Fragment Based Druggability (Ligandability) Screening to Predict Lead Discovery Success

FBLD case story – Non-covalent inhibitors of rhinovirus 3C protease: Pursuing the needle in the haystack

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Fragment Based Lead Discovery at AstraZeneca

- Fragment based approaches alive and well within AstraZeneca!
  - Structure driven FBLD
  - Often run in parallel with HTS
  - Fragment assisted approaches gaining in importance – structure support not required

- Either Biophysical or Biochemical fragment screening – or both
- Large fragment library (~20k compounds)
- Large corporate collection – mine for rapid follow-up

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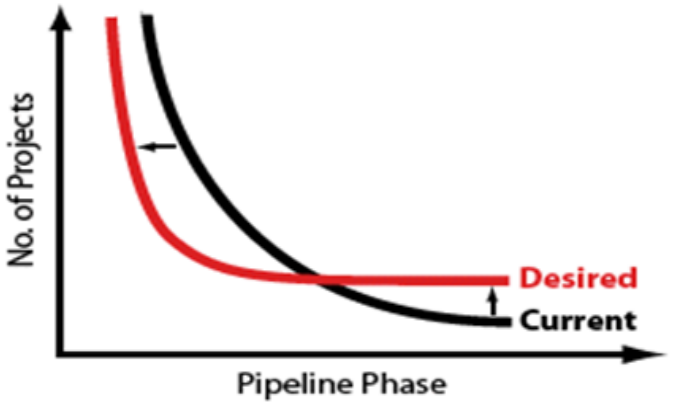
Increasingly more difficult playing field for Pharma industry

- Only ~1% of drug discovery projects make it to market industry-wide
- Lead discovery success rates only at ~50%
- Late stage failure more costly
- High volume - high attrition models not successful
- Modest HTS success rates
- Few new targets successfully addressed with small molecule drugs each year
- Increased regulatory pressures
- Novel mechanisms sought



- What to do?

More informed target selection – pick winners early on

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Target druggability - ligandability

- "Druggability" usually refers to the likelihood of finding orally bioavailable small molecules that bind to a particular target in a disease-modifying way
- Useful to distinguish ability of a target to bind small molecules from more complex pharmacokinetic and -dynamic mechanisms
- Term "ligandability" refers just to ability of a target to bind small molecules

- Accurate predictions of ligandability has potential to greatly influence both selection of targets & lead discovery strategy

- A number of computational methods described
  - Ligand based – requires prior ligand knowledge
  - Structure based – requires 3D protein structure

Fragment based druggability screening

- Experimental method introduced by Abbott Laboratories in 2005
- Fragments sample chemical space more efficiently than large drug-like molecules
- NMR fragment screening hit rate correlates with presence of high affinity ligand
- Fragment screens appear to be predictive of small molecule druggability/ligandability

Table 1. Targets, Binding Sites, and Hit Rate Data Derived from Heteronuclear-NMR-Based Screening against 23 Protein Targets

protein no.	pocket no.	target[a]	binding site[b]	no. tested[c]	no. hits[d]	no. series[e]	hit rate	K_D range (μM)[f]	high-affinity ligand?[g]	log(HR) expt[i]	log(HR) pred[j]
1	1	AK	adenosine	4600	10	9	0.22	80−5000	yes	−0.66	−0.42
2	2	Akt-PH	IP3	8090	1	1	0.01	–		−1.91	−1.98
3	3	Bcl-xL	Bak	9373	73	59	0.78	10−5000	yes	−0.11	−0.64
4	4	bir3	peptide	8640	8	8	0.09	600−260 0		−1.03	−0.72
5	5	CMPK	CMP	8090	6	3	0.07	30−240		−1.13	−0.81
5	6	CMPK	other[h]	8090	4	3	0.05	30−440		−1.31	−1.27
6	7	E2-31	DNA	1532	3	3	0.20	1000−4200		−0.71	−0.72
6	8	E2-31	other[h]	1532	3	3	0.20	30−2300		−0.71	−0.42
7	9	ErmAM	SAH	7233	7	7	0.10	50−3800		−1.01	−0.87
8	10	FBP	DNA	8090	2	2	0.02	200−1700		−1.61	−1.04
9	11	FKBP	FK506	6950	65	60	0.94	10−5000	yes	−0.03	−0.24
9	12	FKBP	other[h]	6950	4	1	0.06	100−2100		−1.24	−1.22
10	13	HI-0065	ADP	8640	13	10	0.15	10−2500		−0.82	−1.28
11	14	LCK	pTyr	6953	43	38	0.62	200−5000	yes	−0.21	−1.07
12	15	LFA	IDAS	11029	44	23	0.40	10−1000	yes	−0.40	−0.35
13	16	MDM2	p53	8640	28	14	0.32	10−420	yes	−0.49	−0.35
14	17	MurA	UDPNAG	9600	4	2	0.04	30−600		−1.38	−1.44
15	18	MurI	Glu	8640	1	1	0.01	2000		−1.93	−2.00
16	19	PAK4	ATP	11450	19	17	0.17	20−1000		−0.78	−0.63
17	20	Pin1	peptide	7842	9	9	0.11	50−1900		−0.94	−1.49
18	21	PSD95	peptide	11759	0	0	0.00	–		−2.00	−1.99
19	22	PTP1B	catalytic pTyr	11892	25	20	0.21	50−5000	yes	−0.68	−1.15
19	23	PTP1B	noncatalytic pTyr	11892	2	2	0.02	1000−5000		−1.77	−1.66
20	24	SARS	RNA	8440	1	1	0.01	1000		−1.93	−1.92
21	25	SCD	substrate	622	5	5	0.80	20−5000	yes	−0.09	−0.55
22	26	survivin	Bir3	9370	1	1	0.01	130		−1.97	−1.99
22	27	survivin	other[h]	9370	33	30	0.35	10−5000	yes	−0.45	−0.35
23	28	UK	peptide	1252	5	5	0.40	10−240	yes	−0.40	−0.81

total hits at known sites: 375
total hits at all sites: 419
percent of all hits at known sites: 89.5

From: Hajduk *et al.*, J. Med. Chem. (2005) **48,** 2518

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In-house validation of method

- 36 drug discovery projects from 2001-2008 analyzed
  - Both conventional HTS & fragment screening by NMR conducted
  - Overall Lead Discovery success rate 50% - agrees well with AsreaZeneca HTS success rate for same period
  - Mix of kinases, proteases, nuclear receptors & bacterial enzymes – but a clear bias towards soluble targets
  - Library size – 768-2000 fragments
- AstraZeneca ligandability scoring definition
  - Not simply hit rates
  - Low – no or few fragment hits, best affinity generally >1mM, low/no diversity
  - Medium – numerous hits with best affinity 0.1-1mM,
  - High – high fragment hit rate with best affinity <0.1mM, high level of diversity
- Projects binned according HTS success rate and overall Lead Discovery success rate

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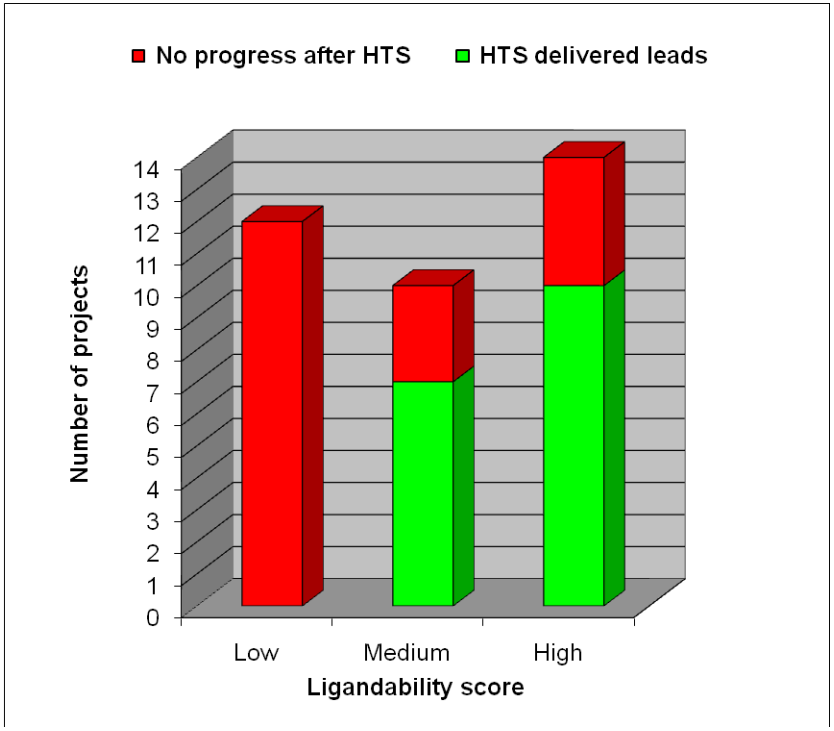
Ligandability score versus HTS outcome

- Comparison between fragment screens and project success based on HTS output
- When ligandability score was low the HTS always failed to deliver
- When the ligandability score was medium or high the HTS success rate was ~70%
- Low ligandability score predictive of failure

Results from 36 fragment screening projects



Ligandability Score Definitions:
Low – no or few fragment hits, best affinity generally >1mM
Medium – numerous hits with best affinity 100uM-1mM
High – high fragment hit rate with best affinity <100uM

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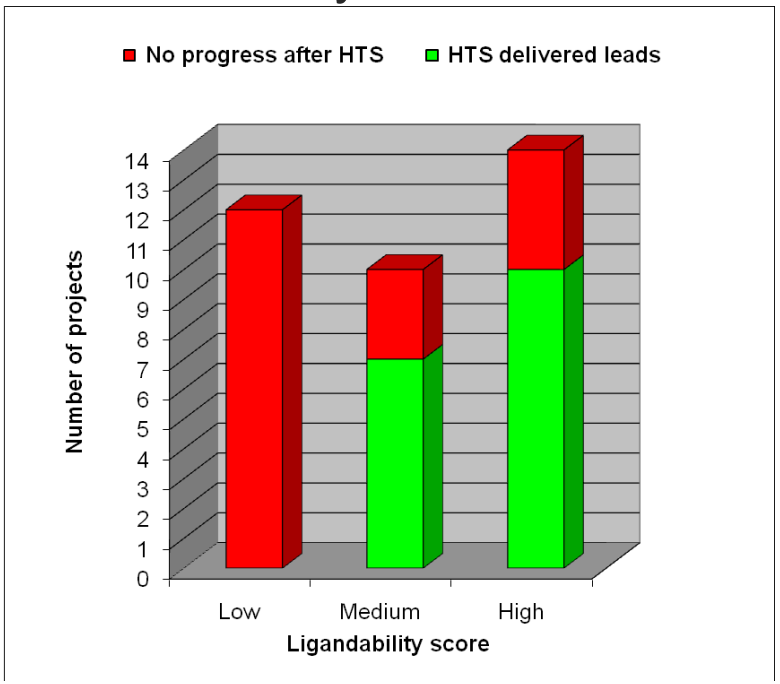
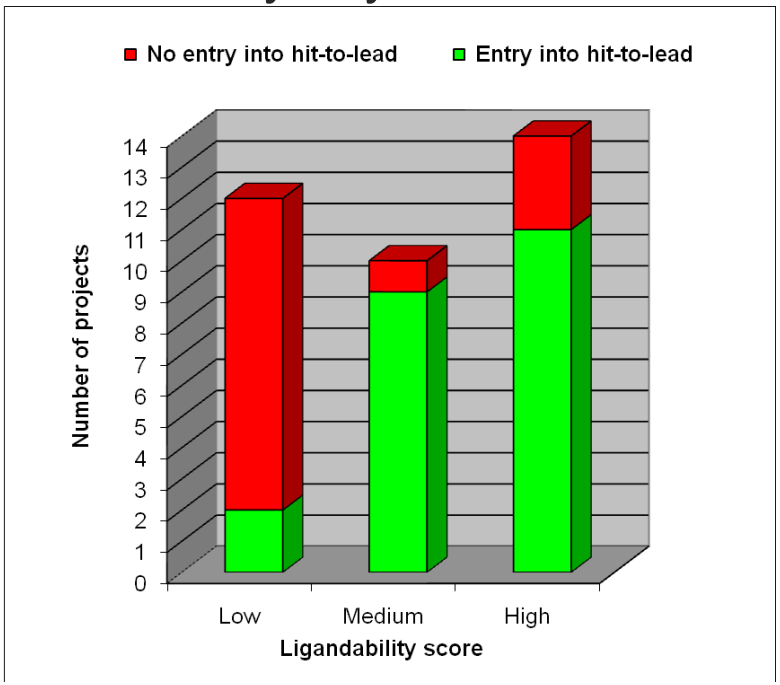
Ligandability score versus entry into hit-to-lead program

- Alternate lead generation strategies (e.g. FBLD, fast follower) increase success rates
- High/medium ligandability targets should be successful with appropriate strategy - could be HTS, FBLG, FF or mix thereof)
- It may be possible to succeed with low ligandability targets – but it will be extremely difficult

By HTS

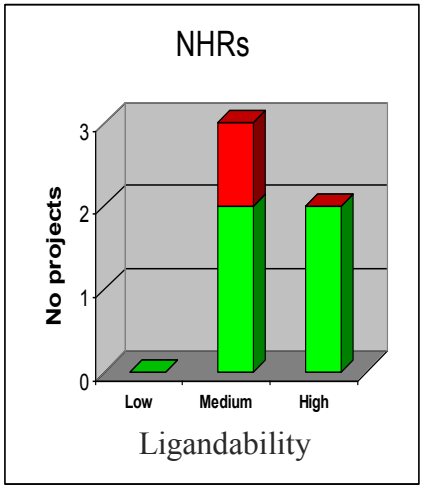


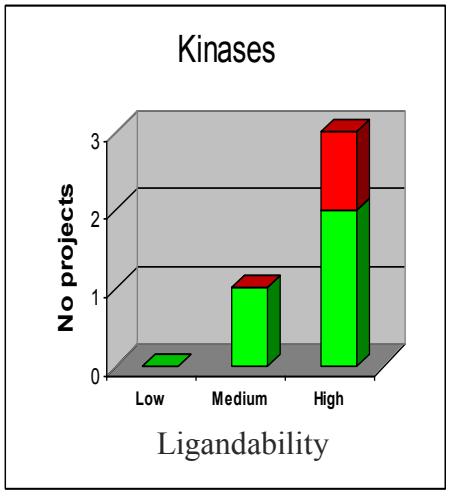
By any means

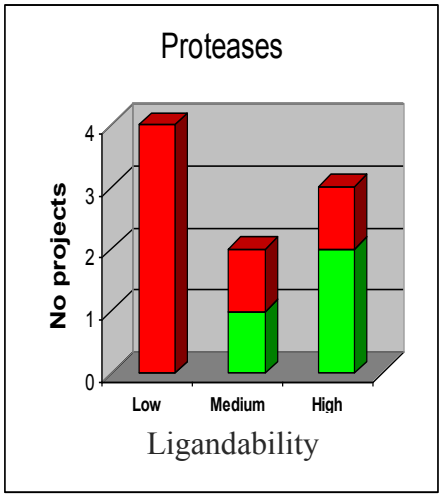


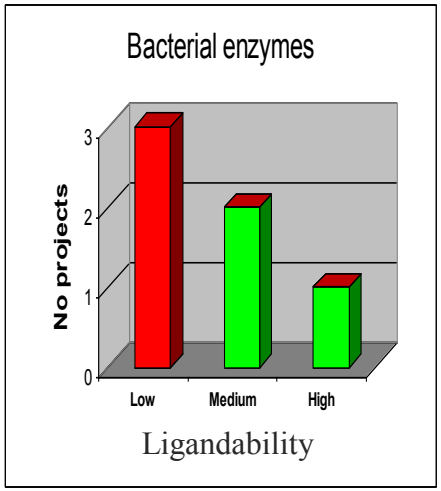
Target class view

- Some target classes clearly more druggable/ligandable than others – reflected in fragment screening data
  - NHRs & kinases are generally druggable
  - Proteases and bacterial enzymes tend to be more difficult
- Reality check
  - Estrogen receptor β (ERβ) – medium ligandability & drugs on the market
  - Protein tyrosine phosphatase 1B (PTP1B) – low ligandability, extremely challenging target, no drugs on market in spite of considerable efforts

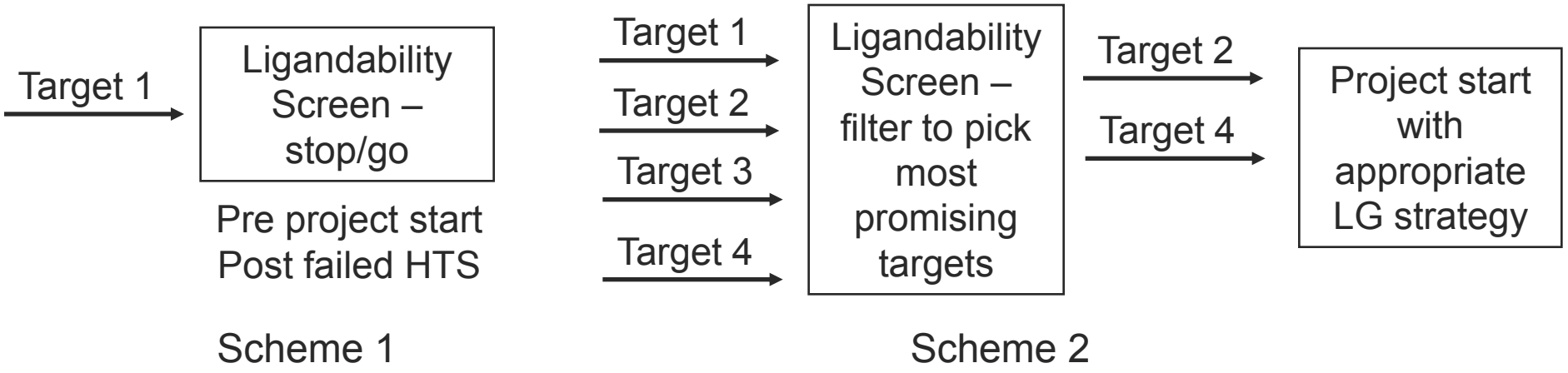








Recommended ways of using Ligandability screening



- Information needs to be considered as part of an overall tractability assessment
- Most value for unprecedented / novel / previously intractable targets
- Early access to ligand information - 'fingerprint' for downstream phys-chem. property space
- Establish feasibility of FBLD approach
- Requires robust & reliable screening method - NMR & SPR
- Critical to avoid false positives & false negatives

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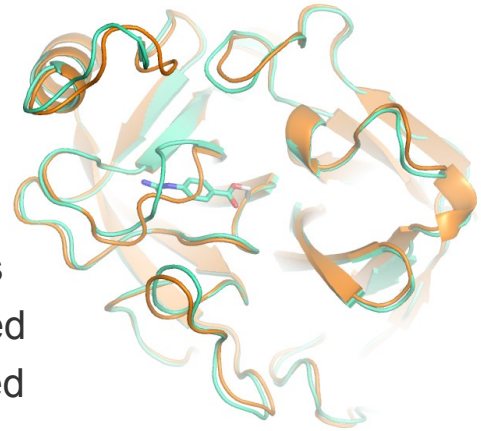
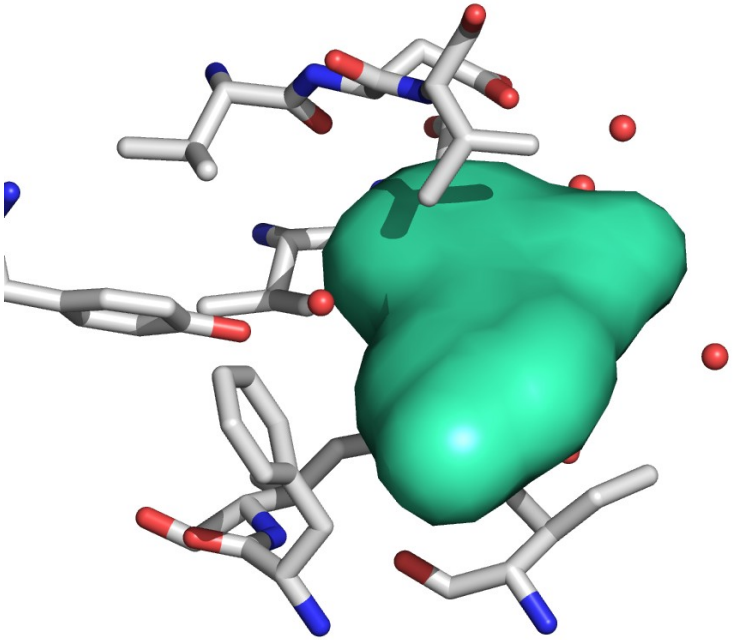
Ligandability screening example – Ser-protease example



Apo vs complexed protein showing collapsed S1-pocket

- **Target:** trypsin-like serine protease
- **Indication:** Chronic obstructive pulmonary disease (COPD)
- **Prior knowledge:**
  - Crystal structures with non-specific covalent Ser-protease inhibitors
  - Low nM specific covalent & reversible peptide-like inhibitors reported
  - Asp-residue at bottom of S1-pocket but access to S1-pocket blocked
  - Competitor claims their HTS failed to yield any viable leads
- **Question asked: Is target really ligandable?**
- **Type of screen:** 1D NMR screen with competition
- **Outcome:**
  - Low hit rate (0.4%); classic amide, amidine, guanidine fragments do not bind competitively; fragment hits show no resemblance to classic Ser-protease binders
- **Ligandability score: low**
- **Conclusions & comments** :
  - Druggability score delivered prior to project launch
  - Information used to devise strategy for project - FBLD initially planned put on-hold
  - Limited initial scoping including synthesis & pre-screen yielded nothing
  - HTS has gone ahead – output is looking meager

Ligandability screening – dehydrogenase example



- **Target class**: NADH dependent dehydrogenase
- **Indication**: Oncology
- **Prior knowledge**:
  - no hits from HTS (turnover assay)
  - binders known for NADH pocket
    - too weak to show inhibition
  - druggability for NADH pocket unknown
- **Type of screen**: 1D NMR, NADH competition to confirm binding site, 2D characterization
- **Outcome**:

  3.5 % hit rate; ~40 fragment hits; affinity <1mM;

  several clusters with drug like structures; soaking of fragment hit gave a crystal structure
- **Ligandability score: medium**
- **Conclusions & comments**:
  - Fragments interesting but weak -> not active in enzyme assay
  - Project restarted as FBLG campaign using fragments as start points

➢ Fragment linking has generated cell active, sub μM tool compounds

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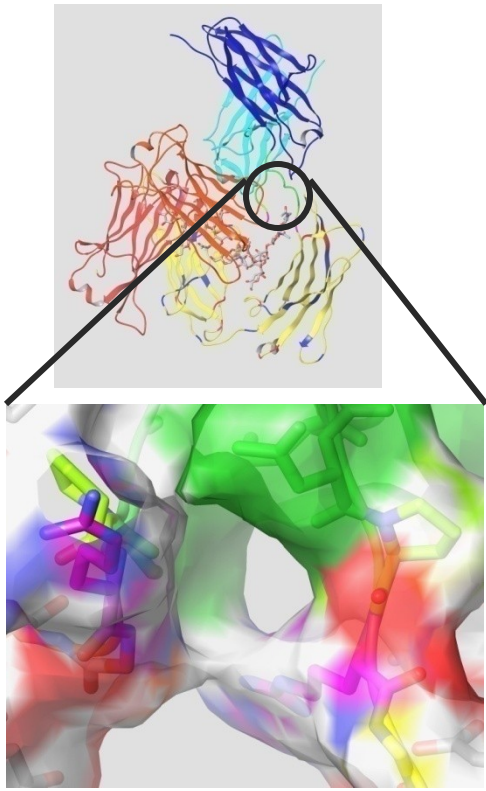
Ligandability screening example - immunoglobulin E (IgE)

- **Target:** immunoglobulin E (IgE)
- **Indication:** inflammation, validated target – anti-inflammatory antibody therapies on market
- **Prior knowledge:**
  - Antibodies and peptide vaccines with epitopes outside receptor binding interface
  - Crystal structures suggest conformational change required for receptor binding
  - Potentially ligandable cavity in hinge region between domains
- **Question asked: Can this target be addressed with small molecules?**
- **Type of screen:** 1D NMR with cross-wise competition of binders
- **Outcome:**
  - Low hit rate; hits not cross-wise competitive
- **Ligandability score: low**
- **Conclusions & comments:**
  - No evidence for distinct binding site (hot spot)
  - Ligandability score combined with other negative data (VS based pre-screen) – **no project start-up**



Structure of IgE with postulated binding site in hinge region

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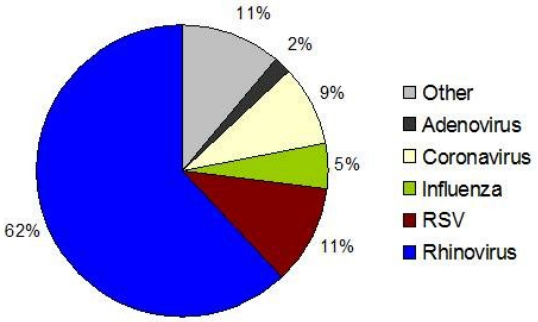
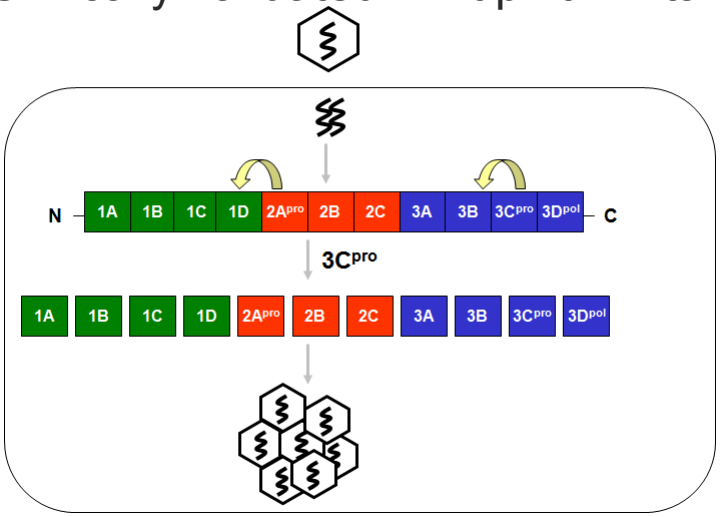
Pursuing the needle in the haystack – RV3CP

- Human rhinovirus (HRV) triggers respiratory infections & related diseases
- 75% of chronic obstructive pulmonary disease (COPD) and asthma exacerbations triggered upper respiratory virus infection – HRV accounts for 2/3rds



- Stop the virus – stop the exacerbation
- Several rhinovirus inhibitors in clinic – none on the market

- Rhinovirus 3C protease (RV3CP) involved in processing of viral polypeptide
- Clinically validated – Rupintrivir taken to phase II trials



3C^{pro} performs **8/10** essential cleavages

<u>No mammalian homolog</u>
- Active site Cysteine
- Trypsin-like serine structure
- Cleaves Glu-Gly (unusual)

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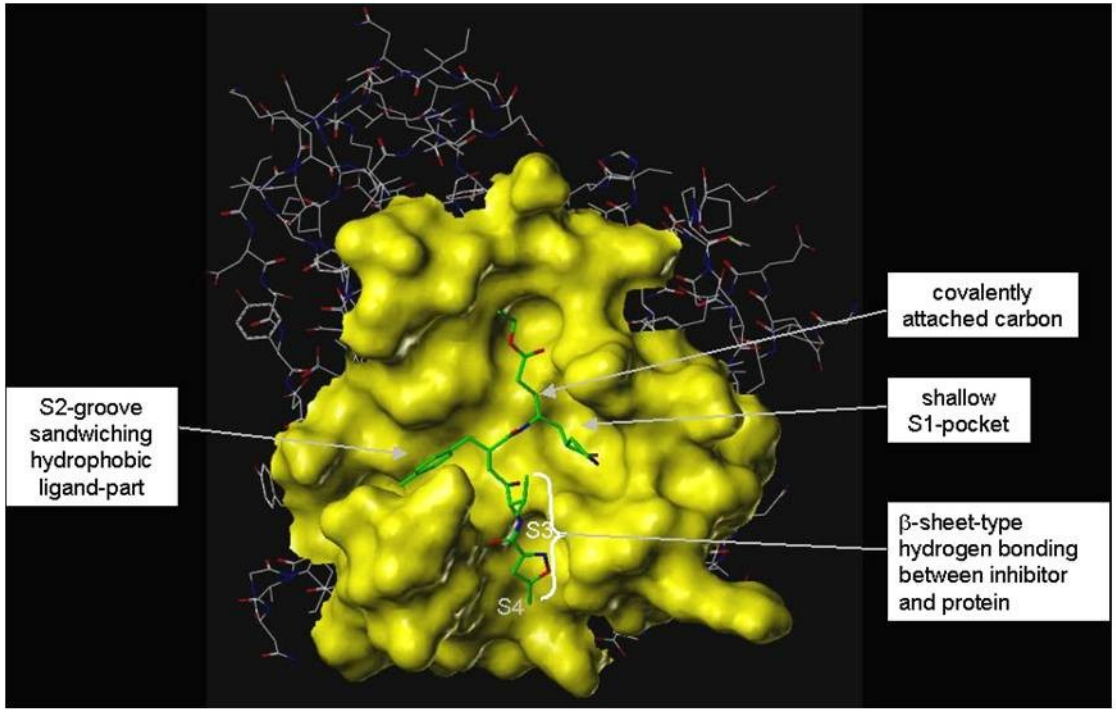
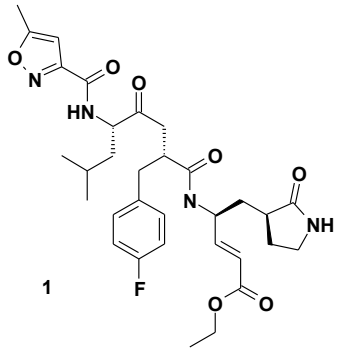
Crystal structure of RV3CP

- All reported potent 3C protease inhibitors contain reactive groups that bind covalently to active site cysteine
- Reactive groups lead to unfavorable DMPK properties of compounds and result in serious side effects in patients - non-covalent inhibitors highly desirable
- Based on published crystal structure hit-finding predicted to be very challenging
- Binding pockets in the active site are shallow and affinity is very much driven by the reactive group – high chemical risk





Rupintrivir
Covalent 3CP inhibitor
Pfizer/Agouron
Stopped in Phase II
Developed through SBDD
J. Med. Chem. 41, 2806

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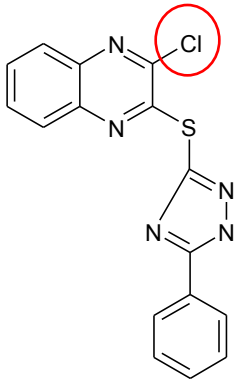
High Throughput Screening (HTS) output

- Straight forward biochemical assay – monitor cleavage of fluorescent substrate
- Corporate collection screened (at 10μM)
- Basically no tractable non-covalent starting points identified
- Potentially interesting series turned out to be covalent inhibitors
  - Quinoxaline series – covalent attachment with irreversible loss of Cl (mass spectrometry)



- 20k fragment library screened as part of HTS (at 100μM)
- Handful of compounds showing some inhibition of RV3CP

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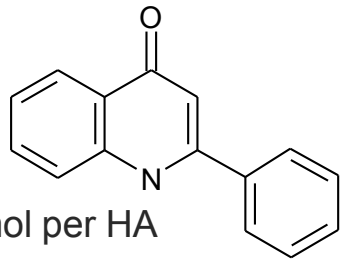
Initial quinolone structure activity relationship

- 2D NMR binding assay used to confirm compound binding, generate Kds, binding site information & to drive fragment evolution
- Quinolone – only confirmed fragment

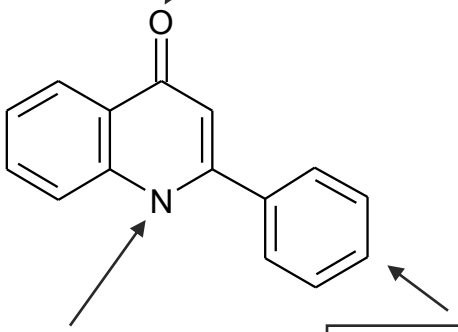


NMR pKd= 3.7
Ligand Efficiency= 0.30 kcal/mol per HA

- Initial SAR by NMR from in-house analogs

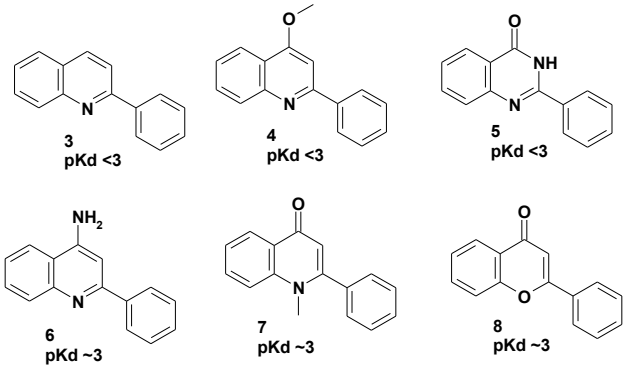
Carbonyl required – makes critical H-bond interaction?

C6 extension results in imoproved affinity but in-house analogs quite insoluble



Nitrogen required, oxygen tolerated with some loss in affinity

Phenyl required, acid functionality can be added to improve solubility



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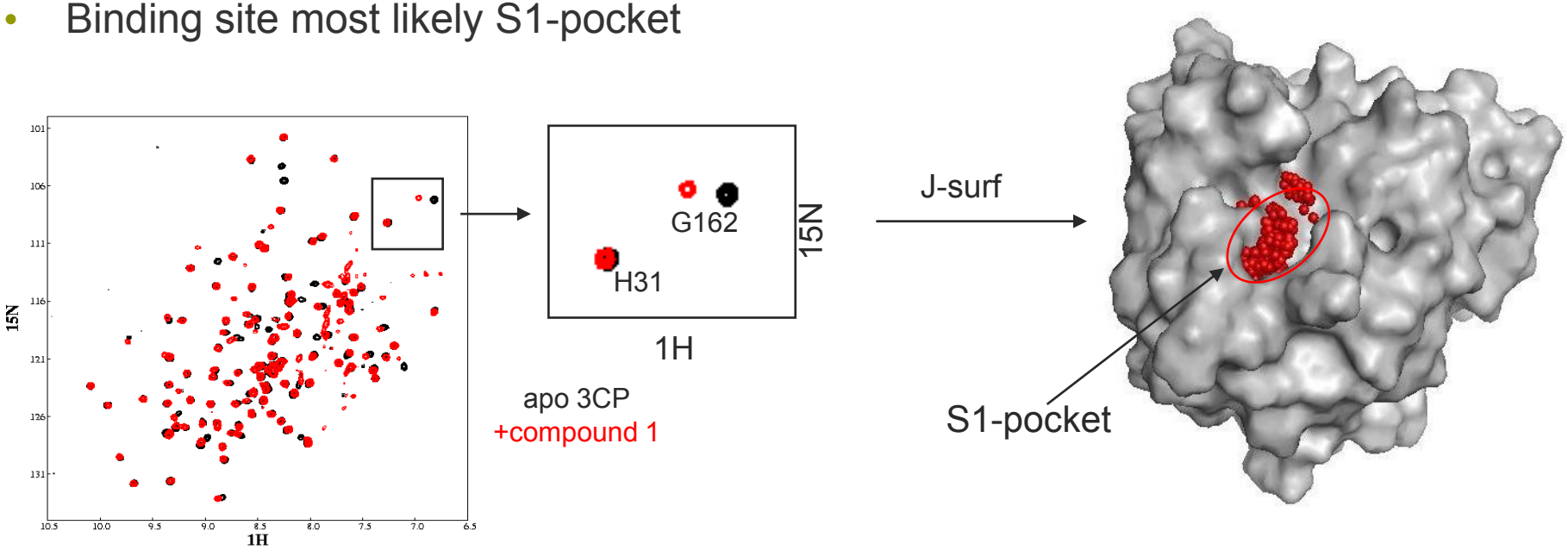
Quinolone binding mode

- Chemical shift mapping
- Program J-surf (JACS, 124, 11758-63) used to calculate most probably location of ligand
- Binding site most likely S1-pocket



- Additional NMR fragment screening
  - 1600 general fragments screened at 1mM
  - Low hit rate – 0.2%
  - No useful hits – only three chemically unappealing fragments identified

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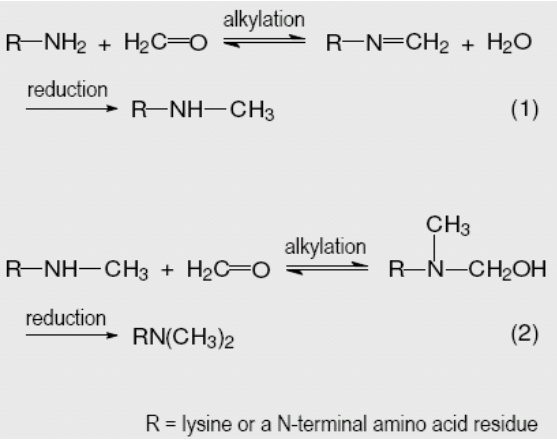
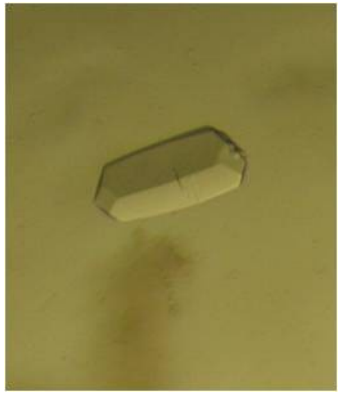
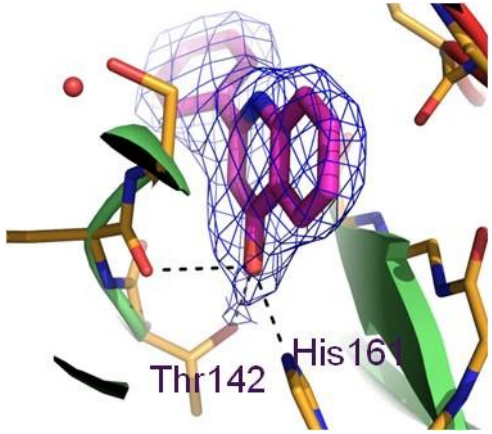
Crystal structure with quinolone fragment

- Extremely challenging system – small flexible protein
- Large peptidic inhibitors stabilize active site & sub-pockets
- Extensive work: >2 years, >20 constructs, exhaustive screening
- System never iterative – only one structure determined
- Quinolone fragment crystallized using reductive methylation
- Quinolone binds in S1-pocket – carbonyl makes hydrogen bonding interactions with His161 & Thr142







2.4 Å resolution at synchrotron

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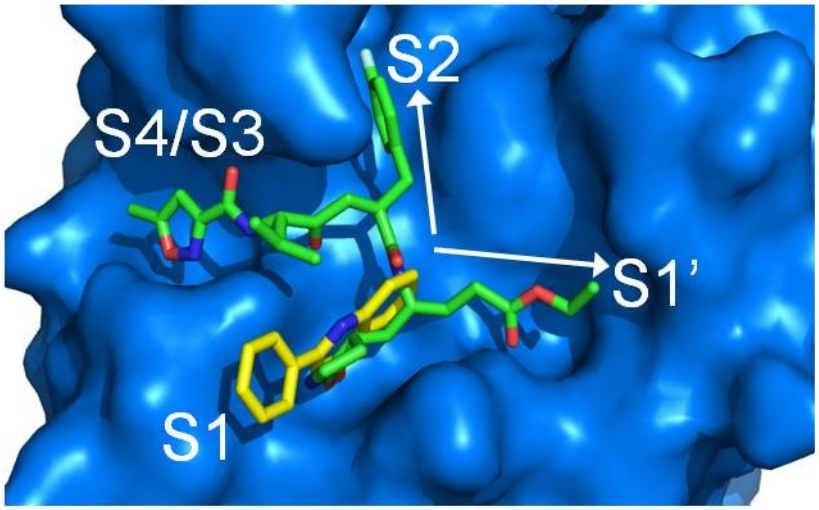
Opportunities for structure based design

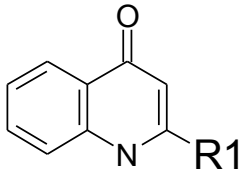
- Published structure with Rupintrivir
  - Peptidic inhibitor covers entire binding site
  - Binding driven by covalent attachment in S1’

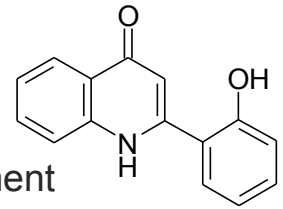
- The in-house quinolone structure
  - Fragment provides an optimal starting point in S1-pocket
  - Extend in direction of S1’ or S2
  - Difficult to reach S3/S4



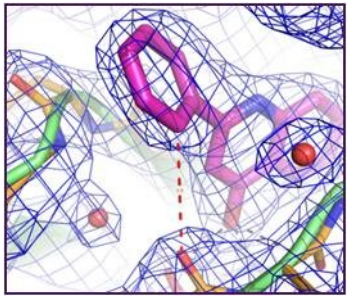
Quinolone fragment evolution – S1 pocket

- Vary C2-phenyl in S1 pocket – opportunities to gain affinity based on structure

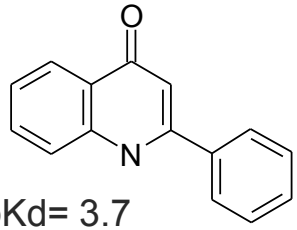




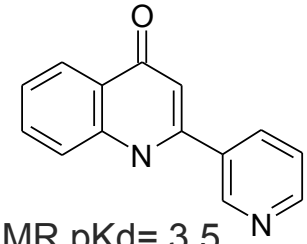
NMR pKd= 3
No improvement



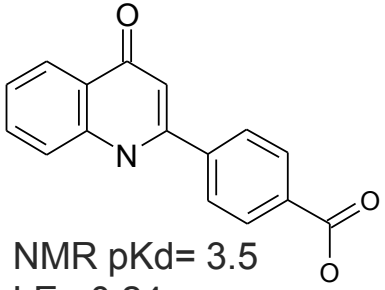
- No improvement in potency achieved but solubilizing groups can be added – phenyl optimal (makes stacking interaction)



NMR pKd= 3.7
Ligand Efficiency= 0.30 kcal/mol per HA
ACD log D= 4.3



NMR pKd= 3.5
LE= 0.28
ACD log D= 3.4



NMR pKd= 3.5
LE= 0.24
ACD log D= 1.3

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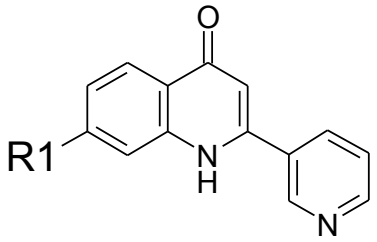
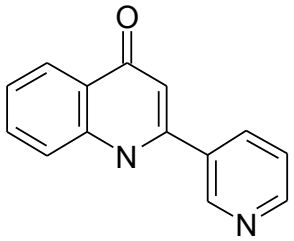
Quinolone fragment evolution – S2 pocket

- Extend from C7 to access S2 and/or S1' pockets – should be most optimal based on crystal structure

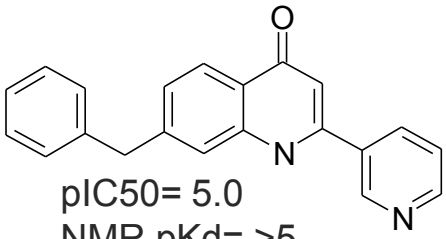


Compd	R1	% effect @ 100uM	pIC50
9	H	35	NA
10	Me	40	NA
11	MeO	<10	NA
12	CONHMe	40	NA
13	CH2CONHMe	45	NA
14	CH2Ph	100	5
15	(+/-)CH(OH)Ph	80	4.7

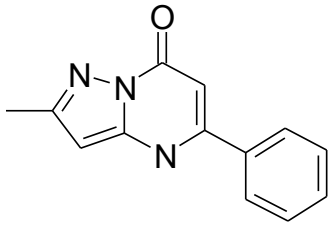
- 30-fold improvement in potency with maintained ligand efficiency achieved with only three different linkers tested



NMR pKd= 3.5
LE= 0.28
Log D= 3.4



pIC50= 5.0
NMR pKd= >5
LE= 0.28
Log D= 5.4



NMR pKd= 3.3
LE= 0.26
log D= 1.7

- Success in developing non-covalent low uM inhibitors for further development
- Prospects for improving affinity through SBDD & solubility

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Concluding remarks

- RV3CP – significant progress on a difficult target with only a single fragment hit and a single crystal structure to guide design
- Druggability/Ligandability screening appears to be predictive of project success based on analysis of historical data
- Ligandabiliy data currently used in truly predictive way

Acknowledgements

Druggability work

Alexander Breeze

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