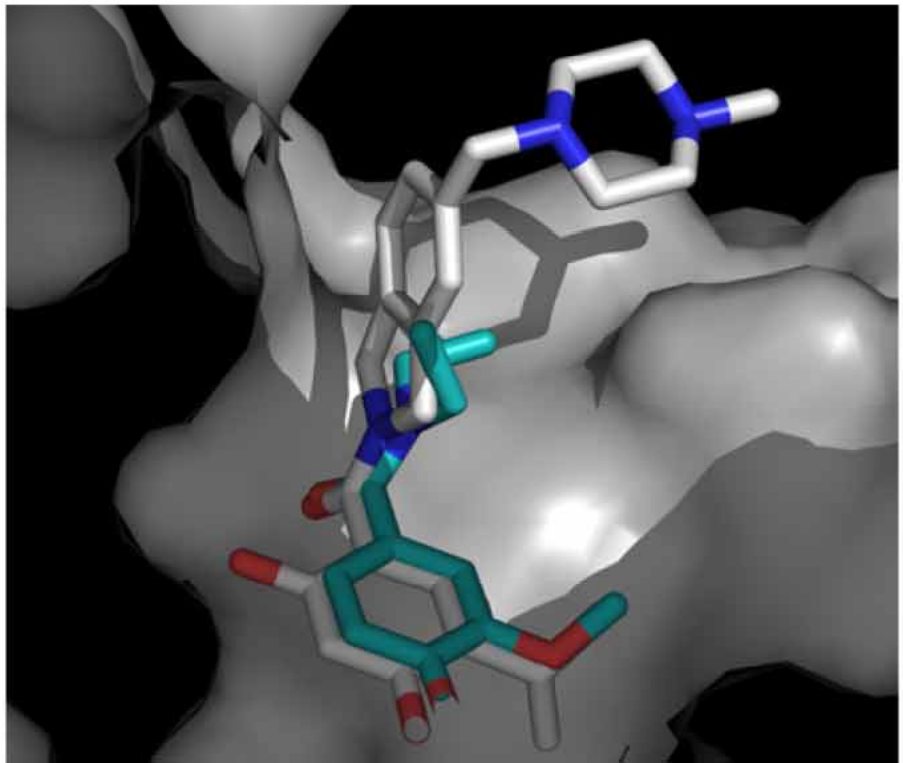
Compound 17
K_d = 7 µM
LE = 0.41

Compound 28
K_d = 0.068 µM
LE = 0.47
IC_{50} (cell)= 17 µM

Compound 31
K_d = 0.00054 µM
LE = 0.57
IC_{50} (cell)= 0.031 µM

AT13387
K_d = 0.00071 µM
LE = 0.42
IC_{50} (cell)= 0.048 µM

AT13387
Phase 1: multiple
Phase 2: GIST

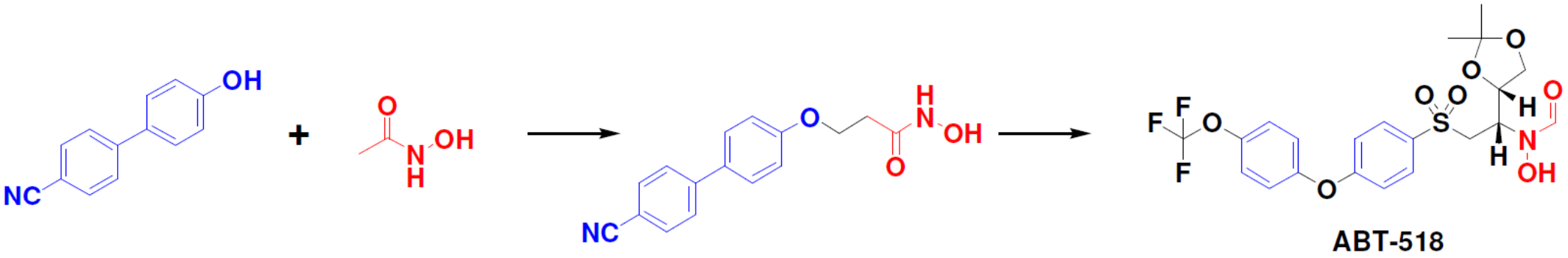


Murray et al. *J. Med. Chem.* 2010, *53,* 5942-5955
Woodhead et al. *J. Med. Chem.* 2010, *53,* 5956-5969

Fragment Linking – The "Poster Child" of FBDD

FBDD of stromelysin (MMP-3) inhibitor

- Fragment linking is very attractive because of the rapid increases in potency that can be obtained due to the "superadditivity" of fragment binding energies



K_d = 20 µM	K_d = 17,000 µM	IC_{50} = 0.025 µM	IC_{50} = 0.00078 µM
LE = 0.43	LE = 0.48	LE = 0.49	LE = 0.37
15 heavy atoms	5 heavy atoms	21 heavy atoms	34 heavy atoms
MW 195	MW 75	MW 282	MW 505

Hajduk, P.J. *et al., J. Am. Chem. Soc.* 1997, **119**, 5818-5827; Wada, C.K. *Curr. Top. Med. Chem.* 2004, **4**, 1255-1267.

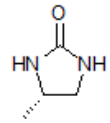
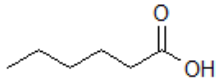
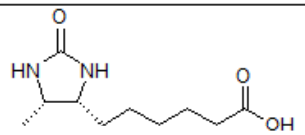
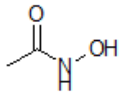
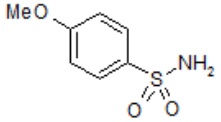
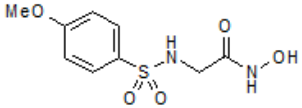
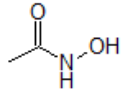
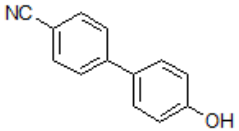
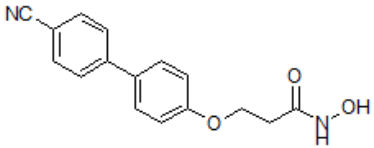
Fragment Linking can give great improvements in potency

Examples of super additivity?

- The “linking efficiency co-efficient” (*E*) (also known as theoretical linker factor (f_L)) can be used to score success of fragment linking

$$K_D^{AB} = K_D^{A}\, K_D^{B}\, \boldsymbol{E}$$

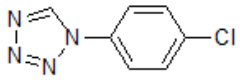
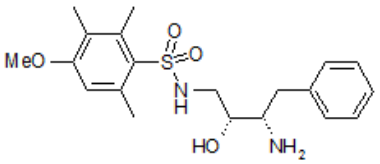
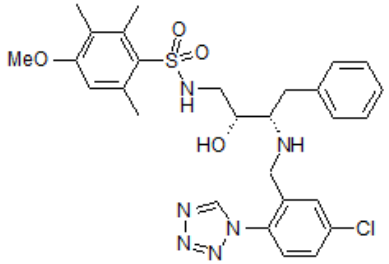
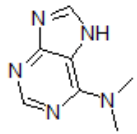
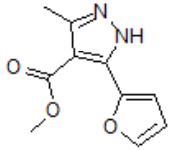
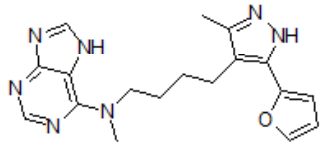
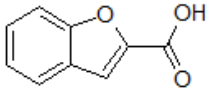
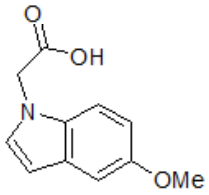
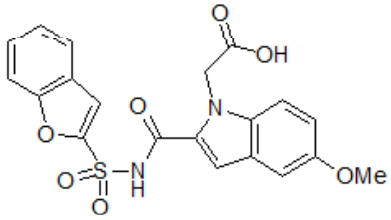
- Superadditivity is indicated by $E < 1$ when the free energy of the linked compound exceeds the sum of the binding energies of the corresponding component fragments

Entry (Target)	Fragment A	Fragment B	Linked Compound	Linking coefficient *(E)*
1 (avidin)	 K_i=34 µM	 K_i=260 µM	 K_i=0.00041 nM	4.6×10^{-5}
2 (MMP-12)	 K_D=6.2 mM	 K_D=1.5 mM	 K_D=20 nM	2.1×10^{-3}
3 (MMP-3)	 K_D=17 mM	 K_D=20 µM	 K_D=25 nM	0.07

Borsi, V., *et al., J. Med. Chem*. 2010, **53**, 4285-4289.
Röhrig, C.H., *et al., ChemMedChem* , 2007, **2**, 1054-1070.
Ichihara O., *et al.* , *Molecular Informatics*, 2011, **30**, 298-306.

Fragment Linking can give satisfactory increases in potency

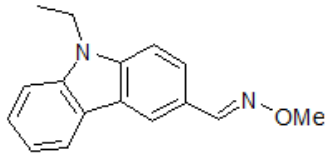
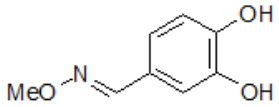
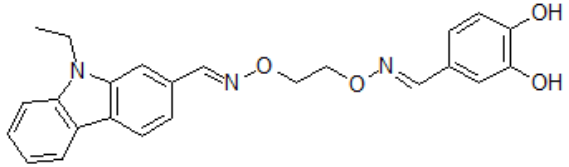
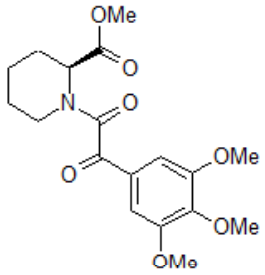
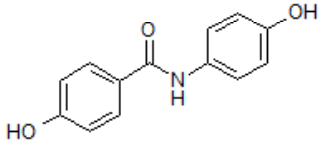
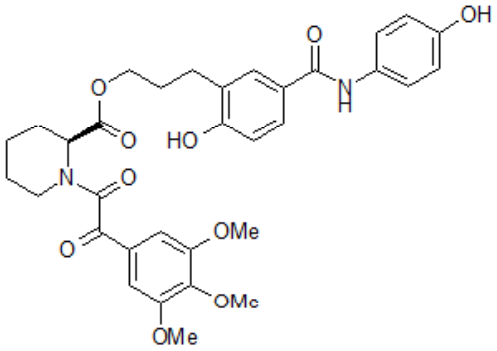
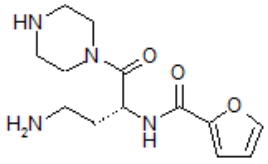
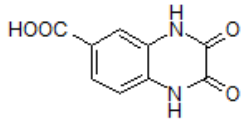
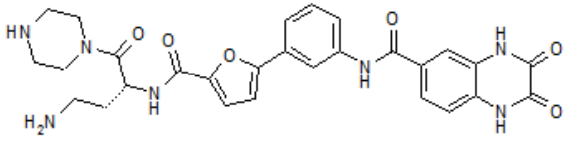
Examples that are neutral in terms of supper additivity

Entry (Target)	Fragment A	Fragment B	Linked Compound	Linking coefficient *(E)*
4 (Thrombin)	 IC_{50}=330 μM	 IC_{50}=12 μM	 IC_{50}=1.4 nM	0.35
5 (Hsp90)	 IC_{50}=1.5 mM	 IC_{50}=1 mM	 IC_{50}=1.5 μM	1.0
6 (Pantothenate sythase)	 K_D=1000 μM	 K_D=500 μM	 K_D=1.8 μM	3.6

Ichihara O., *et al.* , *Molecular Informatics*, 2011, **30**, 298-306.

Fragment Linking can give suboptimal potency improvements

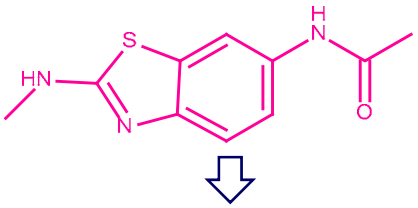
Examples where fragment free energy of binding is not maintained

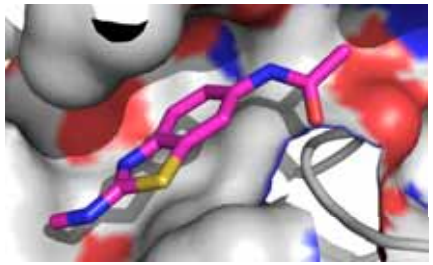
Entry (Target)	Fragment A	Fragment B	Linked Compound	Linking coefficient *(E)*
7 (c-Src)	 IC_{50}=40 µM	 IC_{50}=41 µM	 IC_{50}=~64 nM	~39
8 (FKBP)	 K_D=2 µM	 K_D=100 µM	 K_D=49 nM	245
9 (23S rRNA)	 K_D>100 µM	 K_D>100 µM	 K_D=6.5 µM	~650

Ichihara O., *et al.* , *Molecular Informatics*, 2011, **30**, 298-306.

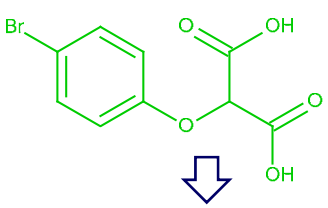
- Example of fragment linking in lactose dehydrogenase (AstraZeneca)

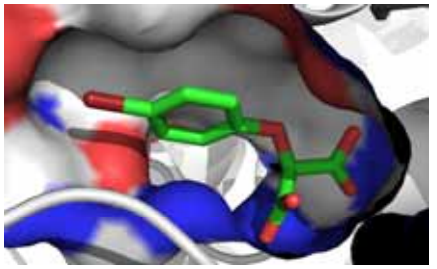
Fragment 12
K_D = 770 μM
LE = 0.21
Enzyme IC_{50} > 500 μM



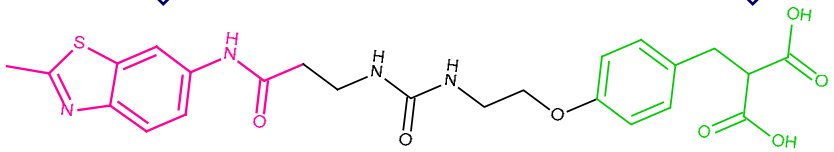


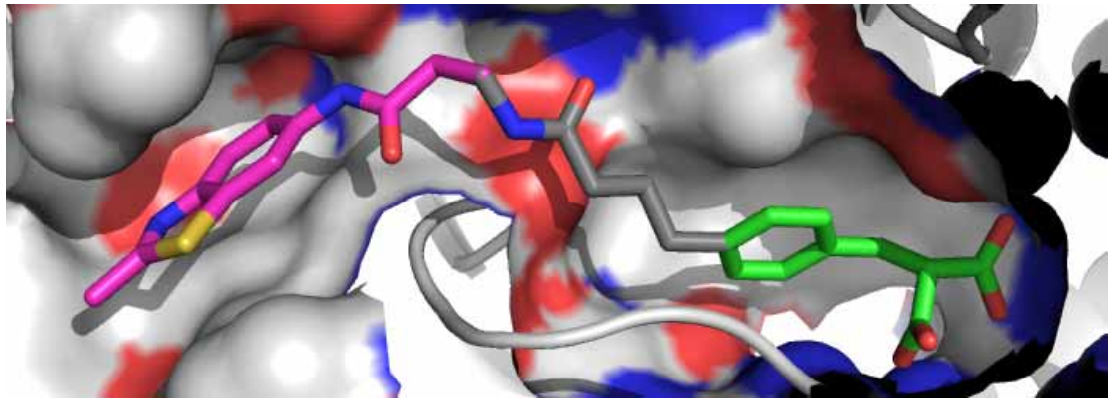
Fragment 20
K_D = 210 μM
LE = 0.24
Enzyme IC_{50} > 500 μM



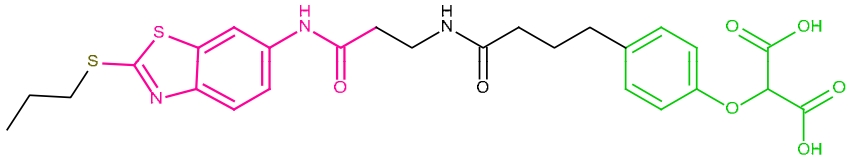


Compound 26
K_D = 0.13 μM
LE = 0.19
Enzyme IC_{50} = 4.2 μM





Compound 34
K_D = 0.008 μM
LE = 0.21
Enzyme IC_{50} = 0.27 μM

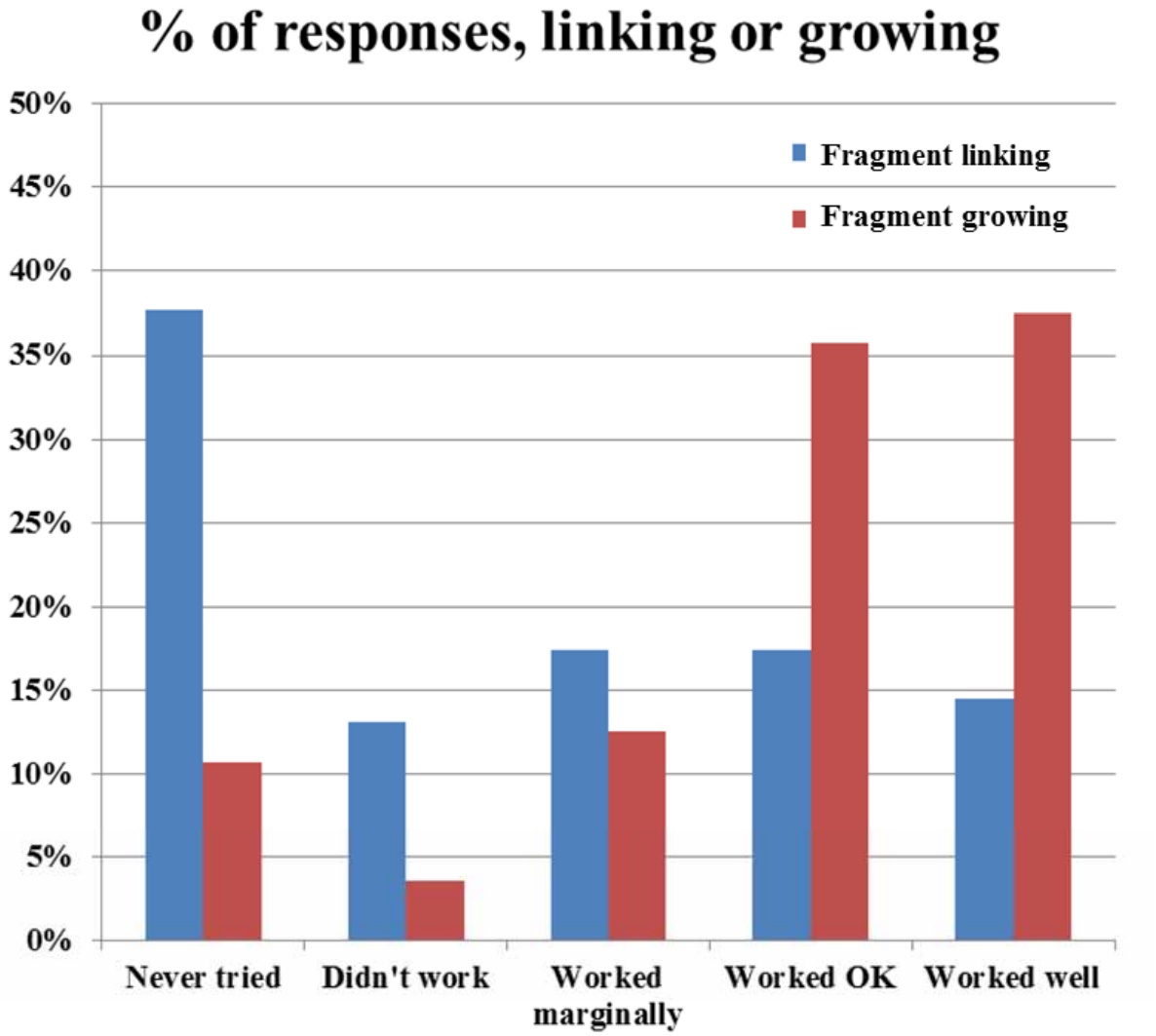


Ward, R.A., *et al., J. Med. Chem.*, 2012, **55**, 3285–3306.

Fragment linking

Practical Fragments poll result of linking vs growing: September 2014

- Linking two fragments together is usually more difficult to do than growing the best fragment hits
- Requires that the binding pose of each fragment is effectively maintained in the linked molecule particularly if the binding of each fragment is enthalpicaly driven
- Perhaps best applied to situations where there are distinct and separate binding sites such as protein-protein interactions

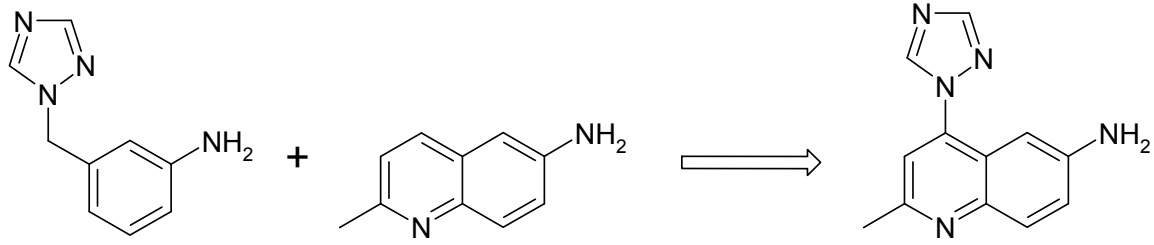


http://practicalfragments.blogspot.co.uk/2014/09/fragment-growing-vs-fragment-linking.html

Fragment Merging

Example: *Mycobacterium tuberculosis* P450 CYP121 inhibitors

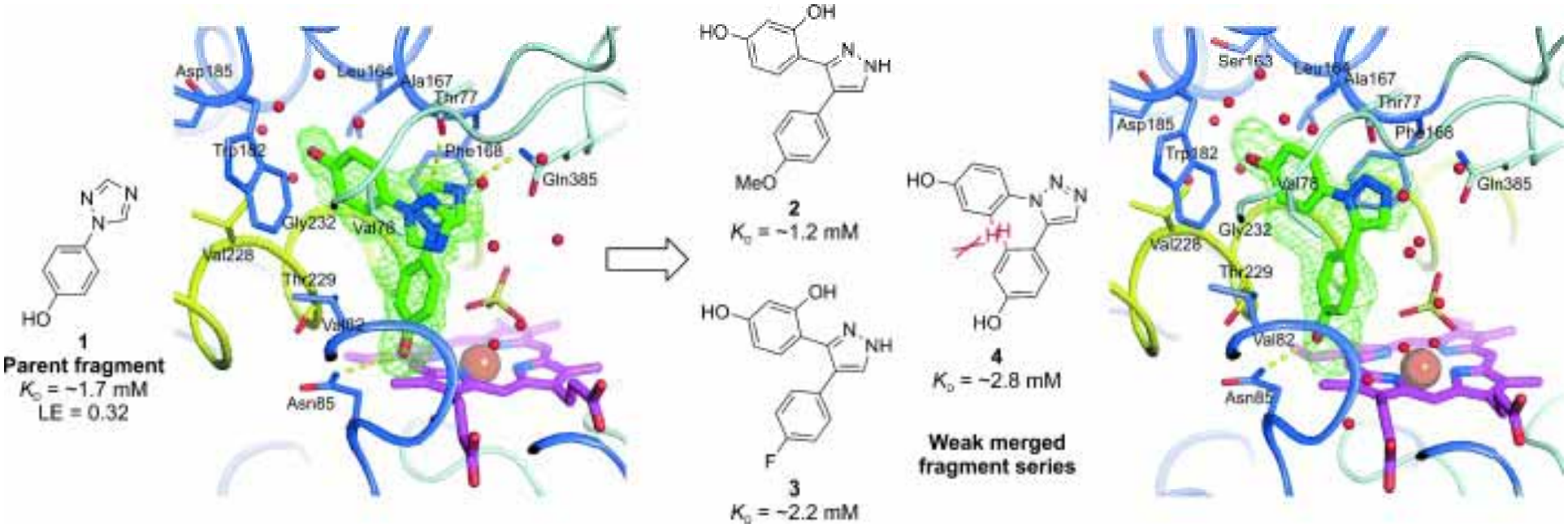
- Where multiple fragment hits are available and there is insight into similarities in their binding modes then new fragments can be designed that combine key features
- Can be difficult to get to work as technique may force subtle changes in binding mode



Fragment 1
K_D ~1.6 mM
LE 0.29

Fragment 2
K_D 400 μM
LE 0.39

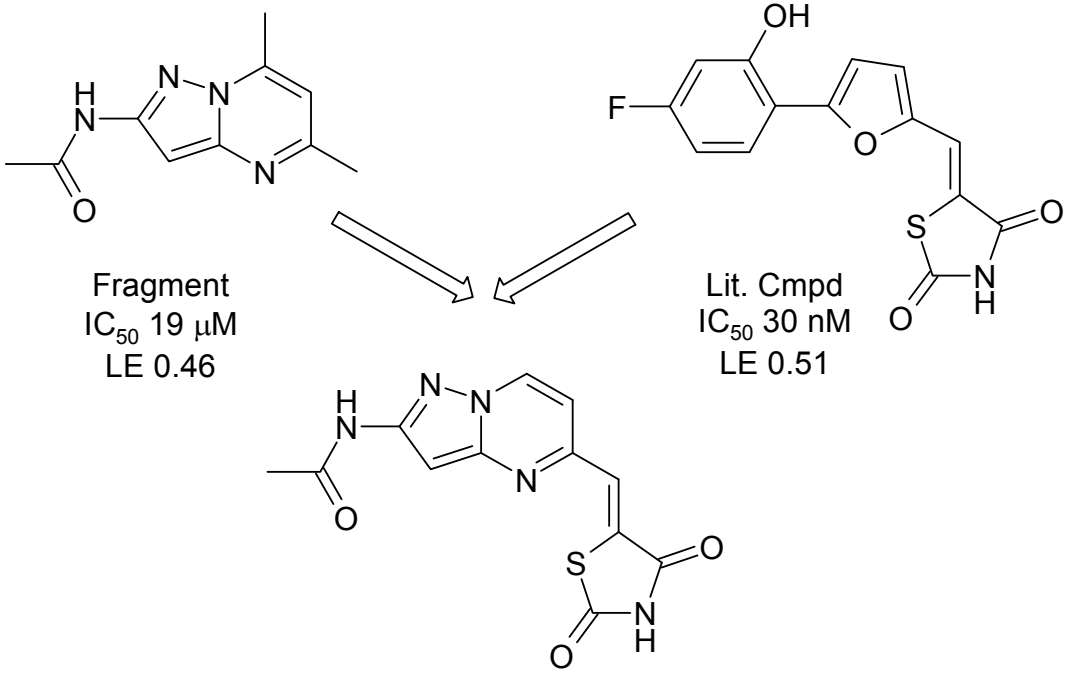
Merged fragment
K_D 28 μM
LE 0.39



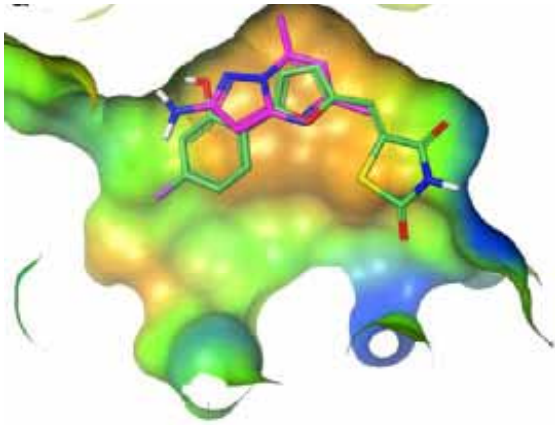
Hudson, S.A. *et al., Angew. Chem.Int. Ed.*, 2012, **51**, 9311-9316; Hudson, S.A. et al., *ChemMedChem*, 2013, **8**, 1451-6.

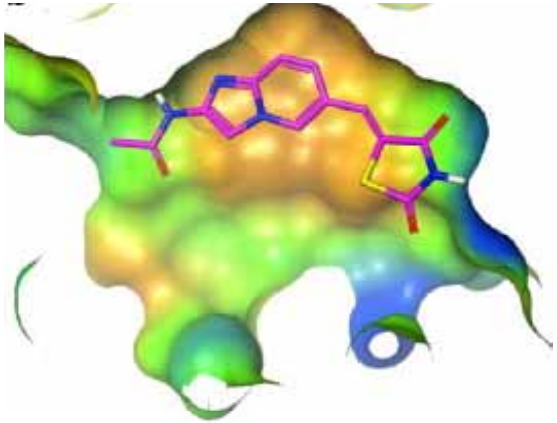
Fragment hybridisation

Novel PI3Kγ inhibitor obtained by hybridisation



- Analysis of the X-ray crystal structure of fragments and the X-ray crystal structure for a known literature inhibitor a hybridised inhibitor can be designed
- In this example for PI3Kγ inhibitors the binding mode of the hybrid compound, determined by X-ray crystallography, was as predicted from the component fragments





Hughes, S.J., *et al., Bioorg. Med. Chem. Lett.*, 2011, **21**, 6586-6590.

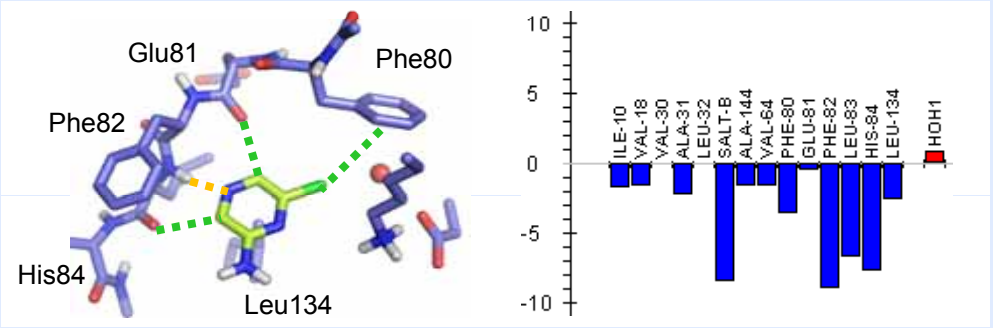
Leveraging Computational Chemistry

Extracting additional value from ligand-protein structures

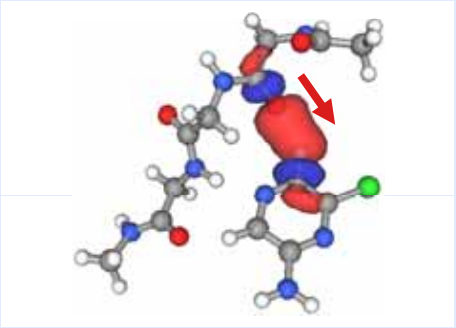
Quantum mechanic calculations are used to assess the enthalpic contribution to small molecule-protein binding

- Analysis of the interacting molecular orbitals and by the analysis of energy contributions to binding can give valuable insight into what are the key interactions
- Maintaining the right electrostatic/dispersive balance in medicinal chemistry is important
- Ratio of electrostatic and dispersive interactions predicts which fragments are good to expand on, and which a good to link to

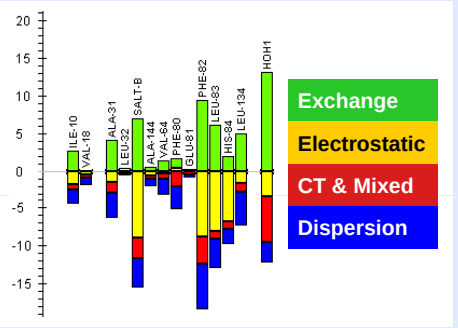
PDB: 1WCC, IC_{50}=350, μM / -48.40 kcal/mol



Molecular orbital analysis



Energy analysis



Mazanetz M.P. *et al., J. Cheminform.*, 2011, **3**:2.

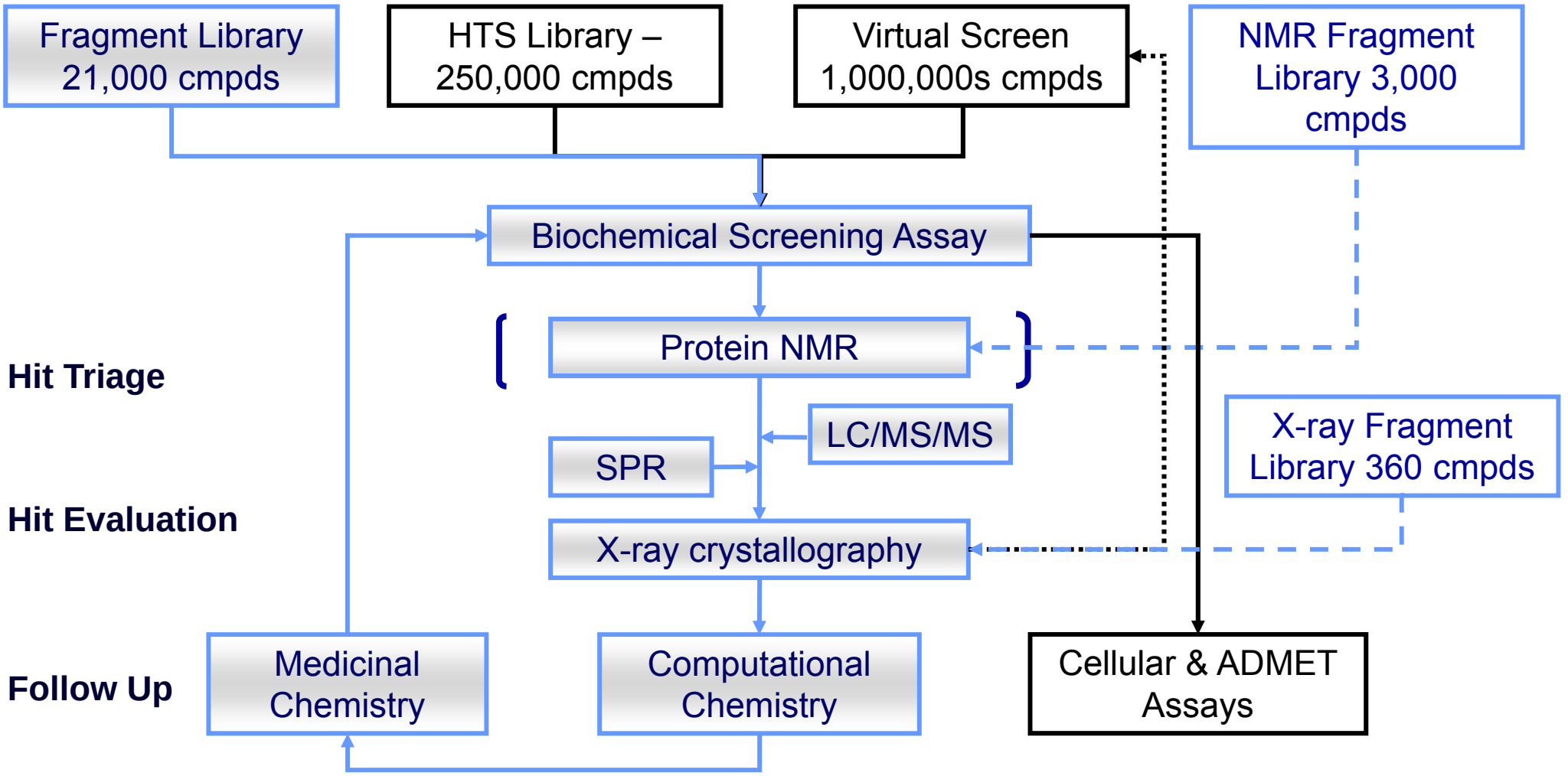
So what do you do if you can't get a structure?

Give up or press on?

- Some companies rigorously place a decision gate that if no fragment structures are obtained the project is terminated
  - The advantage is to only focus on projects which are tractable for fragments
  - The disadvantage is letting the technology approach select which targets to work on rather than the biological rationale (e.g. membrane proteins may be less tractable for a fragment approach but are not necessarily less druggable)
- Options for progression in the absence of structure include:-
  - In silico fragment expansion selecting compounds which retain key scaffold elements but add functionality to explore potential optimisation vectors
  - Integration of fragment hit data with information from other screening methods (Fragment Assisted Drug Discovery)

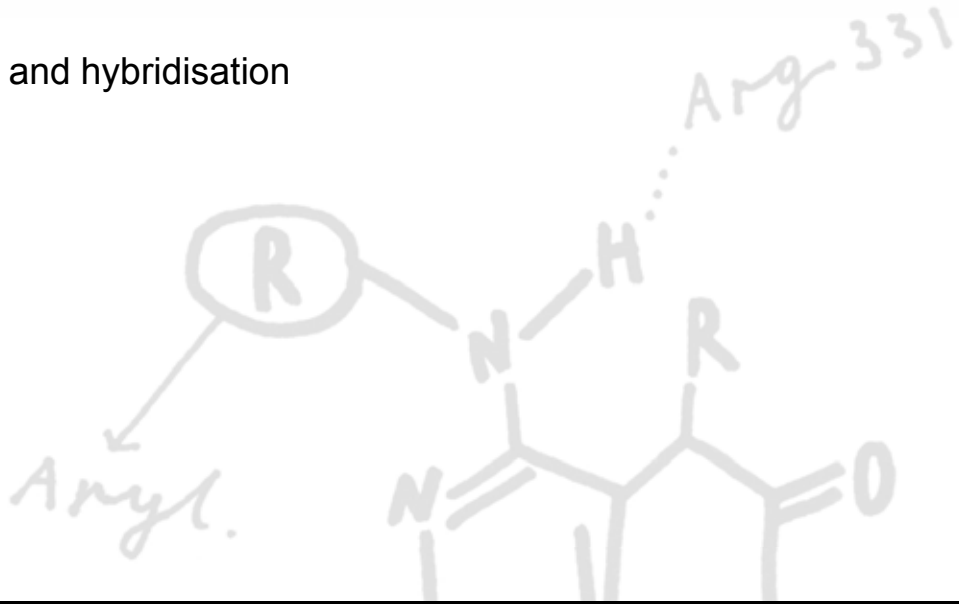
Fragment screening as part of an integrated approach to hit finding

Maximising chances of finding high quality hits



Agenda

- Fragment optimisation in an ideal world
- **Fragment optimisation in reality**
  - Metrics for fragment hit assessment and optimisation
  - **Selecting the best fragment hits to work with**
  - Fragment expansion
  - Growing, linking, merging and hybridisation
- Summary



Some limitations of fragment discovery

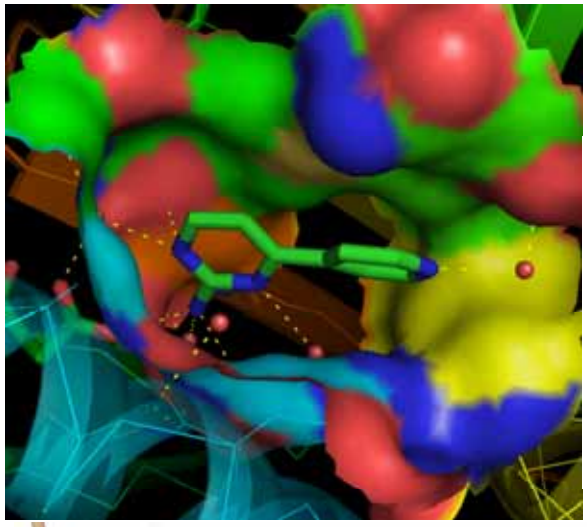
Best suited to soluble protein targets

- Targets are ideally amenable to Structure Based approaches
  - Preference for *E. coli* and insect cell protein production routes
- Fragments have to be soluble in aqueous buffers at concentrations of > 1 mM
- Target affinities of hits are two orders of magnitude lower than in HTS
- Biophysical and biochemical screening techniques dominate
  - Hit follow-up with cell-based assays not expected to work
- Multiple technologies required to fully execute optimisation programme

Fragments – Future

A mainstay of drug discovery

- FBDD provides a clear path for rapid optimisation from multiple start points
- Novel hits can be found in crowded regions of IP
- Promising, ligand efficient hits for notoriously difficult targets, including PPIs
- Use of multiple computational methods in tandem with fragment structures to guide medicinal chemistry, e.g. QM[1]
- Application of fragment methods to membrane proteins
  - Potential starting points for medicinal chemistry (e.g. H3/H4)
  - Use of detailed GPCR modelling to aid structure-based design[2]



1) Alexeev Y. *et al., Curr. Top. Med. Chem*., 2012, 12(18),. 2013-2033; 2) Heifetz A. *et al., Biochemistry*, 2012, **51**, 3178-3197;

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- Dan Erlanson: Permission to use content from Practical Fragments (http://practicalfragments.blogspot.co.uk) and from FBLD 2014 Introductory Tutorial “I’ve got a fragment – now what?”

- The *Fragments 2015* committee

  – Mike Hann, Samantha Hughes, Thorsten Nowak, Gordon Saxty

- Colleagues at Evotec involved in FBDD and FADD

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Selected References

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- Experiences in fragment-based drug discovery. Murray, C.W., *et al., Trends Pharmacol. Sci*., 2012, **33**, 224-232.
- Introduction to fragment-based drug discovery. Erlanson, D.A. *Top. Curr. Chem.*, 2012, **317**, 1-32.
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‘RESEARCH NEVER STOPS’

Building innovative drug discovery alliances

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