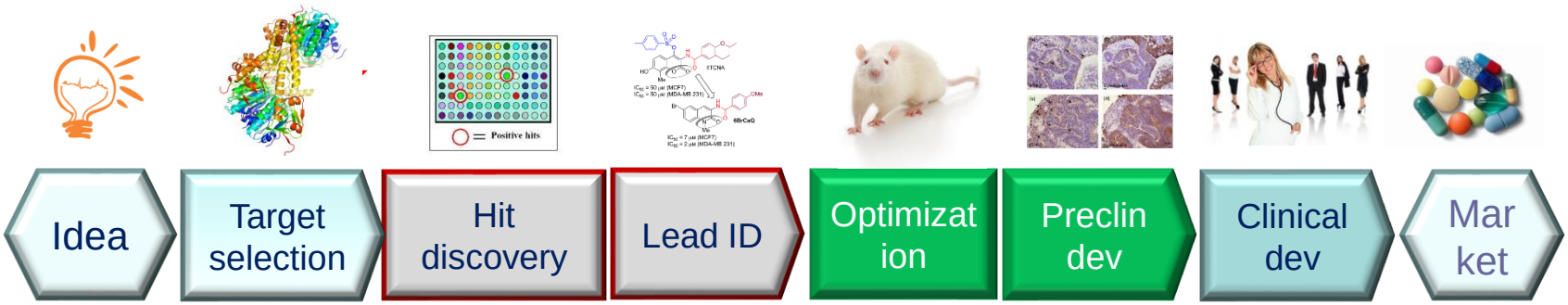
Fragment based drug discovery

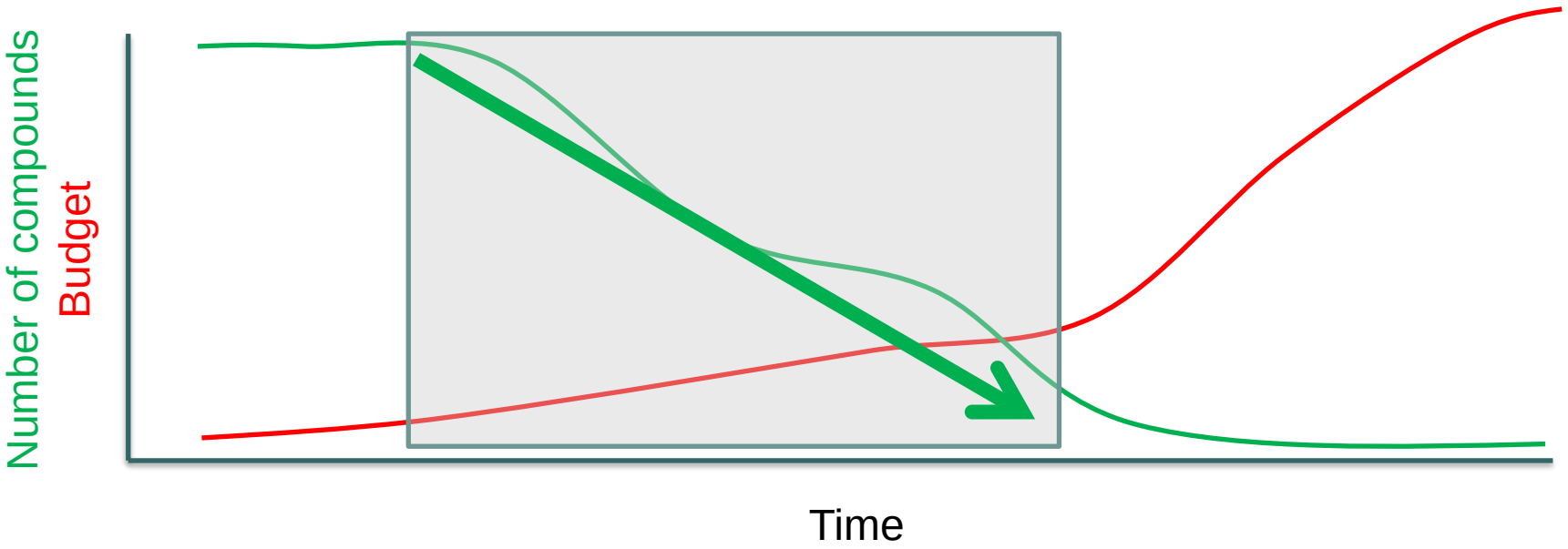
György M. Keserű

RCNS Hungary

FRAGNET Innovative Training Network

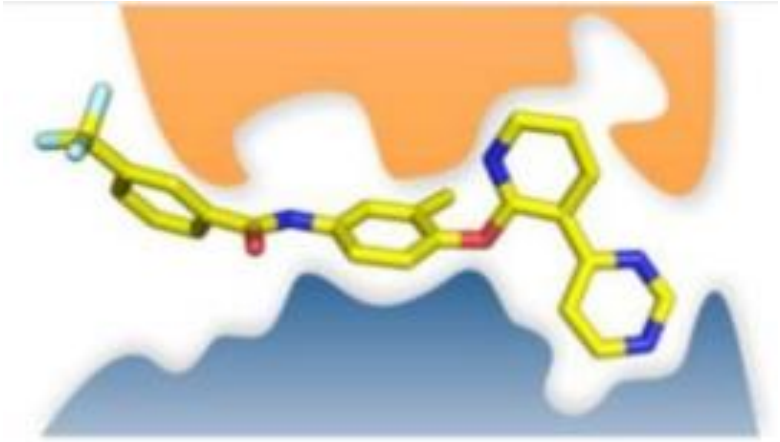
Drug discovery process





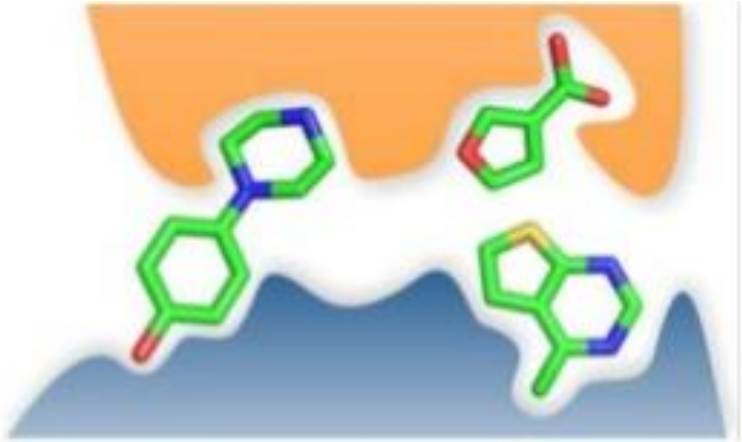
Hit discovery from screening

Druglike library



- Large molecules (MW>300)
- Large library (10^6 compound)
- Biochemical testing
- Less diverse hits
- High affinity (1-10 μM)

Fragment library

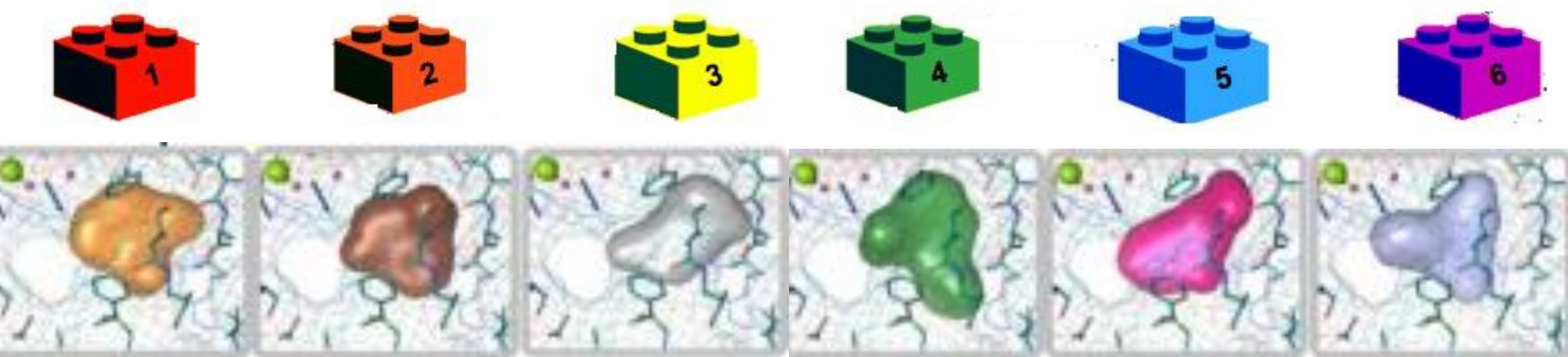


- Small molecules (MW<300)
- Small library (10^3 fragment)
- Biophysical testing
- Diverse hits
- Low affinity (<100 μM)

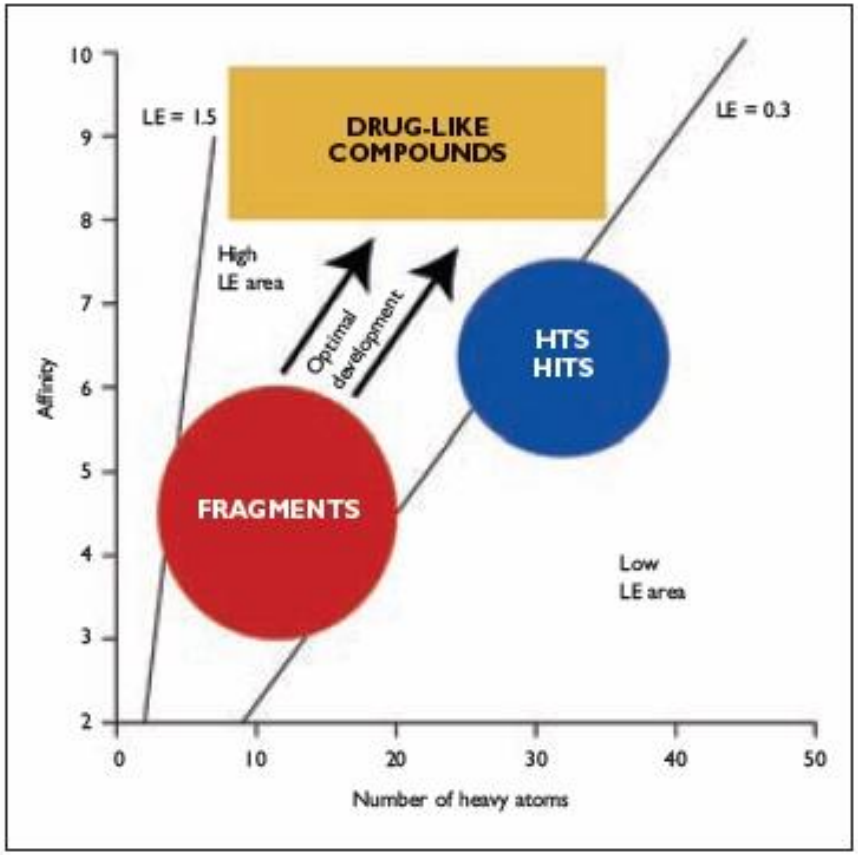
What do we call ‚fragments'?

Simple, small, polar molecules

- Rule of 3:
  - MW $\leq$ 300 ($N_{heavy} \leq 22$)
  - Log P $\leq$ 3
  - Number of H-donors $\leq$ 3
  - Number of H-acceptors $\leq$ 3
- Further properties:
  - Number of rot. bonds $\leq$ 4
  - Polar surface $\leq$ 60 Å^2
  - Good solubility



Why fragments are beneficial?



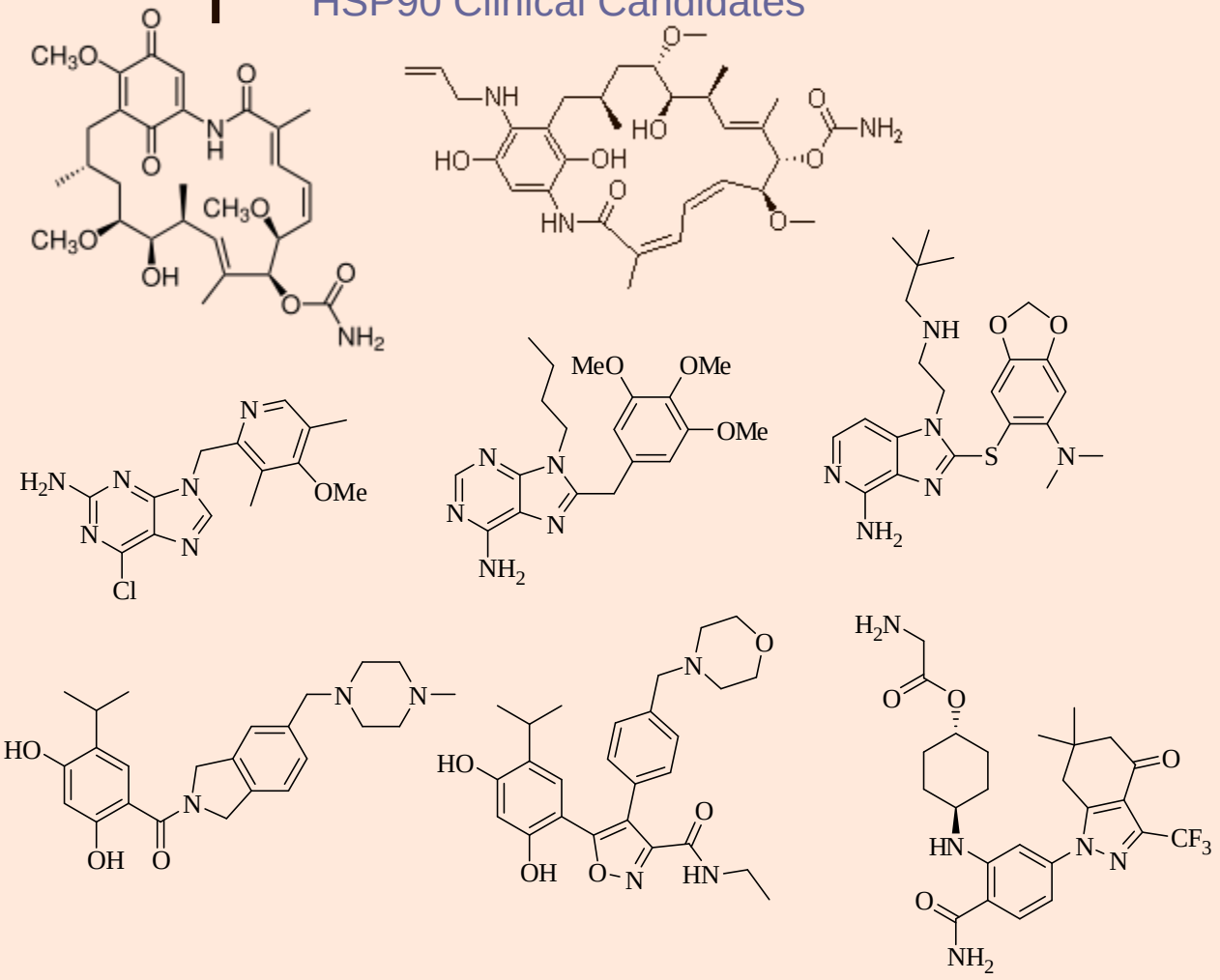
- They have good physchem profile
- A smaller library contains more chemical information
- Their chemical space is smaller: better sampling
- They bind to protein hot spots
- They provide rational optimization towards drugs

Fragment space is smaller

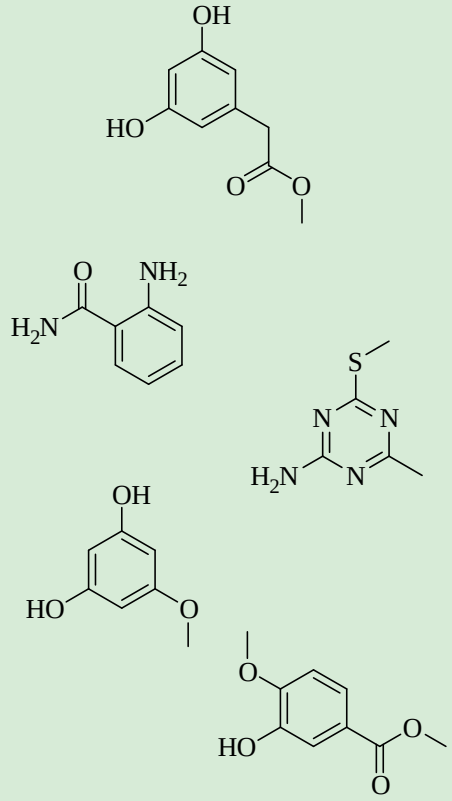
- Druglike compounds
  - Estimated number: 10^{60}
  - Compounds in Chemical Abstract Registry: 150×10^{6}
  - Compounds commercially available: 10^{6}
  - Number of compounds screened: 10^{5}
- Fragments
  - Estimated number: (max 17 C, O, N, H, S, Cl atom):10^{11}
  - Fragments commercially available: 10^{5}
  - Number of compounds screened: 10^{3} - 10^{4}

Sampling is more efficient

HSP90 Clinical Candidates



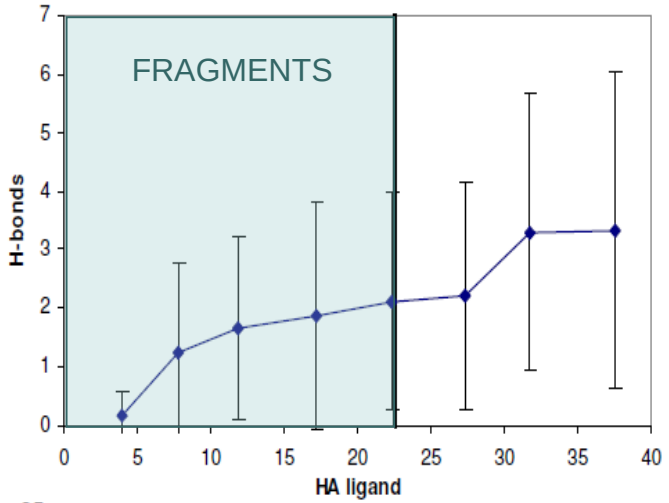
Fragment hits from a screen of 719 fragments

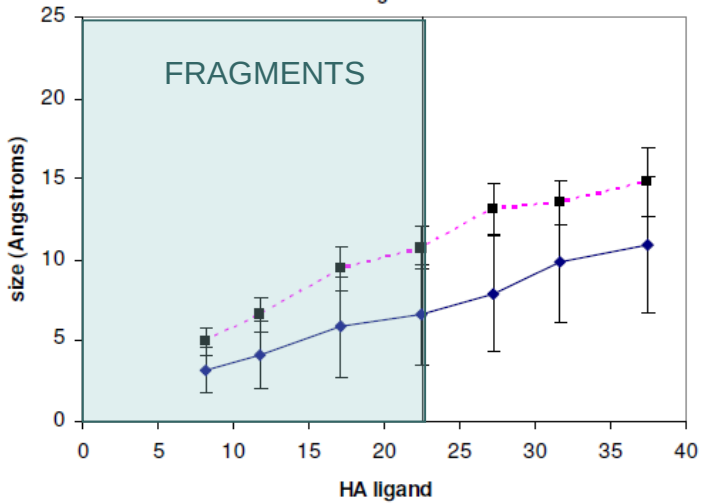


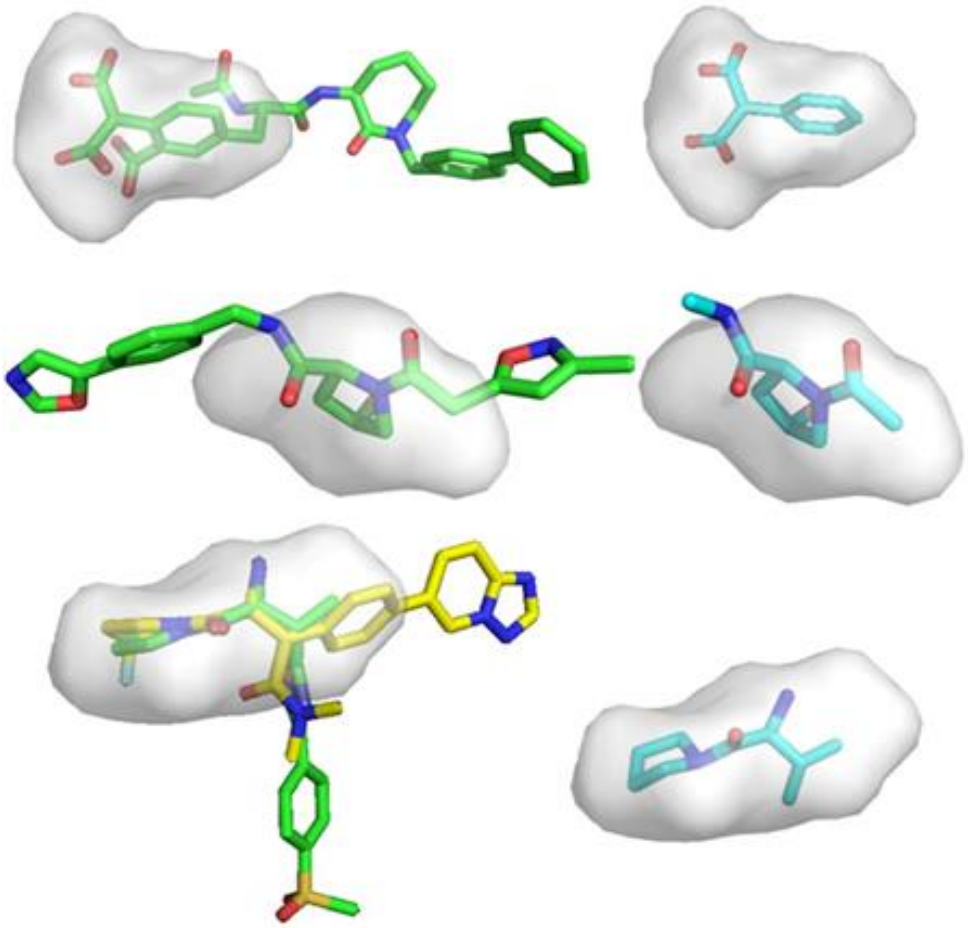
J. Med. Chem. **2011**, 54, 3989.

Key features of all known clinical candidates are represented in hits from a small fragment library

Fragments bind to hot spots





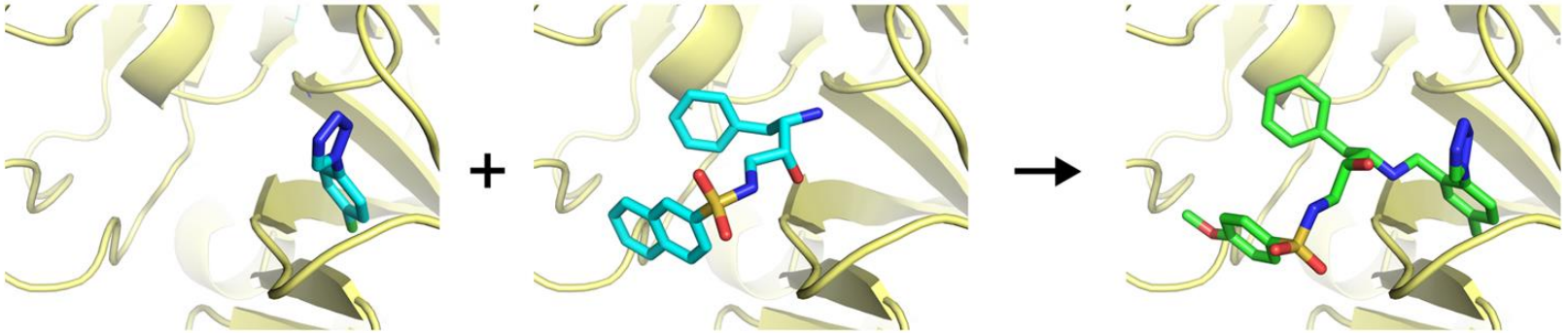


Fragments form limited number of polar interactions within a small region of protein binding sites

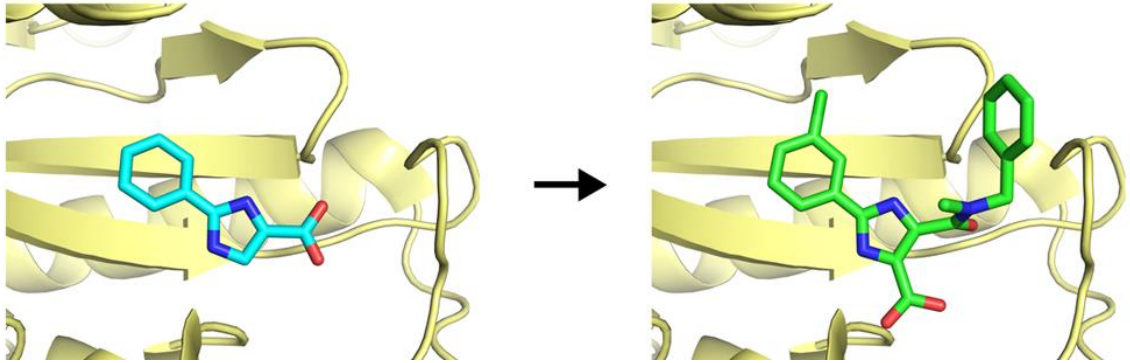
Keserű JCIM 2012, Vajda PNAS 2015, Shaw JMC 2019

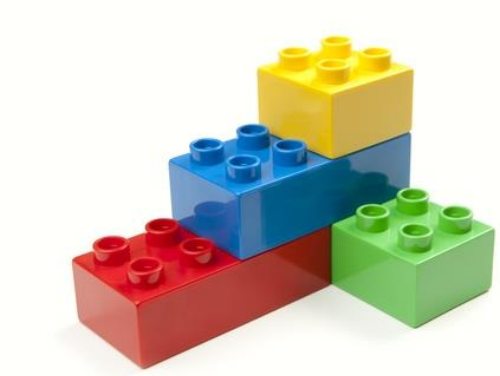
Rational optimation strategies

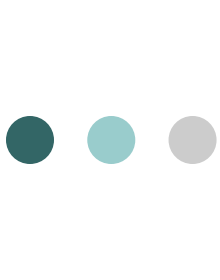
Linking



Growing



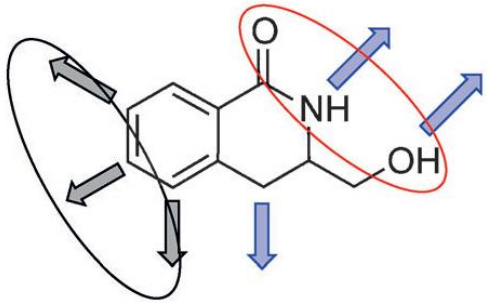




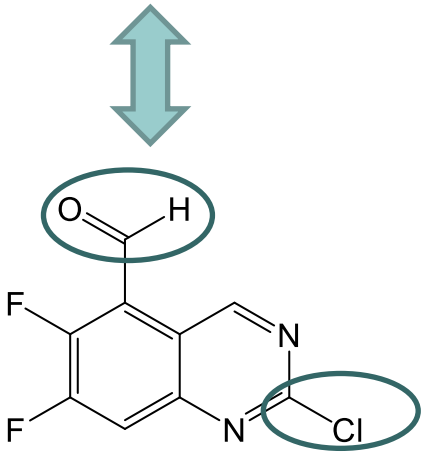
DESIGNING FRAGMENT LIBRARIES

What are the general features of a good fragment?

- Interactions
  - Diverse polar groups
  - Pharmacophore variety
  - Scaffold variety
- Physicochemical properties
  - Size, complexity
  - Shape
  - Lipophilicity
  - Solubility
- Synthetic vectors for growing
- Reactivity, stability, aggregation
- Synthetic tractability, cost



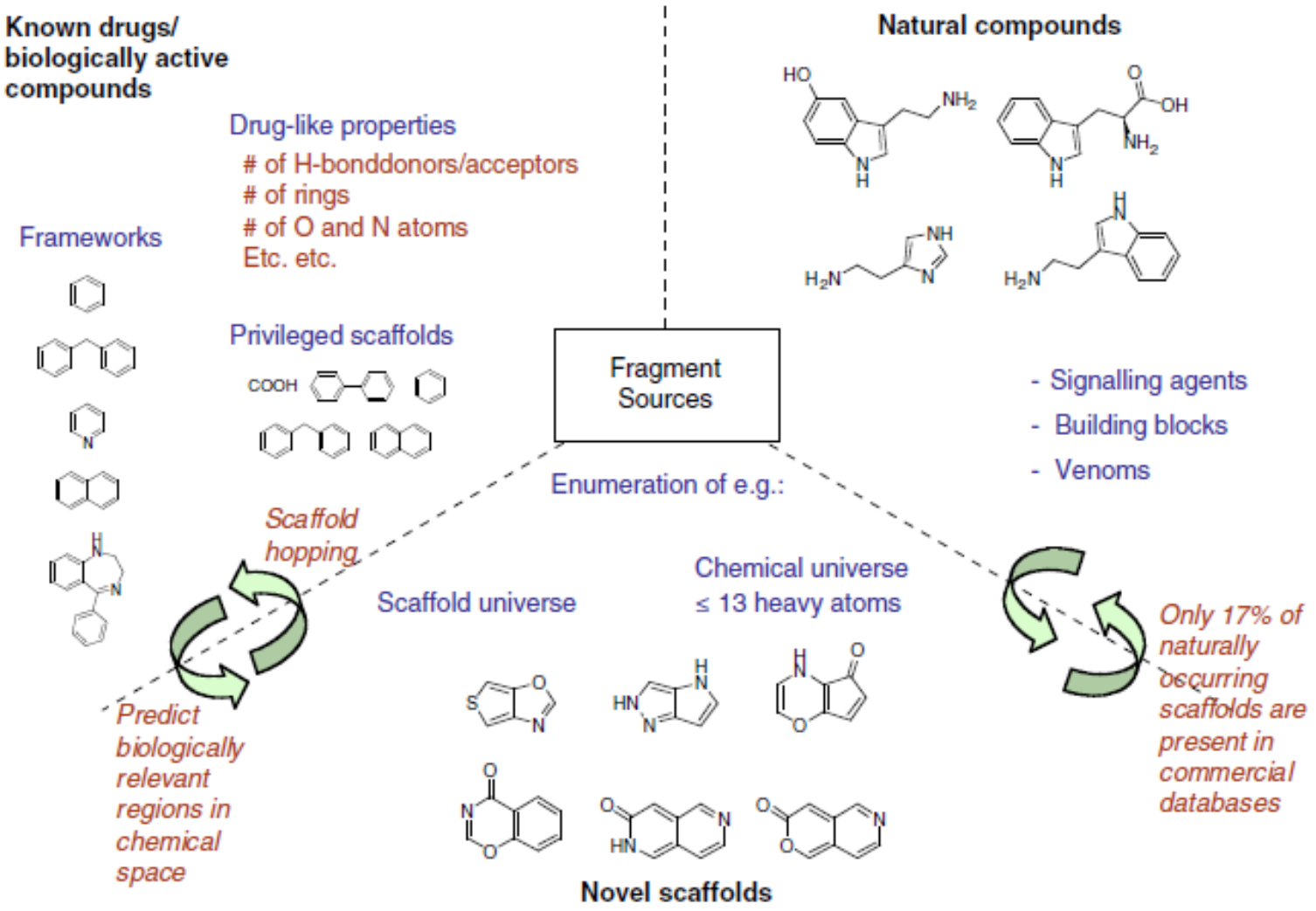
MW = 177; cLogP=0.32



MW = 278 cLogP=1.84

Murray, Rees Angew. Chem. Int. Ed. 2015, 54, 2–7

Fragment sources



Boyd, Kloe DDT Technologies 2010, 7, e173

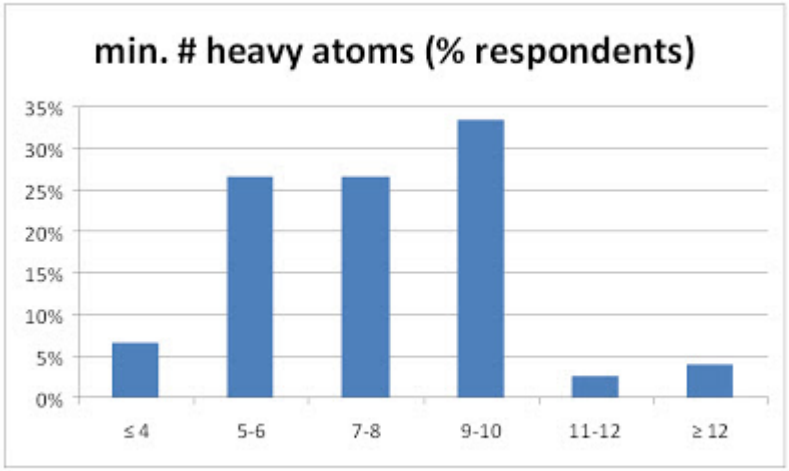
General design principles

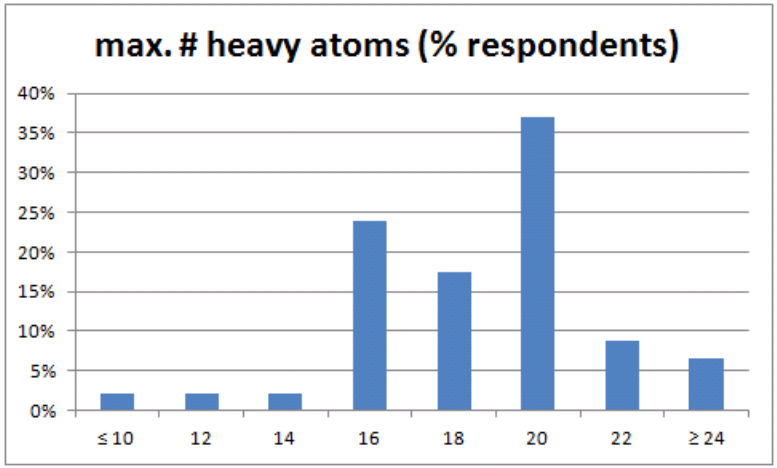
Level 1 Compound properties	Level 2 Sampling	Level 3 MedChem	Level 4 Experimental
Physicochemical	Priviledged structure	Analogue accessibility	Purity Solubility Stability
Reactivity	Pharmacophore diversity	Synthetic tractability	
Aggregator, assay interference	Scaffold diversity	Stability	
Availability	Shape diversity		
Cost			

Chris Swain (CMC): 1216 fragment hits, 240 publications, 174 molecular targets, 26 detection technologies

Fragment size

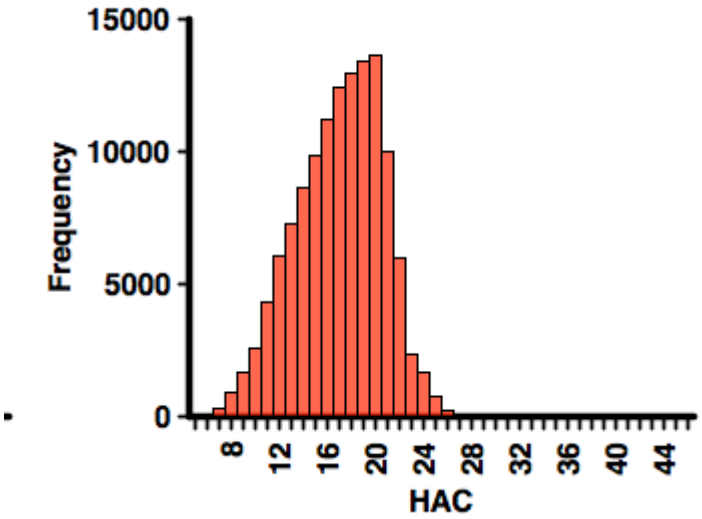
Polls



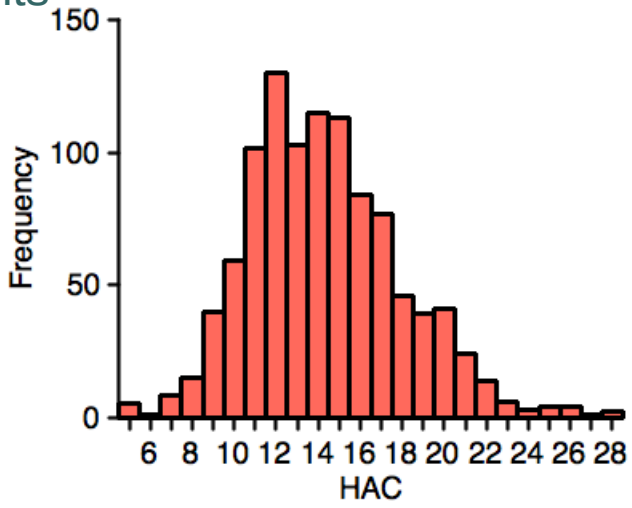


Practical Fragments Blog

Libs



Hits

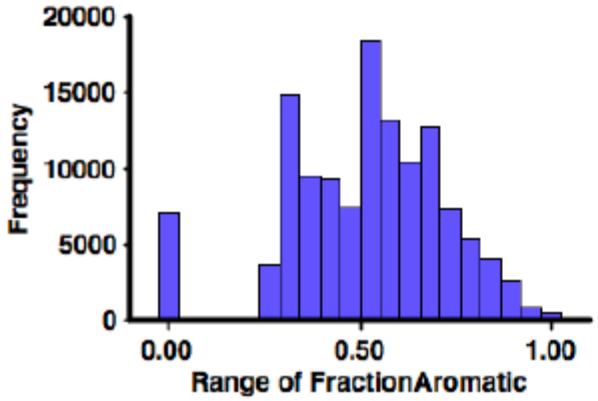


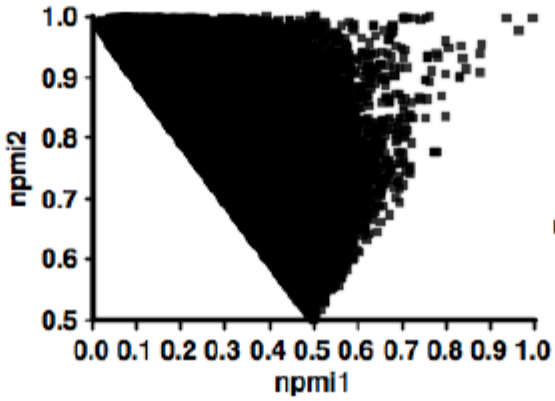
Chris Swain, CMC

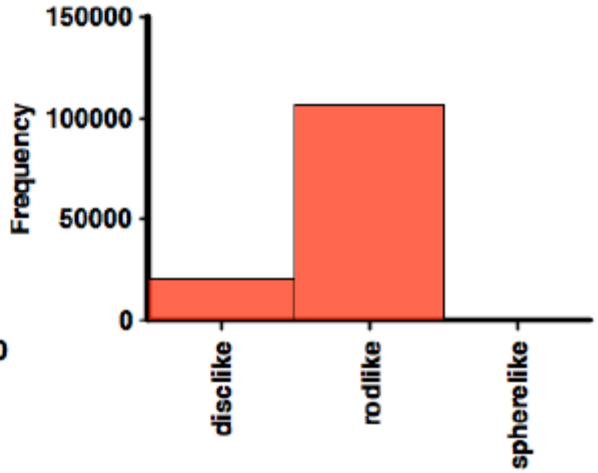
Physicochemical

Fragment shape

Libs







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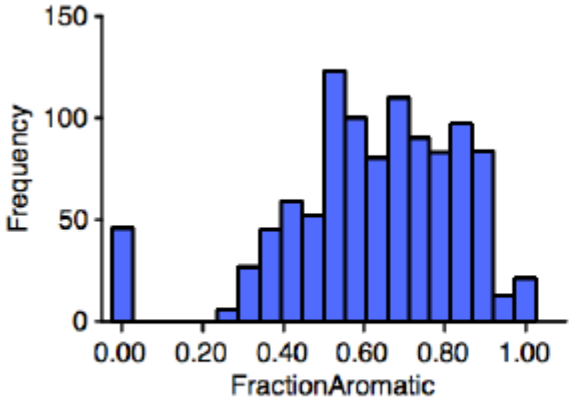
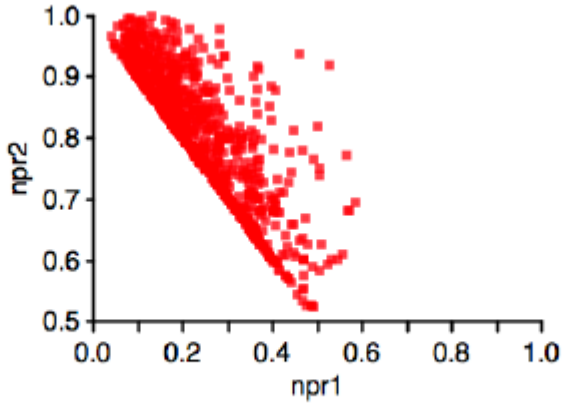
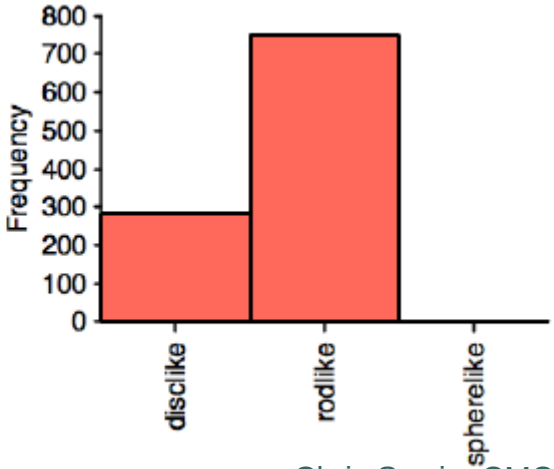


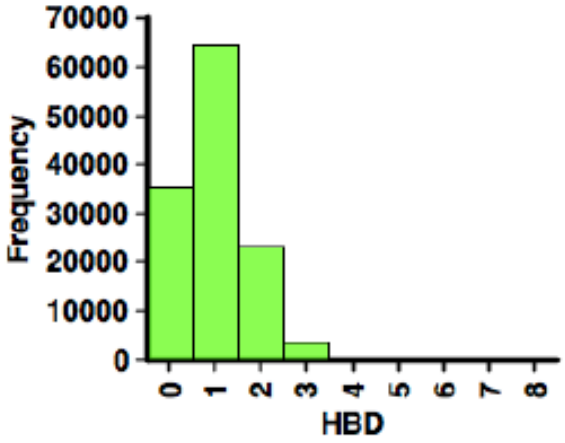


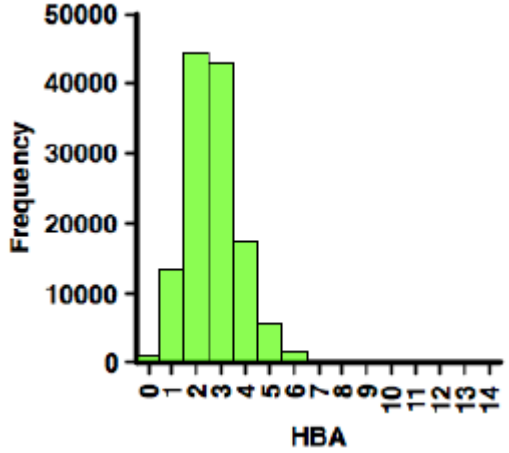


Chris Swain, CMC

Donors and acceptors

Libs





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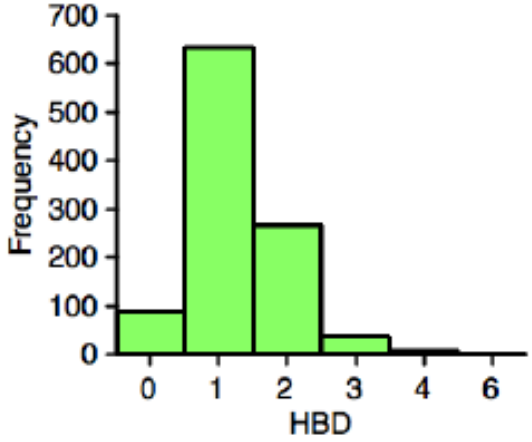
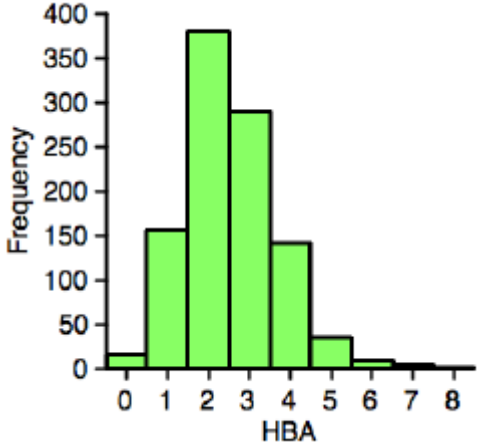
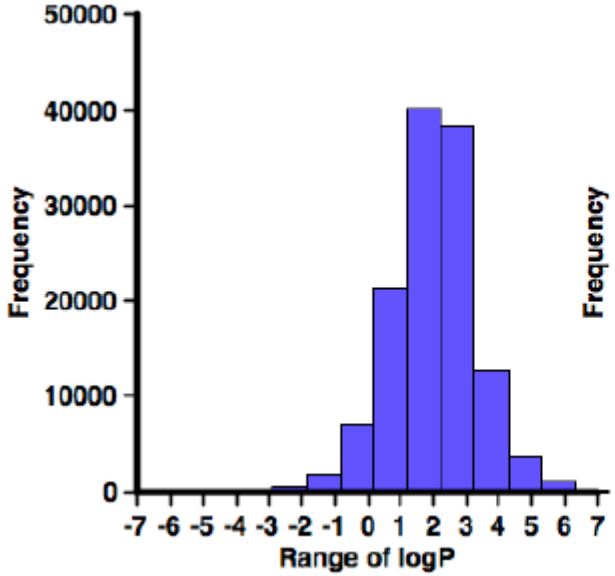


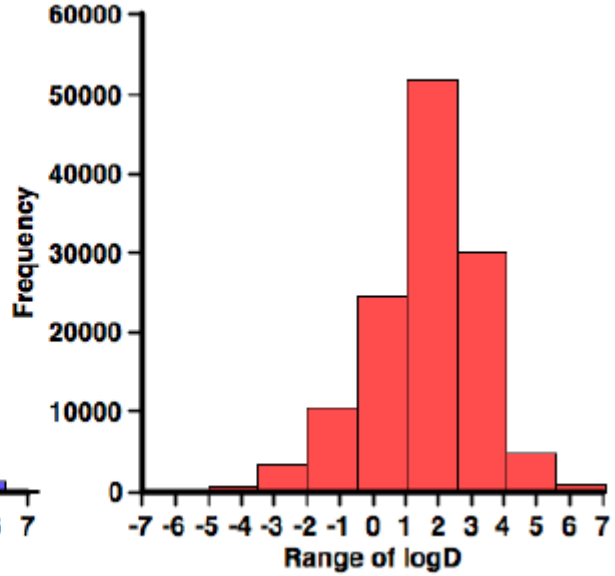


Physicochemical

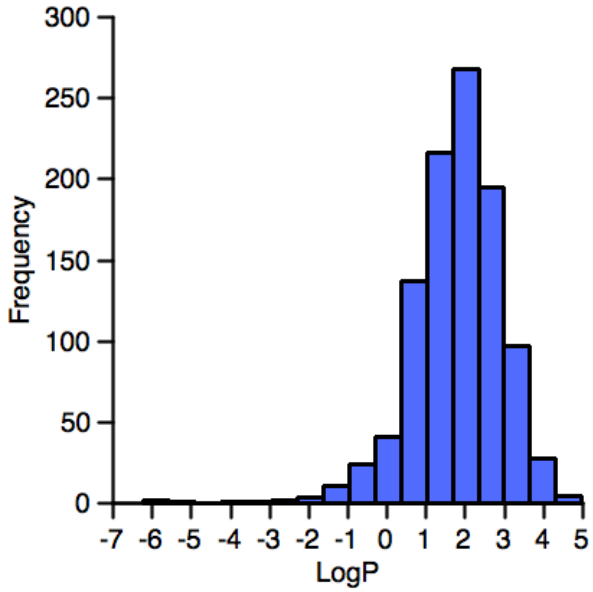
Lipophylicity

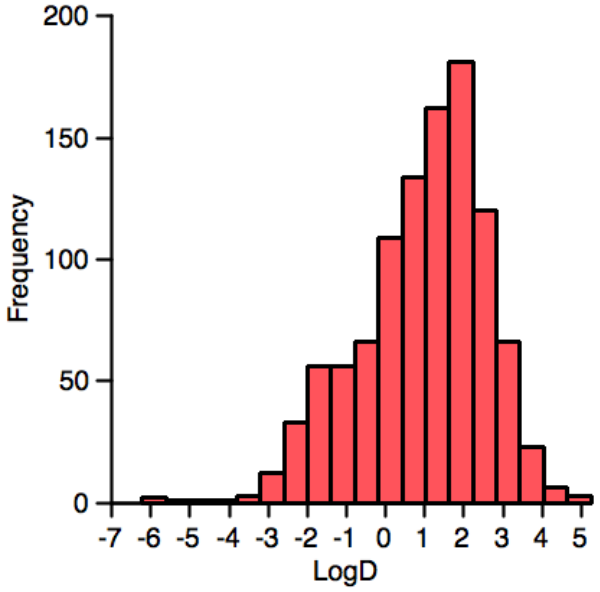
Libs





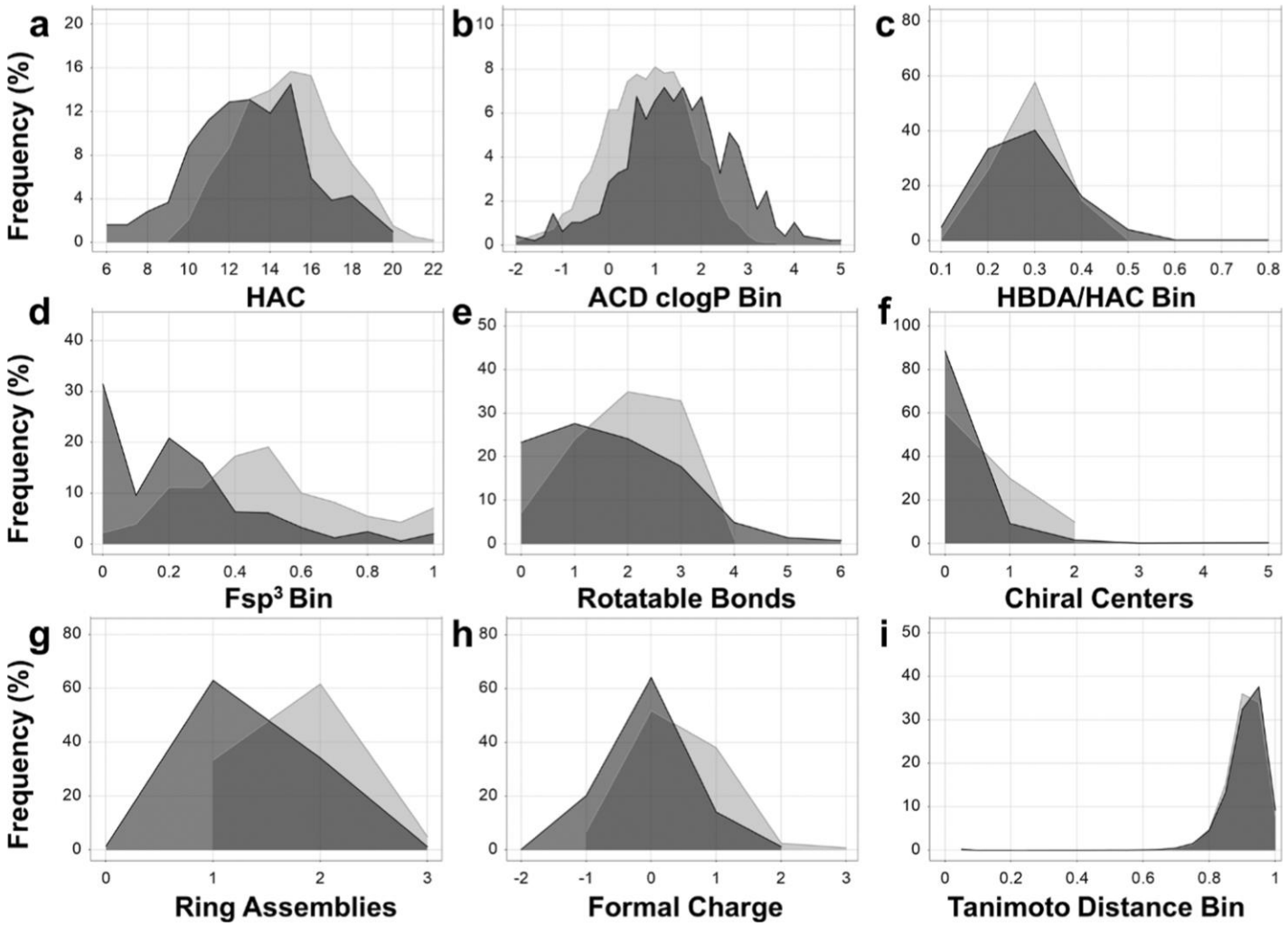
Hits





Chris Swain, CMC

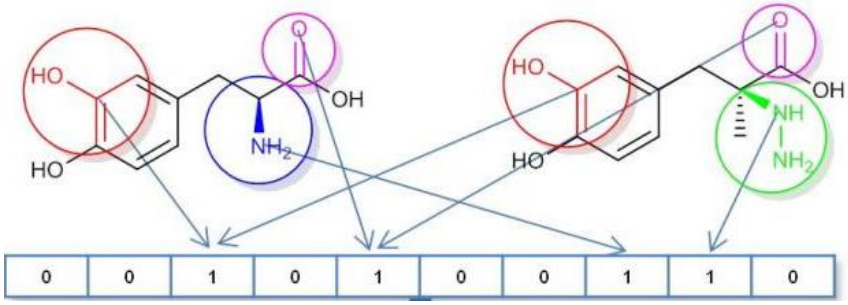
Characteristics of fragment hits



Shaw et al. J. Med. Chem. 2019, 62, 3381−3394

Sampling of the fragment space

- Scaffold diversity



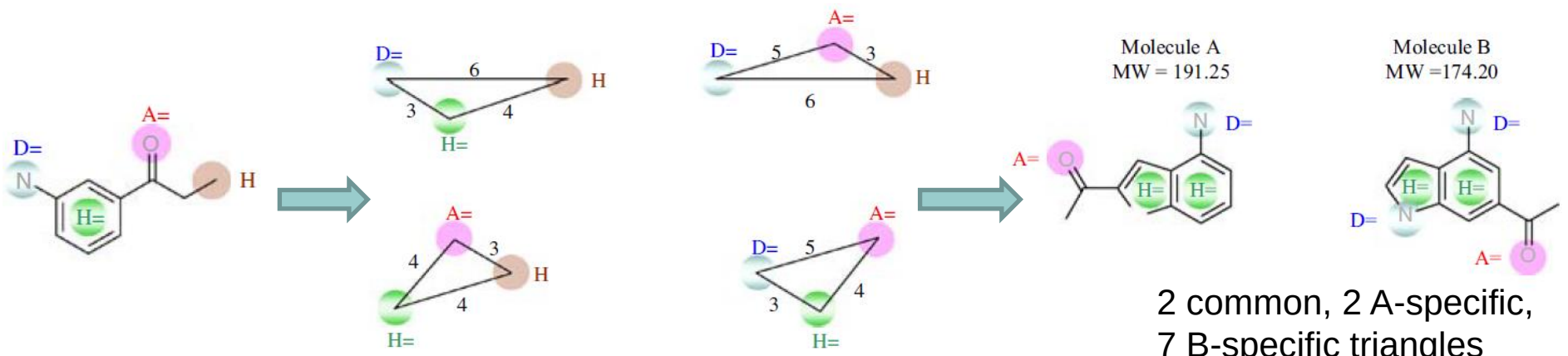
Tanimoto coefficient for binary bit strings

$$SIM_{RD} = \frac{C}{R + D - C}$$

- *C* bits set in common in the reference and database structure
- *R* bits set in reference structure
- *D* bits set in database structure

2 common bits, 3 bits in reference and database mols

- Pharmacophore diversity



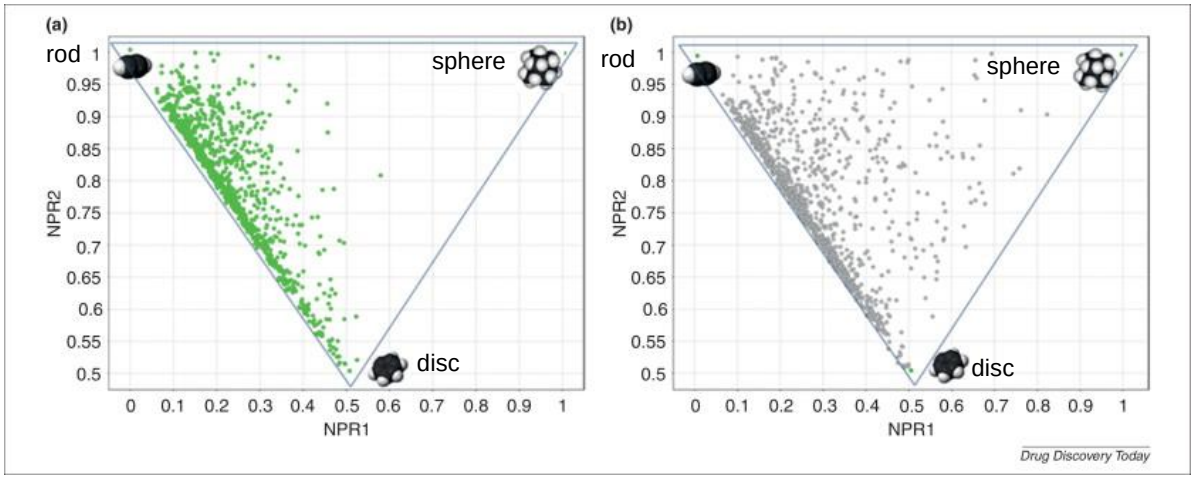
2 common, 2 A-specific, 7 B-specific triangles

Hubbard et al. J Comput Aided Mol Des (2009) 23:603–620

Sampling of the fragment space

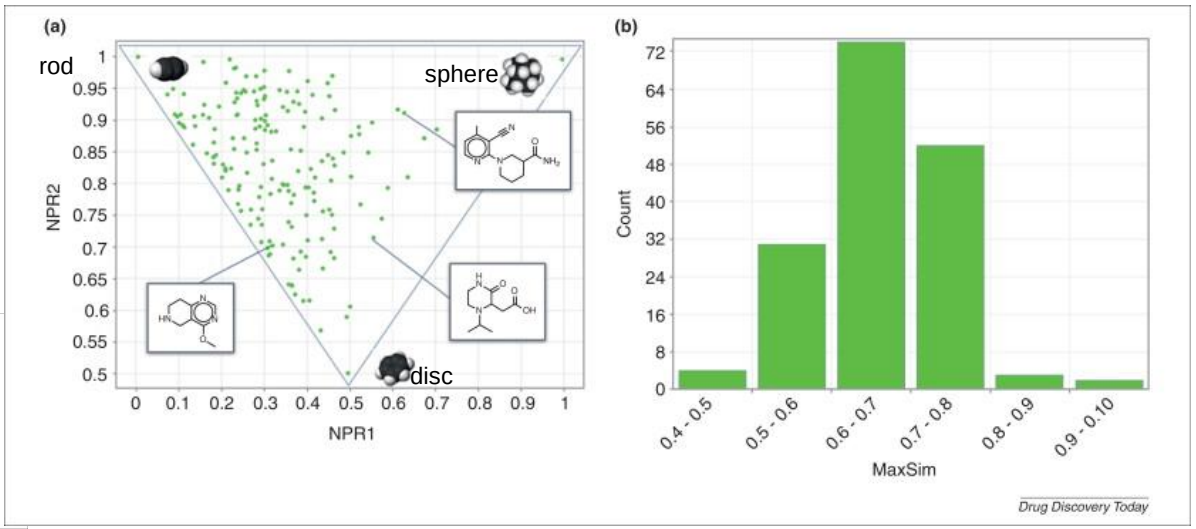
- Shape diversity

Conventional



„Clinical”

3D



3DFrag Consortium
DDT 2013, 18, 1221

MedChem

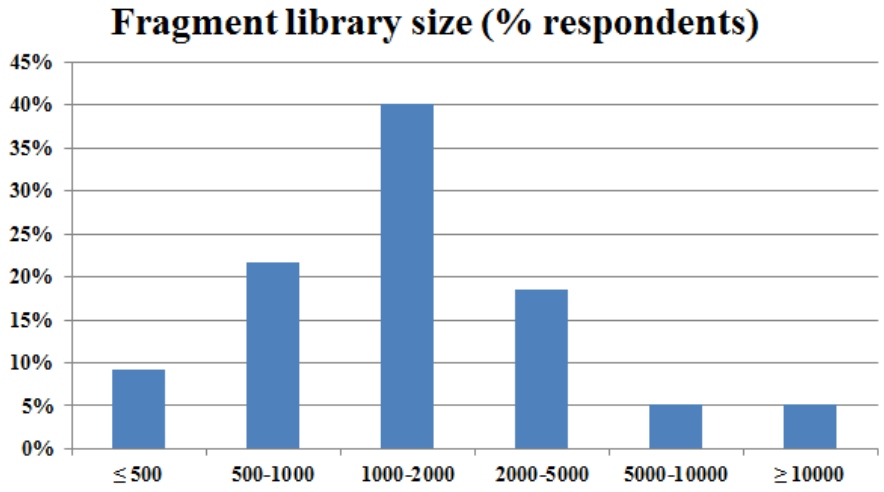
MedChem and experimental

Experimental

- MedChem considerations
  - Multiple synthetically accessible vectors
  - Should be synthesizable in <4 steps
  - Analogues should be available
  - Use racemates
- Experimental evaluation
  - Purity should be 95% or higher
  - Aqueous solubility (preferably ≥5 mM in 5% DMSO, or other screening co-solvents)
  - Stability (>24 h in solution)

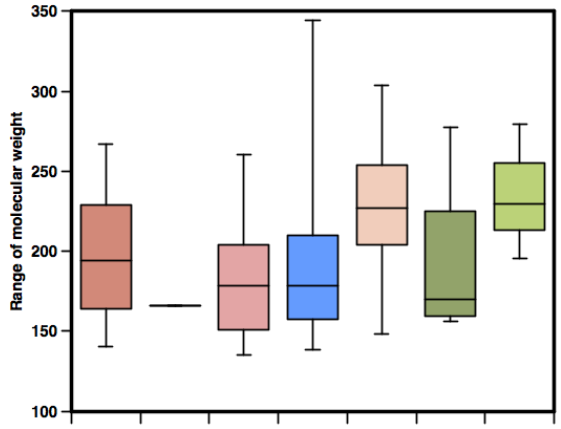
Further considerations

- Screening technologies
- Target related aspects
- Synthesis related aspects
- Library management aspects

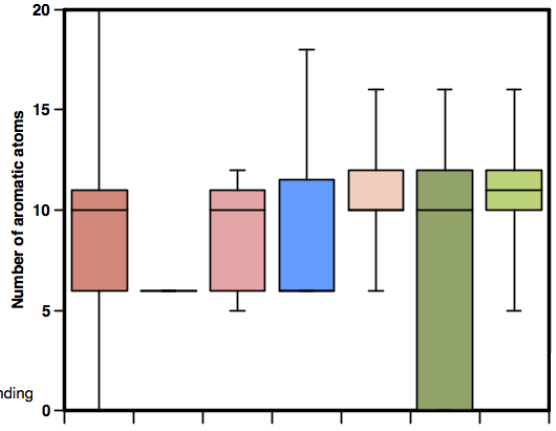


Target related aspects

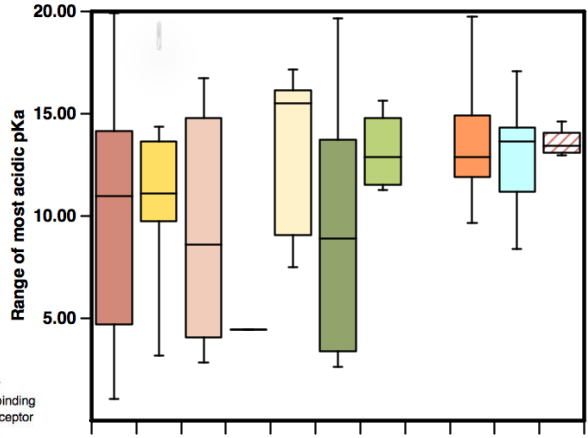
MW

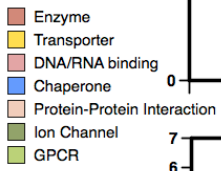


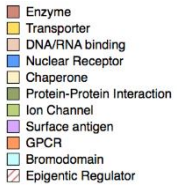
#Ar

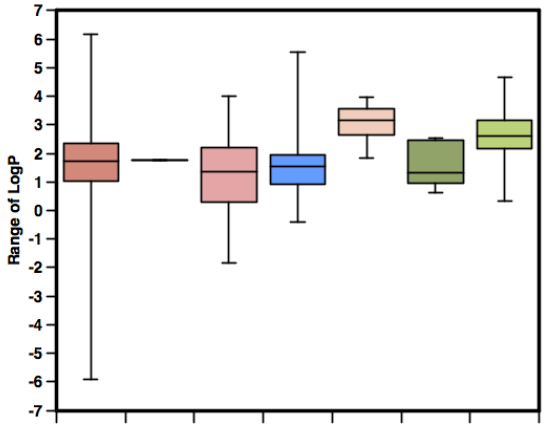


acid pK_a

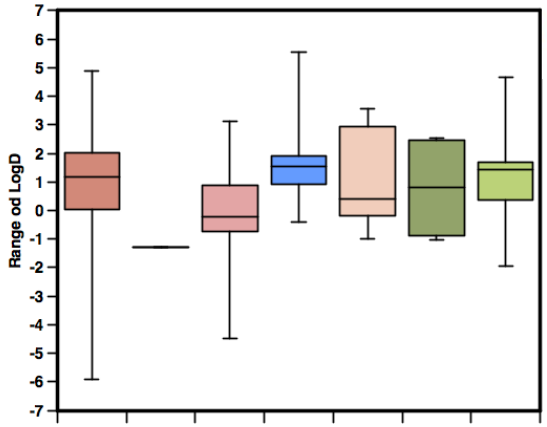




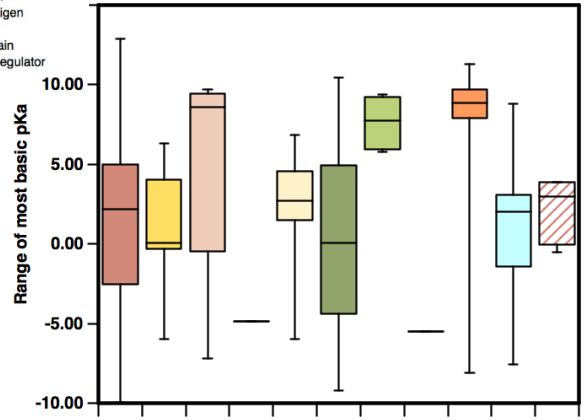




logP



logD

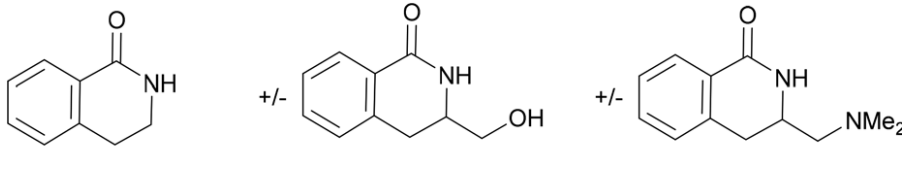


basic pK_a

Chris Swain, CMC

Synthesis related aspects

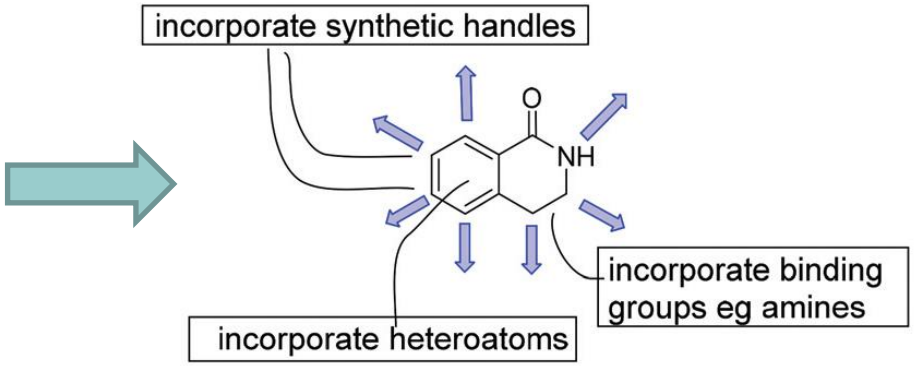
- Development of dihydroisoquinolone fragments

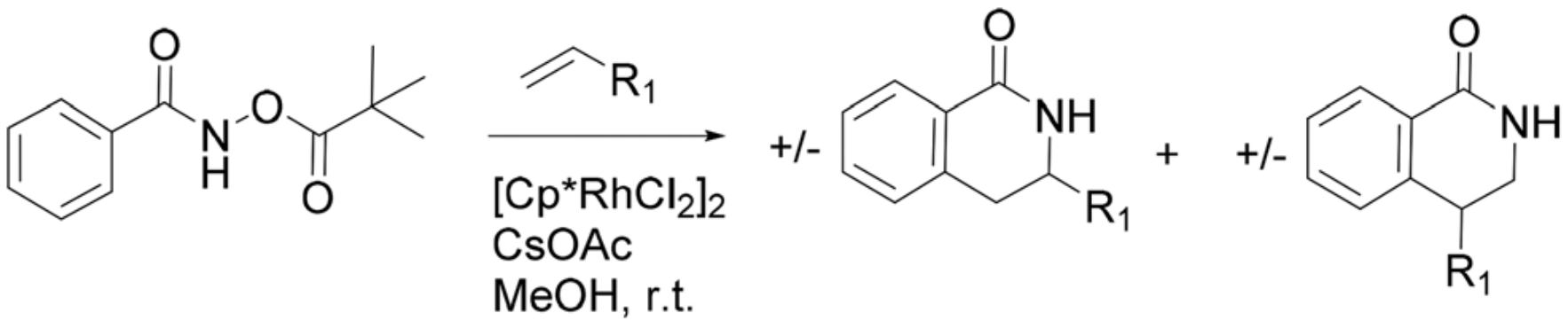


1
MW=147
cLogP=1.0
sol ≥ 5mM

2
MW=177
cLogP=0.32
sol ≥ 5 mM

3
MW=204
cLogP=1.3
sol ≥ 5 mM



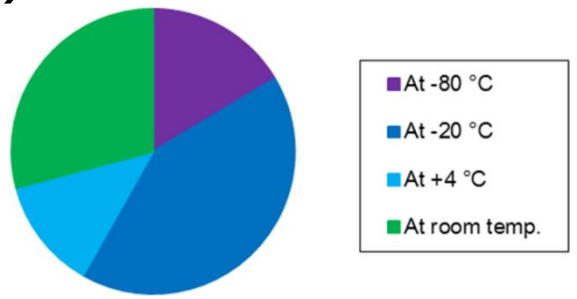


Substituted aromatic and heteroaromatic analogues

Rees et al. Org. Biomol. Chem., 2016, 14, 1599–1610

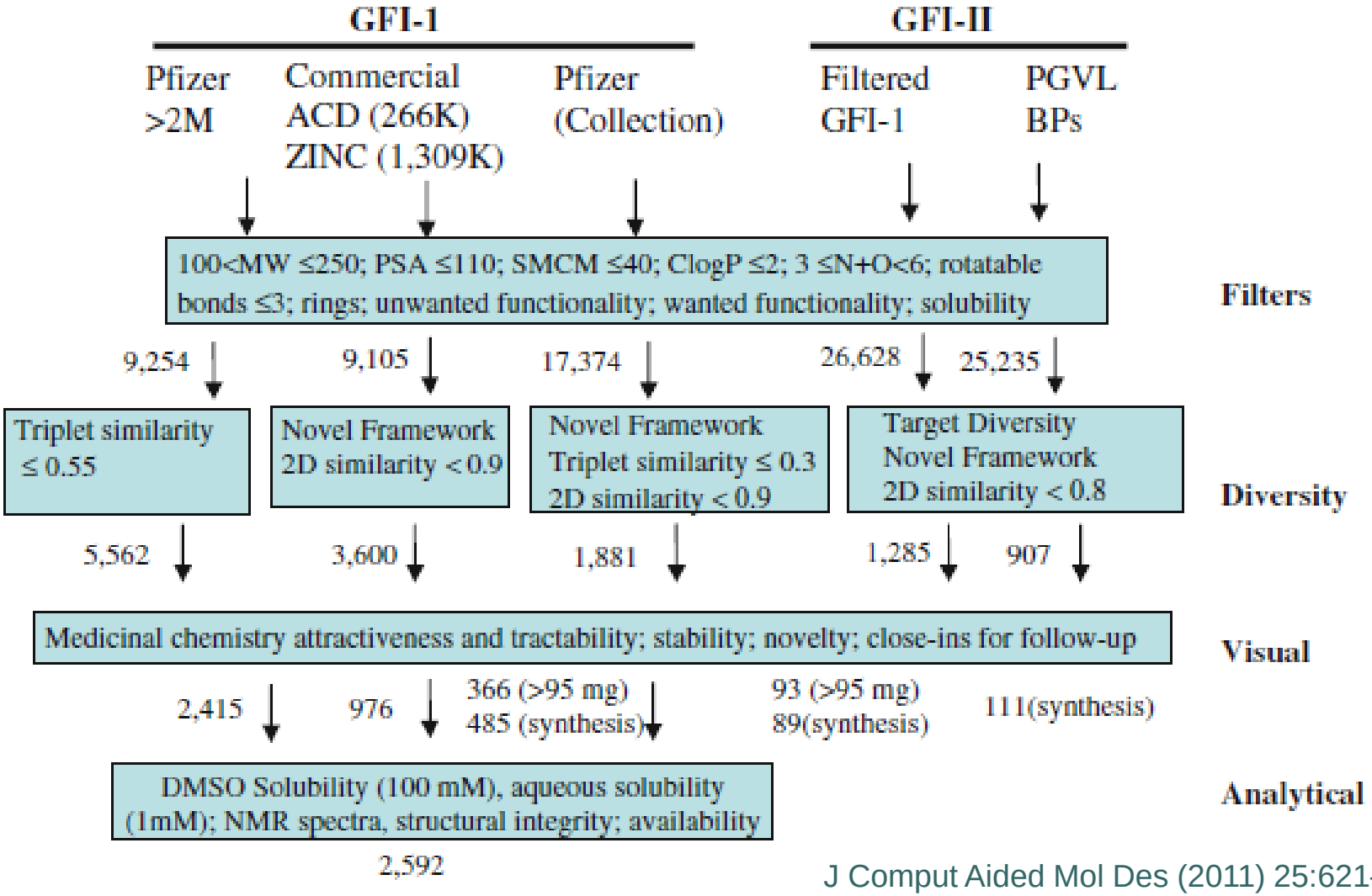
Library management aspects

- Purity should be > 90-95% LC/MS
- Identity should be checked by NMR
  - ~15% fails QC from vendors
- DMSO stability (concentrations from 50-200 mM)
- Regular QC is needed (P&G study)
  - 8% had degraded after 3 months
  - 17% after 6 months
  - 48% after 12 months
- Solution storage at low temperature as liquids



Keserű et al. J. Med. Chem. 2016

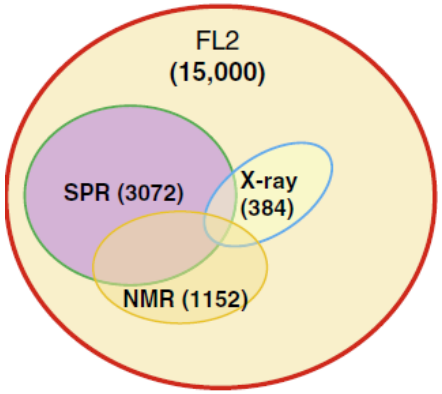
A case study from Pfizer



J Comput Aided Mol Des (2011) 25:621–636

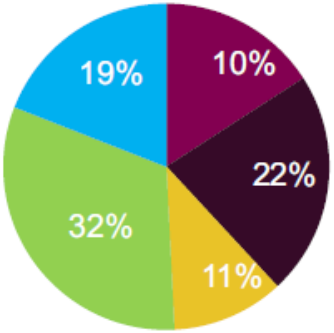
A case study from AstraZeneca

- From HCS to biophysical screening: from larger to smaller fragment library for X-ray, NMR, SPR
- Eliminating decomposing and assay interfering fragments
- New criteria for aqueous solubility of >500 μM
- Removal reactive fragments, unattractive structures for follow-up, undesirable chemical functionalities
- Program to design and synthesize novel fragment libraries
- Replace Ro3 with pharmacophore representation and structural diversity

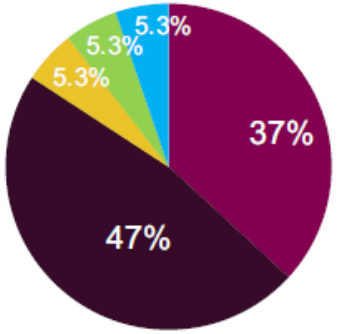


Physicochemical property guidelines for FL2 members	
cLogP	≤ 3
MW	≤ 300
Purity	>95%
Solubility	≥ 500 μM
DMSO Sol	≥ 100 mM

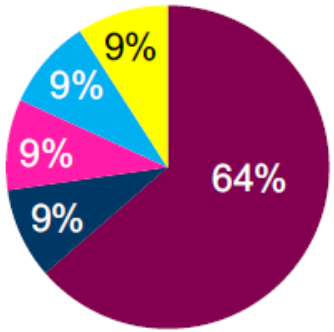
FBLG 2002-2008 (63 projects)



FBLG 2009-2011 (19 projects)



FBLG 2012-2014 (11 projects)

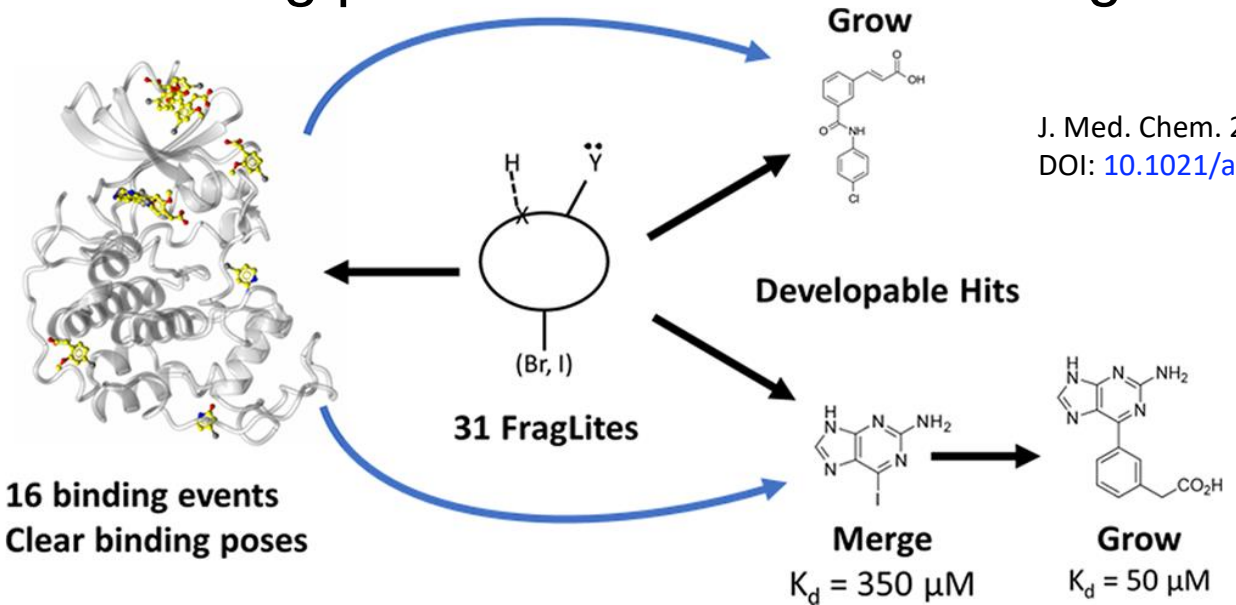


- Successful
- Failed: Portfolio reason/target validation
- Failed: Hit progression could not achieve desired potency
- Failed: Hits not progressable
- Still active in hit progression

DDT 2016, 21, 1273

New trends in library design 1

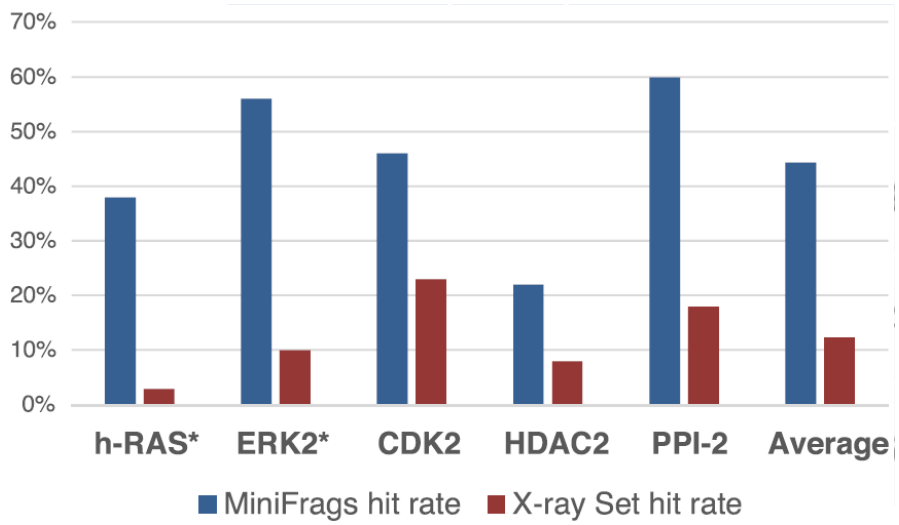
- FragLite: a set of halogenated compounds expressing paired hydrogen-bonding motifs
- Maps interaction sites by X-ray crystallography, exploiting the anomalous scattering of the halogens
- Provides an assessment of druggability and can identify efficient starting points for the de novo design

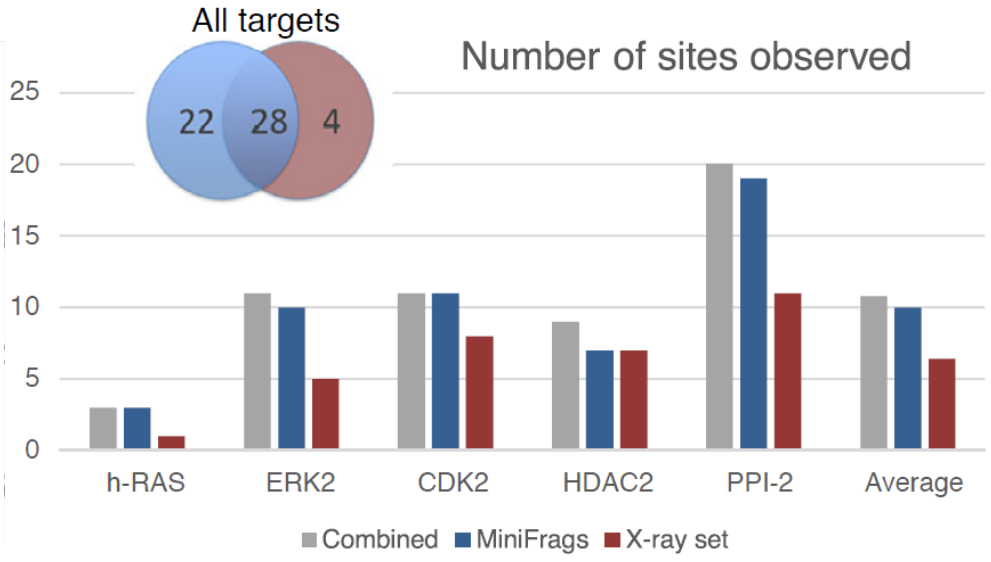


J. Med. Chem. 2019
DOI: 10.1021/acs.jmedchem.9b00304

New trends in library design 2

- Minifrags: a novel crystallographic screening methodology (Astex)
- High concentration aqueous soaks with a chemically diverse and ultra-low-molecular-weight library (HAC 5–7)
- Identifies ligand-binding hot and warm spots on proteins

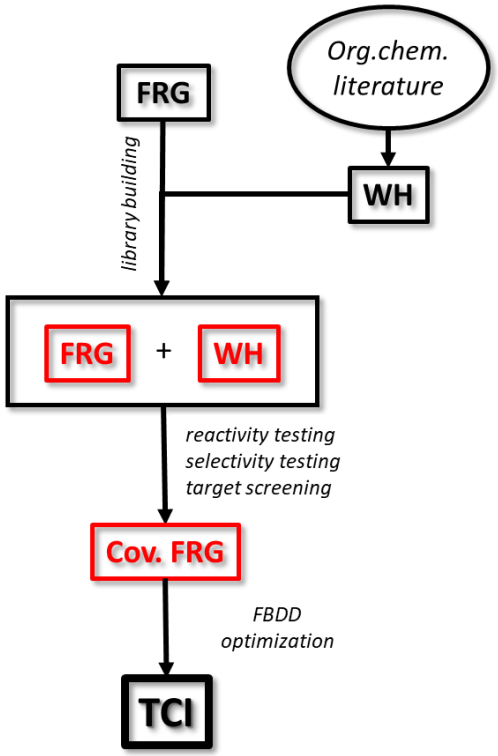




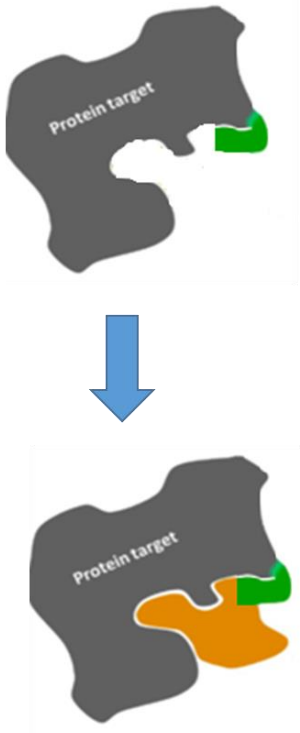
Drug Discov Today (2019), https://doi.org/ 10.1016/j.drudis.2019.03.009

New trends in library design 3

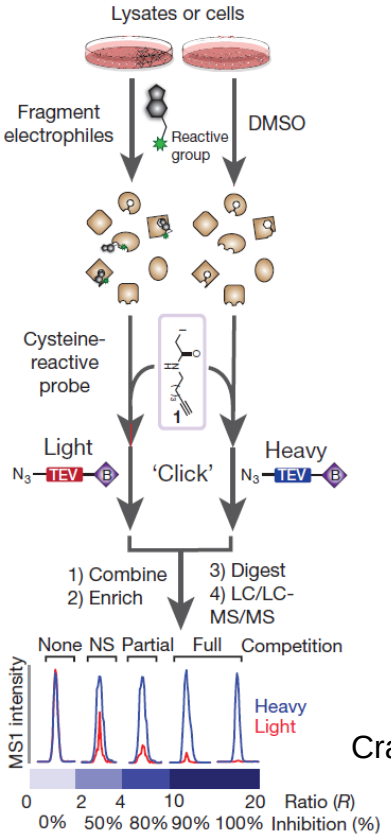
- Covalent fragments are equipped by electrophilic functionalities
- Useful to asses druggability and provides starting points for TCIs



Keseru, EJMC, 2018, Arch. Pharm 2018
MedChemComm 2019



Med. Chem. Commun., 2016, 7, 576-585
Chem. Soc. Rev., 2018, 47, 3816-3830



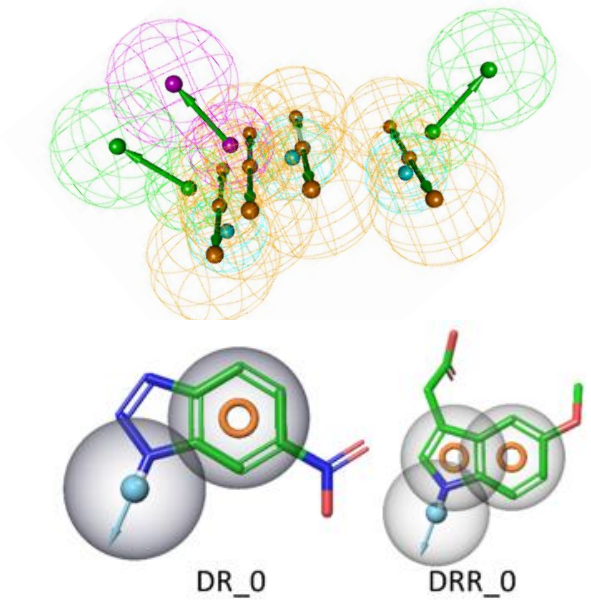
Cravatt, Nature, 2016

New trends in library design 4

- SpotXplorer: pharmacophore optimized fragment library

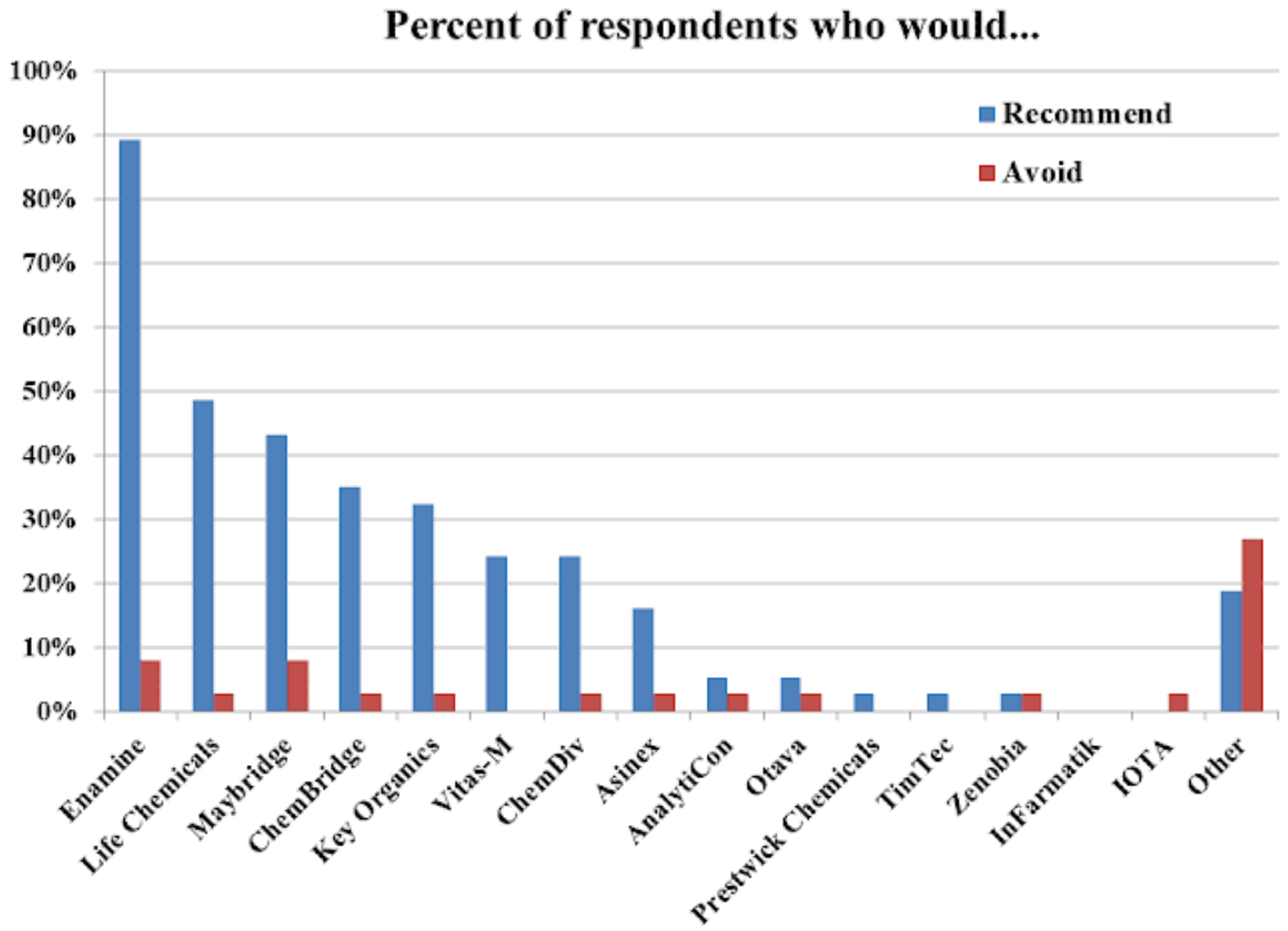
  - Select protein fragment complexes from the PDB
    - Non-Hydrogen atoms: 10-16
    - (MW: 140-230 Da, logP: 0-2, rotB: 0-3)
  - Binding Pharmacophore(s)
    - Features responsible for fragment binding to protein hot spot
    - 2-, 3- and 4-point pharmacophores identified from x-ray structures
    - Ranked by their free energy contribution estimated by docking
    - Most relevant pharmacophores are collected and clustered
    - Non-redundant set of fragment binding pharmacophores identified
  - Library Design

    Design a library with a diverse set of pharmacophores that covers most of the experimentally validated set of non-redundant fragment binding pharmacophores



Keseru, EJMC, 2018, Arch. Pharm 2018
MedChemComm 2019

Library providers



FRAGMENT SCREENING

Fragments and ligand efficiency

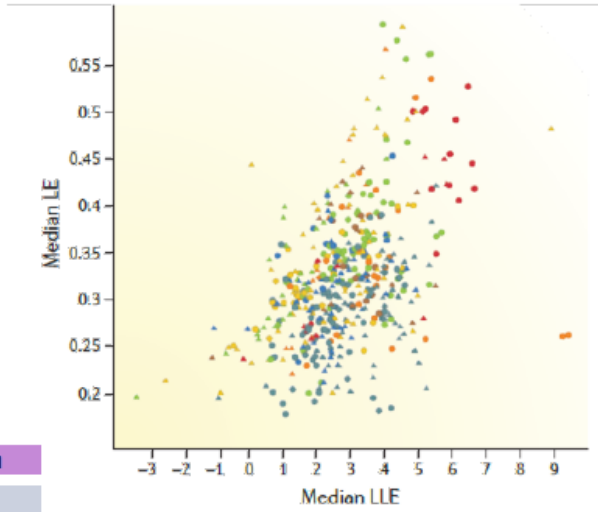
- Ligand efficiency
  - Key concept for fragments
  - Binding energy per heavy atom
- Low MW startpoint will have lower affinity
  - Typically K_D 10uM – 1mM
- Defining feature of FBLD
  - Low affinity is a result of small size
  - Fragments are no different to any other hit (*with caveats …*)
- *Those caveats in more detail …*
  - Low affinity has major implications for Hit ID and evolution
    - Careful experimental design
      - Robust assays, reliable validation
    - Strategies for fragment evolution
      - Transition from low affinity "fragment" to more potent "hit"
      - Fragment optimisation, elaboration, evolution

$$LE = \frac{(-2.303RT)}{HAC} logK_D$$

Intrinsic LE of the binding site

- "Intrinsic" ligand efficiency of a binding site varies from protein to protein
  - Linked to "druggability"
  - Calculate LE of known inhibitors or substrates
- LE varies from at least 0.6 to 0.15
  - Low druggability (0.2-0.35)
  - High druggability (> 0.4)
- Predict expected K_D
  - Assay must be robust and reliable over this range

480 target–assay pairs with more than 100 compounds covering 329 human drug targets



Colour and shape by target class

Carbonic anhydrase	Ion channel
Dehydrogenase	Kinase
GPCR: amine	NHR
GPCR: lipid	Other
GPCR: other	P450
GPCR: peptide	Protease
Hydrolase	Transporter

Hopkins et al. (2014) Nat Rev Drug Disc. 13 1474-1776

K_D

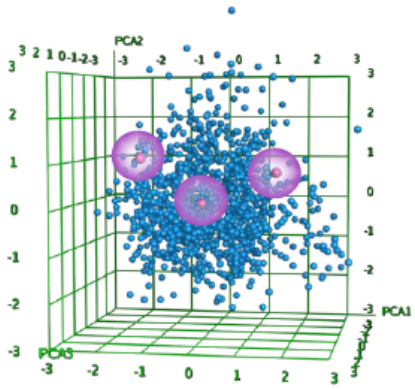
LE ((kcal/mol)/HA) \ HAC	10mM	1mM	100uM	10uM	1uM	100nM	10nM	1nM
0.15	18	27	36	45	55	64	73	82
0.20	14	20	27	34	41	48	55	61
0.25	11	16	22	27	33	38	44	49
0.30	9	14	18	23	27	32	36	41
0.35	8	12	16	19	23	27	31	35
0.40	7	10	14	17	20	24	27	31
0.45	6	9	12	15	18	21	24	27
0.50	5	8	11	14	16	19	22	25
0.55	5	7	10	12	15	17	20	22
0.60	5	7	9	11	14	16	18	20

Why are fragments different to other hits?

- They're not - a fragment is just a small hit
  - Low affinity is purely a result of small size

$$\Delta G = LE.HAC$$

- Why can't we just screen them the same way as normal compounds ?
  - Easy to mistake artefacts for weak binding
  - Low affinity requires careful experimental design
- Fragment evolution strategies apply constraints on screens
  - "Optimal" fragment almost certainly not present in screening library
    - Complete hit dataset required – low false negative rate
    - Fragment optimisation is key – search close NN of initial fragment hits
  - Fragment characterisation and optimisation
    - Resource intensive – low false positive rate
    - Relatively small library compared to other screening methods



- Most FBS (Fragment Based Screening) methods will find fragment hits
  - As confident as possible in validity of hits
  - As complete as possible a dataset

Detecting fragment binding

- Fragments typically 8-18 HAC
- Predicted K_Ds in the region of 10mM – 10nM
  - Typically 1mM – 10µM
- Choice of assay will depend on expected K_D
  - Reliability range of assay
    - High LE targets : *e.g. K_D 10µM*
    - Low LE targets : *e.g. K_D 1-10mM*
  - Choose assay which is appropriate for the expected affinity

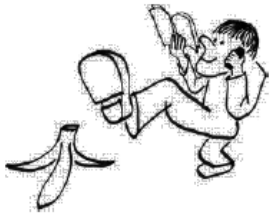
K_D

LE	10mM	1mM	100uM	10uM	1uM	100nM	10nM	1nM
0.15	18	27	36	45	55	64	73	82
0.20	14	20	27	34	41	48	55	61
0.25	11	16	22	27	33	38	44	49
0.30	9	14	18	23	27	32	36	41
0.35	8	12	16	19	23	27	31	35
0.40	7	10	14	17	20	24	27	31
0.45	6	9	12	15	18	21	24	27
0.50	5	8	11	14	16	19	22	25
0.55	5	7	10	12	15	17	20	22
0.60	5	7	9	11	14	16	18	20

LE ((kcal/mol)/HA)

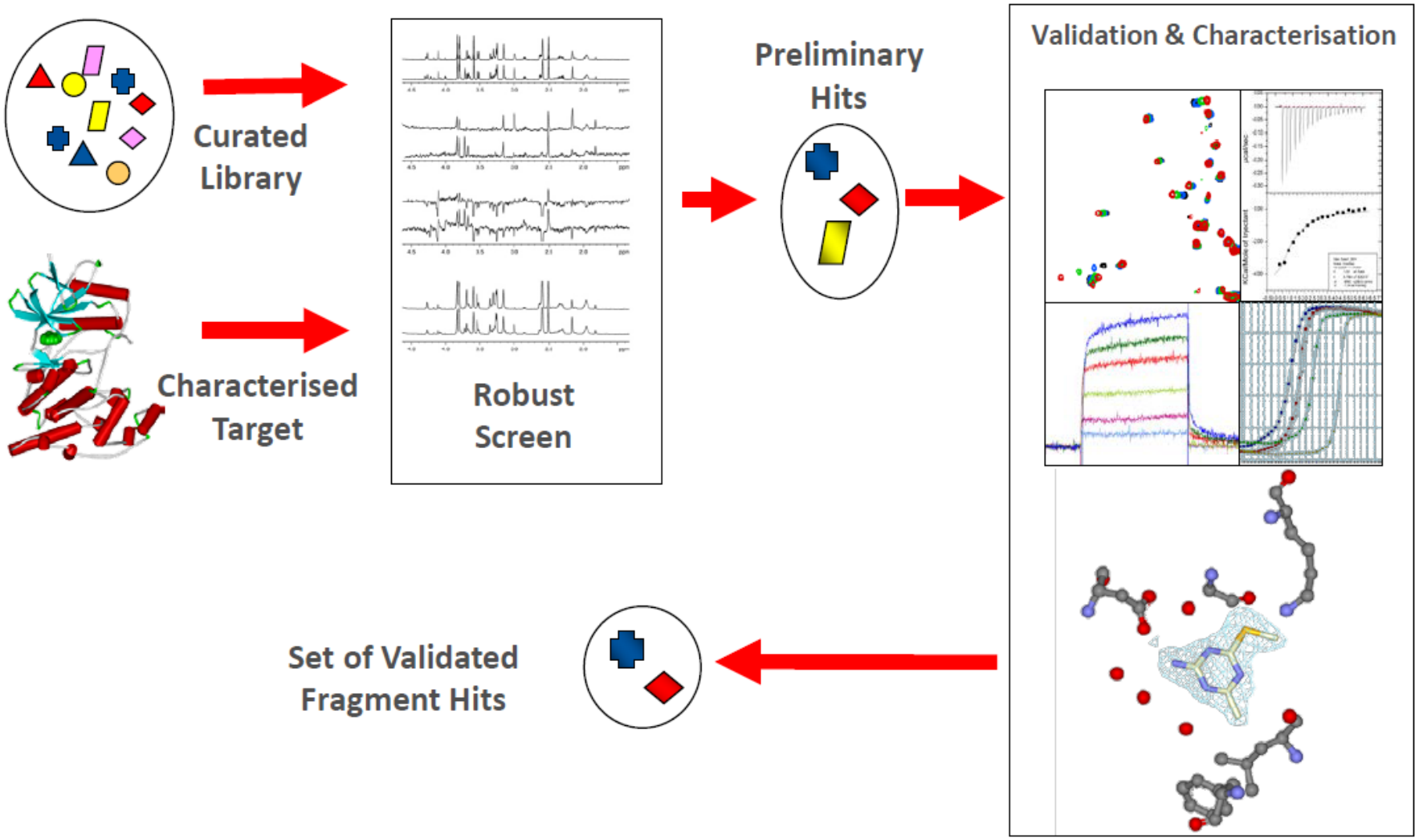
HAC

- <u>False positives are the main issue</u>
  - Easy to mistake artefacts for weak binding
    - At [L]=1mM a 1% contaminant is 10 µM
    - Assay interference from high concentrations of compounds
      - pH, redox behaviour, DMSO, metal chelation, detergents, fluorescence or absorption, interference with secondary/coupled detection system
    - Compound solubility & aggregate formation



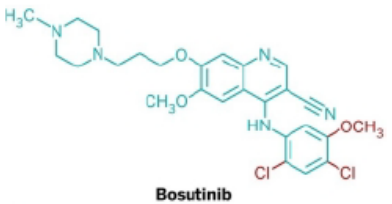
Learning from our mistakes: the 'unknown knowns' in fragment screening
Davis & Erlanson (2013) Bioorg Med Chem Lett. 23(10):2844-52

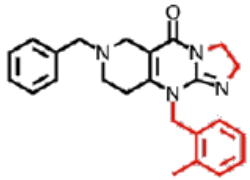
Generic FBS protocol

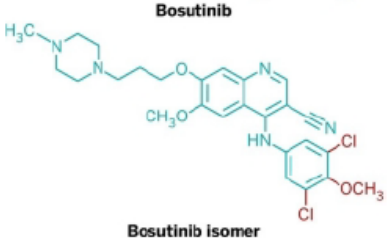


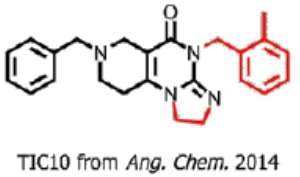
Curated library

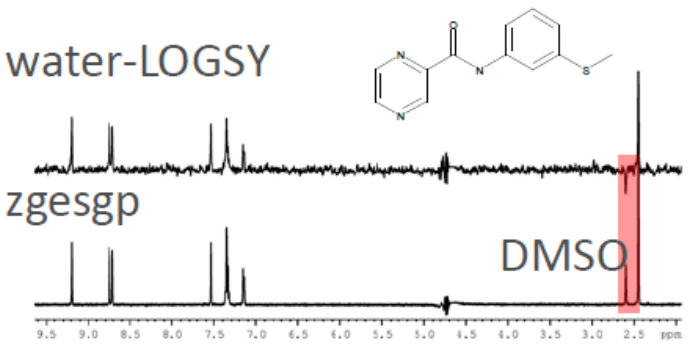
- Correct compound ?
  - Vendors & chemists do make mistakes
  - Correct isomer (bosutinib, TIC10)
- Impurities
  - Low levels of potent impurities
  - Metal contaminations
- Compound stability
  - Long term DMSO, 24 hour aqueous
- Reactive molecules
  - PAINS (pan-assay interference compound)
    - Baell, Chem. Inf. Model. 2013, 53, 39
  - Redox cyclers
- Aggregators & self-associators
  - Particulate formation







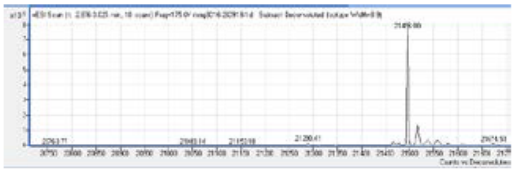


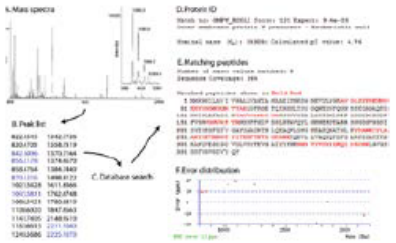


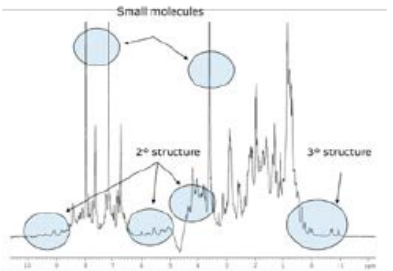
Characterised target protein I

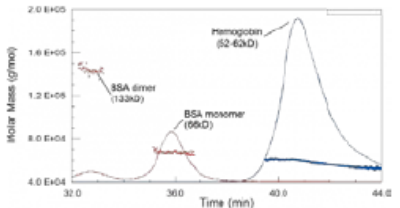
Expression and purification

- Most basically:
  - Is this the correct protein ? LCMS, PMF
  - Are there post translational modifications ? Intact mass
- Is the fold correct
  - (Specific) activity, 1D ^{1}H NMR
- Is the oligomerisation state correct ?
  - SEC, SEC-MALS, DLS, AUC, NMR
- Is the protein stable ? How long is the FBS process ?
  - DSF (including buffer screening); NMR
  - Binding of control ligand (SPR, MST)
- Is the experimental configuration optimised ?
  - SPR – immobilisation conditions and tag; buffer …
  - MST – interactions with capillary, detergents, dyes …





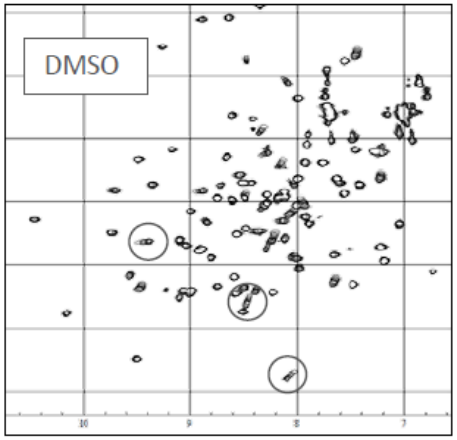


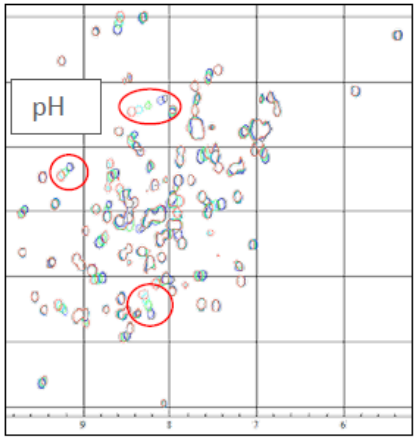


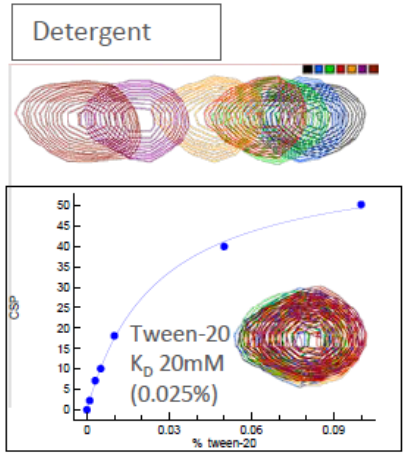
Characterised target protein II

Interactions

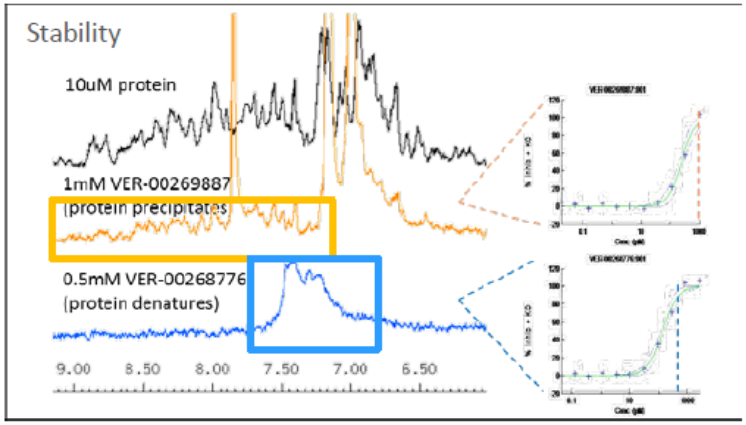
Interactions with
- Buffer components
  - Phosphate buffer
- Reducing agents
- Metal ions
- .....







Interactions with compounds
- 2 compounds with activity in biochemical assay
- Mode of inhibition
  - **Precipitation**
  - **Denaturation**

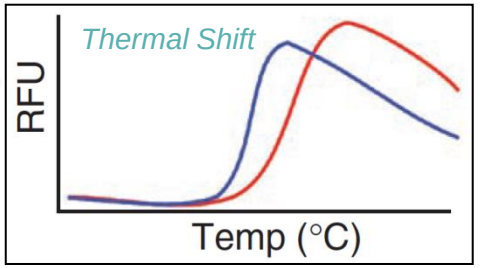


Roboust assay

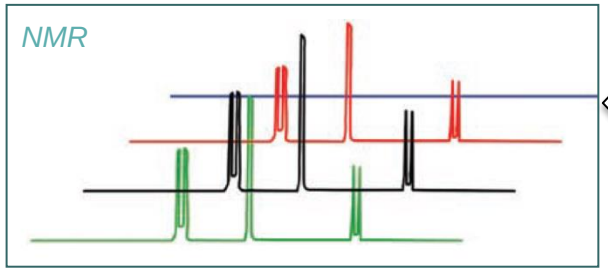
- Biochemical assay
  - Indirect detection of fragment:protein interaction
    - Functional assay, displacement of probe molecule, disruption of donor:acceptor interaction
  - Can work very well
    - Specific to protein (enzyme) or protein class
    - Optimised for specific protein
    - Artefactual effects of high concentrations of fragments
      - Careful controls are key
      - Detergents
- Biophysical methods
  - Direct measurement of a binding interaction
  - Information rich
  - Typically more tolerant & sensitive than biochemical or functional assays
  - Robust & (relatively) generic

Fragment screening methods

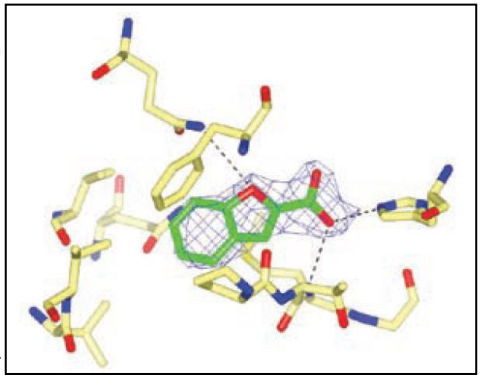
Primary Screening
Thermal Shift / SPR / NMR



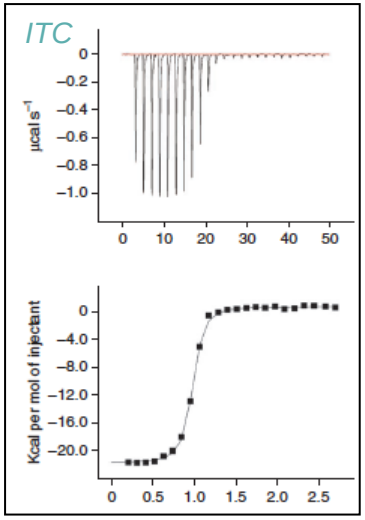
Secondary Screening
NMR Spectroscopy

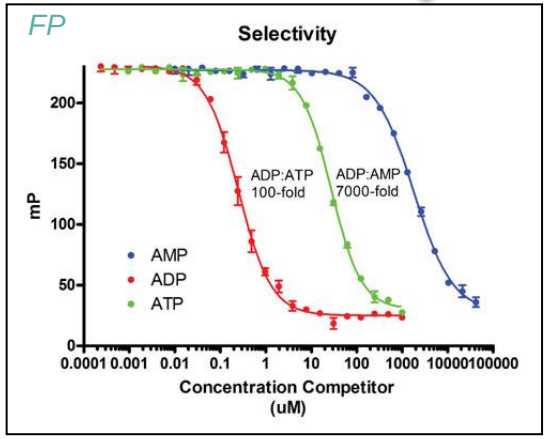


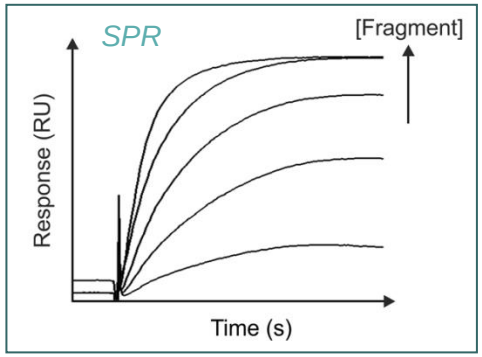
X-Ray



Binding Affinity
ITC / SPR/ FP





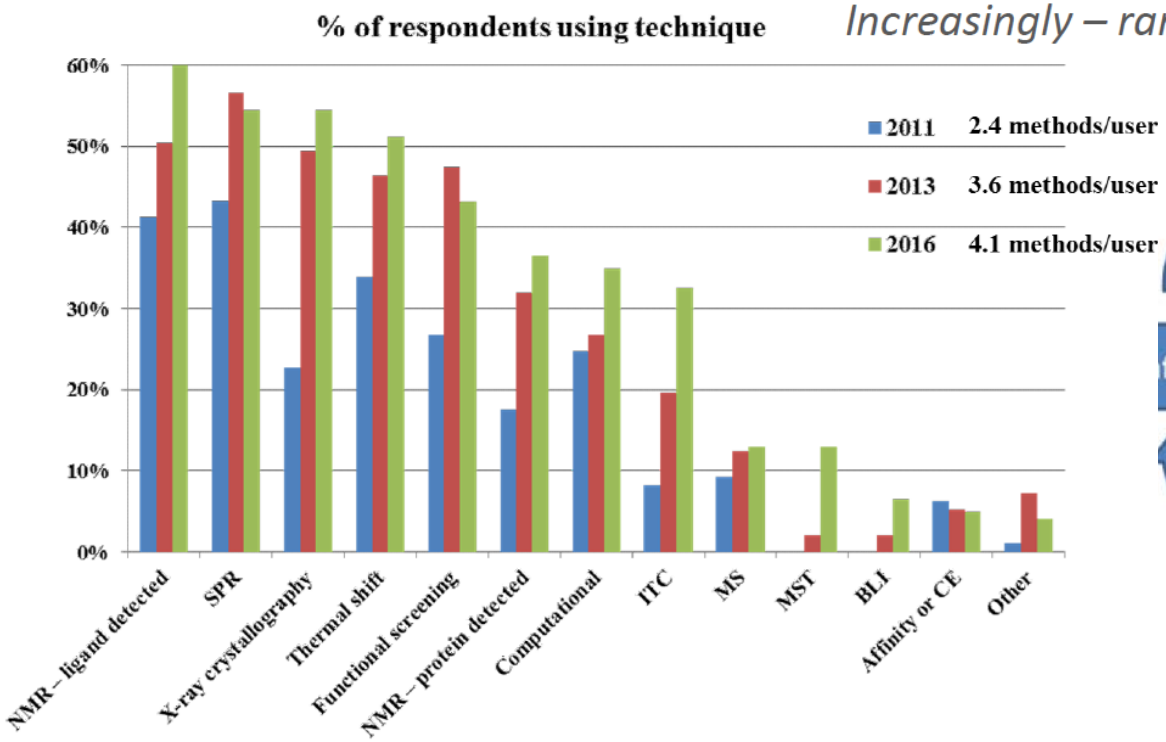


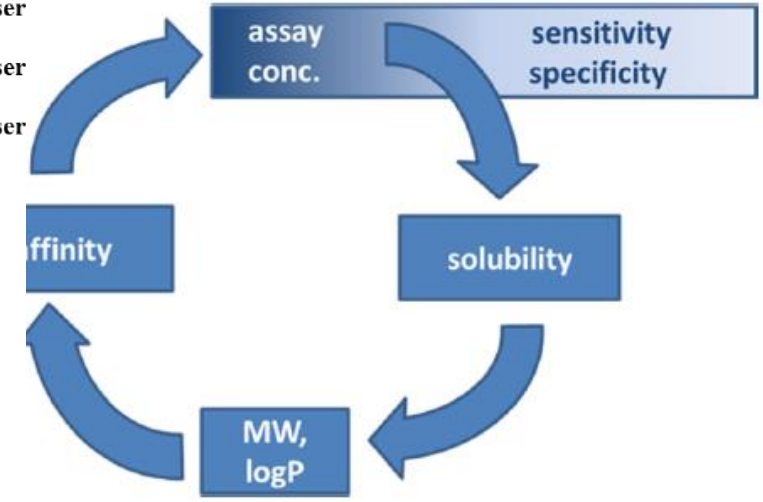
Christina Spry

The impact of screening technologies

Biophysical methods widely used for FBS

Increasingly – range of orthogonal methods are used

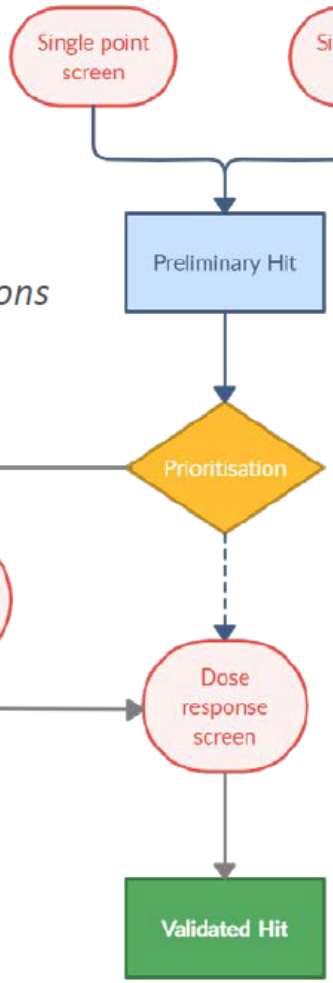




Keserű et al. J. Med. Chem. 2016

method	sensitivity limit	specificity assessment	throughput	structural information	propensity for false positive/false negative
biochemical	high μM	+	high	none	high FP/FN
ligand-NMR	low mM	–	medium	some	medium FP
protein-NMR	low mM	+	low	high	low FP/FN
SPR	high μM	+	medium	none	medium FP
thermal shift	high μM, low mM	–	high	none	high FP/FN
X-ray	mid mM	+	low	high	low FP/high FN

Fragment validation



One or more of : SPR, LO-NMR, PO-NMR, ASMS, crystallography, biochemical assay, virtual screening, thermal shift, MST …

Hit rate (ligandability) & downstream resource implications

Number of hits, diversity, throughput, project requirements

Another of SPR, LO-NMR, PO-NMR, ASMS, crystallography, biochemical assay, virtual screening, thermal shift, MST …

Typically SPR, PO-NMR, biochemical assay

Fragment characterisation & elaboration

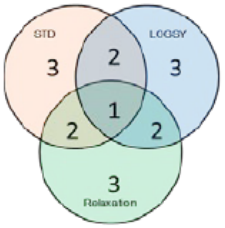
Characterisation of hits

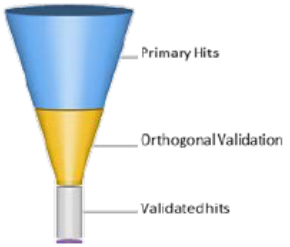
- **Validation of preliminary hits**
  - Initially "data are consistent with binding" …
- Orthogonal screening methods
- "Single point" versus "Dose response"
- Prioritisation when orthogonal techniques disagree ?
  - Dogmatic or pragmatic
  - Resource requirements and alternative ligands

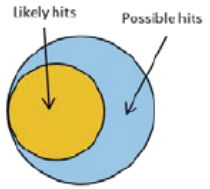
- **Characterisation of validated hits**
- Affinity
  - K_D [k_{on}, k_{off}, ΔG, ΔH & TΔS]
- Structure of protein:ligand complex
  - Crystal [*solution, dynamics*]

Problems in orthogonal assays

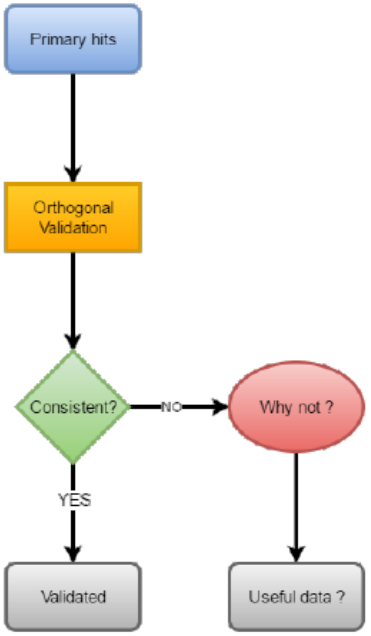
- Inconsistencies observed between results from different NMR experiments
  - Same sample, same conditions, same time
- Soft filter required to assess overall data package
  - "NMR Binding Class" used for prioritisation, not exclusion







- More generally …
  - What is the best way to combine output from orthogonal validation?
    - Different techniques, different samples, different conditions, different times
  - Why are orthogonal methods inconsistent ?
    - Compound issues
    - Differences in conditions
    - Experimental error
    - Different measured parameters
  - Synergy between techniques

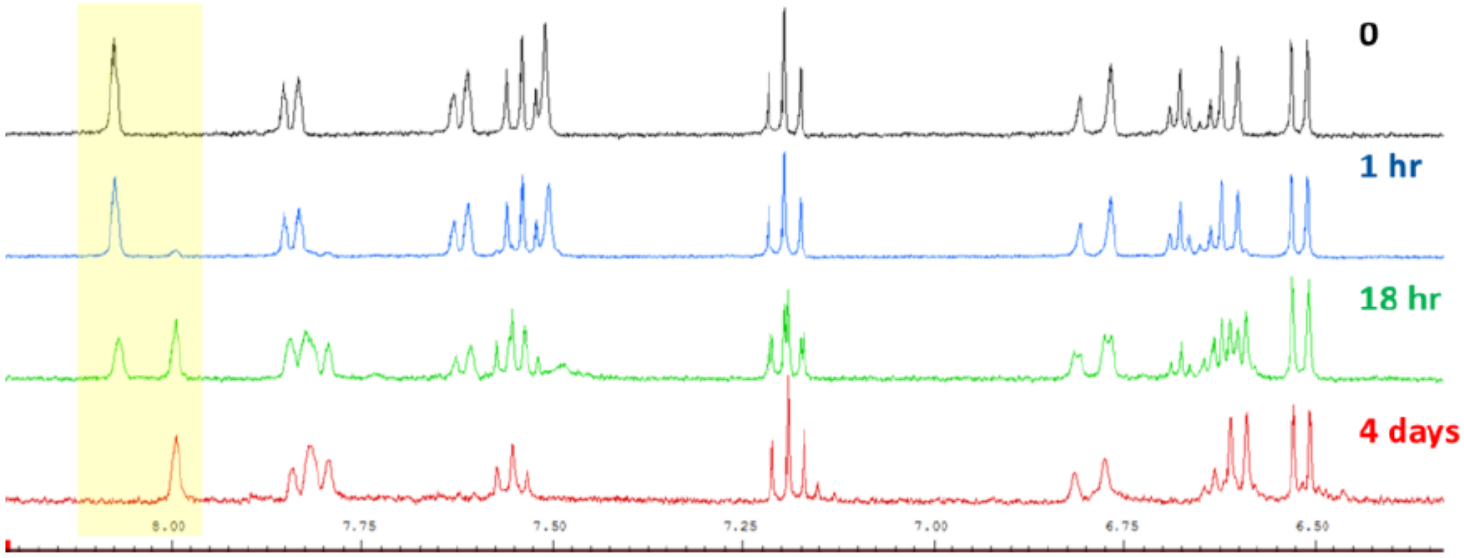


Compound issues

- Batch variation
  - Most common compound-related reason for inconsistent results
    - Different physical sample
  - Stability of stock solutions
  - Contamination
    - Synthesis
    - Stock preparation
- Compound behaviour
  - Rigorous fragment QC prior to inclusion in library
    - ^{1}H NMR in DMSO and aqueous solution (20mM KP pH 7.5)
    - Regular (annual) QC of DMSO stocks (LCMS, NMR)
  - Issues still arise
    - Fragment near neighbours
    - Unexpected behaviour of fragments

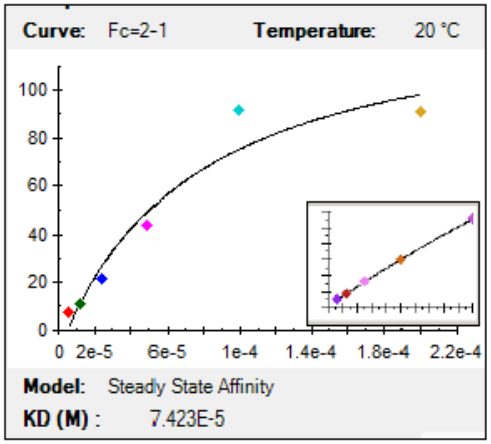
Compound stability I

Degradation product is inactive



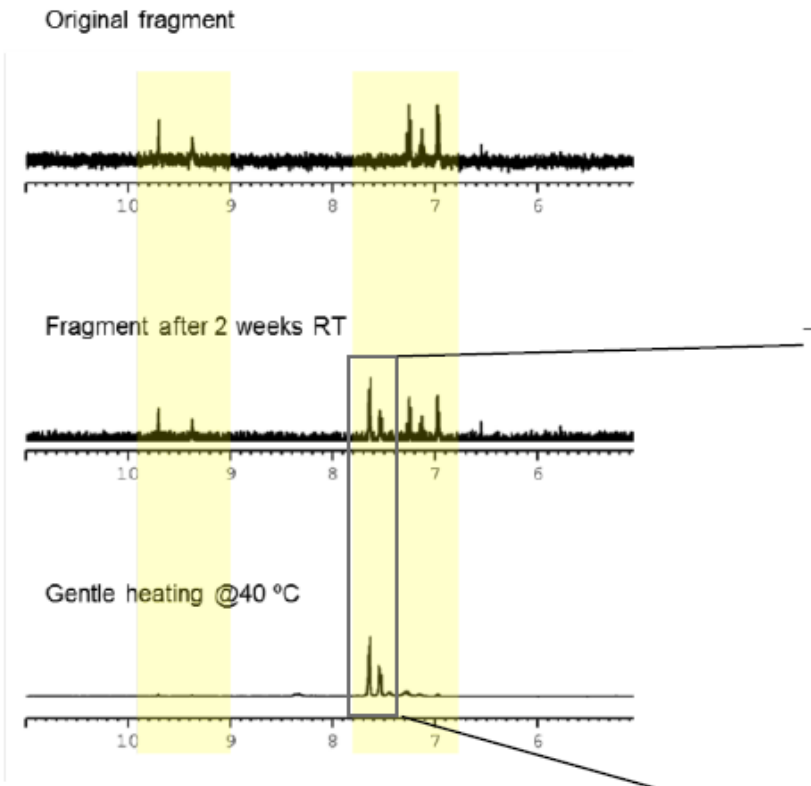
Fragment near neighbour

- Active in initial screen (SPR)
- Decarboxylates on standing in DMSO
- Parent compound binds (K_D 75uM)
- Degradation product does not



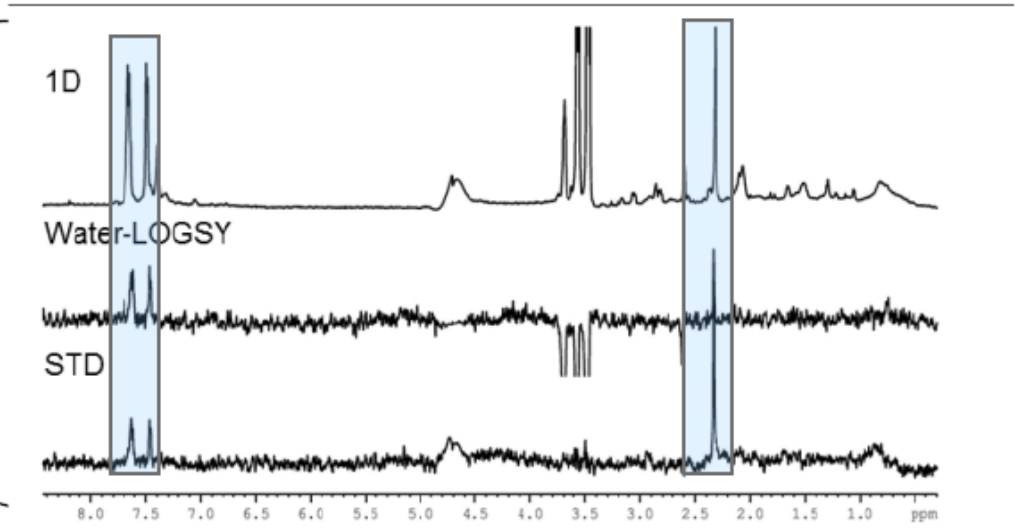
Compound stability II

Parent compound is inactive



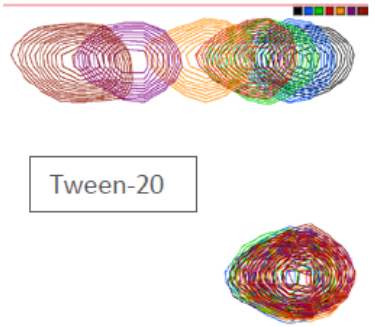
Fragment near neighbour

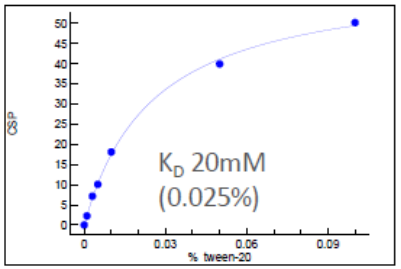
- Parent shows no binding by NMR
- Stock solution QC'd, degradation observed
- Dehydrates and ring closes on standing in DMSO
- Parent compound does not bind
- Degradation product does bind

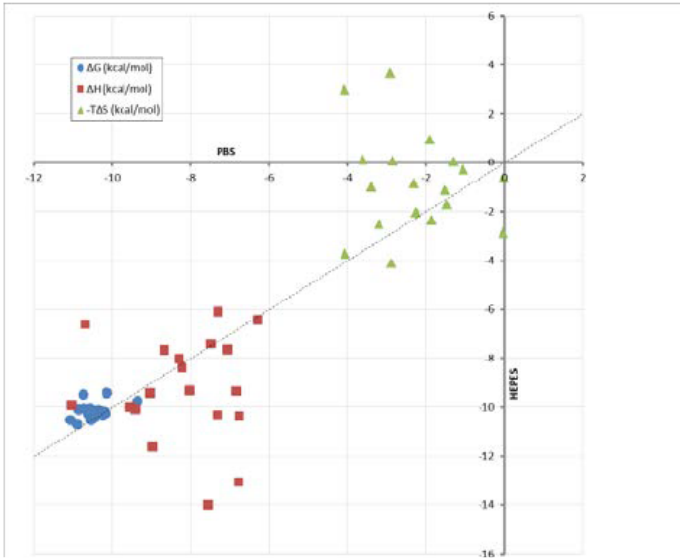


Buffer effects

- Direct
  - Tween-20 required for SPR & FP assays
    - Binds in active site
    - Inhibited compound binding
  - Inconsistencies between assays
  - Reconfigure assays to use non-interacting detergent
- Indirect
  - Binding of 22 compounds by ITC
    - HEPES vs PBS
    - pH 7.4, 150mM NaCl
  - ΔG correlates well
  - ΔH and –TΔS vary substantially



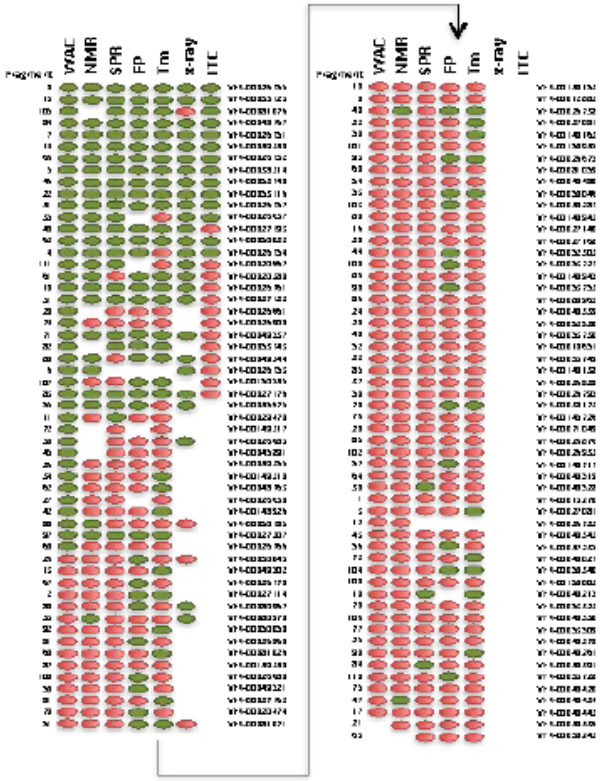




Experimental error

Meiby *et al.* (2013) Anal Chem 85(14):6756-6

	WAC	NMR	SPR	ITC	X-ray	FP	Tm shift
WAC	-	88% (n=103)	83% (n=107)	52% (n=27)	83% (n=30)	66% (n=109)	71% (n=107)
NMR		-	90% (n=102)	63% (n=24)	93% (n=28)	74% (n=104)	74% (n=102)
SPR			-	73% (n=26)	76% (n=29)	73% (n=109)	73% (n=109)
ITC				-	57% (n=23)	63% (n=27)	58% (n=26)
X-ray					-	73% (n=30)	66% (n=29)
FP						-	73% (n=109)
Tm shift							-



- Comparison of techniques to detect binding of 111 fragments to Hsp90α
  - WAC, NMR, SPR, FP, Tm shift
  - Selected fragments tested by crystallography, ITC
- Each of the techniques was operated under optimal conditions for that particular method, rather than attempting to use the same or highly similar conditions for all assays

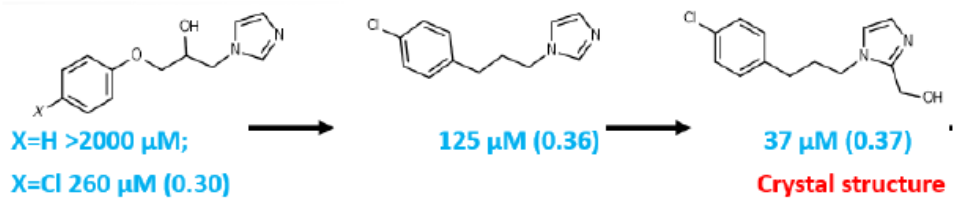
Characterising protein-fragment complexes

- **Why do we need to determine the fragment affinity ?**
  - Identification of potent interactions
  - Track whether subsequent modifications are improving or worsening binding
  - Don't focus purely on affinity – one parameter amongst many

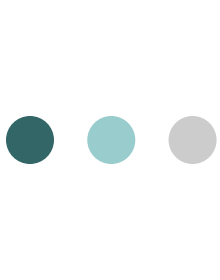
- **Why do we need the structure of a protein:fragment complex ?**
  - Guide medicinal chemistry, fragment morphing and evolution
  - Do you need a X-ray crystal structure ?
    - No, but it really, really helps !
    - Worth investing substantial effort to achieve
  - What resolution structure will help guide medicinal chemistry?
    - X-ray, NMR, NMR based model or purely ligand-driven
    - SAR by Catalogue
  - Timescale of structural data acquisition ?

Advancing w/o X-ray structure

- Straightforward to find & validate fragments that bind
- Evolution requires robust model of fragment binding
  - Guide medicinal chemistry with structural model
  - Best model is from X-ray structure
- X-ray structures not always available
  - 7/27 (2015) & 8/28 (2016) successful FBLD campaigns did **not** have X-ray structures for the initial fragment hits bound to their targets
- Alternative approaches and models can be successful
- Don't rule out a target just because crystallography is challenging

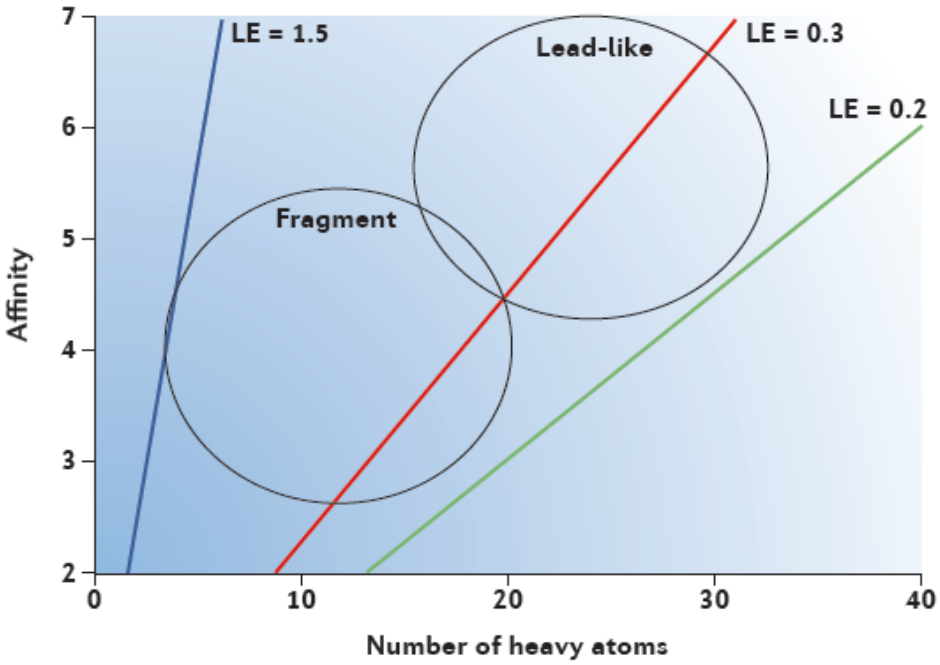


Yamada *et al* (2020) J. Med. Chem, *63*, 14805-20



FRAGMENT OPTIMIZATION

You have a fragment and what?



- Fragment chemical space could be better sampled
- Fragment screening could provide diverse chemotypes
- Fragments are better starting points providing increased operational freedom to medchem optimizations

But fragments are significantly less potent compared to HTS hits and leads

- Their detection is challenging
- They should be effectively optimized to reach the required potency

Fragment Optimization

- Typical properties of starting points
  - Low MW, polar, low affinity (high μM, or mM)
- Optimization workflow
  - Significant increase in size
  - Significant improvement in affinity
  - High operational freedom owing to the small and polar starting point
- Anticipated outcome
  - Balanced affinity, size and lipophilicity

Optimization

Starting point
(HTS, Fragment,…)

→ Clinical candidate

Increase activity
Improve selectivity
Optimize ADME/phys-chem properties

Activity increase ⟷ phys-chem property optimization

Affinity ↗ ☺
lipohilicity↗☹
molecular weight↗☹

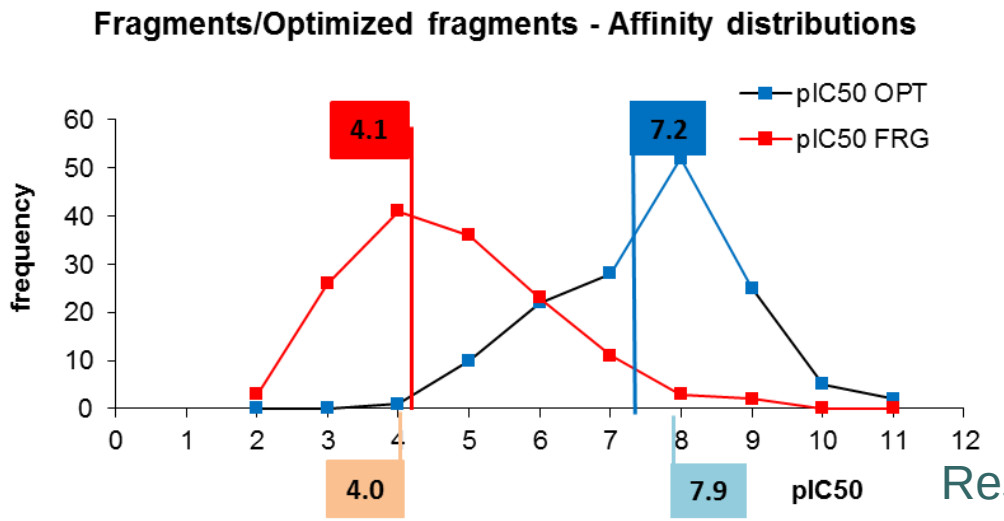
Nat. Rev. Drug Discov., 2007, 6, 881
Nat. Rev. Drug Discov., 2009, 8, 203

Features of fragment optimizations

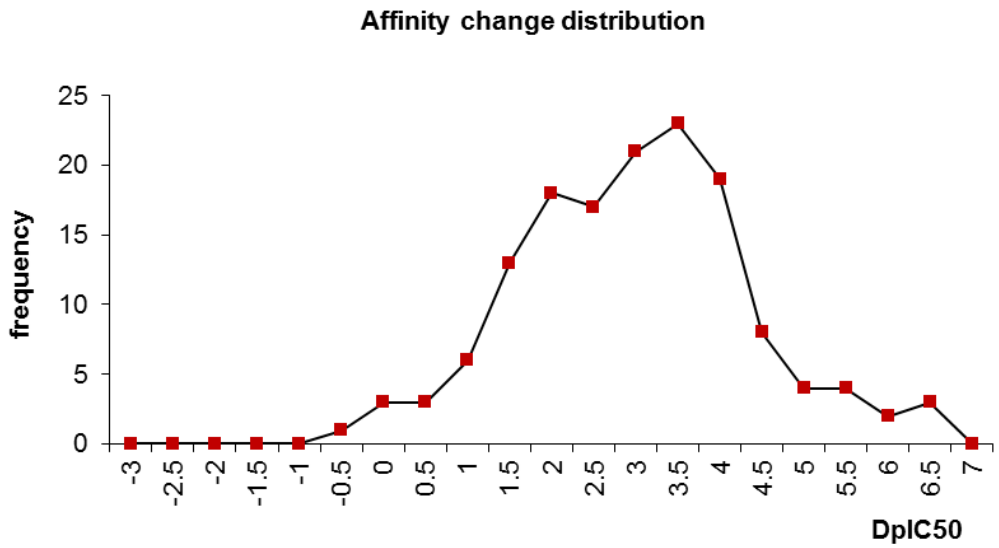
- Significant change in
  - Potency
  - Size (heavy atom count, molecular weight)

- What are the specific features of successful fragment optimizations?
- Analysis of published fragment optimizations

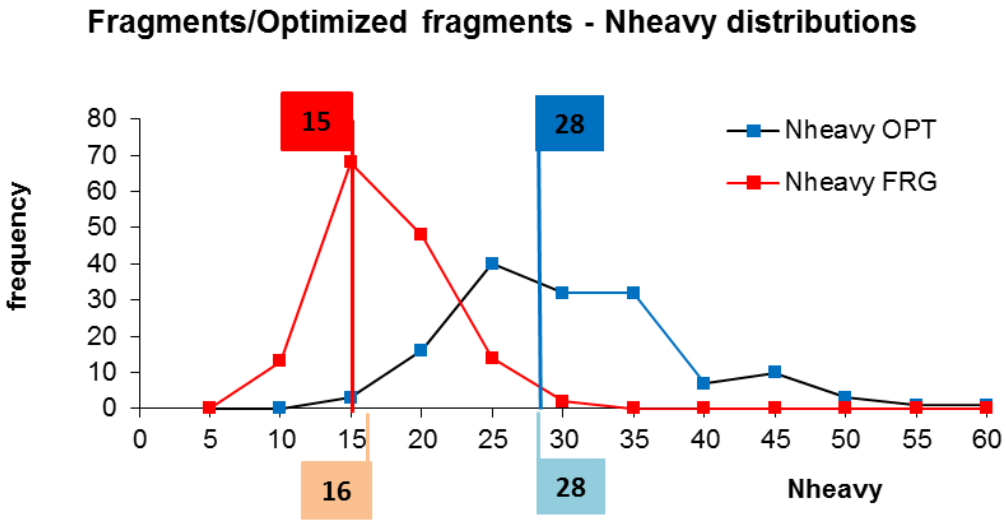
Affinity change

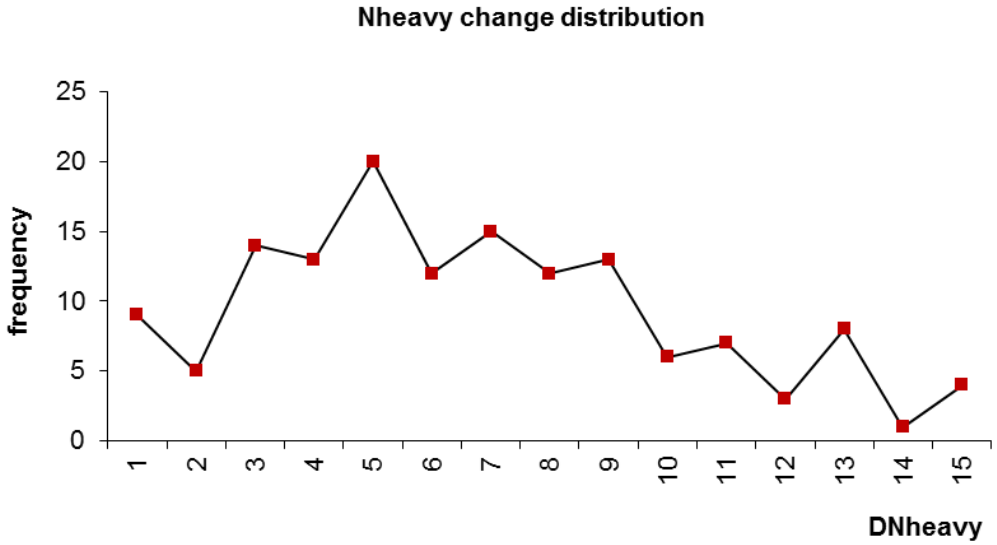


Resulting dev cand

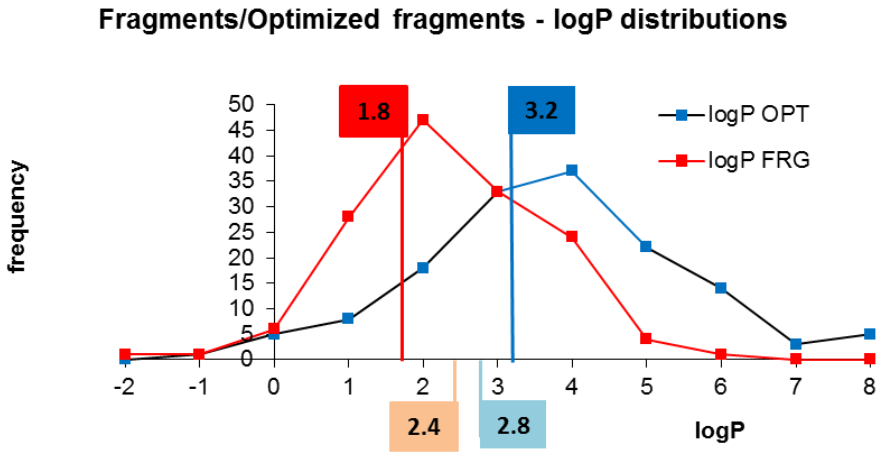


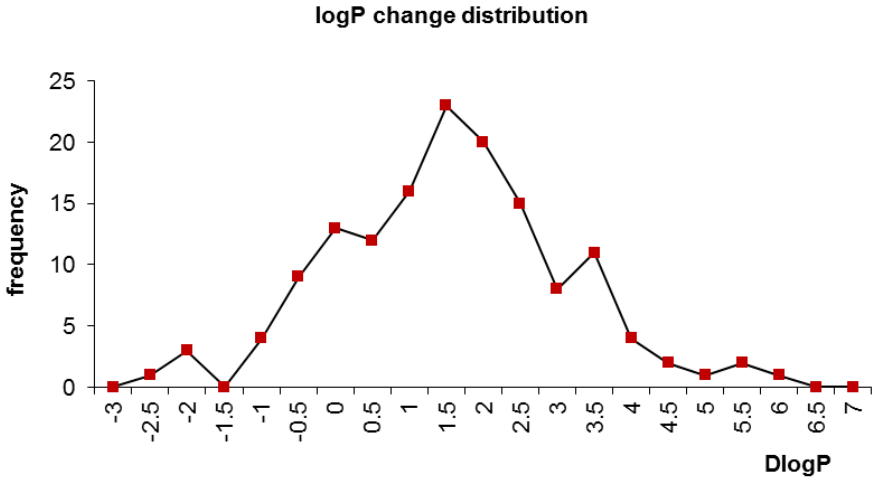
Size change


Fragments/Optimized fragments - Nheavy distributions
frequency
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
55
60
15
28
16
28
Nheavy OPT
Nheavy FRG
Nheavy


Nheavy change distribution
frequency
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
DNheavy

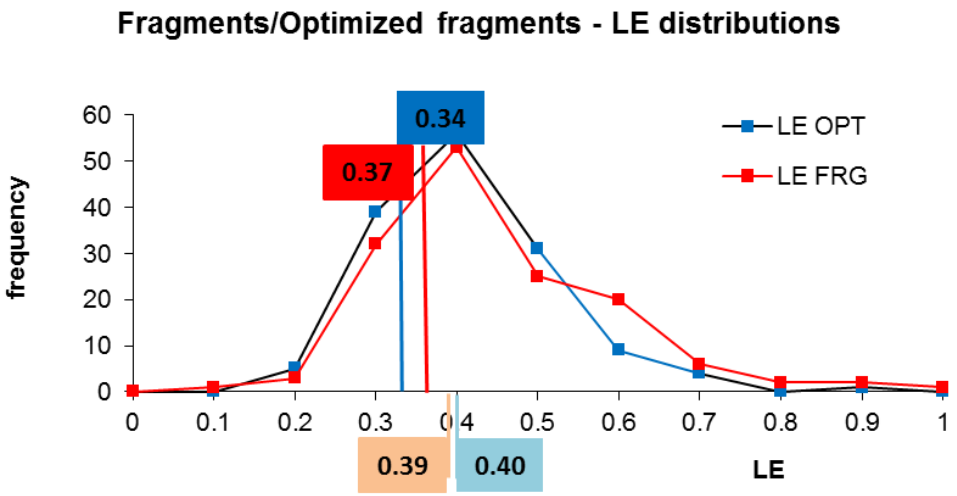
Lipophilicity change



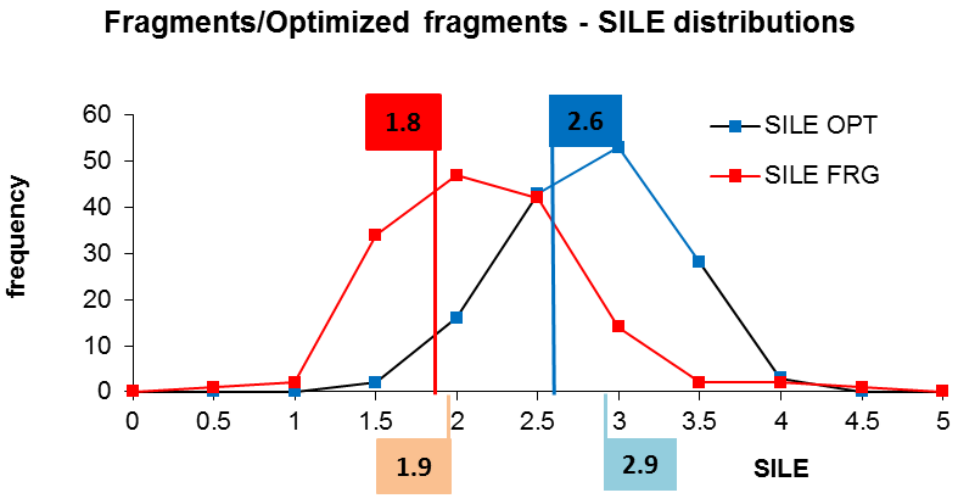


Ligand efficiency change

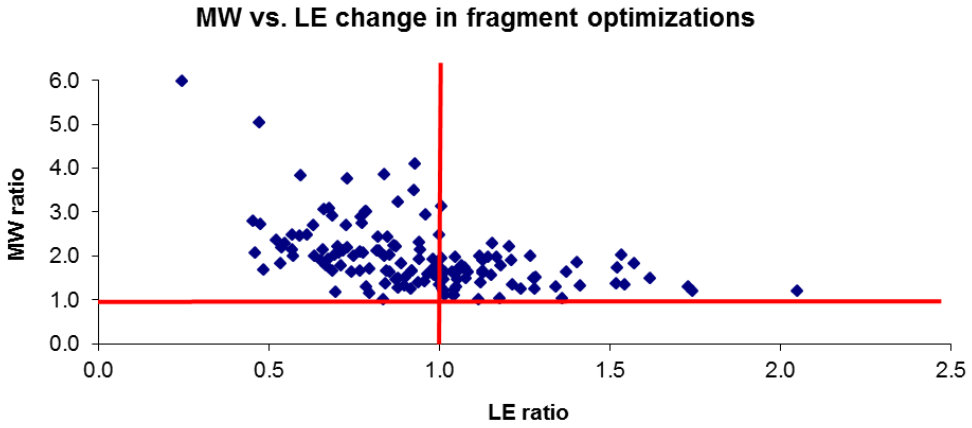
LE - size dependent

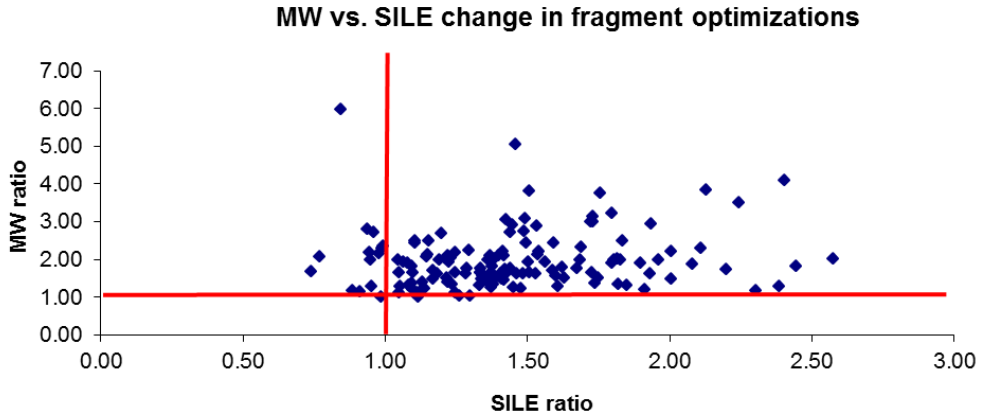


SILE - size independent



LE and SILE

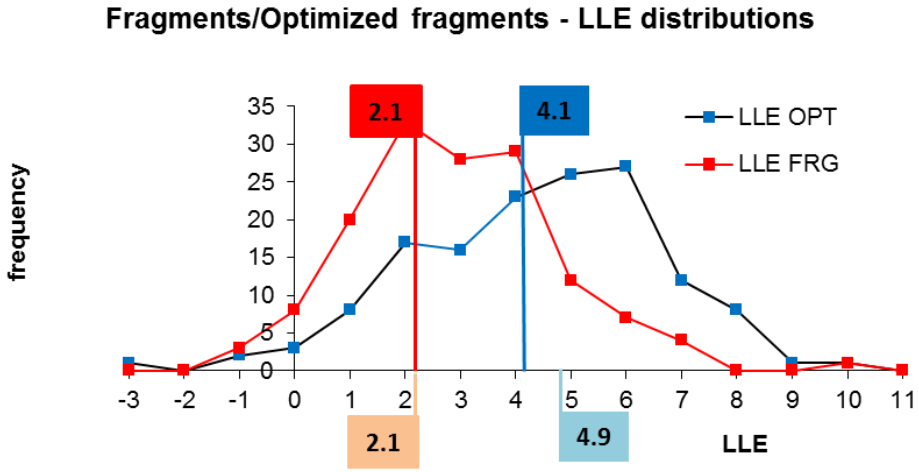




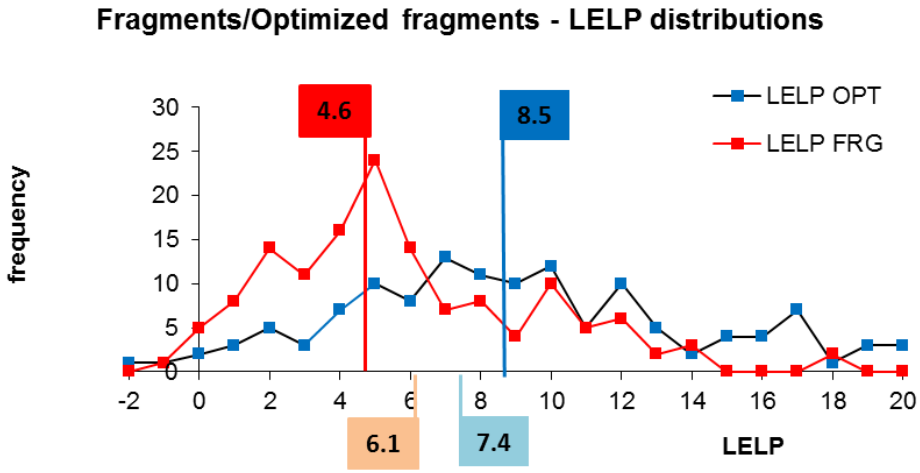
SILE (size-independent) is more informative

LLE and LELP

LLE = pKi - LogP
Ligand-lipophilicity efficiency



LELP = logP/LE
ligand-efficiency-dependent lipophilicity



Ligand efficiency metrics in fragment optimizations

Process	pPot change	MW change	logP change	LE change	SILE change	LLE change	LELP change
HTS based opt.	1.39	51.5	0.27	0.02	0.58	1.1	0.1
Fragment opt.	2.74	186.9	1.33	-0.04	0.70	1.4	4.8
Fragment opt. (successful)	3.10	165.5	0.48	-0.01	0.86	2.6	1.0
Lead opt. (successful)	2.08	89.9	0.05	0.01	0.85	2.1	-1.1

mean of property changes

Keserű, Makara NRDD 2009; Perola JMC 2010; Keserű, Ferenczy JMC 2013, Hopkins, Keserű, Leeson, Rees, Reynolds NRDD 2014, Ferenczy, Keserű unpublished 2014

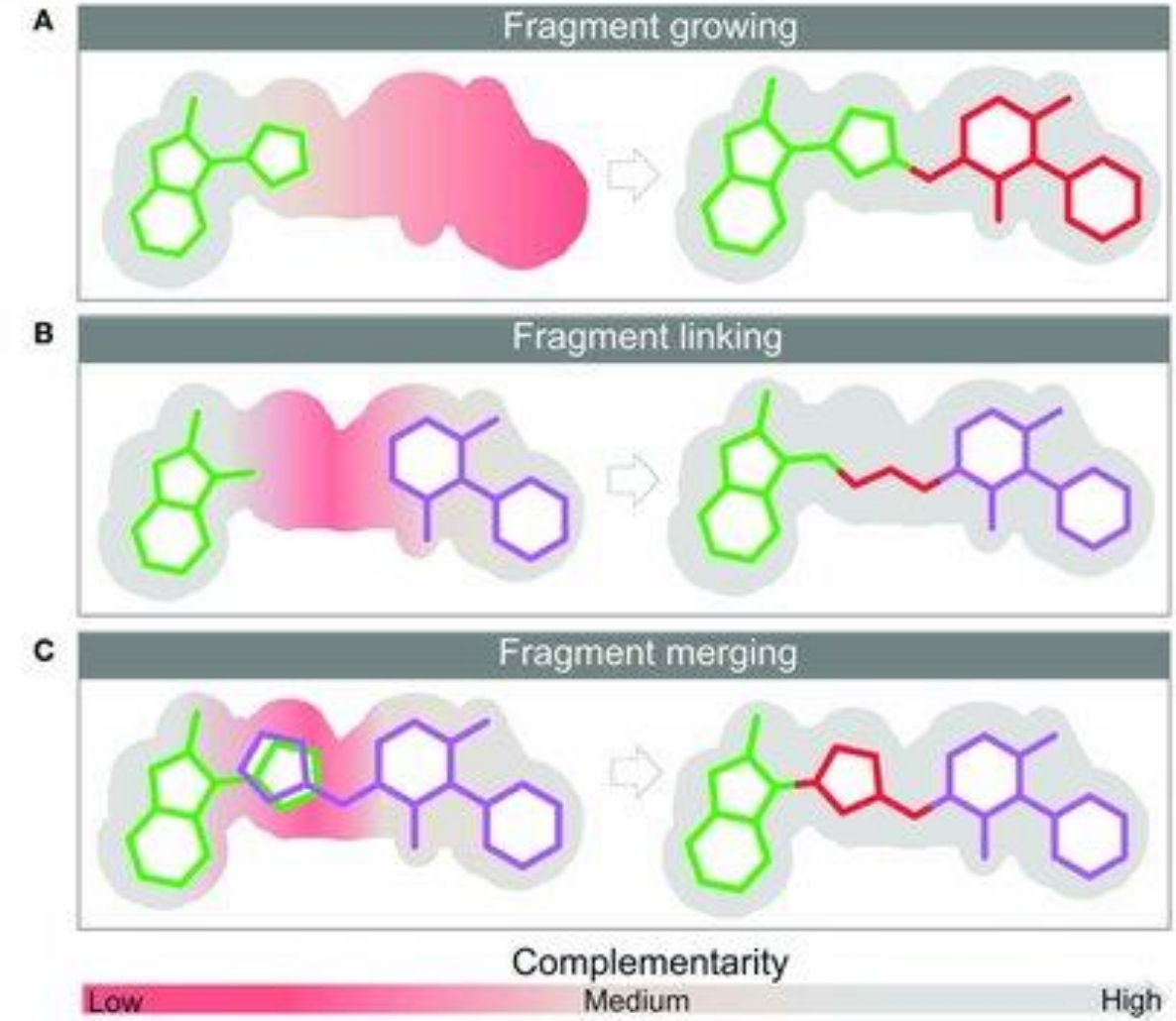
Effect of hit detection method

		Bio-NMR	Bio-Xra	NMR-Vir	NMR-Xra	Vir-Xra
pIC50	hit	4.75-3.53	4.74-3.56	3.53-4.73		4.73-3.56
	opt	7.68-6.70	7.68-7.13[a]			
MW	hit		223-190			235-190
	opt					
HA	hit		15.5-13.0		15.0-13.0	17.0-13.0
	opt					
LE	hit	0.40-0.31			0.31-0.38	
	opt	0.37-0.32				
LLE	hit	3.10-1.36	3.10-1.83	1.36-3.00		3.00-1.83[b]
	opt	4.55-3.17				
LELP	hit	4.05-5.65				
	opt	7.62-10.51				
SILE	hit	2.19-1.53	2.19-1.68	1.53-2.02		
	opt	2.91-2.45	2.91-2.57			

Biochemical
NMR
X-ray
Virtual screening

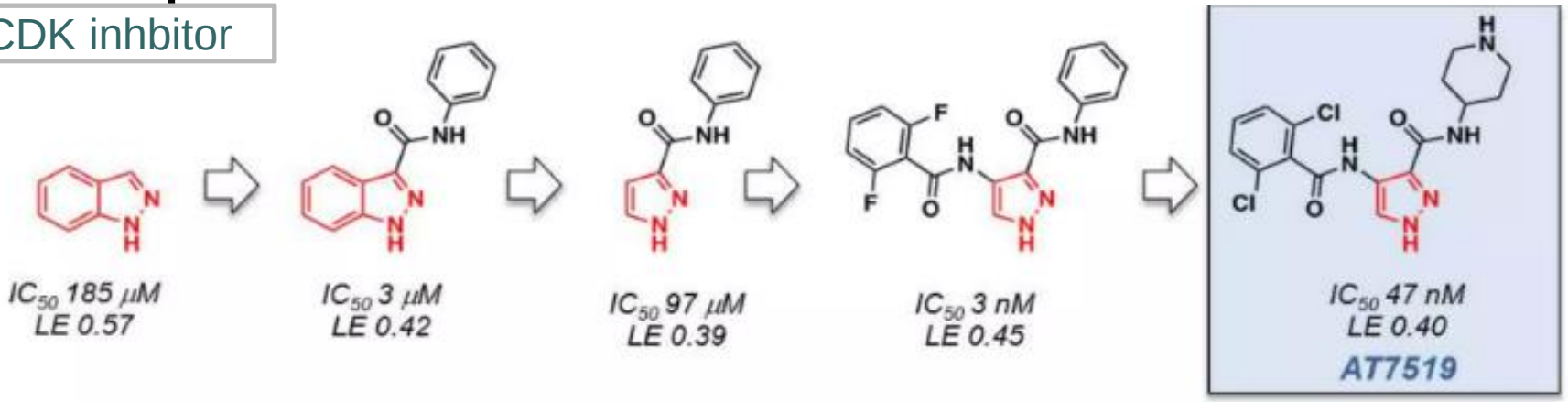
Significant ($p<0.05$) differences in selected metrics

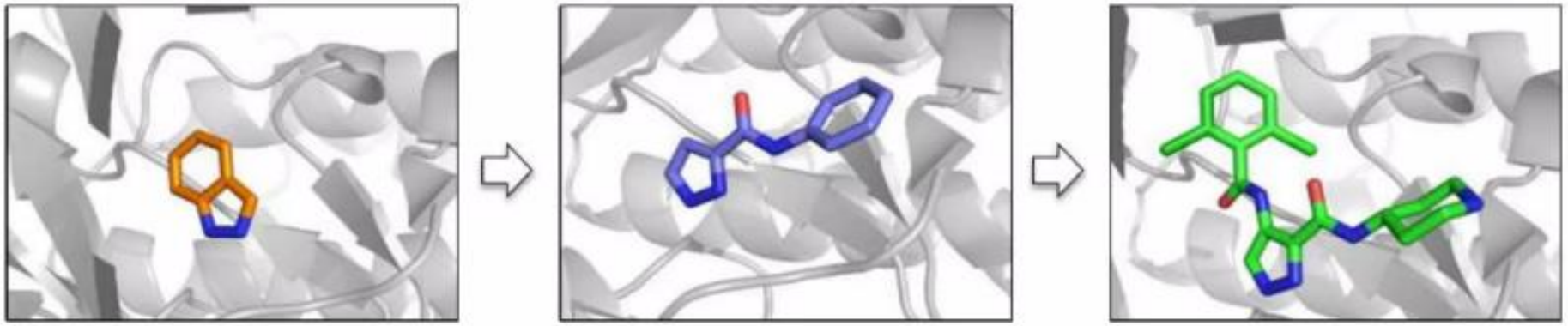
Fragment optimization strategies



Fragment growing

CDK inhbitor

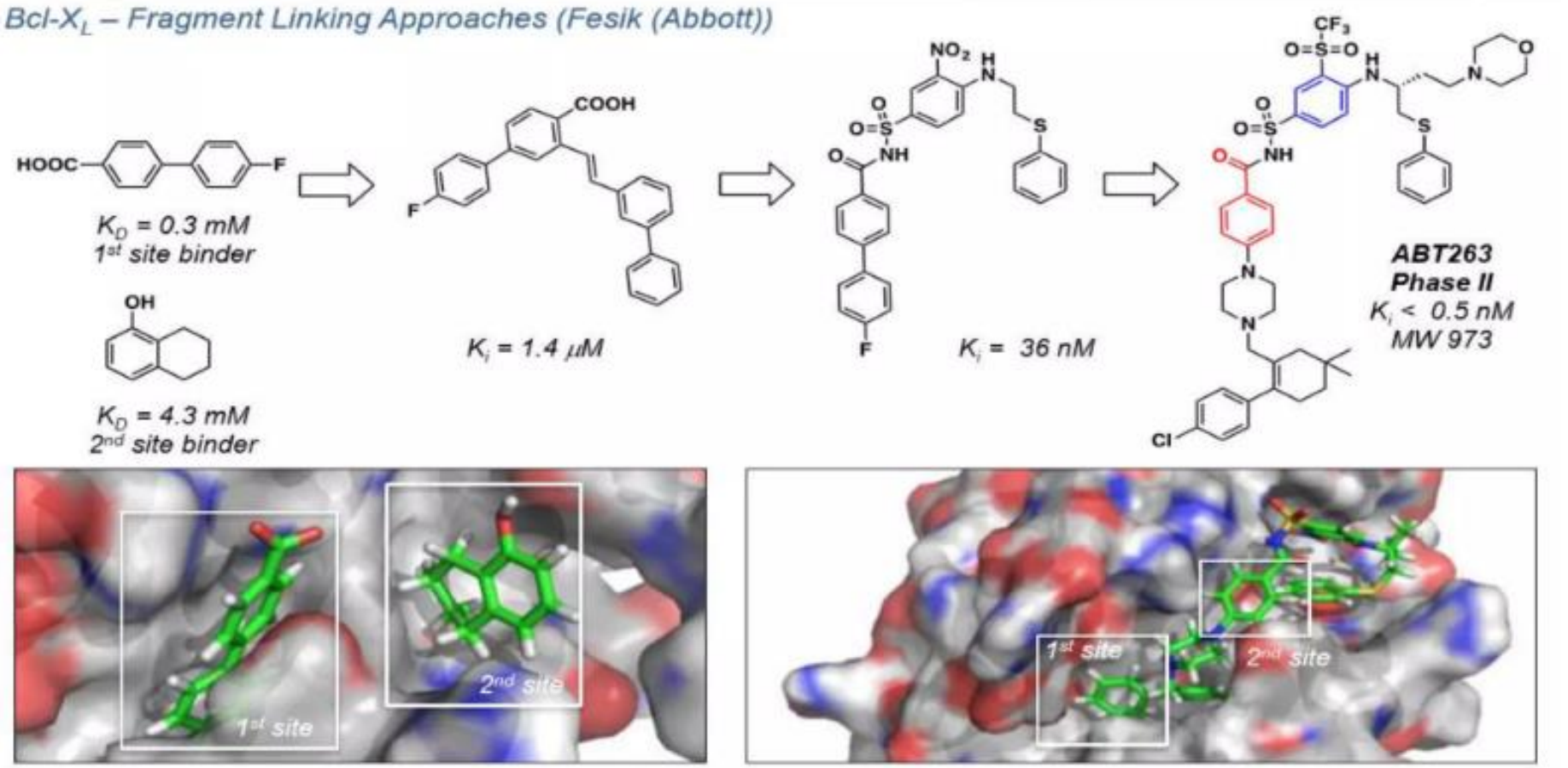




Fragment growing of the initial indazole hit led to a compound with a 50 fold increase in potency. Removal of the phenyl ring of the indazole offered a new startpoint and this was subsequently elaborated to a compound with a IC_{50} of 47 nM with only a small drop in LE (AT7519)

Interestingly the piperidine is protruding out of the pocket toward solvent and the two chlorine atoms in the 2 and 6 position of the phenyl ring fill small hydrophobic pockets on the protein

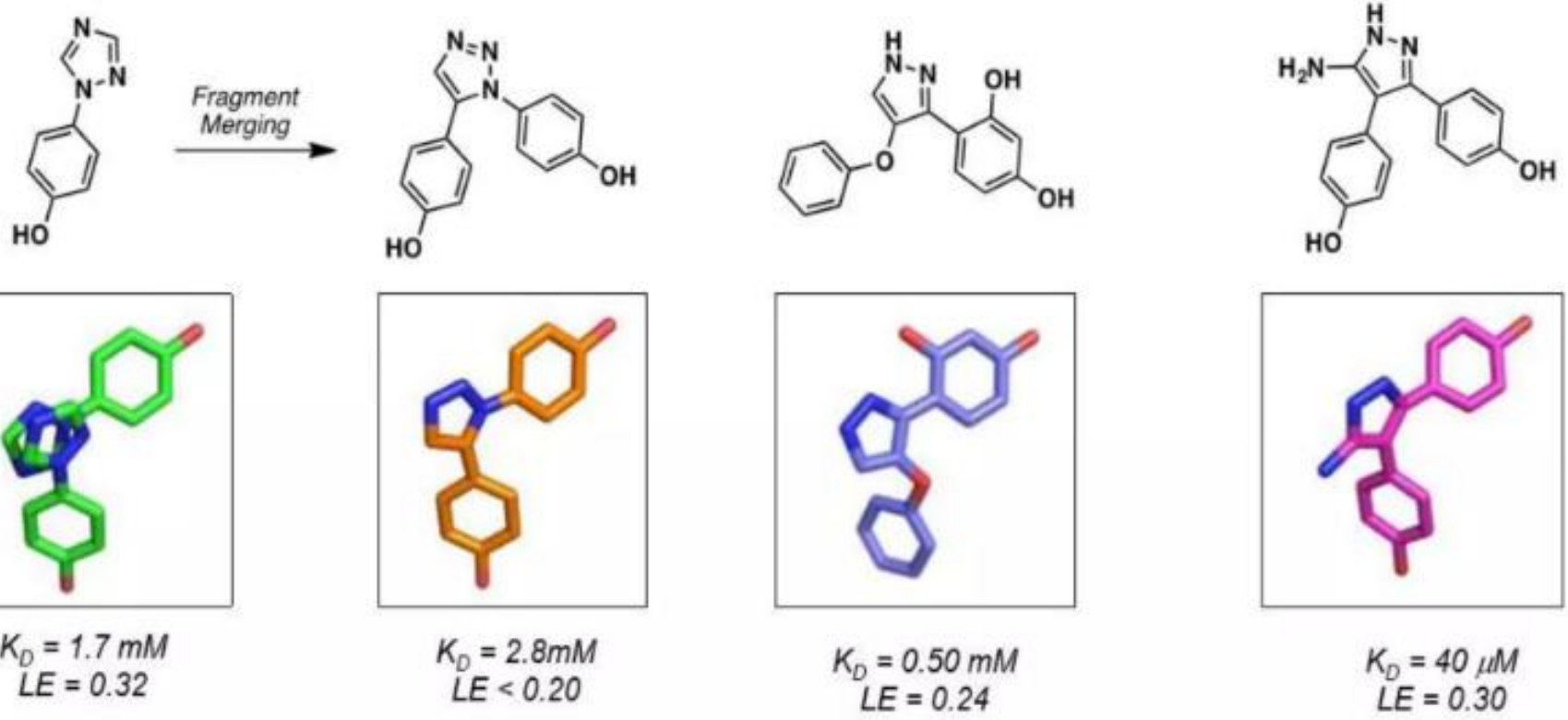
Fragment linking



One of the first successful examples of fragment linking against Bcl-XL where the initial fragment linking with an alkene gave a significant drop in potency. Second site binder discovered through 'SAR by NMR'

Subsequent elaboration led to the development of ABT273 which has a Ki <0.5 nM although the molecular weight of this compound is large (MW 973). Looking at this structure there are still some components of the initial fragment hits present.

Fragment merging



Merging of the two poses of the 1,2,4-triazole into a 1,5 disubstituted 1,2,3-triazole gave a compound which bound in a similar pose as the initial fragment hit however the potency was much poorer

Further elaboration of the triazole ring to a pyrazole and subsequently an aminopyrazole had a significant effect on the potency where this increased to 40 μM with a slight drop in ligand efficiency.

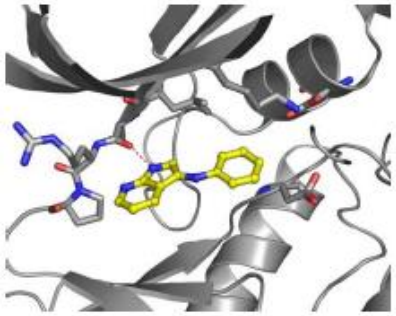
Case studies

- Classic FBDD
- FBDD without structural information
- Covalent FBDD
- Pharmacophore optimized FBDD
- Photoaffinity FBDD

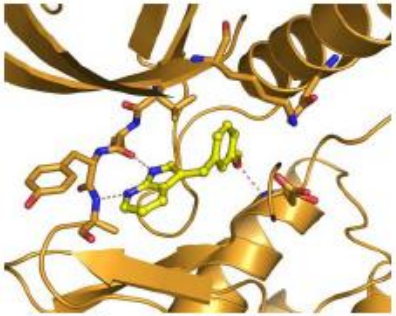
Plexxicon B-Raf inhibitor

- B-Raf^{V600E} is the most frequently observed oncogenic mutation
- Screen of 20,000 compounds (150 – 350 Da) at 200uM against Pim-1, p38, and CSK
- 238 compounds with >30% @ 200uM subjected to crystallography: >100 structures solved
- 7-Azaindole included among hits (IC50 > 200uM) but had different binding modes in the ATP site of the 4 asymetric units of Pim-1
- But its derivatives showed conserved binding mode across 3 different kinases!

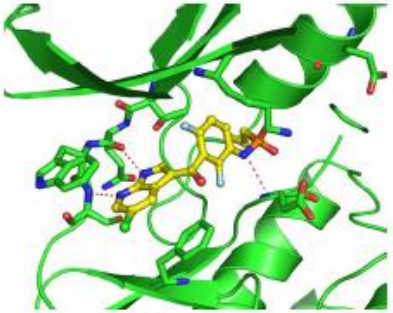
3-aminophenyl
a general motif based
on Pim-1 structure



3-benzyl
binds similarly
to FGFR1



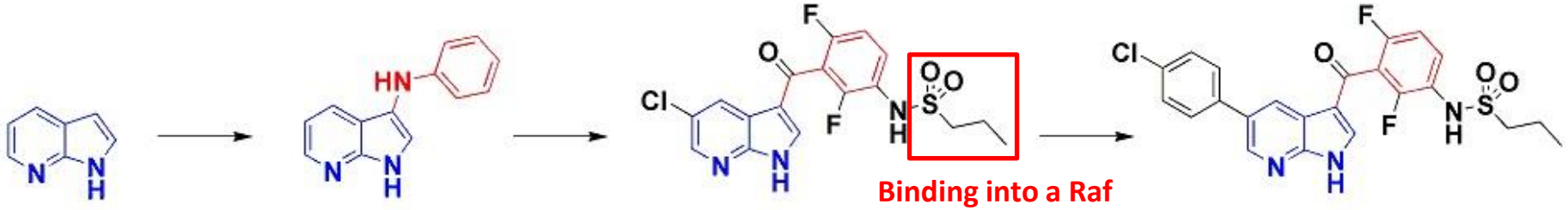
PLX4720
bound to
B-Raf



PNAS, **2008**, 105, 3041.

B-Raf Hit Progression

- Based on structures against 17 kinases the azaindole 3,4 and 5 positions were targeted for chemical derivatizations
- Series & selectivity progression:



7-Azaindole
Pim-1 IC_{50} > 200 μM
Pim-1 LE < 0.56

9 heavy atoms

Multiple crystallographic binding modes

Compound 1
Pim-1 IC_{50} ~ 100 μM
Pim-1 LE ~ 0.34

16 heavy atoms

One binding mode

PLX4720
B-Raf V600E IC_{50} = 0.013 μM
B-Raf V600E LE = 0.40

27 heavy atoms

Pim-1 IC_{50} > 5 μM (also 54 other kinases)
Pim-1 LE < 0.27

Zelboraf (vemurafenib)
B-Raf V600E IC_{50} = 0.031 μM
B-Raf V600E LE = 0.31

33 heavy atoms

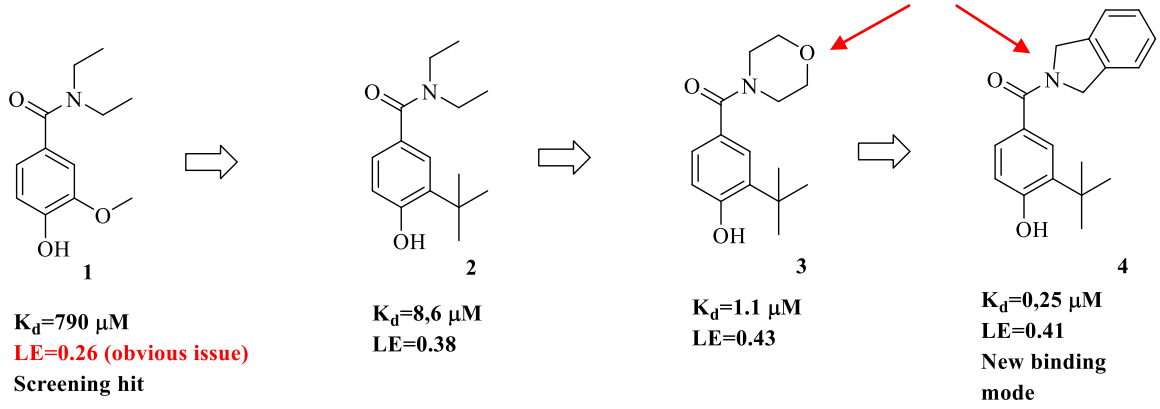
Selectivity of the initial fragment hits is not important!

PNAS, **2008**, 105, 3041.

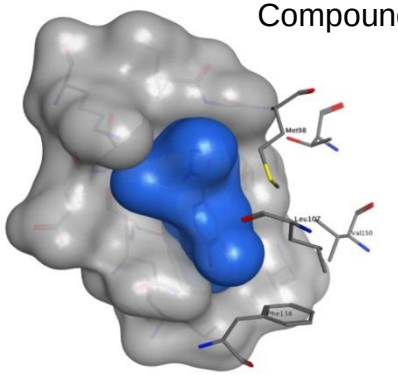
Astex HSP90 inhibitor

- 1,600 fragments were screened with a hit contained motifs similar to those of Radicicol (a natural product)

Design for interaction with Lys58
or displacement of Lys58

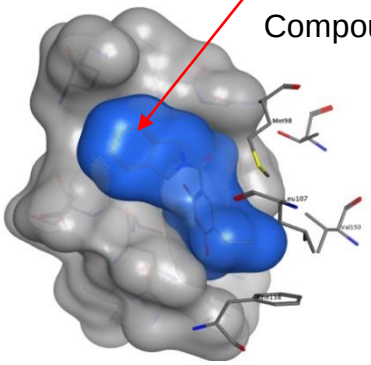


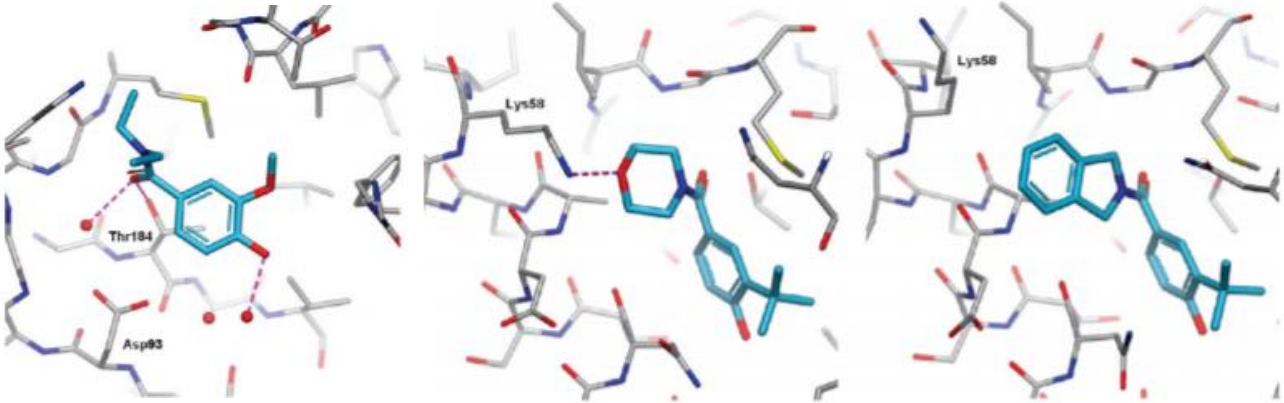
Compound 3



New pocket formed
with Lys58 moving

Compound 4

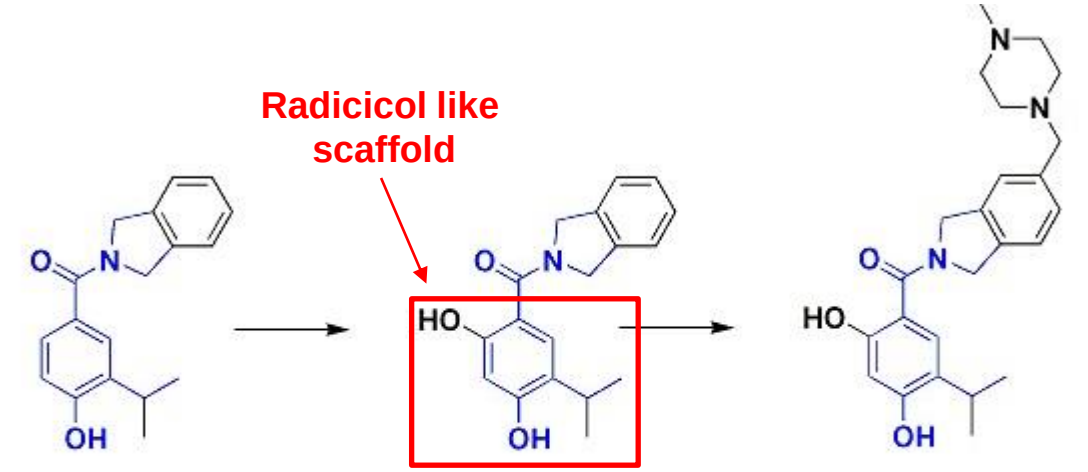




J. Med. Chem. **2010**, 53, 5942
J. Med. Chem. **2010**, *53*, 5956.

Astex HSP90 inhibitor

Radicicol like scaffold



4

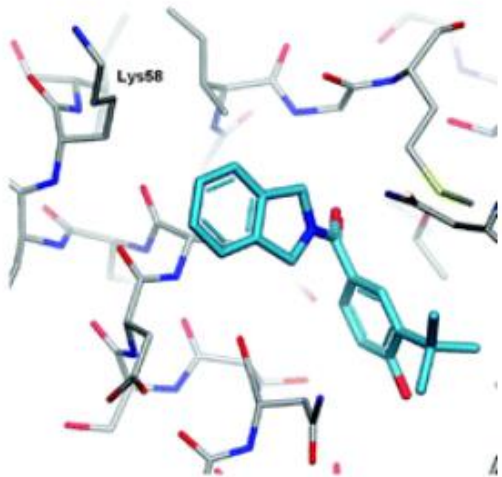
K_d = 0.068 μM
LE = 0.47
IC_{50} (cell)= 17 μM

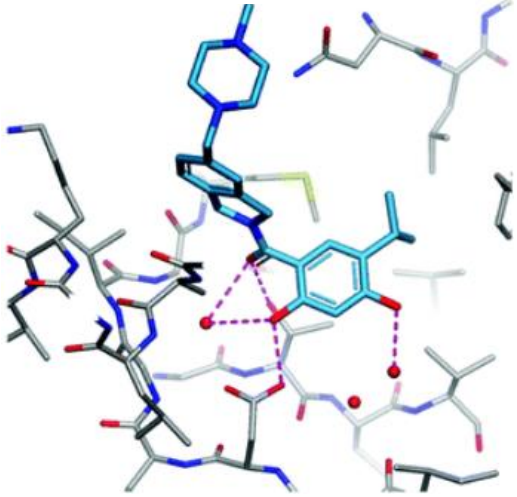
5

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

AT13387

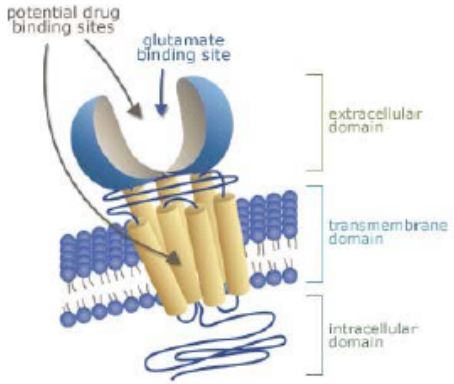
K_d = 0.00071 μM
LE = 0.42
IC_{50} (cell)= 0.048 μM



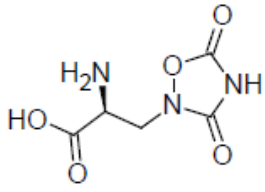


Heptares mGluR5 NAM

- Clinical and pre-clinical evidence for $mGlu_5$ negative allosteric modulators (NAMs) in a range of diseases, including:
  - Anxiety/depression (e.g. basimglurant; Roche/Chugai)
  - Fragile X syndrome (e.g. mavoglurant; Novartis)
  - Episodic migraine (e.g. raseglurant; Addex)
  - Levodopa-induced dyskinesias (e.g. dipraglurant; Addex)



- Glutamate (orthosteric) binding site is difficult to drug
  - Orthosteric ligands are typically glutamate mimics
  - Poor PK properties, often incompatible with CNS exposure
  - Difficult to achieve selectivity (e.g. quisqualate, $mGlu_{1\&5}$, also AMPA)
  - Allosteric site in the transmembrane bundle is more tractable

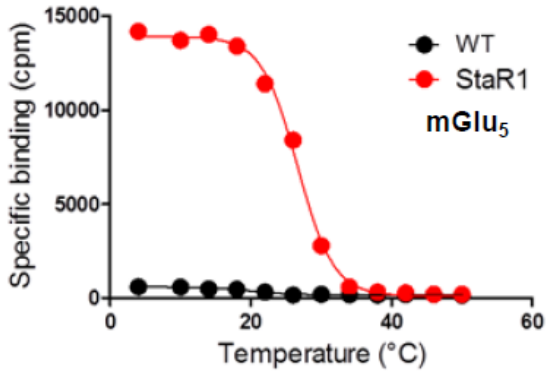


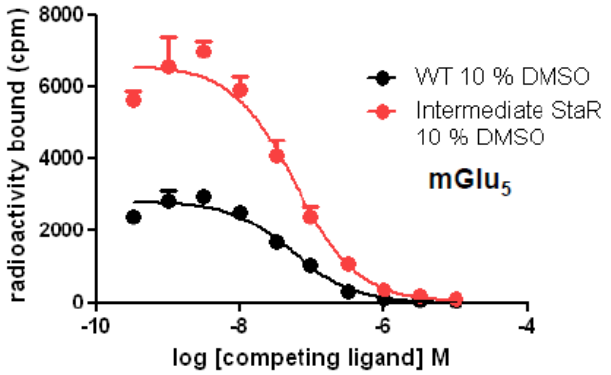
quisqualate

- At the time of starting the project $mGlu_5$ drug discovery was hampered by a lack of structural information needed to enable structure-based drug design
  - Crystal structure of *N*-terminal region (orthosteric site) published but not of the 7 transmembrane bundle (allosteric binding site)

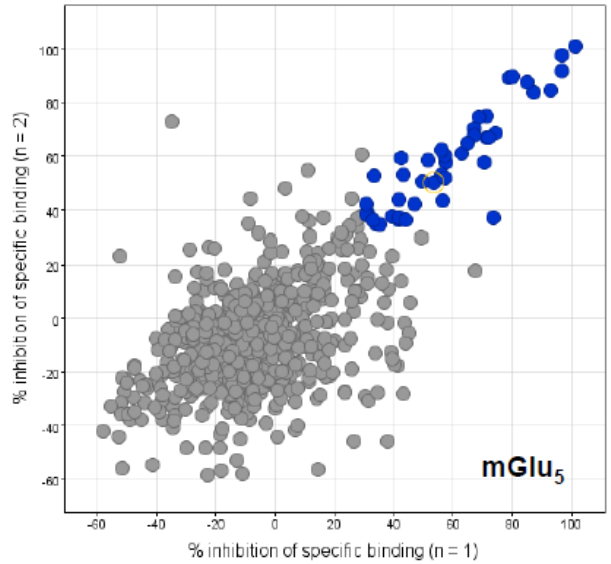
Fragment screening

High Concentration Fragment Screening: $mGlu_5$ and $mGlu_2$

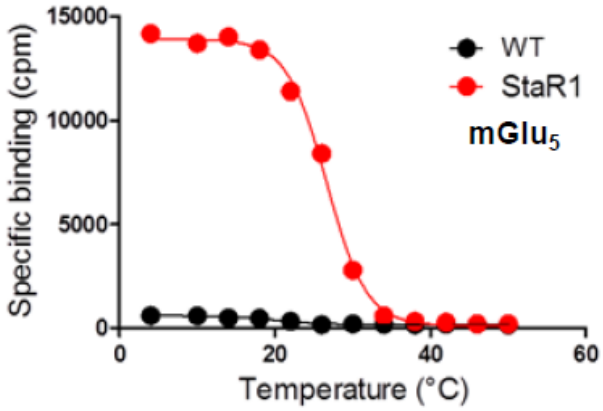


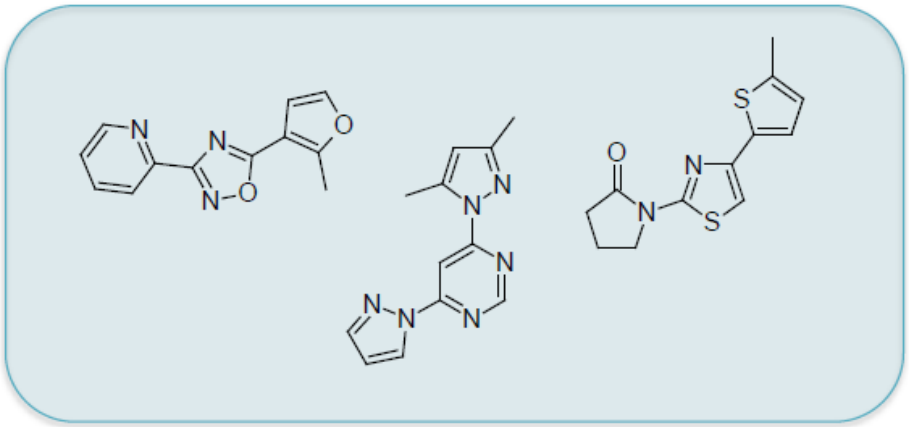


- First example of stabilisation of a Class C receptor
- $mGlu_5$ stabilisation carried out with a negative allosteric modulator (NAM) with binding site in the transmembrane region of the receptor
- Very dramatic increase in expression with the StaR
- StaR has significantly higher DMSO tolerance
- Bespoke Class C fragment set and the general Heptares fragment library yielded tractable hits for $mGlu_5$ and $mGlu_2$ (6-8% hit rate, >30% cut off)



Fragment screening





- First example of stabilisation of a Class C receptor
- $mGlu_5$ stabilisation carried out with a negative allosteric modulator (NAM) with binding site in the transmembrane region of the receptor
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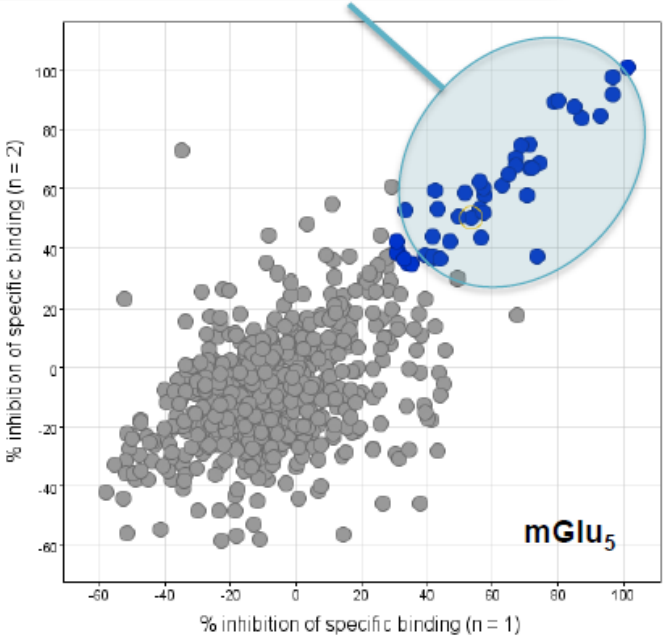
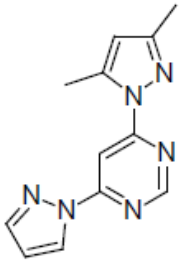
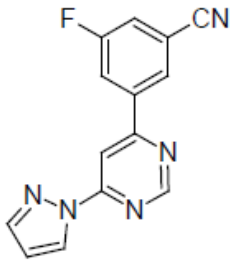


Fragment hit progression

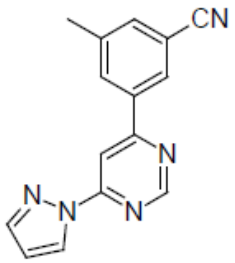
Pyrazole series



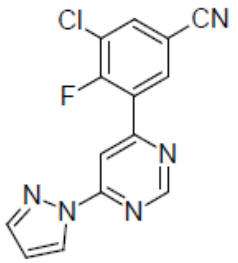
HTL-G1
$mGlu_5$ pK_i 5.2
LE 0.40



HTL-G2
$mGlu_5$ pK_i 7.2
LE 0.49

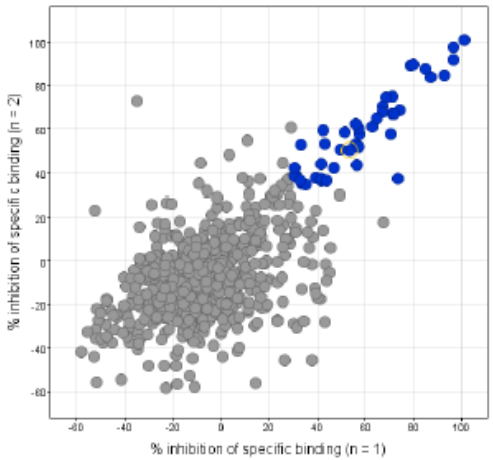


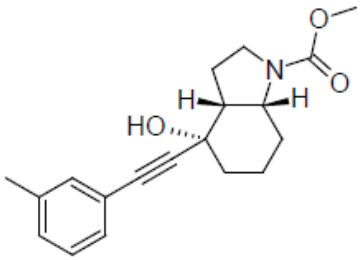
HTL-G3
$mGlu_5$ pK_i 8.4
LE 0.57



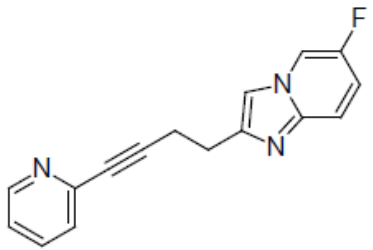
HTL-G4
$mGlu_5$ pK_i 9.3
LE 0.60

High Concentration Fragment Screening





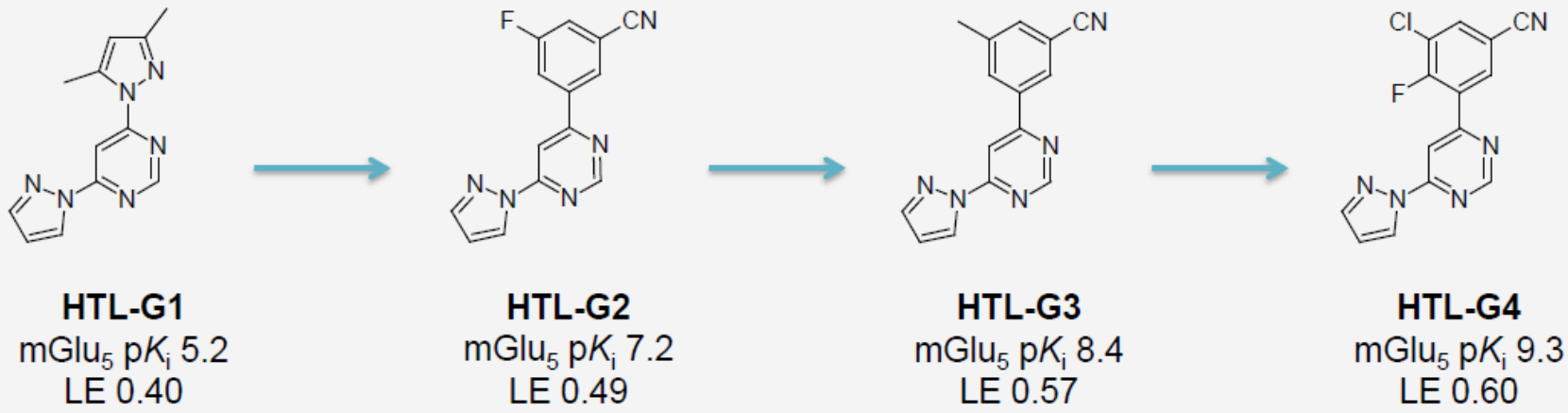
mavoglurant
$mGlu_5$ pK_i 8.0
LE 0.47



dipraglurant
$mGlu_5$ pK_i 6.9
LE 0.47

Fragment hit progression

Pyrazole series

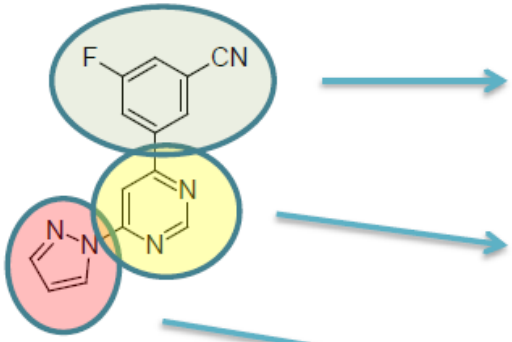


HTL-G1
$mGlu_5$ pK_i 5.2
LE 0.40

HTL-G2
$mGlu_5$ pK_i 7.2
LE 0.49

HTL-G3
$mGlu_5$ pK_i 8.4
LE 0.57

HTL-G4
$mGlu_5$ pK_i 9.3
LE 0.60

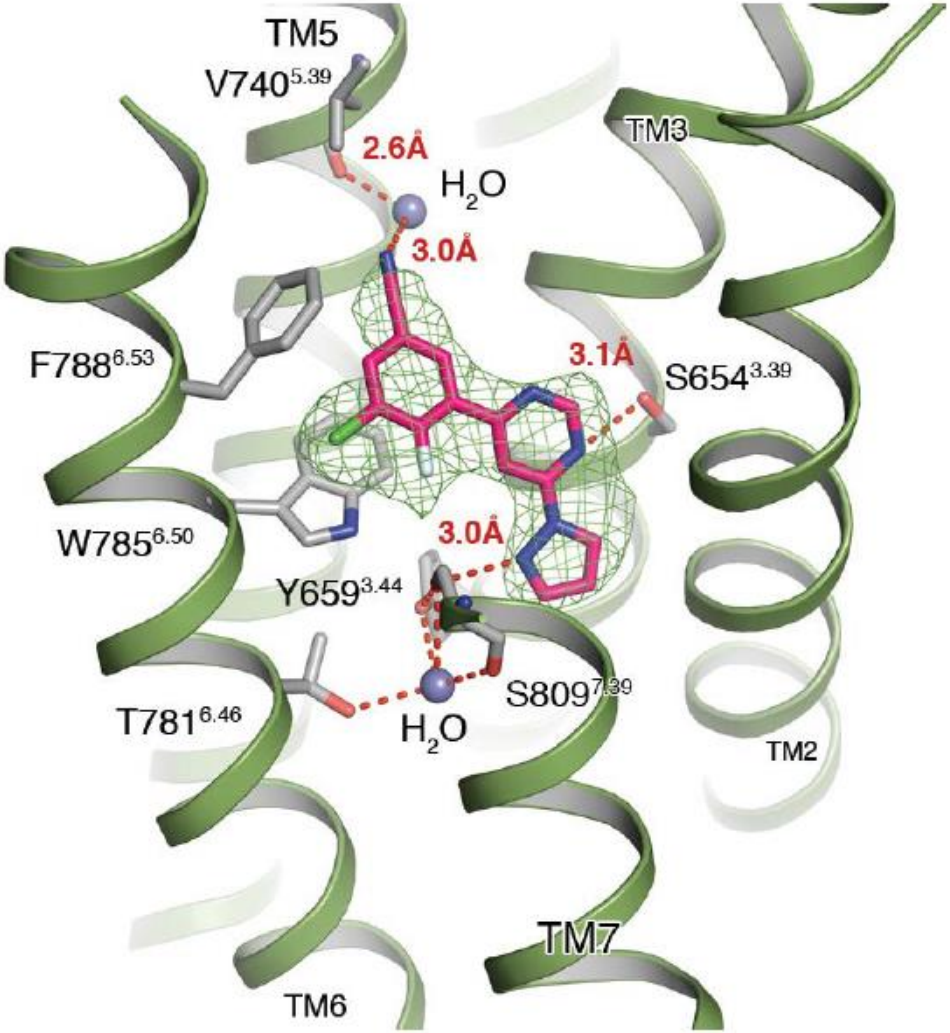


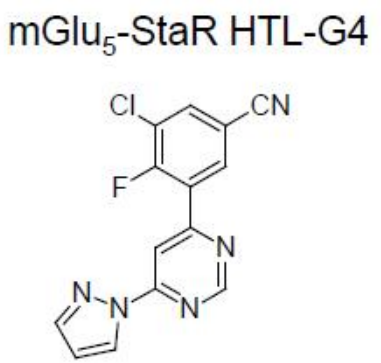
- 3-CN crucial for high affinity (100-fold loss if removed)
- Affinity improved by fine tuning electronics
- No scope to replace top ring by non-aromatics

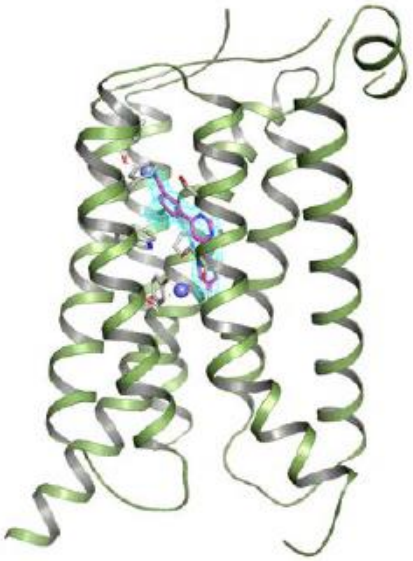
- Removal of either pyrimidine N tolerated (within ~5-fold)
- Substitution poorly tolerated

- Little scope to modify heterocycle; 2-position N required
- Substitution poorly tolerated

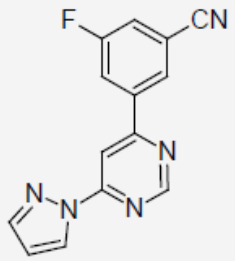
mGluR5 receptor structures



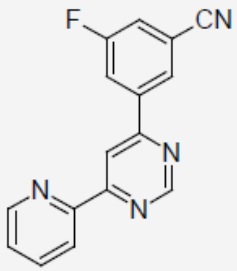




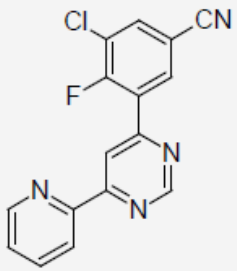
Fragment hit progression



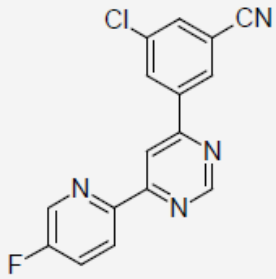
HTL-G2
$mGlu_5$ pK_i 7.2
LE 0.49



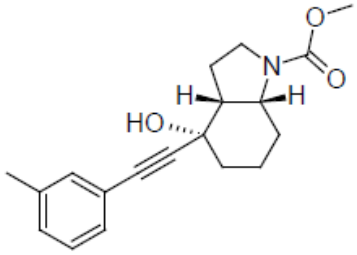
HTL-G5
$mGlu_5$ pK_i 7.1
LE 0.46



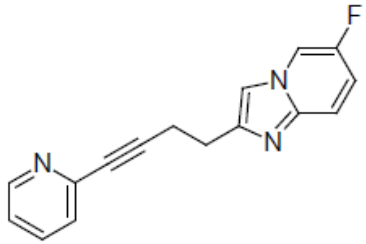
HTL-G6
$mGlu_5$ pK_i 8.9
LE 0.55



HTL14242
$mGlu_5$ pK_i 9.3
LE 0.57

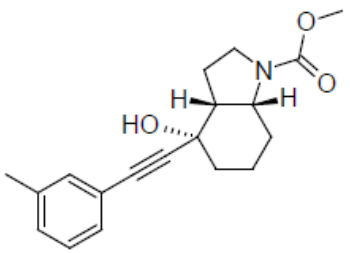


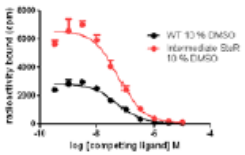
mavoglurant
$mGlu_5$ pK_i 8.0
LE 0.47



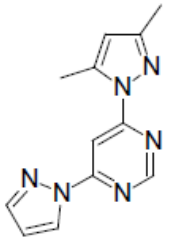
dipraglurant
$mGlu_5$ pK_i 6.9
LE 0.47

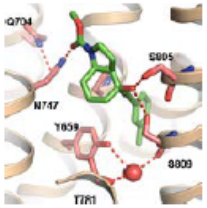
Candidate optimization



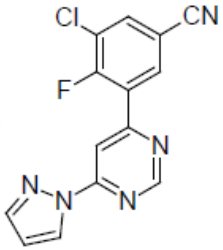


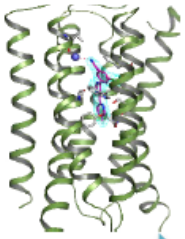
Fragment Screen



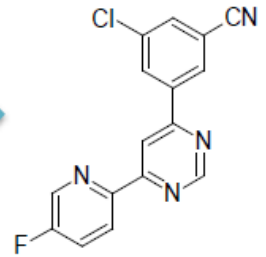


Advanced homology modelling





X-ray driven SBDD



mavoglurant
$mGlu_5$ pK_i 8.0
clogP 3.1
LE 0.47, LLE 4.9
CNS MPO 5.2

Acetylene containing
Poor PK (rat F 22%)

$mGlu_5$ pK_i 5.2
clogP 1.1
LE 0.40, LLE 4.1

Novel non-acetylene containing chemotype
Sub optimal potency & LLE

$mGlu_5$ pK_i 9.3
clogP 2.6
LE 0.60, LLE 6.7

Significant LLE & LE enhancements
Sub optimal metabolic stability

HTL14242
$mGlu_5$ pK_i 9.3
clogP 3.0
LE 0.57, LLE 6.3
CNS MPO 5.5

Good PK
(F%>80% - 2 species)
High RO
(ED_{50} 0.3 mg/Kg)
Clean off-target profile

- X-ray structures enabled SBDD for mGlu receptors and rationalise the historic challenges of allosteric modulator drug discovery
  - Enabled design of high efficiency ligands with no structural alerts

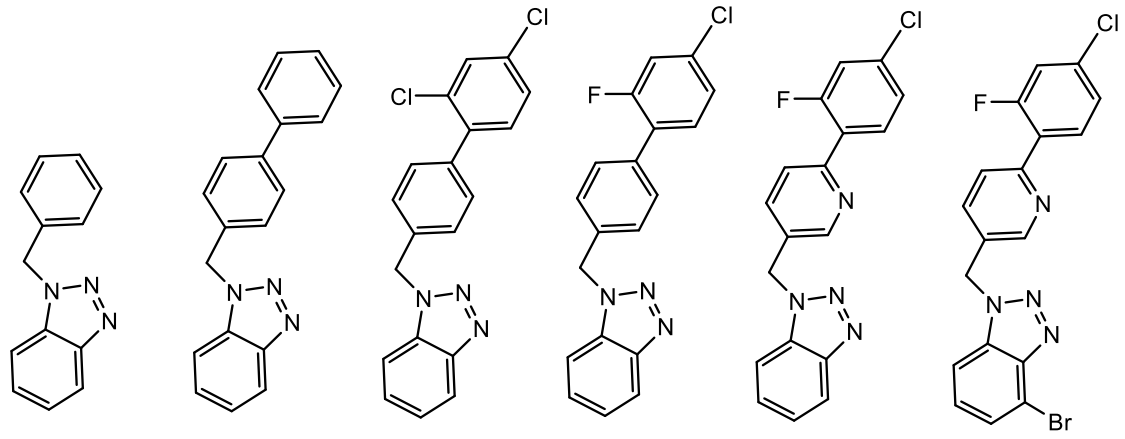
Christopher *et al. J. Med. Chem.* **2015**, *58*, 6653

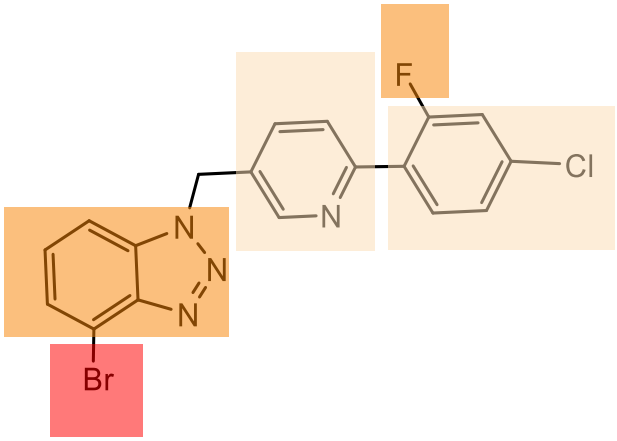
FBDD without structure
Richter mGlu2 PAM

- FBDD was mainly used for soluble proteins
- Structural studies are more challenging on membrane proteins
- Statistical view
  - Unless SAR exists all positions have equal chance for growing or need for modifications
  - Synthetic ease is irrelevant but we can use SAR by catalogue where feasible
- A ligand efficiency based strategy
  - LE = 0.3 – 0.4: fine-tune structure
  - LE > 0.4: grow (synthetic ease is relevant)
  - Keep or increase LE during growing by continuous fine-tuning
  - Follow lipophilic efficiency metrics as LLEat or LELP

Group efficiency analysis

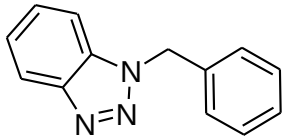
- Assumes the molecules being compared bind in a similar fashion
- Different portions of a molecule are likely to have vastly different group efficiencies, pick up the lowest and modify



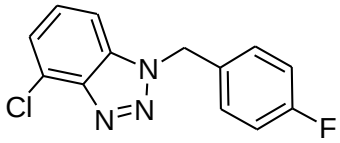


ΔΔG	-10,3	-1,8	-0,3	-0,6	0,3	-1,2
GE	0,6	0,3	0,3	0,6	0	1,2

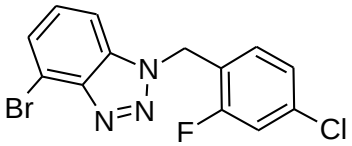
Fragment optimization



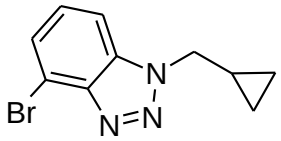
clogP: 3.15
LE/LLEat: 0.47 / 0.31
hmGluR2 PAM EC_{50}: 3 µM
Stability (h/r/m): 13/60/63 %



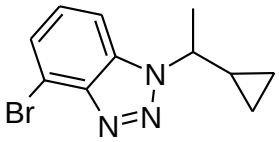
clogP: 3.61
LE/LLEat: 0.53/0.37
hmGluR2 PAM EC_{50}: 0.106 µM
Stability (h/r/m): 67/59/44%



clogP: 4.73
LE/LLEat: 0.56/0.35
hmGluR2 PAM EC_{50}: 0.023 µM
Stability (h/r/m): 72/32/56 %



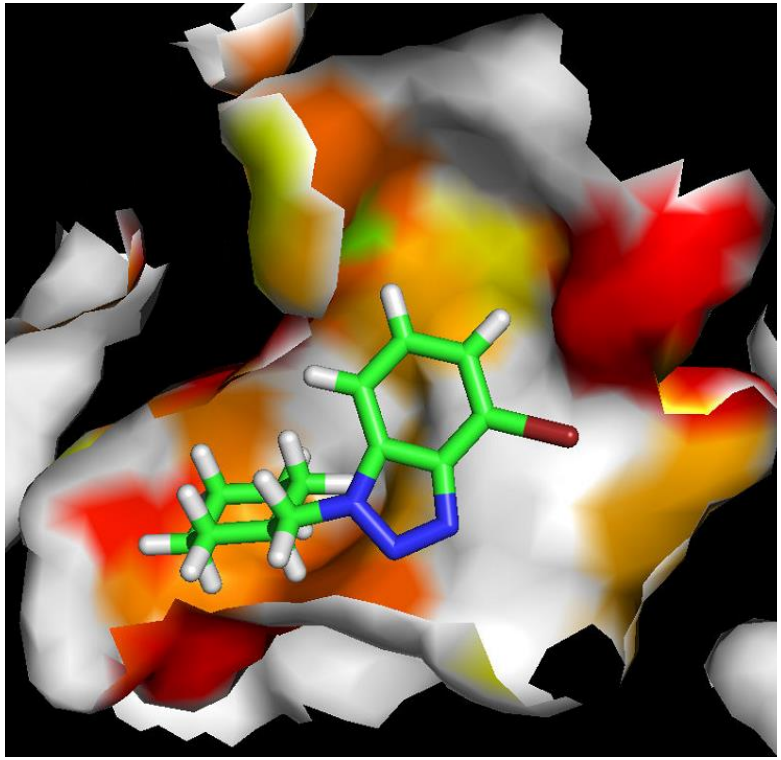
clogP: 2.68
LE/LLEat: 0.70/0.55
hmGluR2 PAM EC_{50}: 0.066 µM
Stability (h/r/m): 47/74/18 %

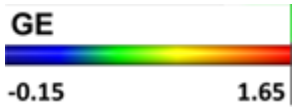


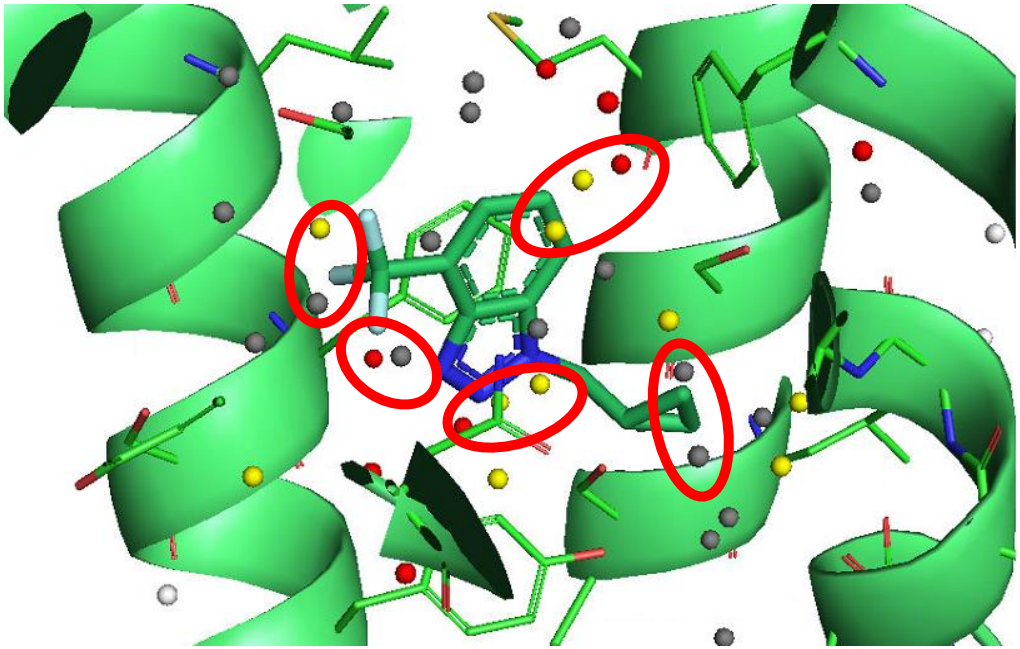
clogP: 3.34
LE/LLEat: 0.60/0.44
hmGluR2 PAM EC_{50}: 0.038 µM
Stability (h/r/m): 55/75/36 %

clogP: 3.50
LE/LLEat: 0.54/0.38
hmGluR2 PAM EC_{50}: 0.083 µM
Stability (h/r/m): 86/94/75 %

Hot spot analysis

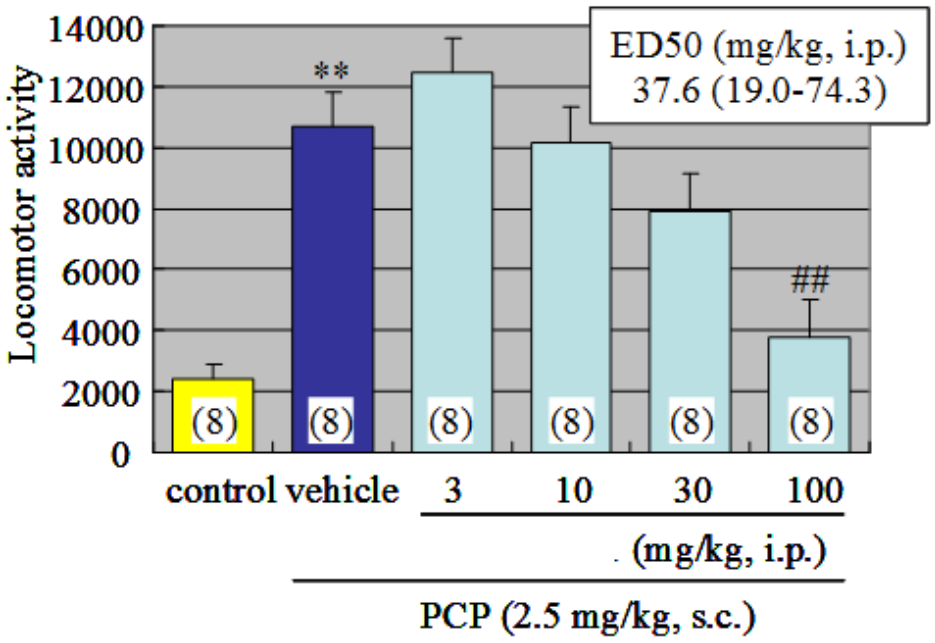






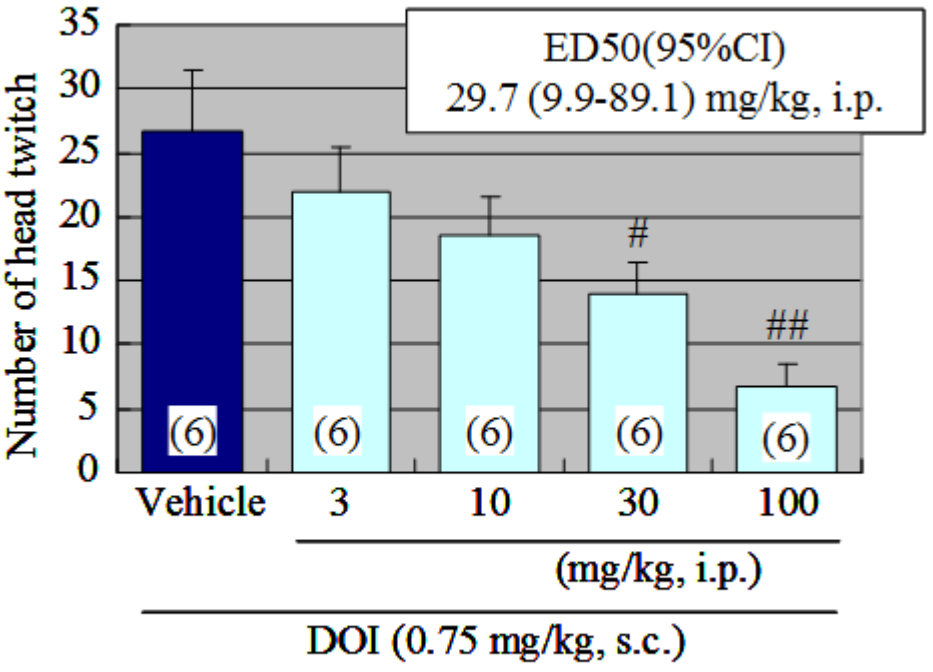
In vivo proof of concept

PCP-induced hyperlocomotion in mice



**p<0.01 vs control (t-test)
##p<0.01 vs vehicle (Dunnett's test)

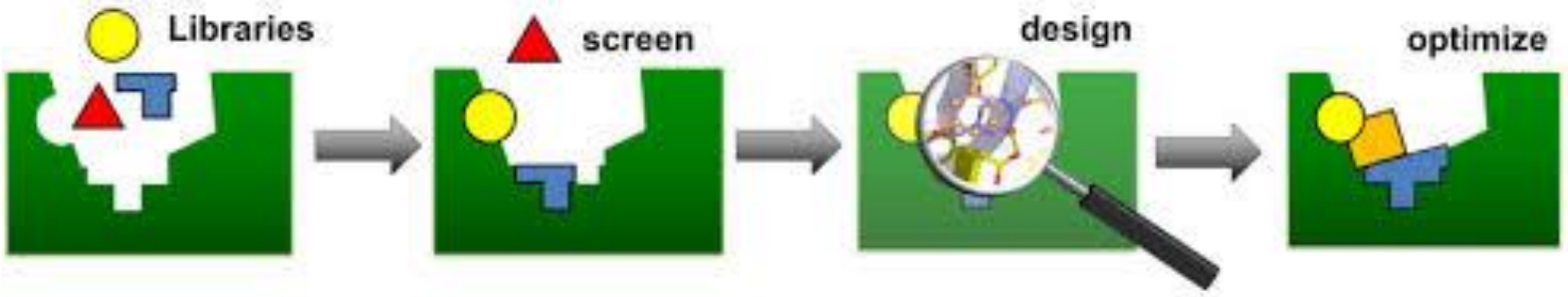
DOI-induced head twitch in mice



#p<0.05, ##p<0.01 vs vehicle (Dunnett's test)

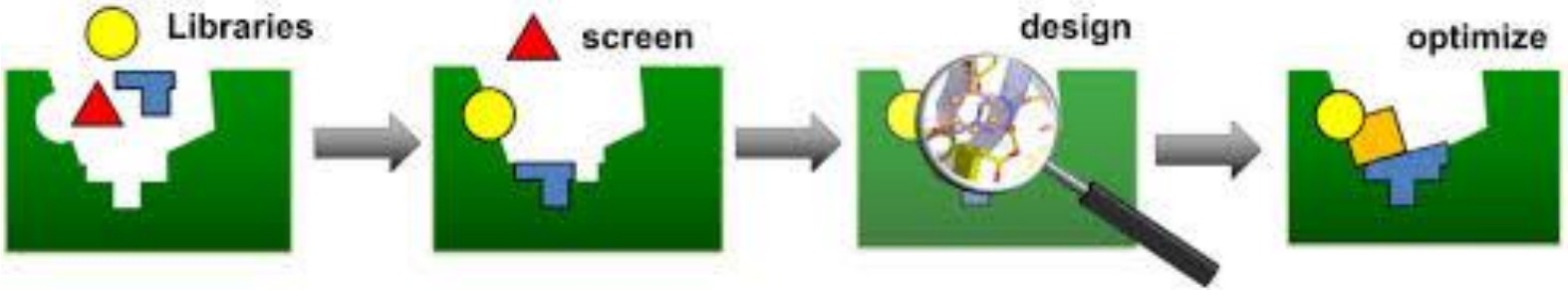
Conclusions

- Effective design of fragment libraries requires a multi-level evaluation protocol
  - Compound properties
  - Coverage of the chemical space
  - Medicinal chemistry aspects
  - Experimental evaluation



Conclusions

- Fragment screening requires biophysical approaches
- Orthogonal assays and robust validation protocols are needed
- Fragment optimizations are best performed with structural information
- Ligand efficiency metrics support the effective optimization of fragments



Evolution of FBDD