The use of target immobilised NMR screening to identify and develop fragment binders to Hsp90

Hot topics in drug discovery: finding the next lead

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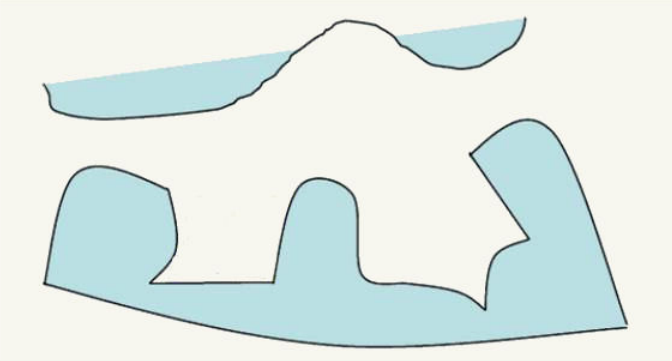
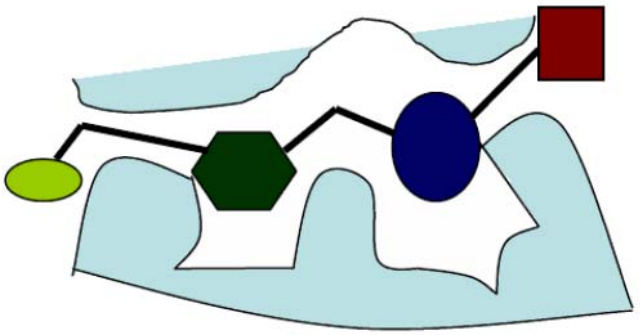
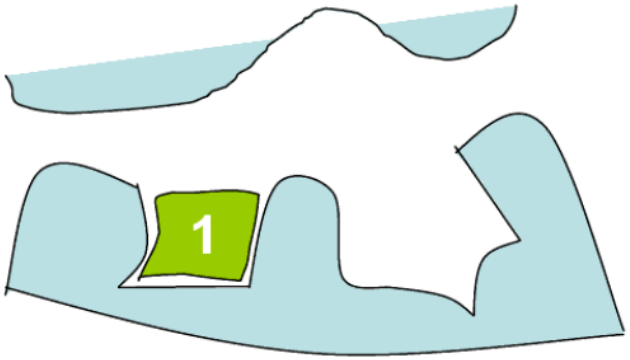
Introduction

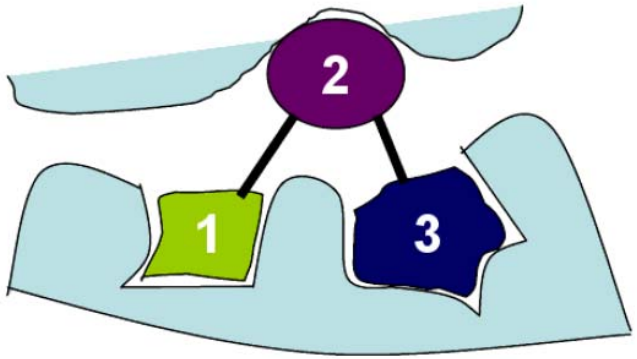
- Fragment based drug design (FBDD) is becoming a popular method of finding starting points for drug discovery programmes
- Wanted to evaluate FBDD in-house
- Key issue is the choice of screening method to identify fragment binders
- One such method is target immobilised NMR screening (TINS).
- Review our experiences in evaluating TINS to find fragment binders to Hsp90
  - Comparison with other screening methods
  - Some of the approaches that we have followed to develop the identified fragment hits

Fragment screening







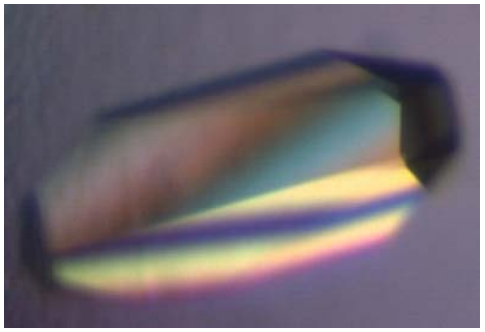


Fragment screening

- Libraries are typically smaller as fragment chemical space is smaller
- Bind with high atom efficiency but with low affinity
- Greater hit rate
- Typically require biophysical screening methods
- Ideally require structural information on how fragments bind for rapid progression

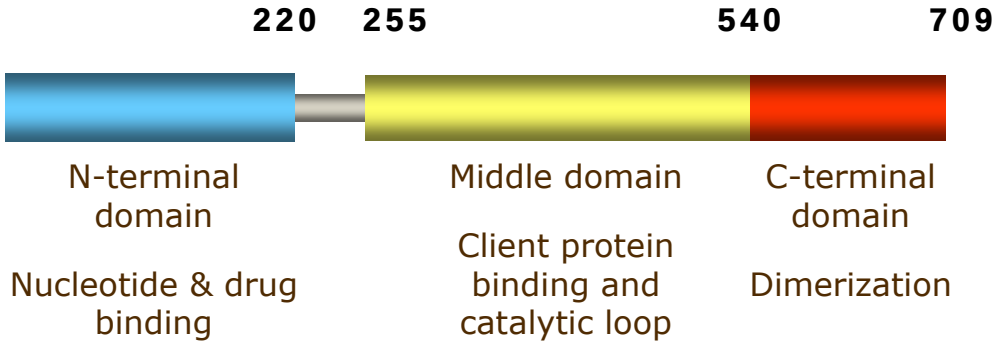
Why Hsp90 to validate fragment screening?

- Protein is well-expressed (>100 mg/L)
- Crystallises readily
- >80 crystal structures in PDB
- NMR solution structure solved
- Precedented target for fragment screening (positive controls available)

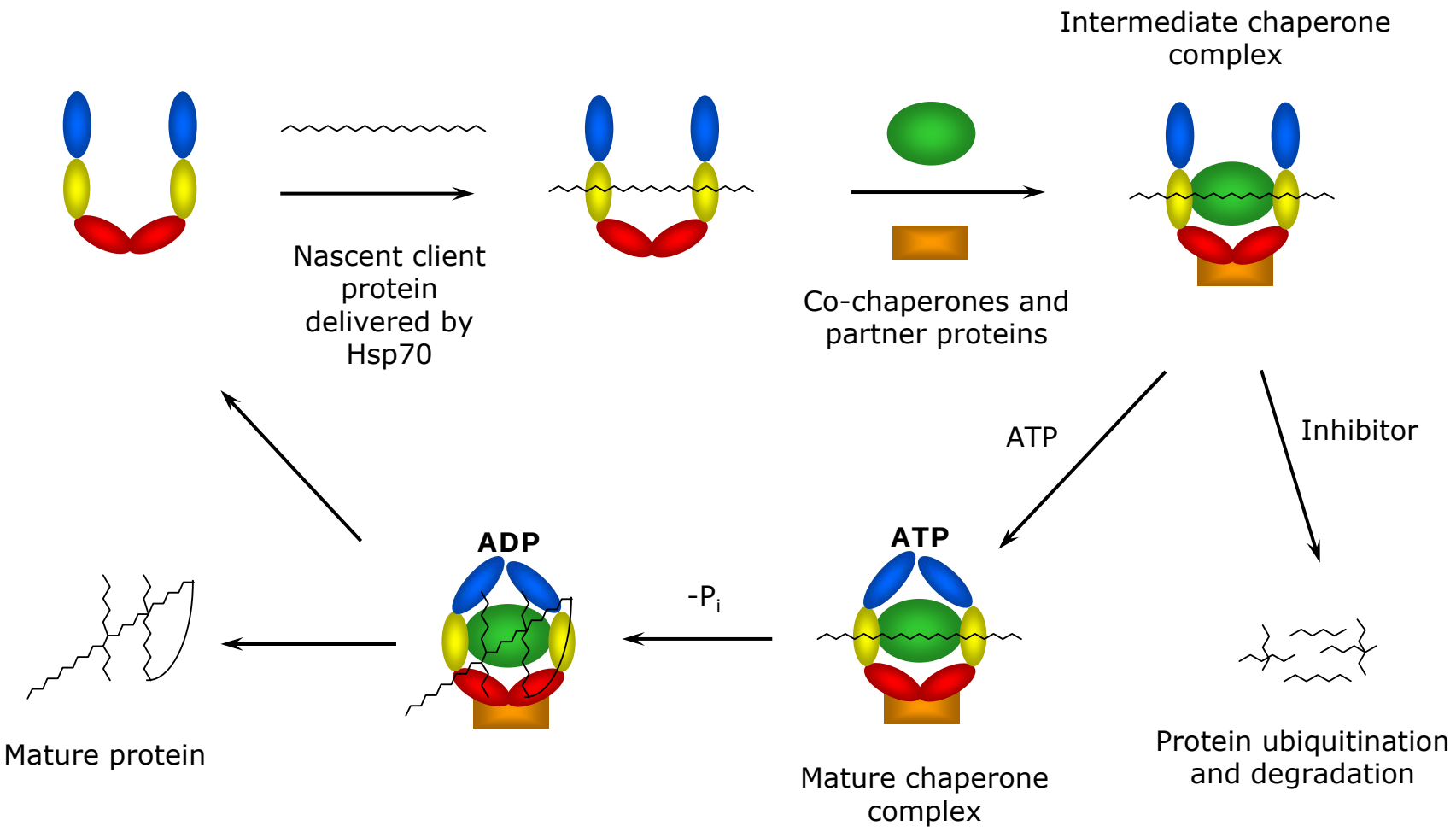


Heat Shock Protein 90 (Hsp90)

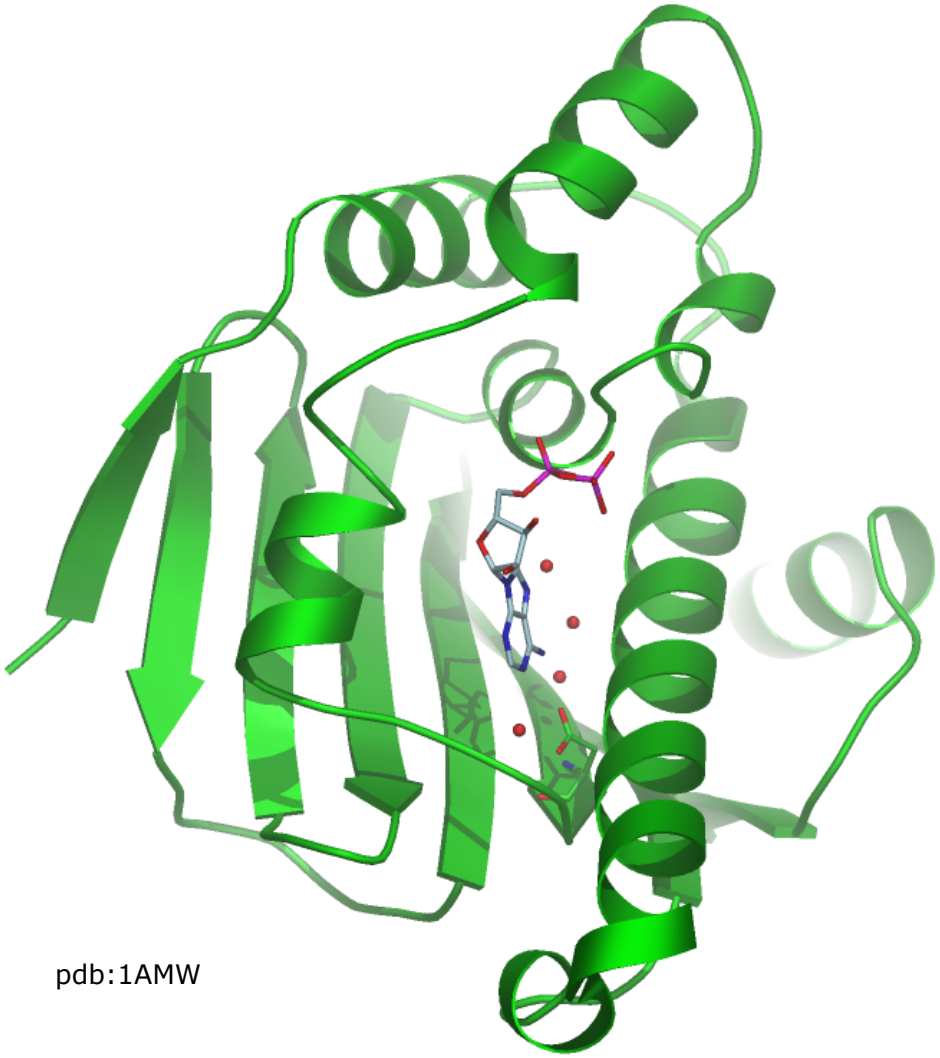
- HSP90 is an ATP-dependent molecular chaperone
- Responsible for conformation and stability of many 'oncogenic' client proteins e.g. RAF, ErbB2, AKT
- Mutant oncoproteins particularly reliant on HSP90 e.g. B-RAF, EGFR, KIT
- Many of these proteins are key for driving cancer phenotype
- Inhibition of Hsp90 leads to ubiquitination and degradation of client proteins
- Potential one step combinatorial treatment for cancer



Mode of action



Hsp90 Structure



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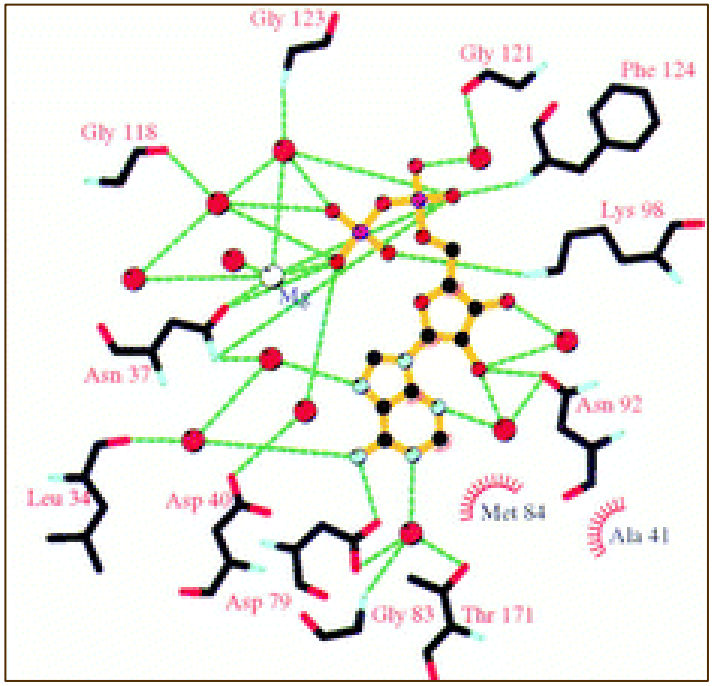
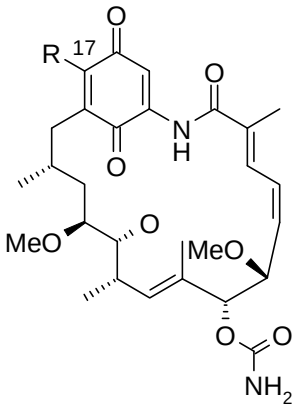
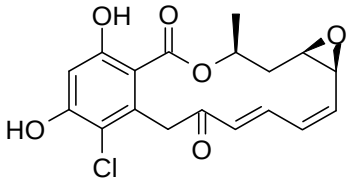


Prodromou *et al*, Cell, 90, 65-70, 1997

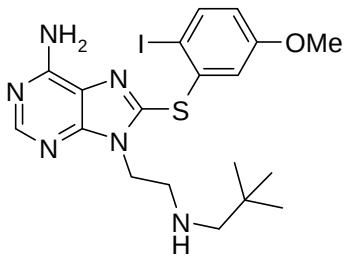
Representative Hsp90 Inhibitors



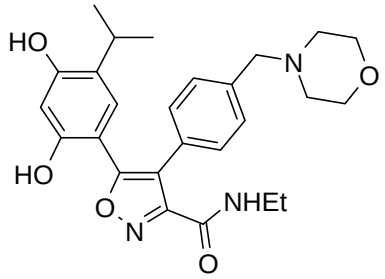
Geldanamycin Analogues



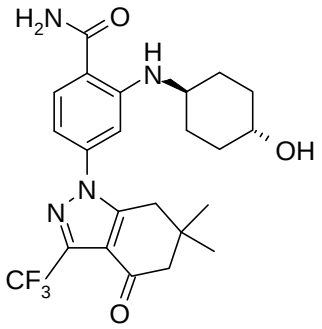
Radicicol analogues



CNF2024



VER-52296 (NVP-AUY922)



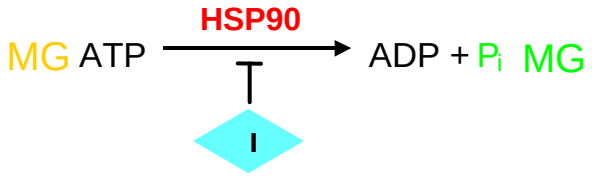
SNX2112

Fragment Screening Deck

- 3 components
  - Commercially sourced
  - Selected by virtual screening and medicinal chemists (kinase focused)
  - In house fragments
- "Rule of 3" criteria
  - MW <300 Da
  - logP <3
  - HBD ≤ 3
  - HBA ≤3
  - Rotatable bonds <3
  - PSA <60 Å^2
- No reactive or toxic functionality
- Screened for solubility
- QC by LC-MS
- Consists of 2389 compounds

Fragment Deck: Biochemical Assay

HSP90 Colorimetric ATPase assay: tolerant to DMSO but lack of sensitivity and colour interference with some fragments



2389 Fragments

Hit-rate= 1.5%

Initial hits

94 Fragments

94 hits > 20% @ 10mM, n=1

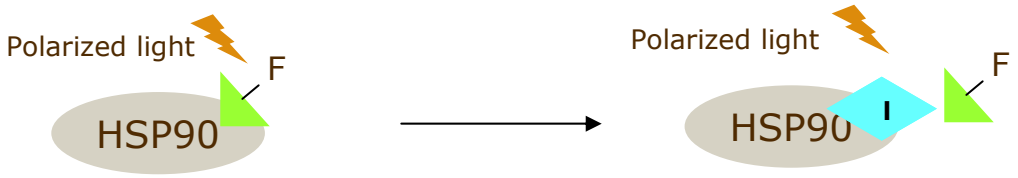
Hit confirmation

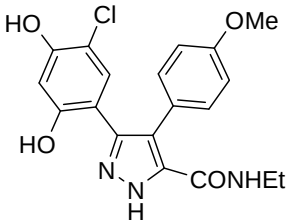
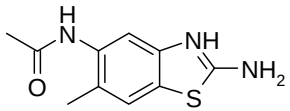
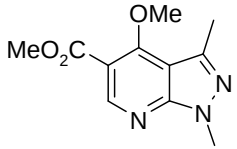
37 Fragments

37 hits > 20% @ 10mM, average, n=2

HSP90 Biochemical screening assays

HSP90 fluorescence polarization competition binding assay

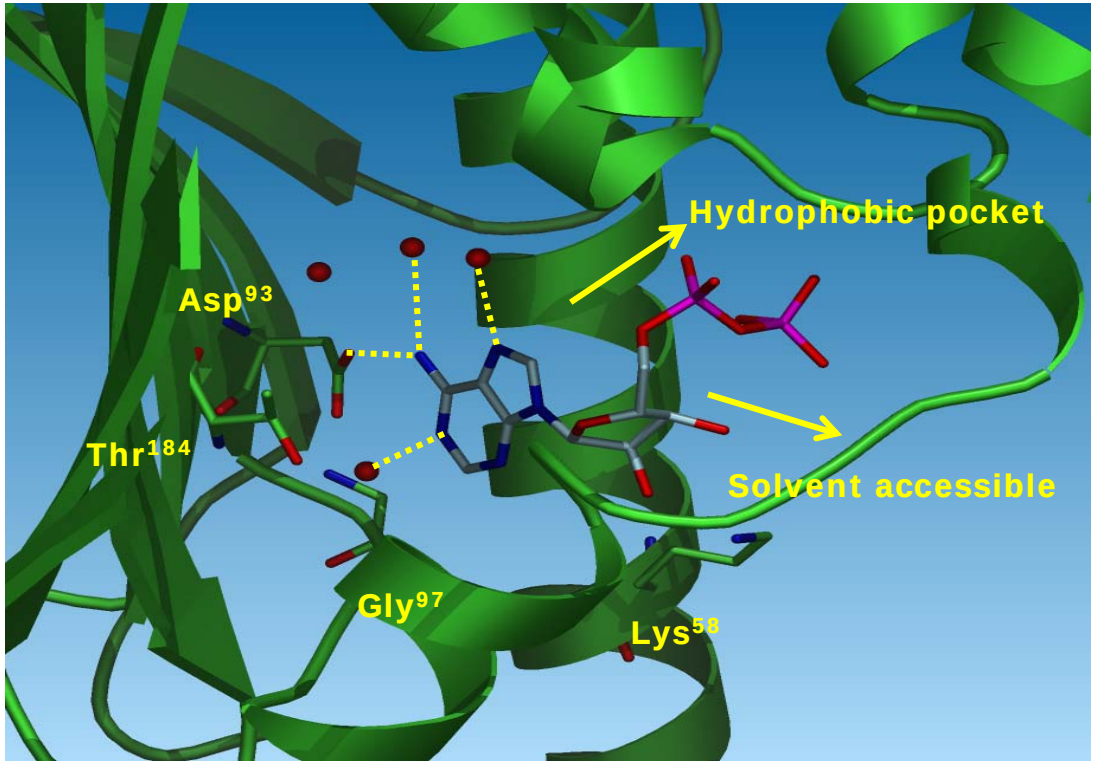


		ATP-ase activity IC_{50} (µM)	FP activity IC_{50} (µM)	ITC K_D (µM)
VER-49009		1.0	0.11	nd
UCB1050452		50% @ 10 mM	952	50
UCB1271054		37% @ 10 mM	160	49

Fluorescence polarization competition assay is able to generate IC_{50}s for fragments (~1mM limit)

STD NMR

- Wanted to compare biochemical screening with biophysical methods
- Chose Saturation Transfer Difference NMR
- Relatively low throughput method requires pre-screening of fragment library



1688 Structures

Dock using GOLD

Check for key interactions

438 Structures

Visual Inspection

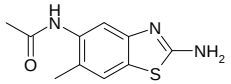
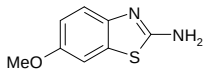
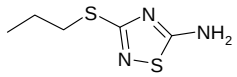
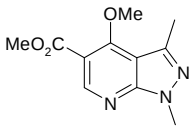
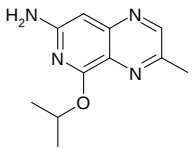
132 Fragments

+ 21 Fragments from Biochemical Assay

153 Fragments Selected

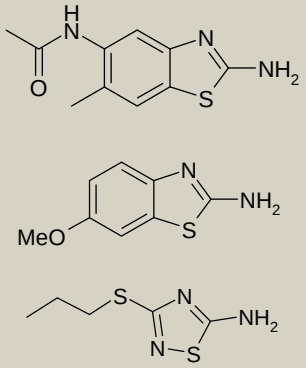
Fragment screening by STD NMR spectroscopy

- Analysis of data is subjective (the magnitude of the STD effect as reflected in the S/N of the response) and time consuming
- Total hits 46 (30% hit rate)
- Includes 6 fragments identified from biochemical assay
- Obtained ligand/protein crystal structures for 5 hits

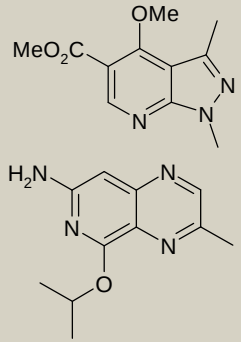
		Binding Site	FP Assay IC_{50} (uM)	Ligand Efficiency
UCB1050452		A	952	0.26
UCB1176735		A	24% @ 5mM	-
UCB1326516		A	nd	-
UCB1271054		B	160	0.33
UCB1326498		B	nd	-

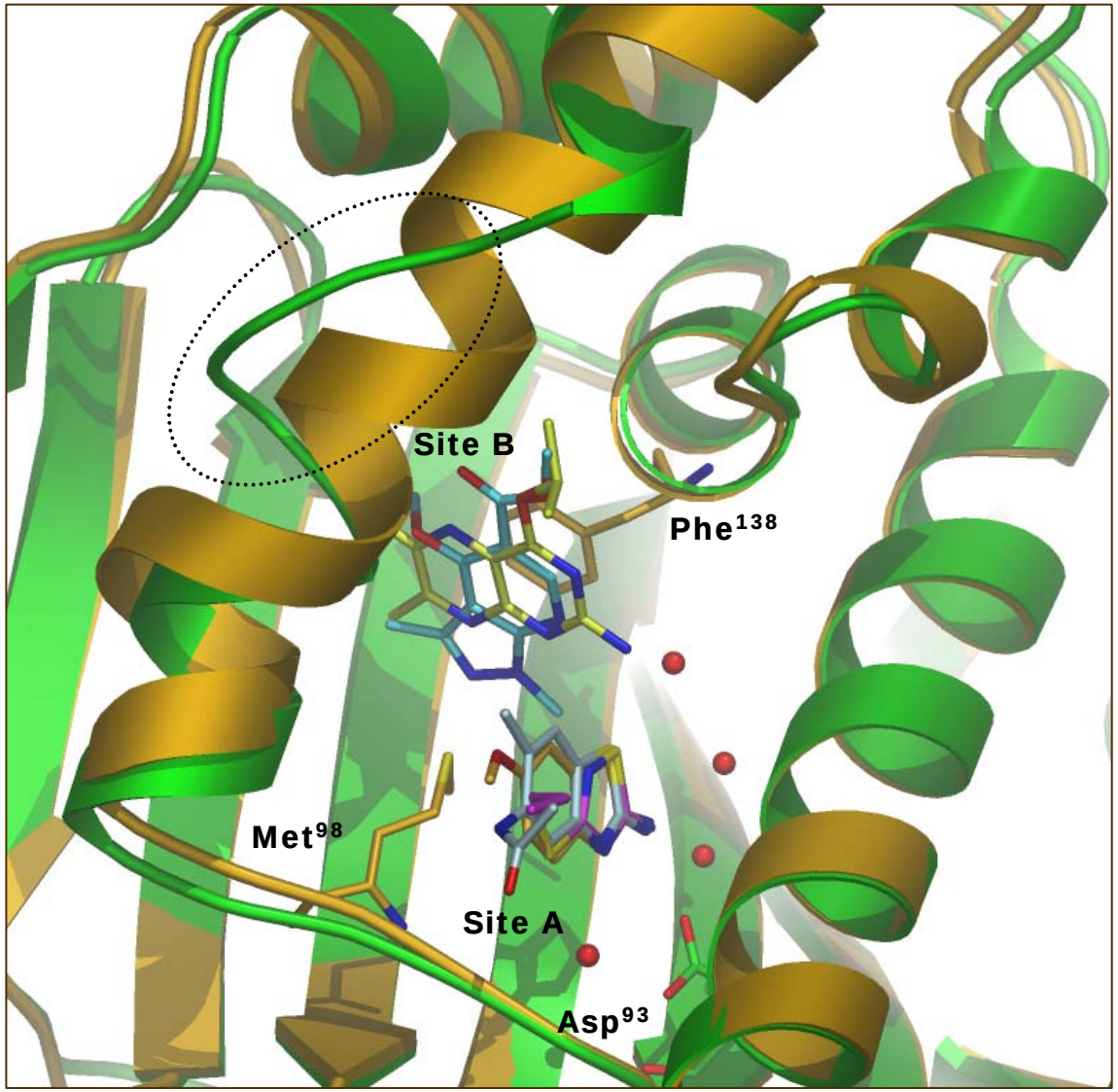
Two binding sites identified

Site A



Site B





Green: Protein conformation for site A binders; Gold: Protein conformation for site B binders

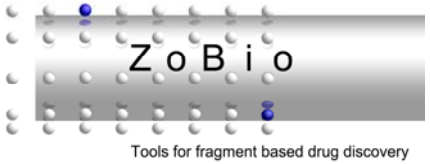
Lessons learned from in-house screening

~150 compounds were made from these starting points but no improvement in binding affinity

- Focused on the 5 binders for which structures were available
- Not enough diversity in starting point structures
- Biochemical assay too insensitive
- Structural information important

Looked for an alternative source of finding fragment starting points

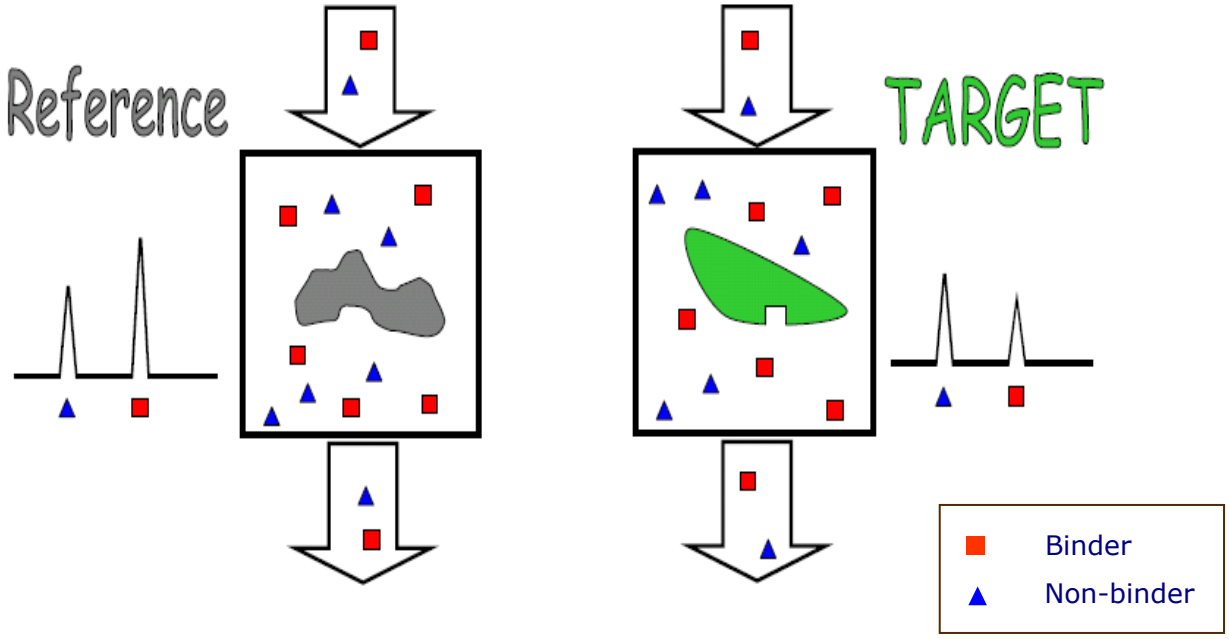
Target Immobilised NMR Screening (TINS)



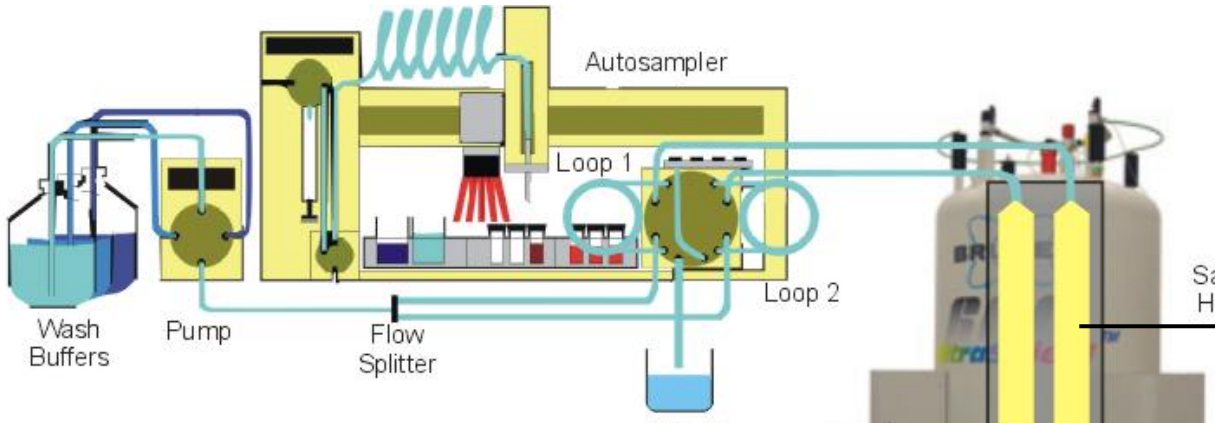
- Zobio BV
- Based at The University of Leiden, The Netherlands
- Offer comprehensive fragment screening service using TINS technology
- Uses a library of ~1400 diverse, commercially available compounds (co-developed with Pyxis Discovery, Delft, The Netherlands)
    - Every compound is aqueously soluble @ 500 µM
    - Every compound has QC 1H NMR spectra recorded by ZoBio
    - Conforms to commonly accepted fragment criteria

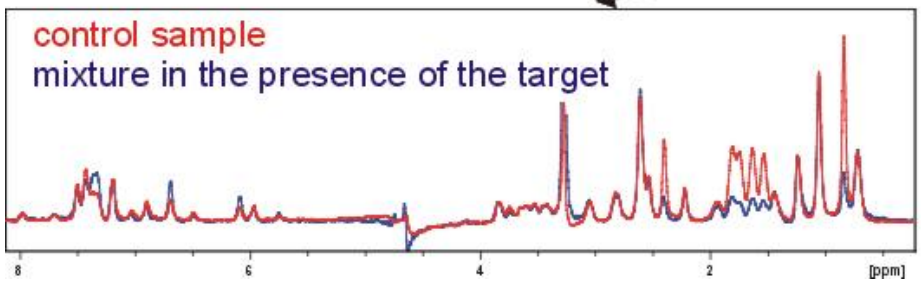
Target Immobilised NMR Screening (TINS)

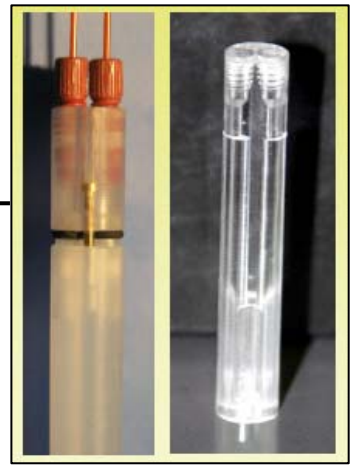
- Protein immobilised on resin and packed into flow cell in NMR spectrometer
- Library of fragments (in pools of 4-8 compounds) flows over protein and reference protein (PH domain of Akt)
- Spectra acquired and processed to identify binders (reduction of signal)
- Reference protein avoids false positives



The TINS Screening Station

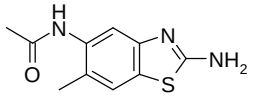
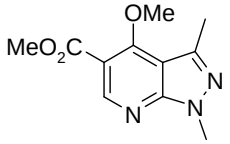
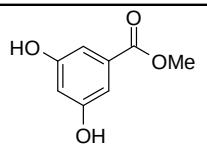
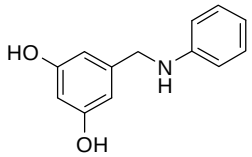
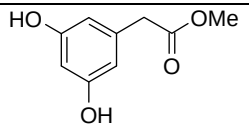
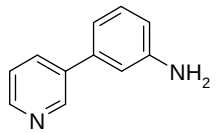






Flow-injection probe capable of holding 2 samples of solid support simultaneously.

Hsp90 TINS Pilot Screen

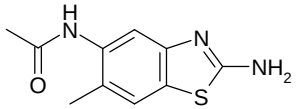
ID #	TINS	WaterLOGSY	FP Activity IC_{50} (µM)	Solubility @ pH 7.4	Structure
UCB1050452	Yes	ND	952	>5 mM	
UCB1271054	Yes	ND	160	>5 mM	
UCB1388097*	Yes	ND	322	>1 mg/mL	
UCB1400374*	Weak	Weak	ND	>1 mg/mL	
UCB1388094*	Yes	Yes	1714	>1 mg/mL	
UCB1349014	No	No	STD NMR Hit	>5 mM	

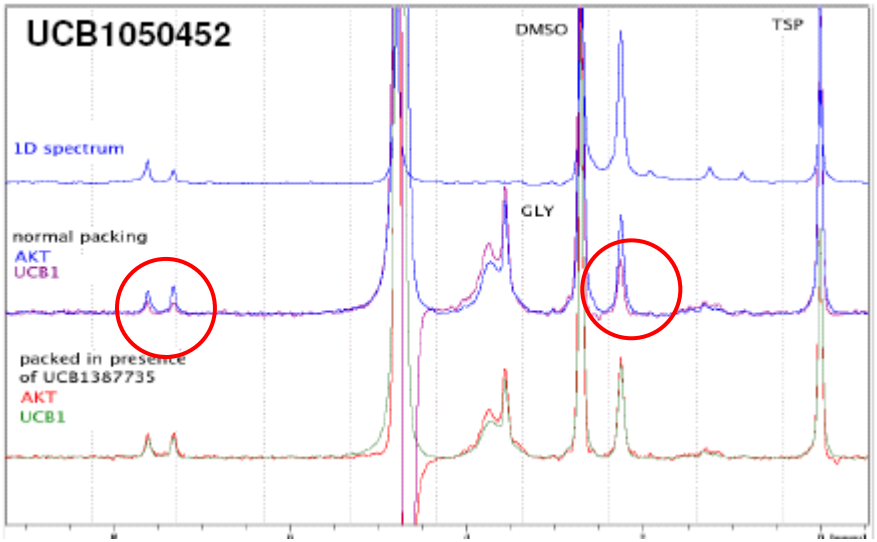
* Aboul-ela et al, AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics, November 17-21, 2003, Boston, USA, Abstract A8

ND Not determined

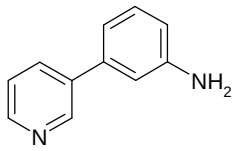
TINS screening of positive controls

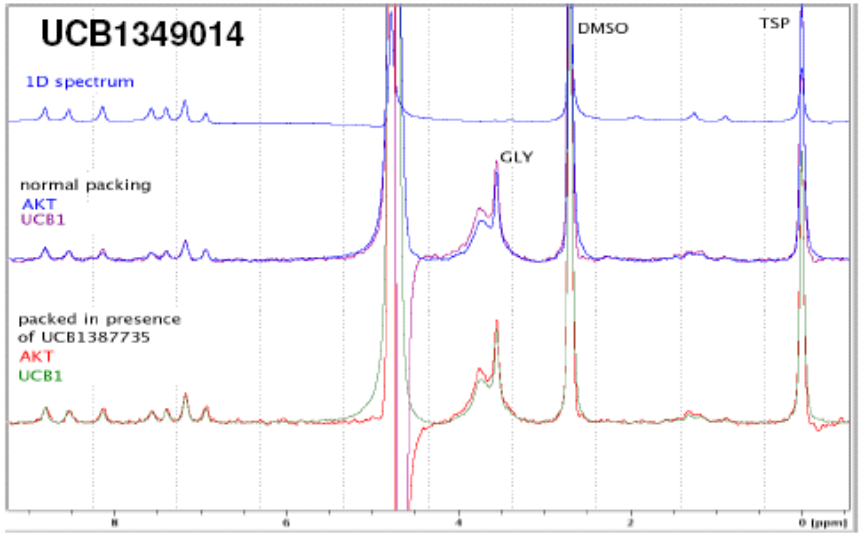
Binding





Non-binding



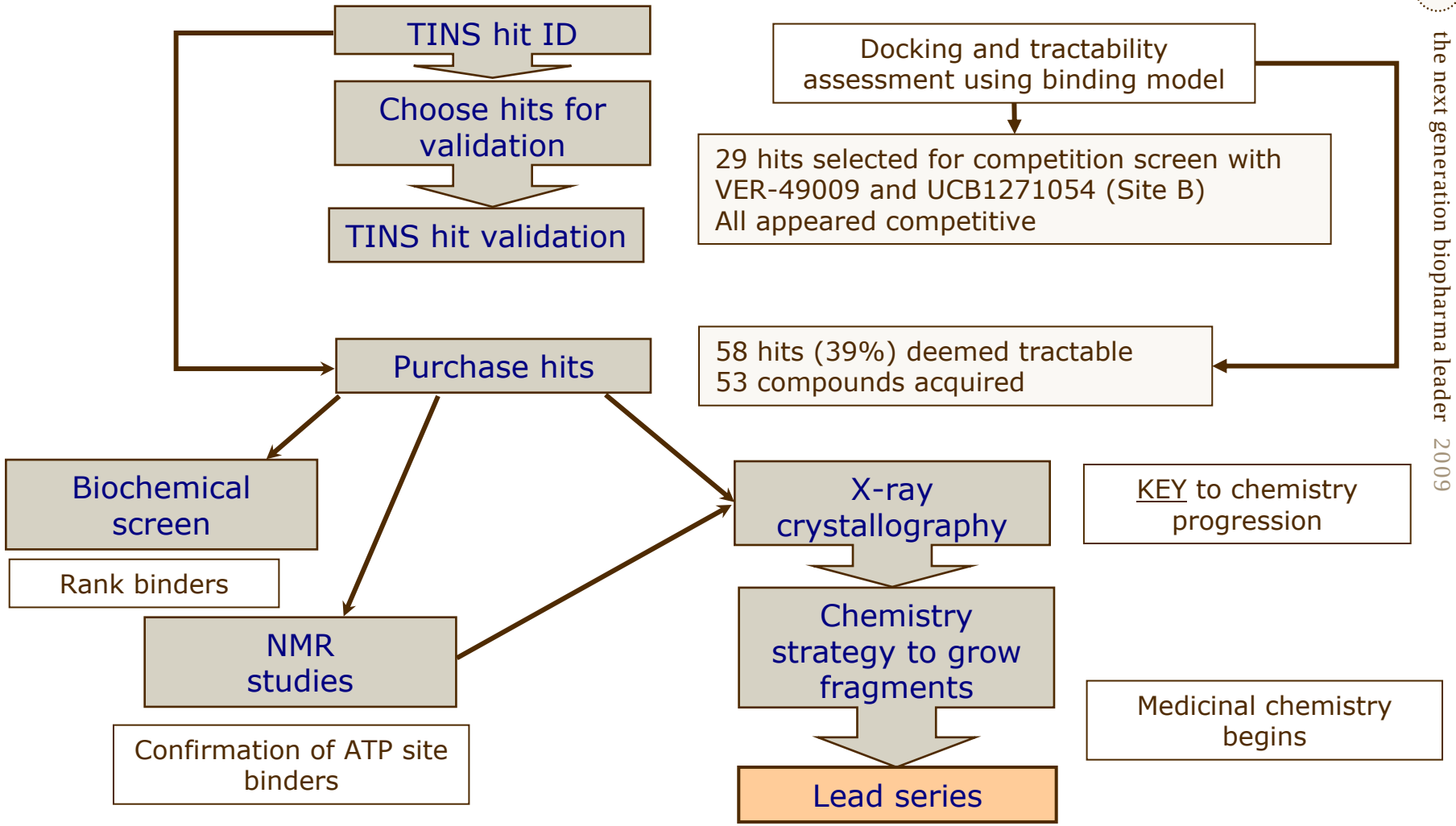


Results

- Confirmation that TINS identifies both Hsp90 binding sites
- 1393 compounds screened
- 3-4 weeks to complete (screening & data analysis)
- 91 hits (>35% difference in signal intensity)
- 6.5% hit rate

	TINS Hsp90	STD NMR Hsp90	Biochemical Fragment Screen Hsp90	Biochemical HTS Corporate Library Hsp90	TINS Kinase	TINS PPI-1	TINS PPI-2
Hits	91	46	37	1	54	106	74
Compounds screened	1393	150	2389	~77000	1439	1414	1459
Hit Rate	6.5%	31%	1.5%	0.000013%	3.8%	7.4%	5.1%

Hsp90 TINS Workflow



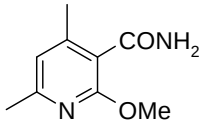
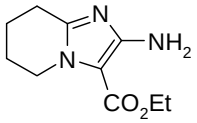
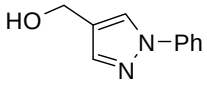
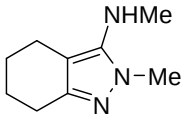
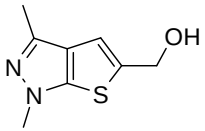
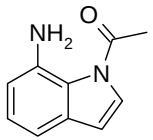
Crystallography

- 53 TINS hits screened in crystallography soaking experiments
- 17 ligand/protein crystal structures obtained (< 2Å)
- Crystallisation success rate 35%
- 6 Site A binders and 11 Site B binders

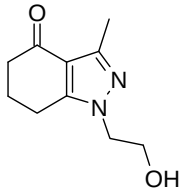
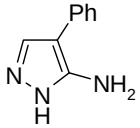
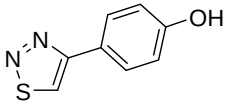
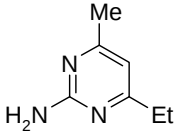
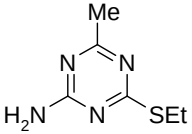
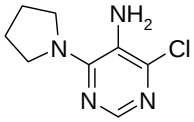
	Attempts	Hit Rate	Site A	Site B
ZB hits	53	32%	6	11
UCB hits	40	12 %	3	2

- Orthogonal screening method to prioritise TINS hits for focused crystallography?
  - HSQC NMR
  - SPR
  - ITC

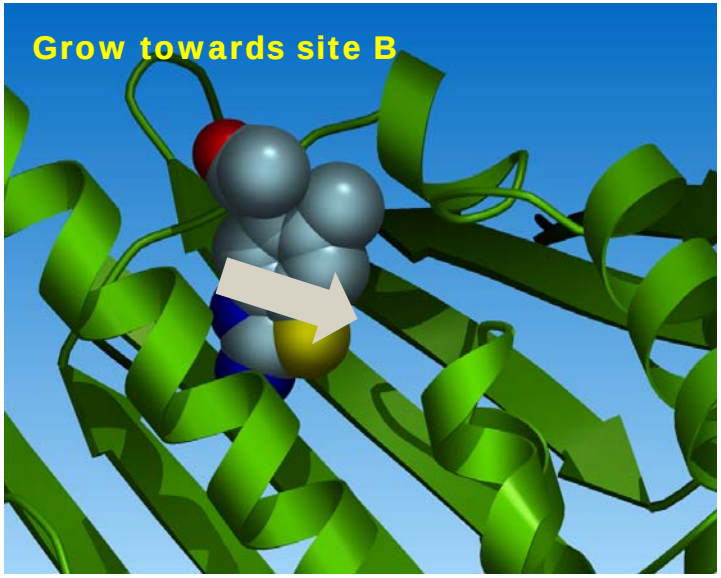
Crystallography Hits

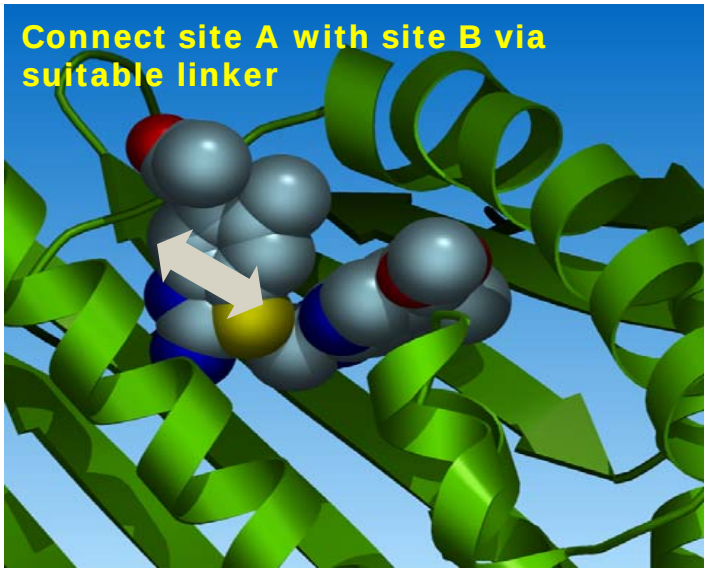
Cmpd		Crystal Structure Binding Site	HSQC Kd mM (s.d)	Bioassay IC_{50} (µM)	Mol wt	Ligand efficiency
1		B	2.75 (1.56)	i/a	180	0.376
2		B	2.65 (0.57)	i/a	209	0.327
3		B	11.7 (1.97)	i/a	174	0.415
4		B	nd	i/a	165	-
5		B	8.89 (4.15)	i/a	182	0.349
6		A	7.11 (4.46)	i/a	176	0.332

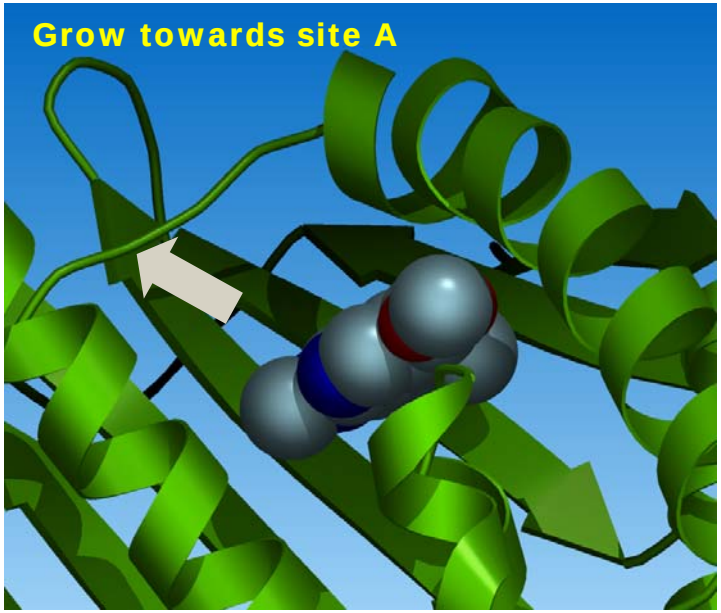
Crystallography Hits

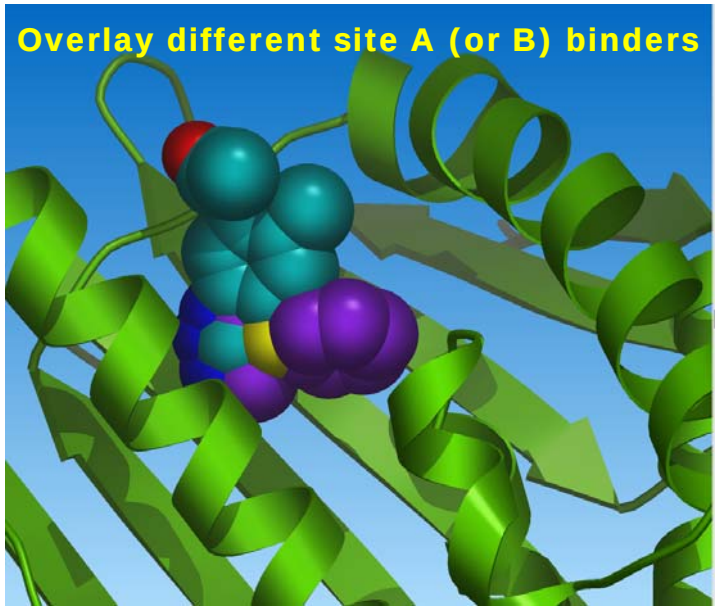
Cmpd		Crystal Structure Binding Site	HSQC Kd mM (s.d)	Bioassay IC_{50} (μM)	Mol wt	Ligand efficiency*
7		B	nd	i/a	194	-
8		A	0.6 (0.085)	i/a	159	0.483
9		A	0.164 (0.032)	i/a	178	0.433
10		A	0.116 (0.034)	715	137	0.540
11		A	0.058 (0.014)	117	170	0.529
12		B	nd	i/a	197	-

Strategies for developing fragment hits

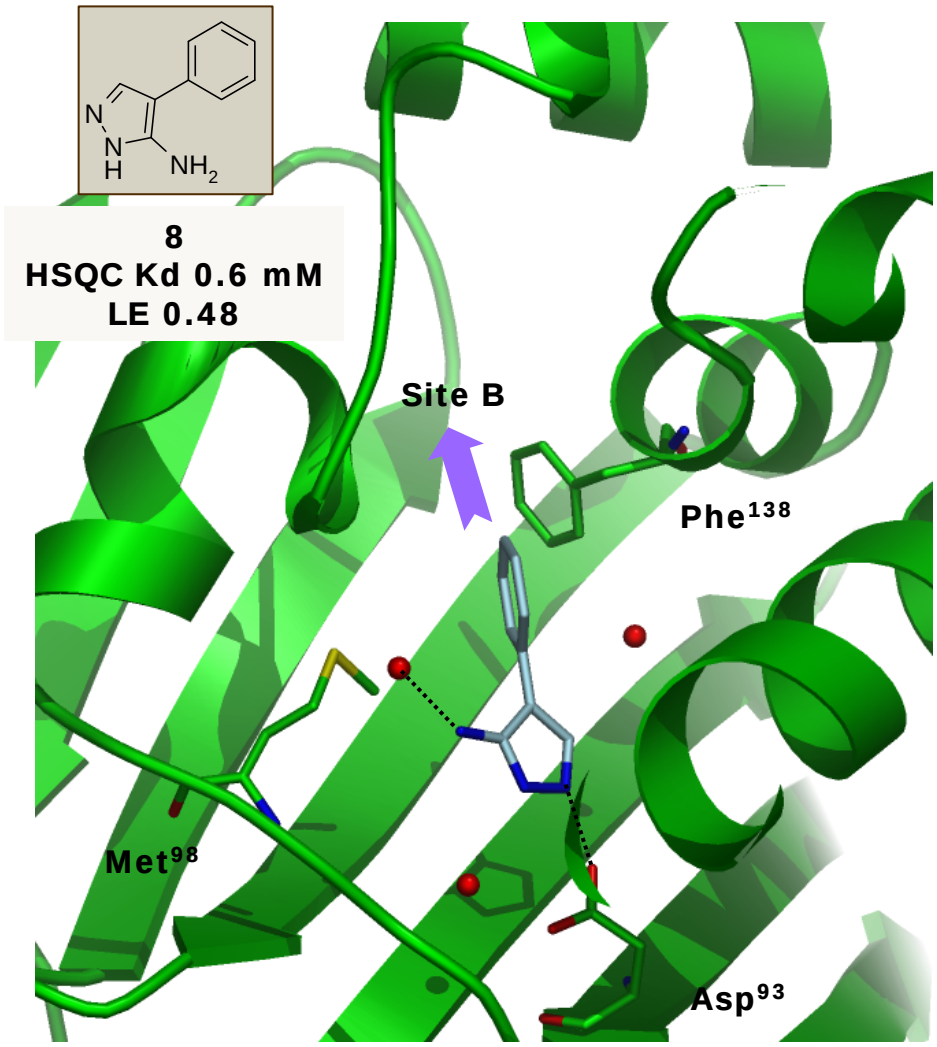


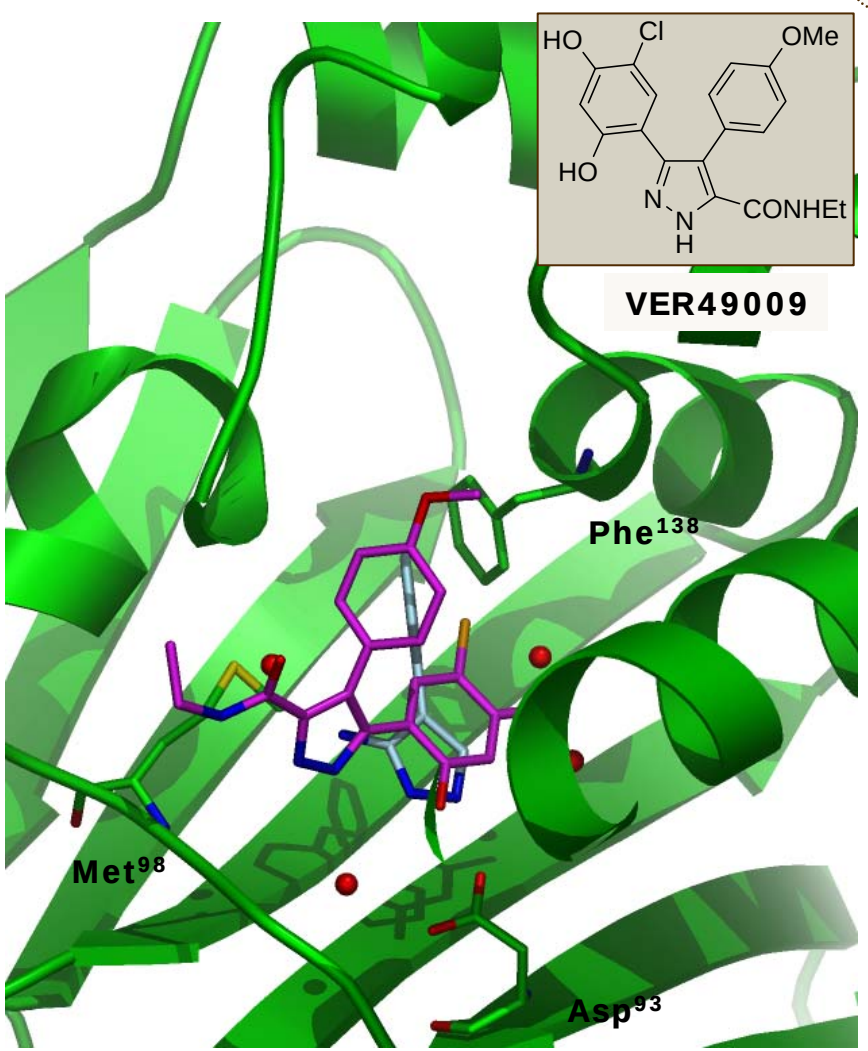




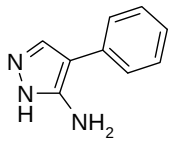
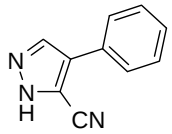
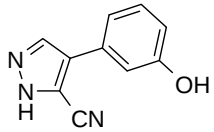
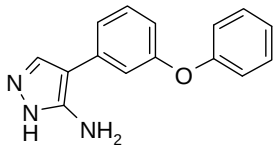
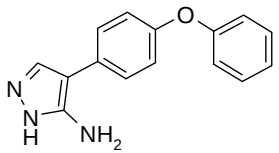


Rational design: growing from Site A to Site B

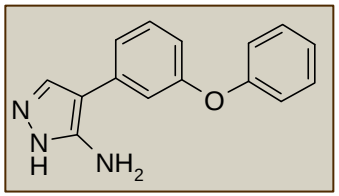




Rational design: growing from Site A to Site B

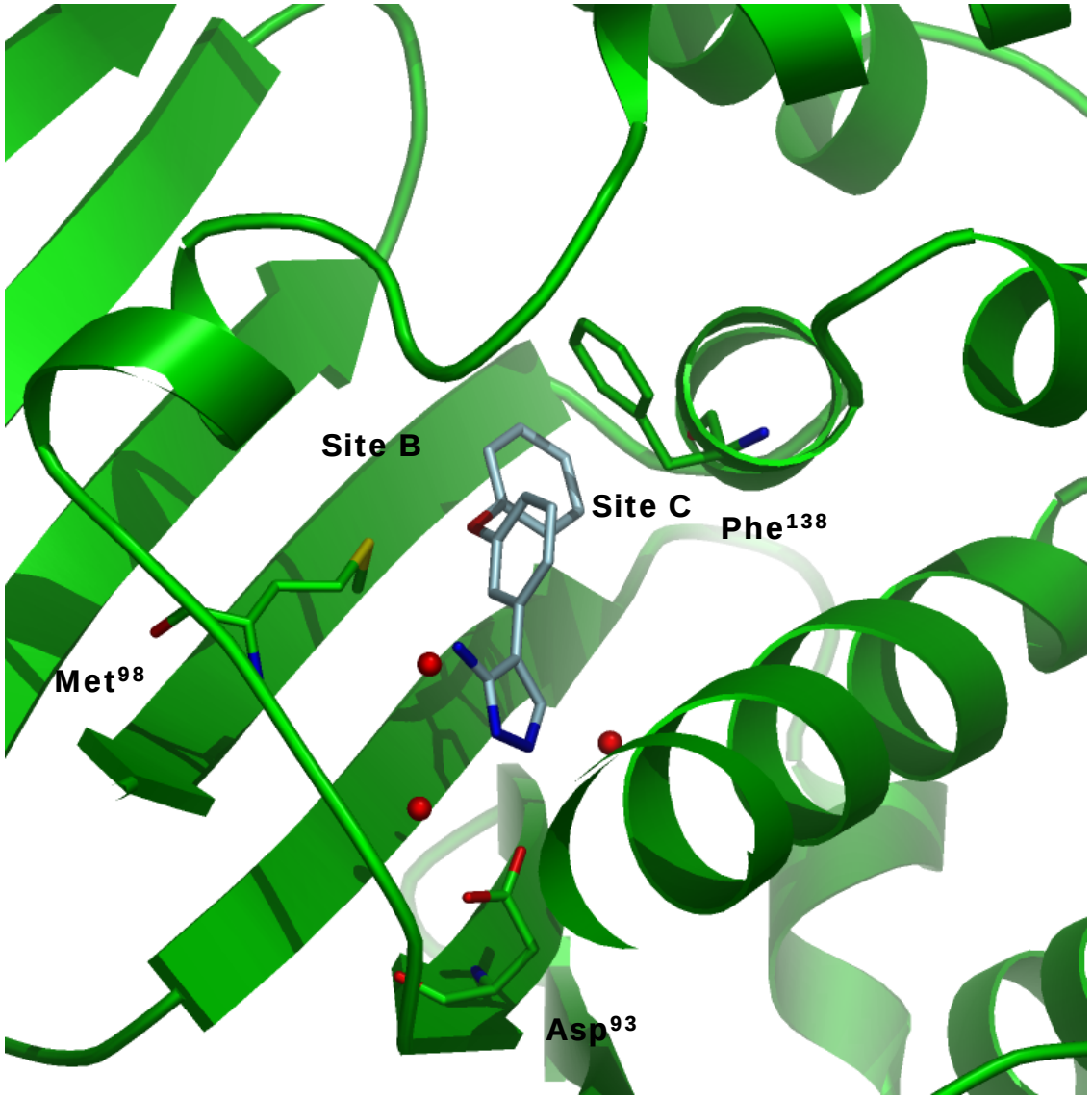
Cmpd		FP activity IC_{50} (μM)	Mol Wt	Ligand Efficiency
8		24% @ 5mM	159	
UCB1423685		435	169	0.355
UCB1425591		436	185	0.329
UCB1423351		235	251	0.262
UCB1423352		22% @ 5mM	251	

Rational design: growing from Site A to Site B

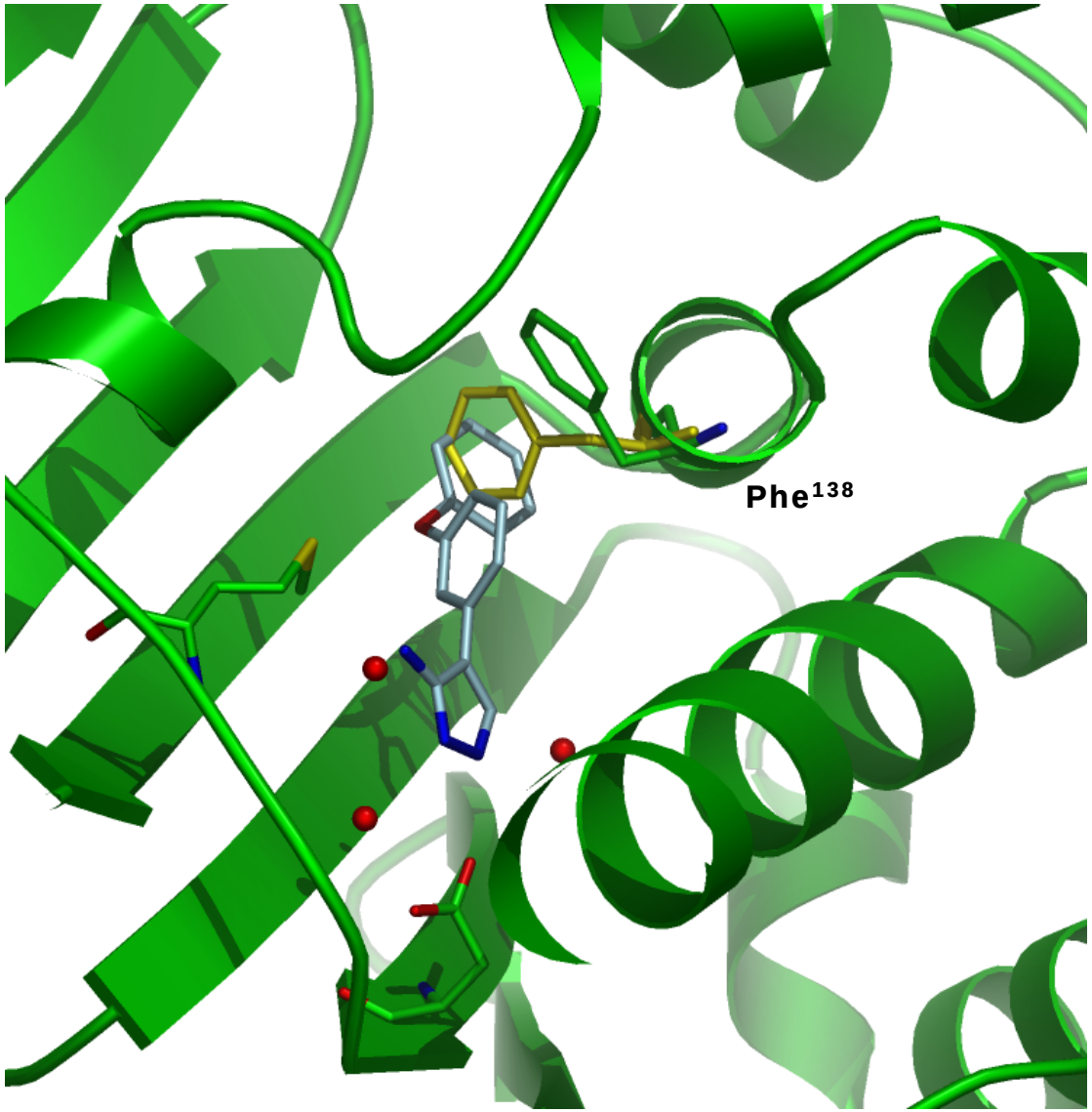


IC_{50} 235 µM

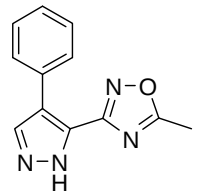
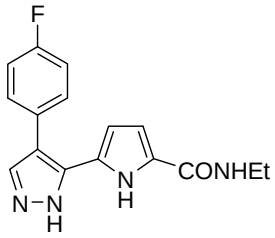
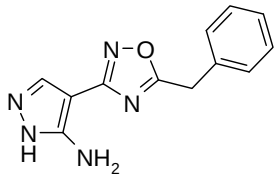
LE 0.262



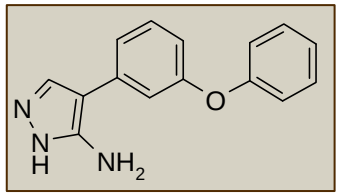
Rational design: growing from Site A to Site B

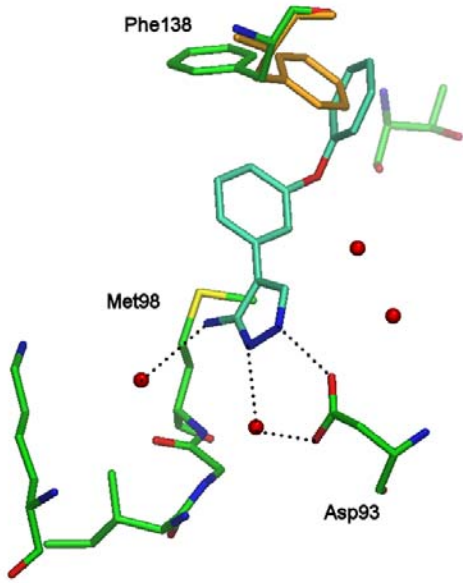


Rational design: growing from Site A to Site B

Cmpd		FP activity IC_{50} (μM)	Mol Wt	Ligand Efficiency
UCB1423761		1165	226	0.237
UCB1424124		3485	298	0.153
UCB1425584		740	241	0.239

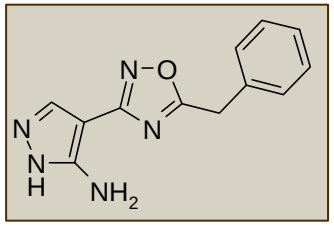
Rational design: growing from Site A to Site B

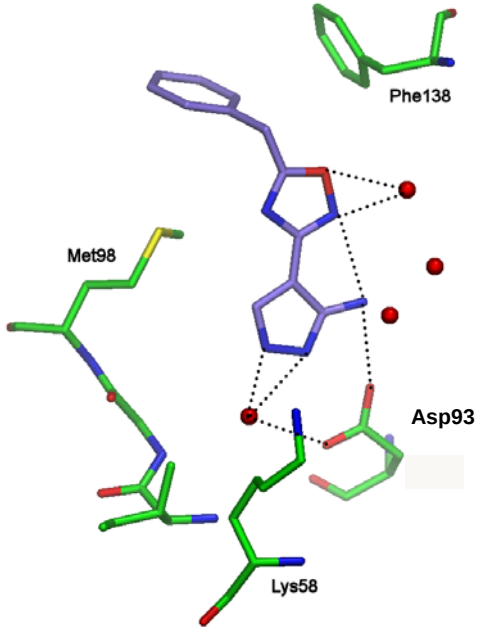




IC_{50} 235 µM

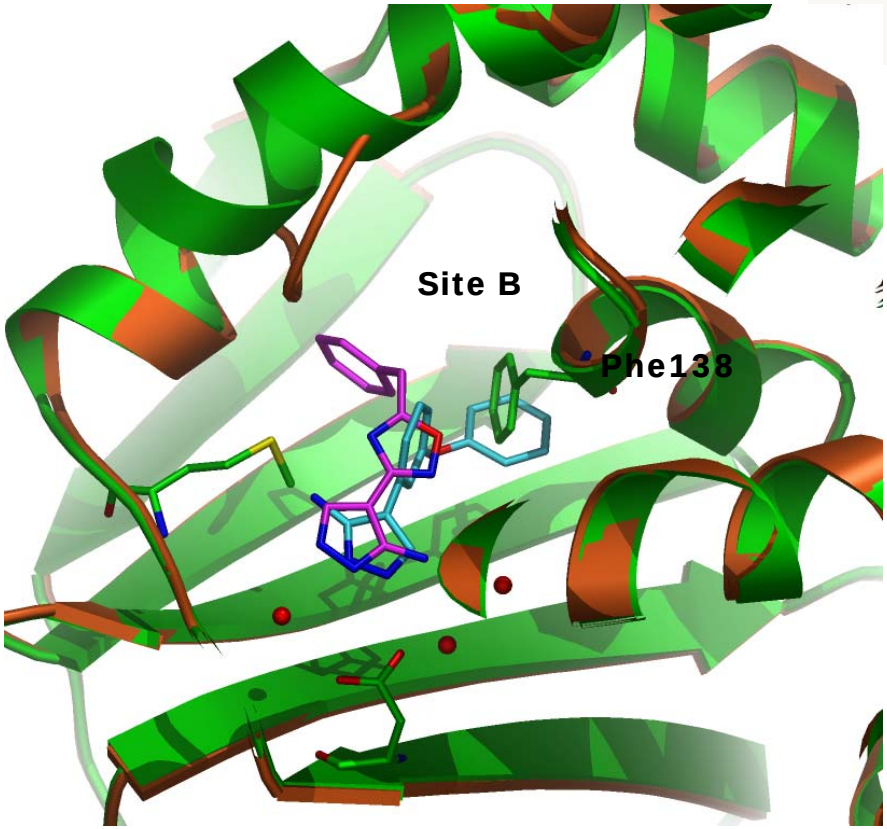
LE 0.262



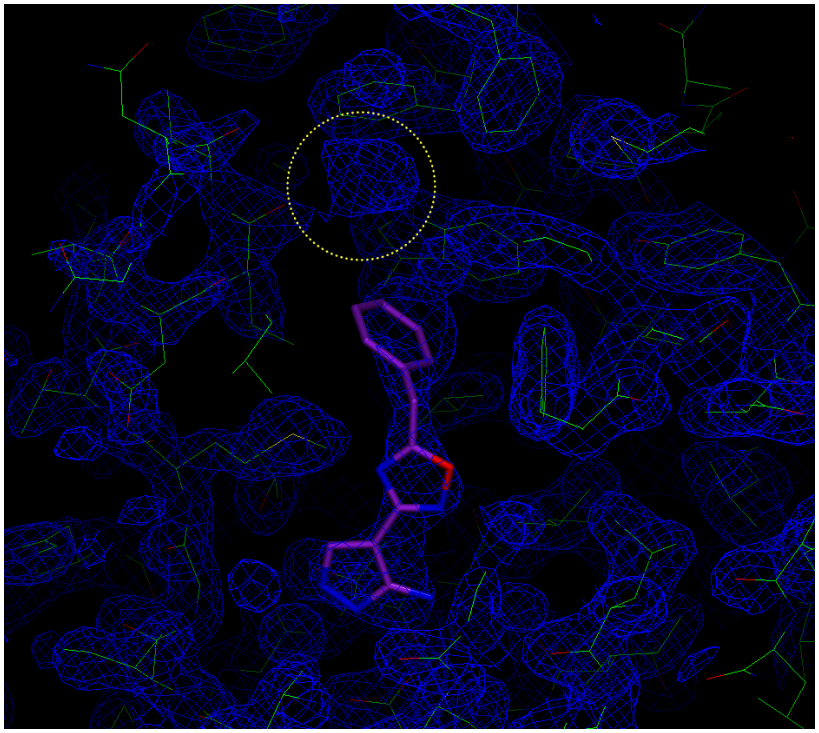


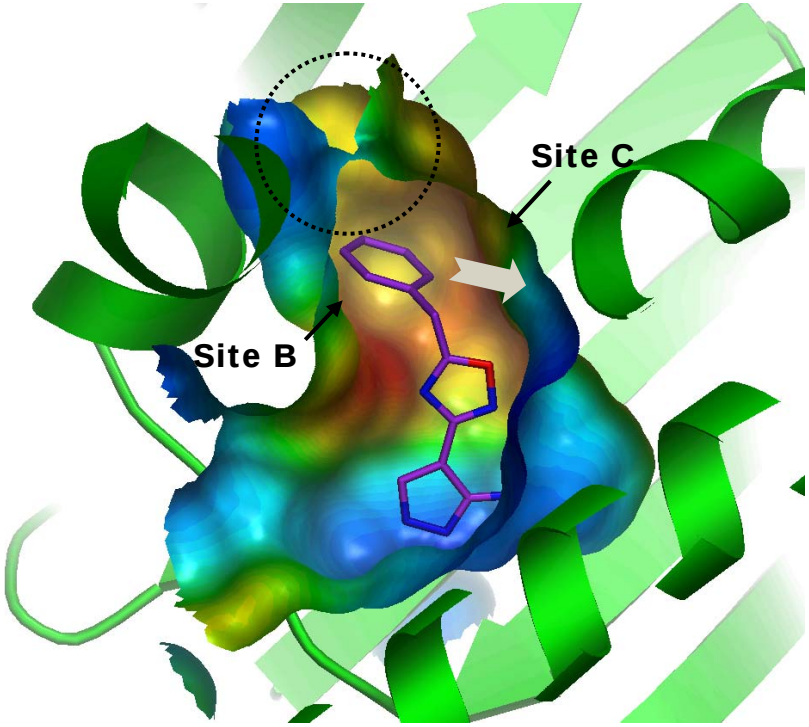
IC_{50} 740 µM

LE 0.239

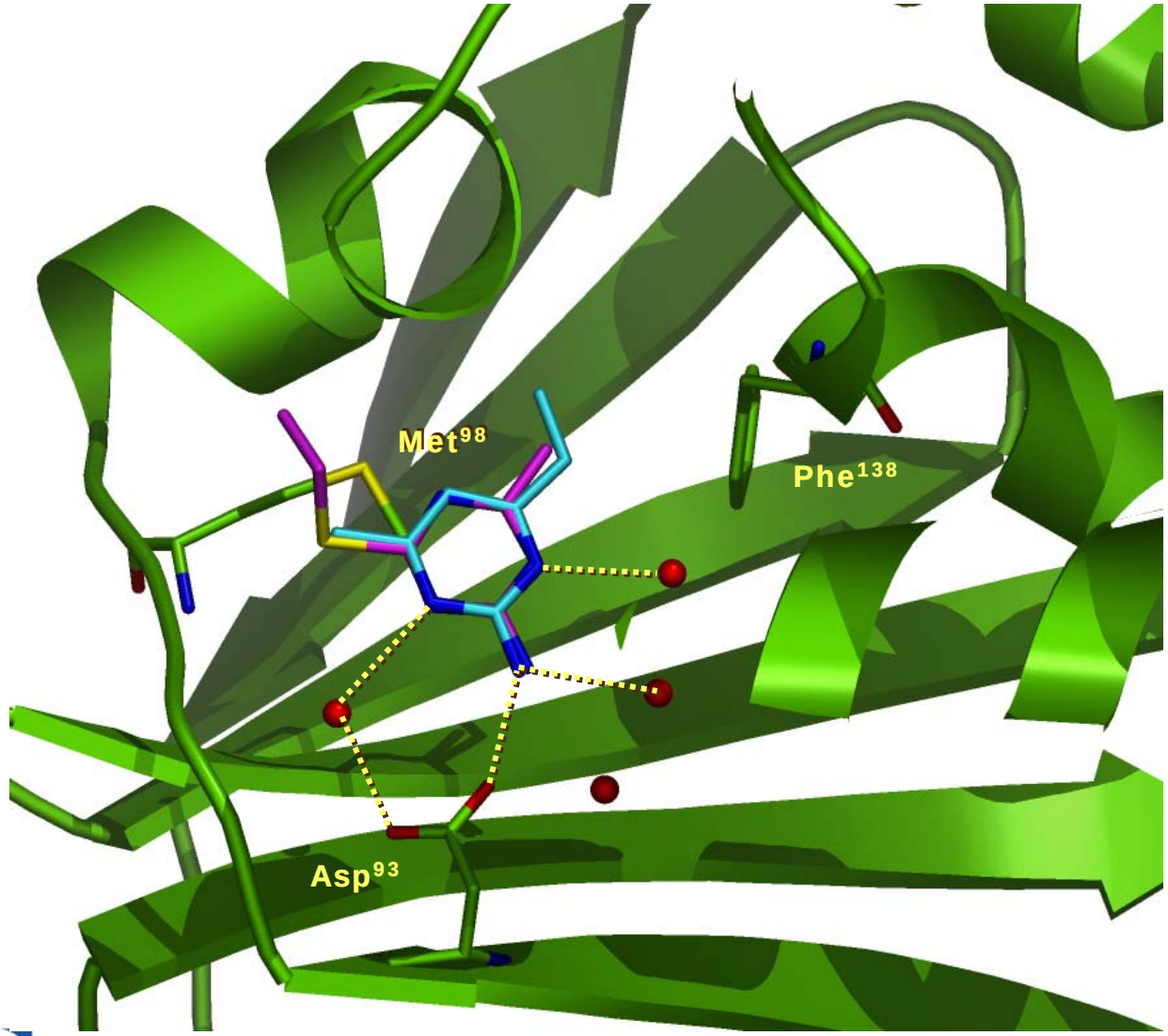


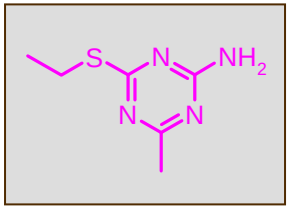
Rational design: growing from Site A to Site B and beyond?



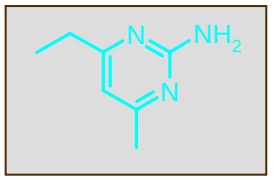


Fragment development: Analoguing

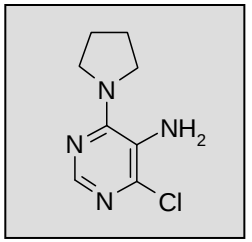




FP IC_{50} 117 uM
Eff 0.540

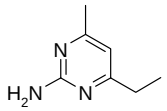
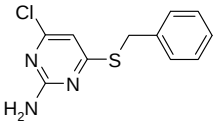
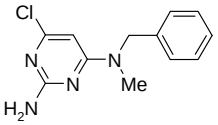
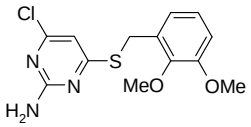
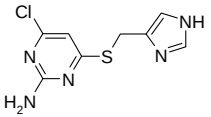
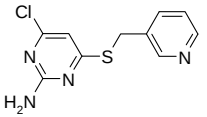
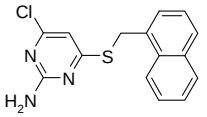
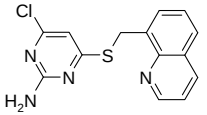


FP IC_{50} 750 uM
LE 0.522

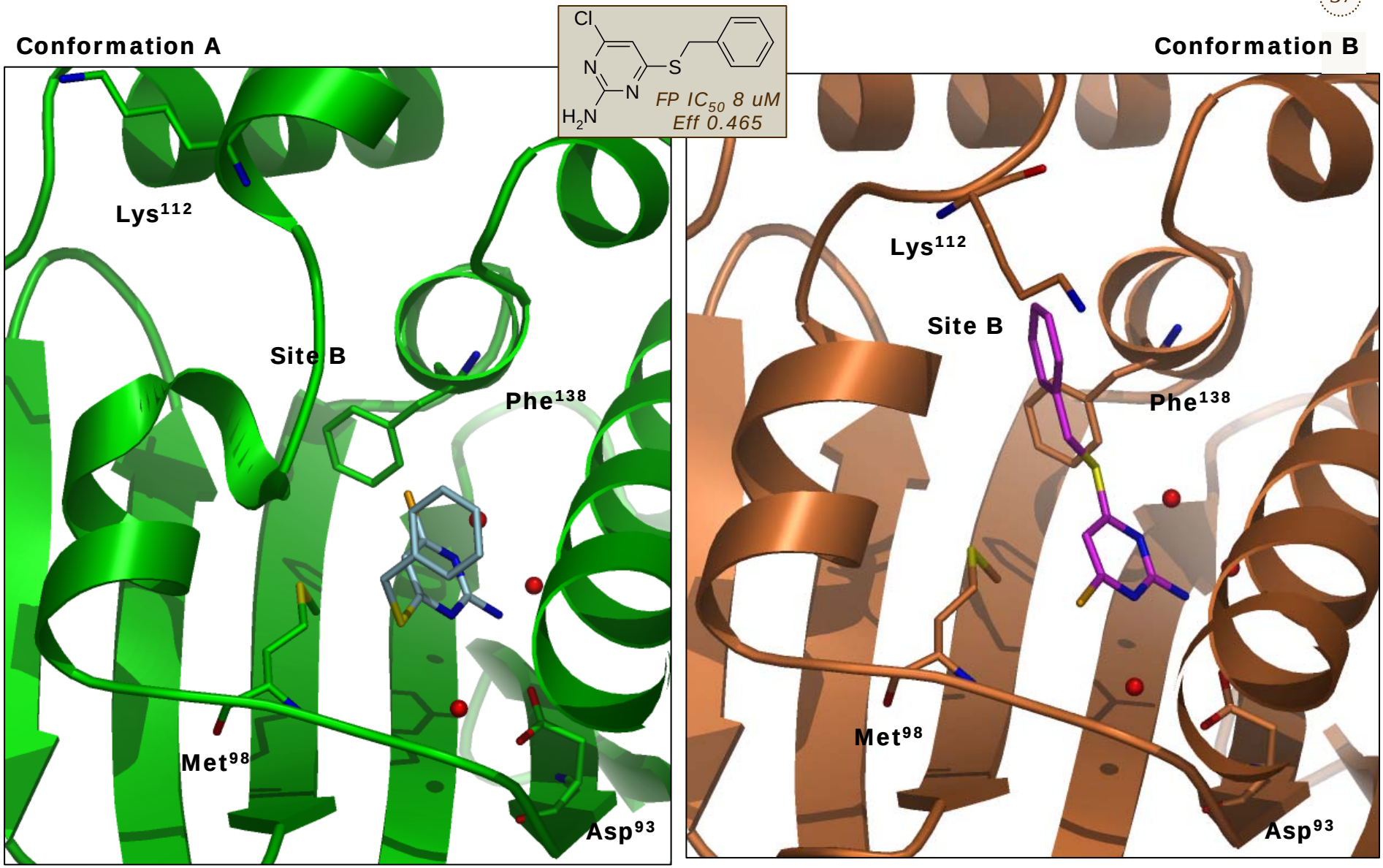


FP IC_{50} inactive

Fragment development: Analoguing

Cmpd		FP IC_{50} (µM)	Mol Wt	Ligand Efficiency
UCB1415551		750	137	0.522
UCB1168620		8.0	251	0.465
UCB1425888		154	249	0.372
UCB1428877		10.5	310	0.362
UCB1430535		15.4	241	0.466
UCB1430219		5.6	252	0.470
UCB1428616		1790	301	0.280
UCB1429640		9.0	302	0.391

Fragment development: Analoguing



Summary

- A number of methods for finding Hsp90 fragment binders have been explored
- TINS methodology has been validated
- Structural information drives understanding of binding modes
  - Crystallography is not always successful-ideally need orthogonal methods
  - Don’t overlook analoguing!
- Potential medicinal chemistry starting points identified
- This target is no longer being pursued because of strategic changes

Acknowledgements

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