



p38 α : A fragment-based drug discovery case study at BioFocus

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MipTec
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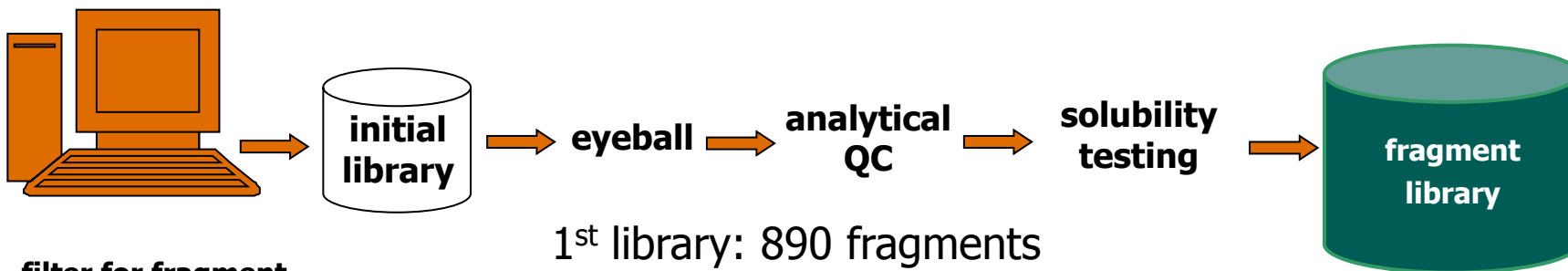




Outline

- BioFocus Fragment library
- Screening technologies
 - surface plasmon resonance (SPR)
 - mobility shift assays (CaliperLS)
 - fluorescence lifetime technology (FLT)
 - X-ray crystallography
- Results of a case study: p38 α

BioFocus fragment library Generation



filter for fragment-like properties

removal of reactive functionality

1st library: 890 fragments
• 260 unique to BioFocus

2nd library: 1159 fragments
• mainly commercial
• rule of 3 compliant
• **QC & solubility ongoing**

Analytical QC
analysed by LC/MS,
90% purity pass

Solubility QC
kinetic solubility tested
>750 μM in 10% DMSO in PBS

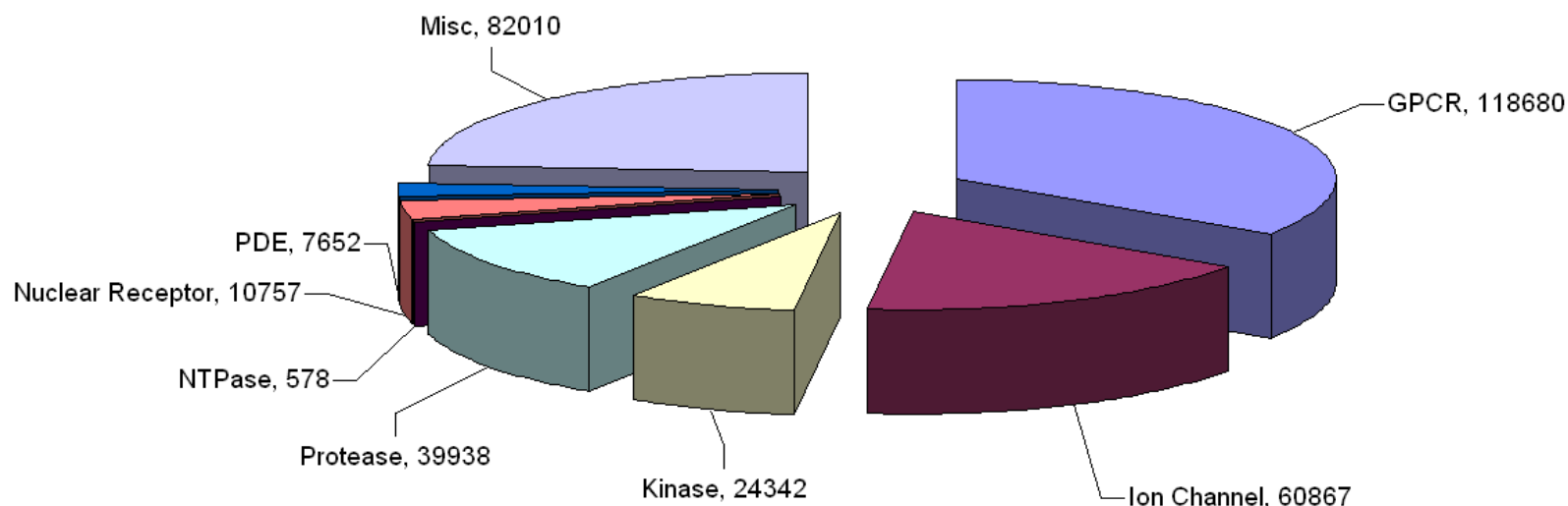


Coverage of chemical space

- Assessment of diversity is not trivial
 - a frame of reference is necessary as diversity is relative
- ChEMBL database designed for data-mining
 - bio-active compounds, biological targets and pharmacological effects
 - >578K compounds, ~7.5K targets, >2.7M data-points
- 190K ligands with affinity $<1\mu\text{M}$ for their respective target extracted as test set (representative for 'drug-like' space)
- Exact match experiment with the BioFocus fragment library

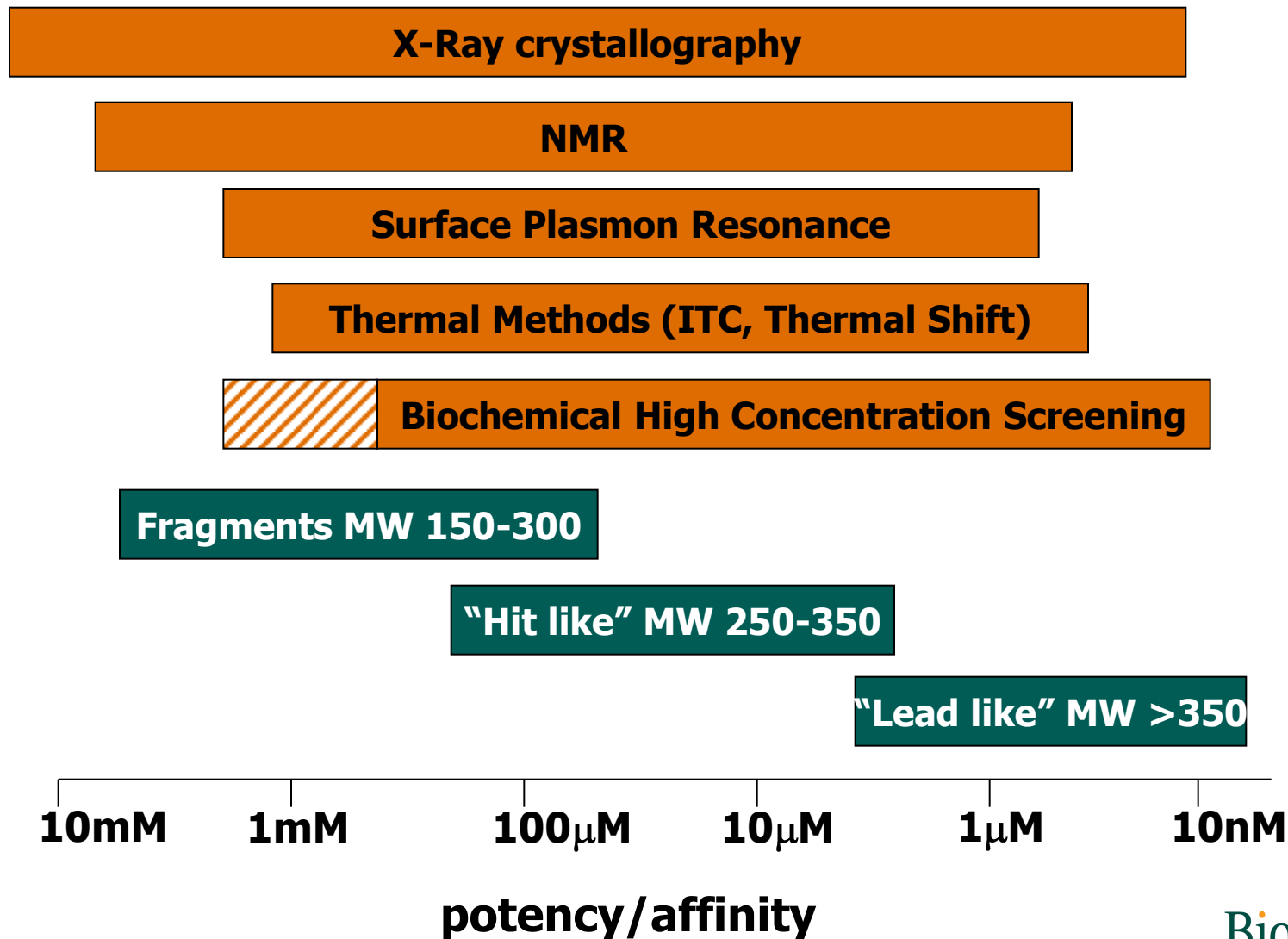
Diversity analysis

- 1060 of the 2051 fragments are present as substructures of test set (52%)
 - covers ~94K diverse ligands and 2,141 targets (one fragment typically occurs in multiple drug-like compounds)





Screening method options



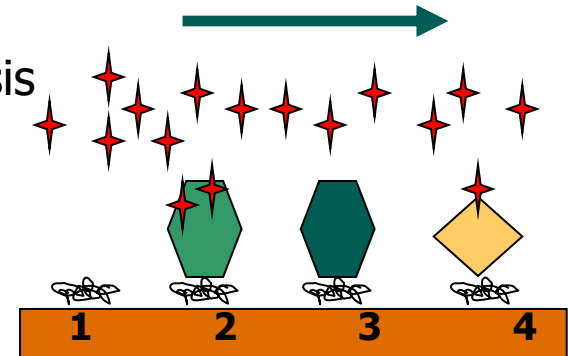


Screening platform at BioFocus

- Biophysical screening methods
 - surface plasmon resonance screening (Biacore)
 - thermophoresis (Nanotemper) (see poster P140)
 - NMR (in collaboration with Zobio)
- Biochemical screening methods
 - high concentration biochemical screening (various platforms, including mobility shift (LC3000) , FLT)
- Structural methods
 - X-ray crystallography (using ESRF or Diamond synchrotron)

Surface plasmon resonance (SPR)

- Biacore offers analysis on multiple flow cells in parallel (T100)
 - blank surface for non-specific surface binding
 - single surface for target protein capture
 - at least two remaining surfaces for analysis
- Options to screen
 - protein targets for non-specific binding
 - E.g. carbonic anhydrase / GST
 - targets selected for specificity
 - different forms of target enzyme
 - active vs unactive kinase
 - zymogen vs fully processed protease
 - wild type vs mutant enzyme



Microfluidic mobility shift assay

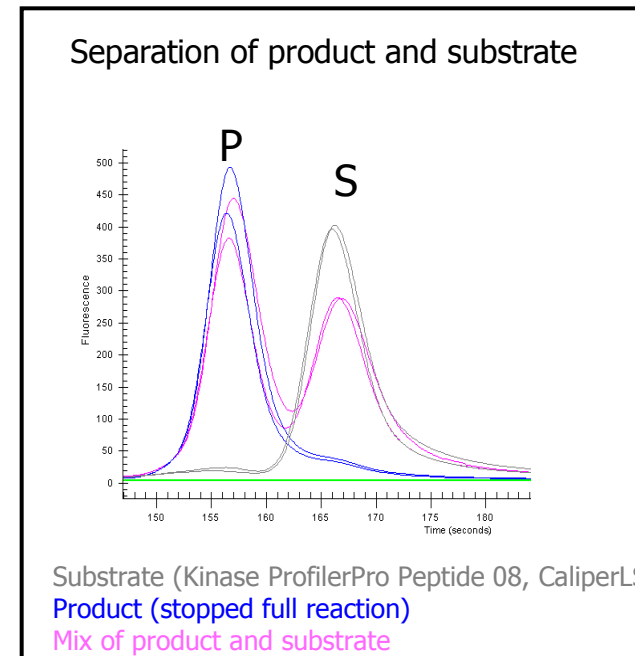
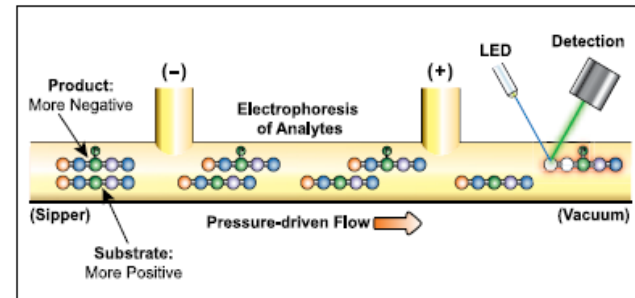
LC3000, Caliper LS

- Principle

- The reaction mixture is sipped from 384-well assay plates into a capillary with a voltage potential
- Substrate and product are therefore separated based on their different charges and masses and are visualized via laser-induced fluorescence

- Target classes:

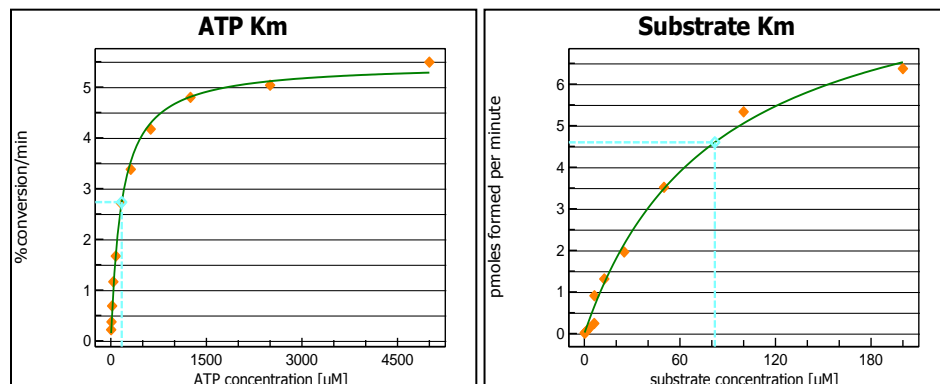
- kinases
- proteases
- phosphatases
- histone deacetylases (HDAC)
- phosphodiesterases (PDE)
- acyl-transferases
- ...



Assay development LC3000

p38 α

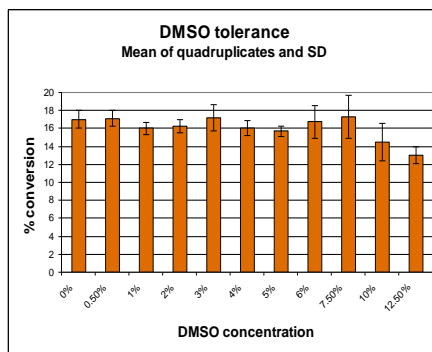
K_m determination



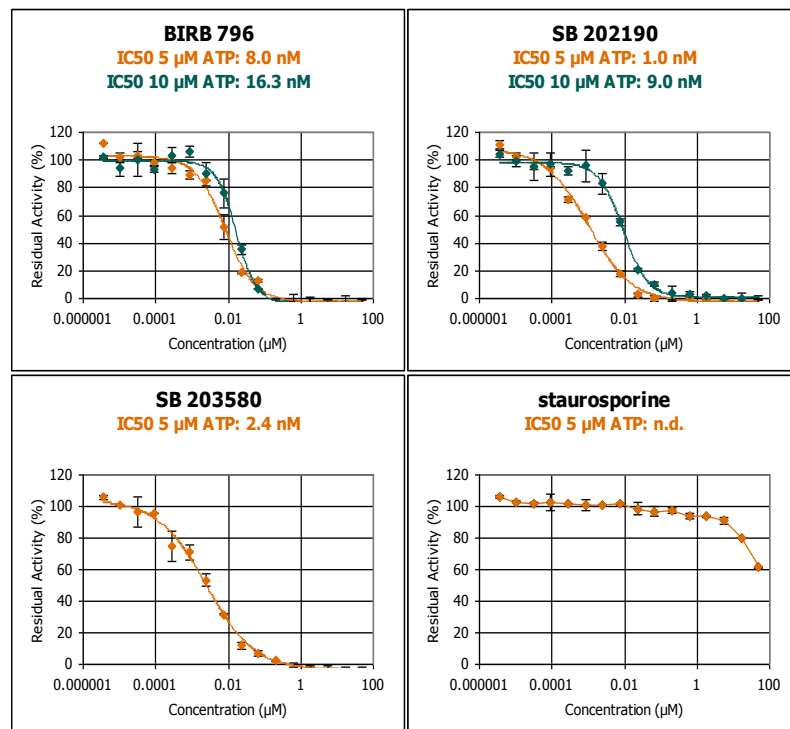
- ATP $K_{m,app}$ 171 μ M
- Substrate $K_{m,app}$ 73 μ M

DMSO tolerance

- Up to 10% tolerated



Reference inhibitors



- Sensitivity for reference cpds in line with literature values

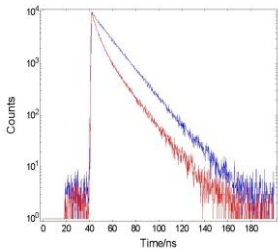
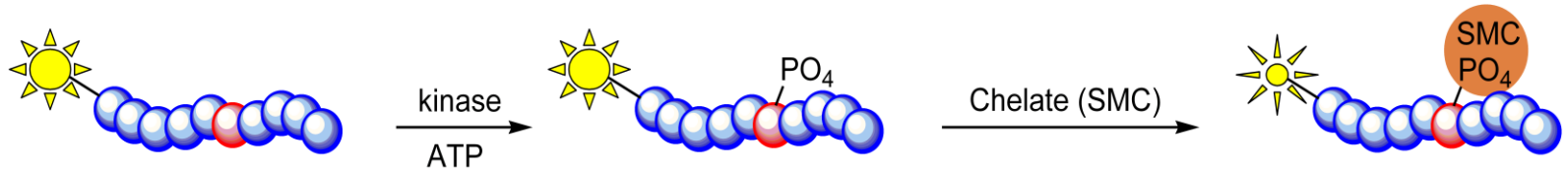


FLT

Almac *FLEXYTE*TM Platform

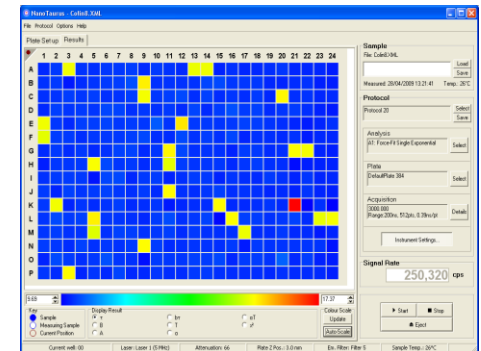
Edinburgh Instruments NanoTaurus Plate reader

• Principle



Example lifetime decay curves for **substrate** and **phosphorylated product**

- 1) 9-aminoacridine (9AA) labelled generic peptide substrate with long fluorescence lifetime
- 2) Kinase phosphorylates Ser or Thr residue
- 3) Small molecule chelate (SMC) complexes phosphate group and effects a reduction in fluorescence lifetime of up to 5 ns
- 4) Phosphorylation dependent decrease in fluorescence lifetime measured on FLT plate reader (i.e. NanoTaurus)



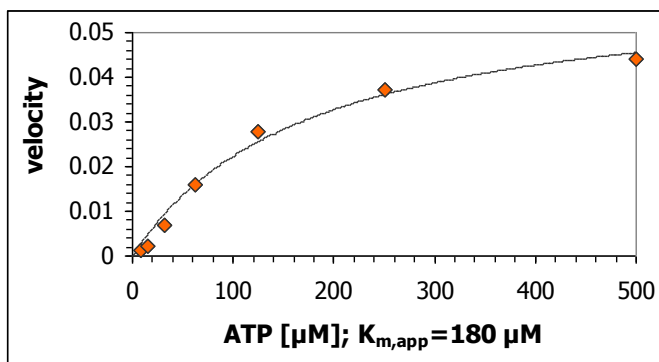
- p38 α outwith scope of *FLEXYTE*TM generic substrates (confirmed)
- A *FLEXYTE*TM peptide substrate tailored for p38 α was developed by Almac and used in all subsequent experiments



Assay development FLT

p38 α

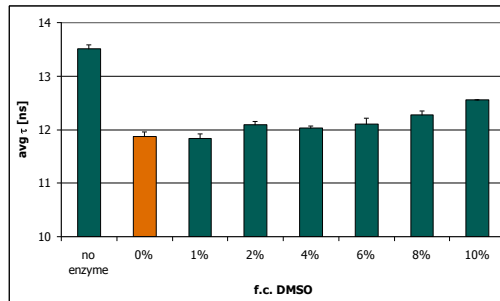
K_m determination



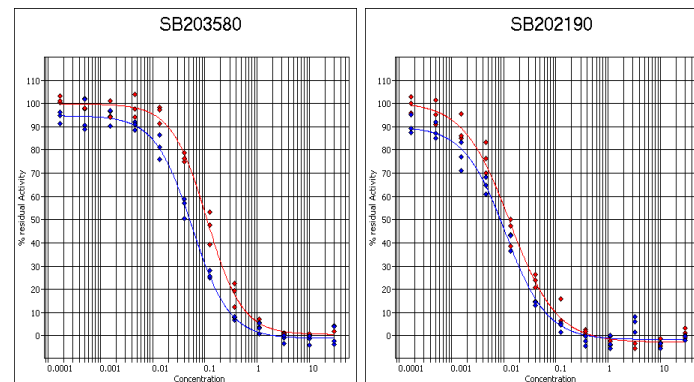
- K_m value comparable to result obtained on LC3000 (170 μM)

DMSO tolerance

- Up to 6% tolerated



Reference inhibitors



IC₅₀ SB203580: 60-100 nM

IC₅₀ SB202190: 10 nM

- Sensitivity for reference cpds in line with literature values



Screening

Case study – p38 α

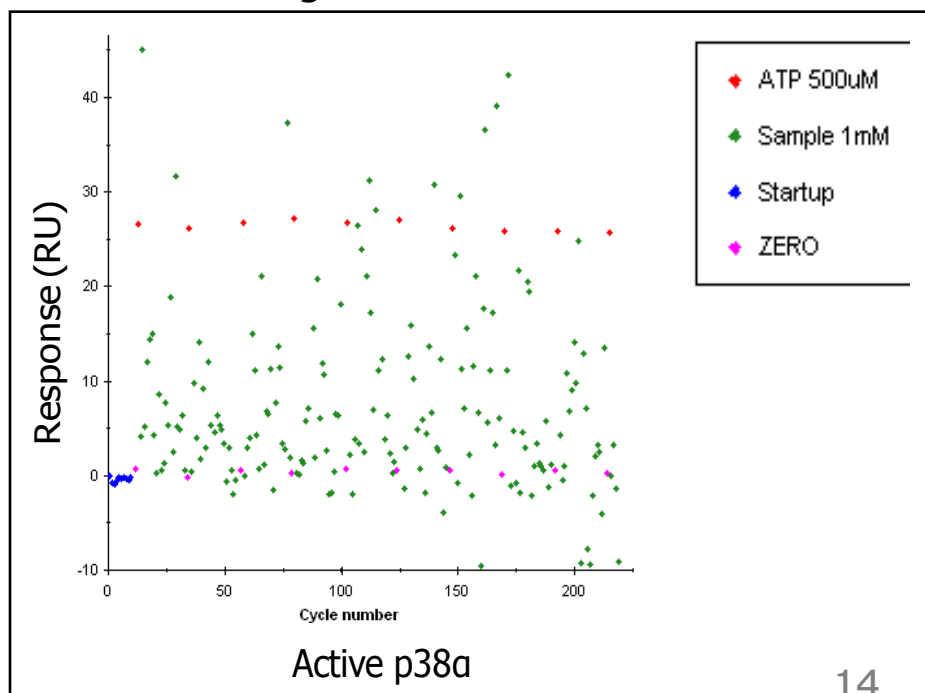
- p38 α : well-studied MAP kinase (autoimmune diseases)
- Can FBDD reveal new insights?
- BioFocus fragment library was screened
 - SPR screen conducted
 - 262 fragments screened at 200 μ M/1 mM (subset of BioFocus library)
 - ~8% hit rate; binding affinity ~0.2 to 10 mM
 - Screen on LC3000
 - 1 mM/ 250 μ M
 - Using a cut-off of >50% inhibition: 5.6% hit rate at 1 mM, 2.1% at 250 μ M
 - Screen subset of 42 fragments in FLT
 - 250 μ M

p38 α case study

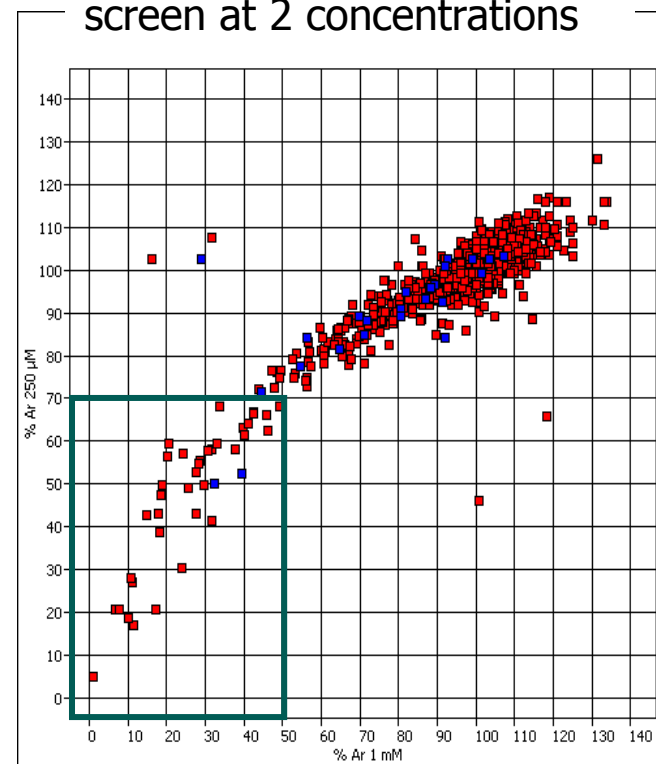
Primary screening data SPR – LC3000

- Robust assay performance:
 - SPR: Z' 0.78-0.92
 - LC3000: all plates with Z' > 0.6

SPR: Screening data at 1 mM



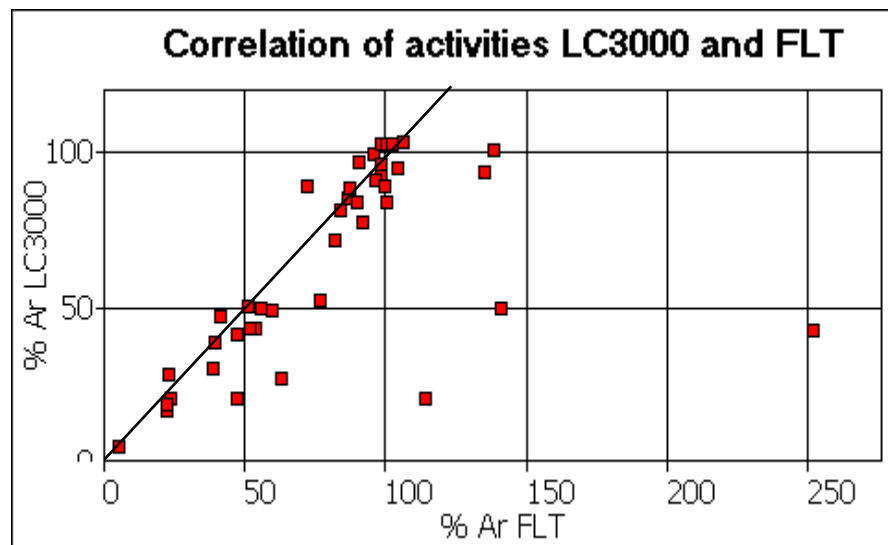
LC3000: Activity correlation screen at 2 concentrations



Blue squares: top 24 cpds
from affinity testing

FLT and Caliper based screens

- 42 fragments were tested in FLT
 - 24 Top fragments SPR + actives in LC3000
- Test conditions FLT:
 - 250 μM cpd conc; 150 μM ATP
- Compared with data from LC3000:
 - 250 μM cpd conc; 10 μM ATP



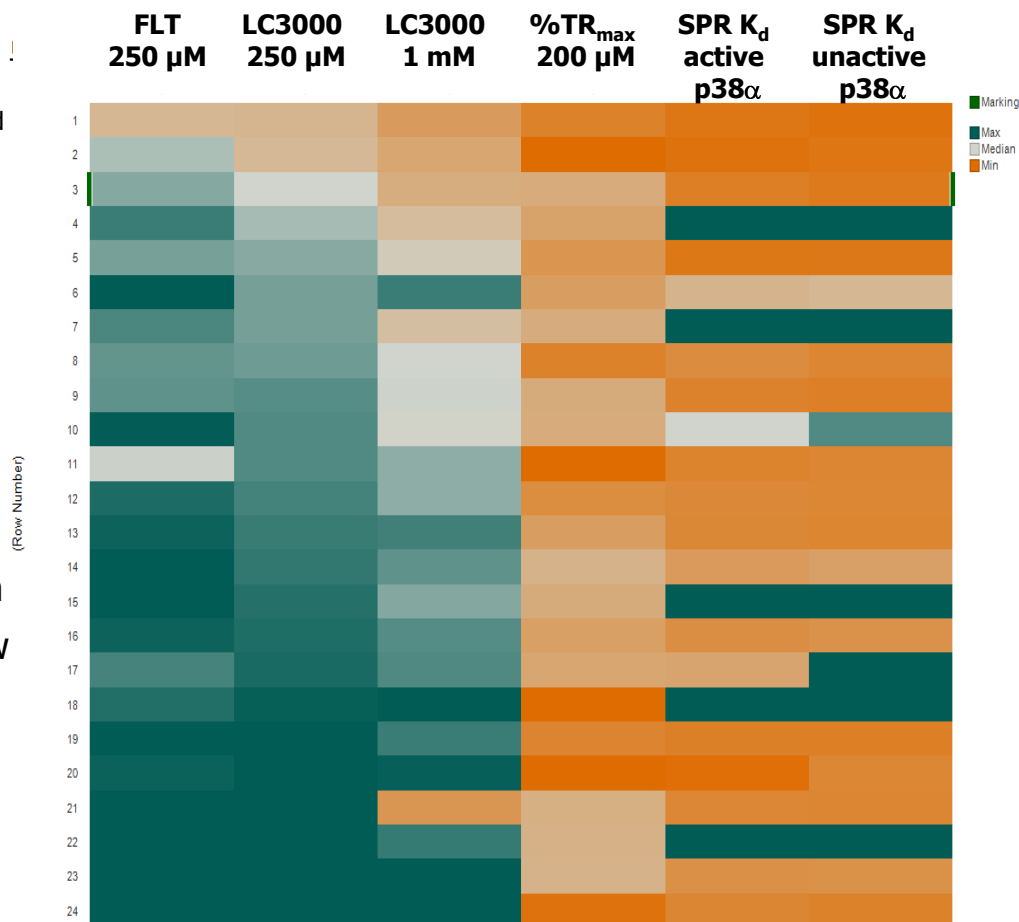
 Good correlation between Caliper LC3000 and FLT screening data



Case study p38 α

Comparison of SPR, LC3000 and FLT

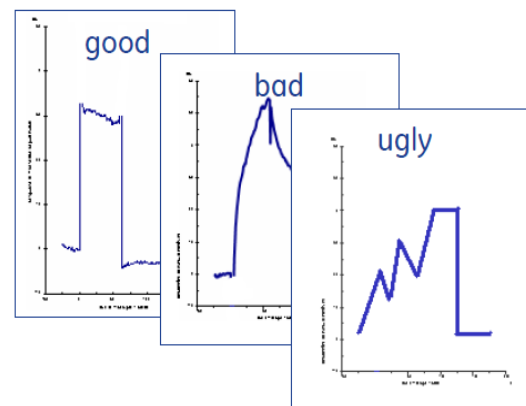
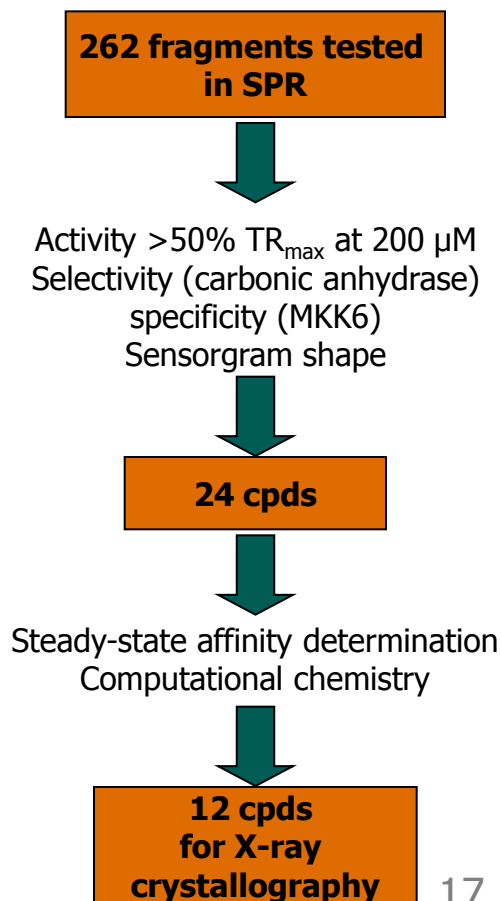
- 24 cpds selected based on SPR results:
 - Data for LC3000, FLT, K_d values from SPR and $\%TR_{Max}$ were available
- The data obtained in the assays were normalized each on scale 1-100:
 - $\%$ effect for FLT and LC3000 or $\%TR_{Max}$
 - K_d values in mM were multiplied by 10, fragments with $K_d > 10$ mM shown in dark green
 - Orange: high activity/low K_d
 - Green: low activity/high K_d



p38 α case study

Prioritization of fragments based on biophysical screen

- Prioritize compounds for structural determination according to binding characteristics





p38 α case study

Confirmed hits – structure determination

Compound	Molecular mass	Active p38 α K _D (mM)	Ligand efficiency
A	195	1.59	0.29
B	214	2.21	0.23
C	234	1.97	0.23
D	235	1.84	0.25
E	240	0.78	0.24
F	208	0.22	0.33
G	218	0.79	0.26
H	236	1.54	0.23
I	212	2.34	0.22
J	185	1.27	0.28
K	245	3.19	0.19
L	210	1.38	0.30

**X-ray crystal
structures
solved to date**

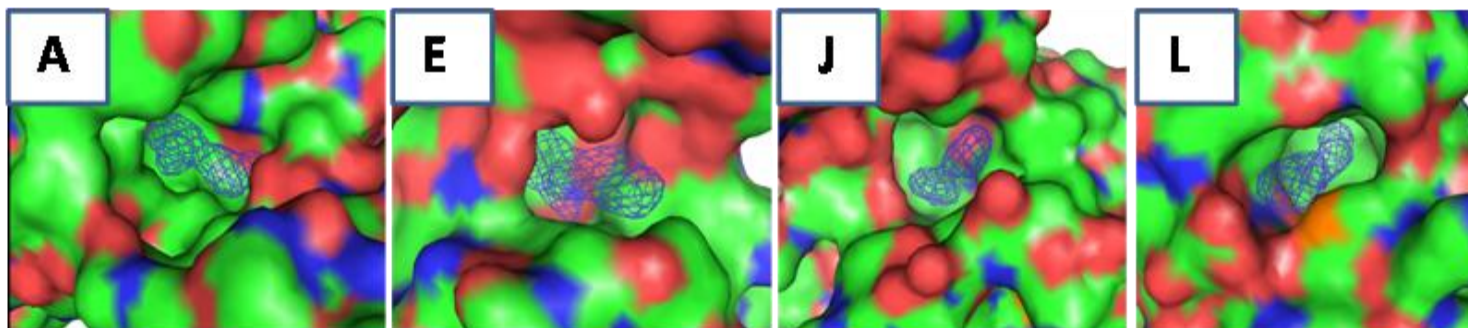


p38 α case study

Structure determination (ongoing)

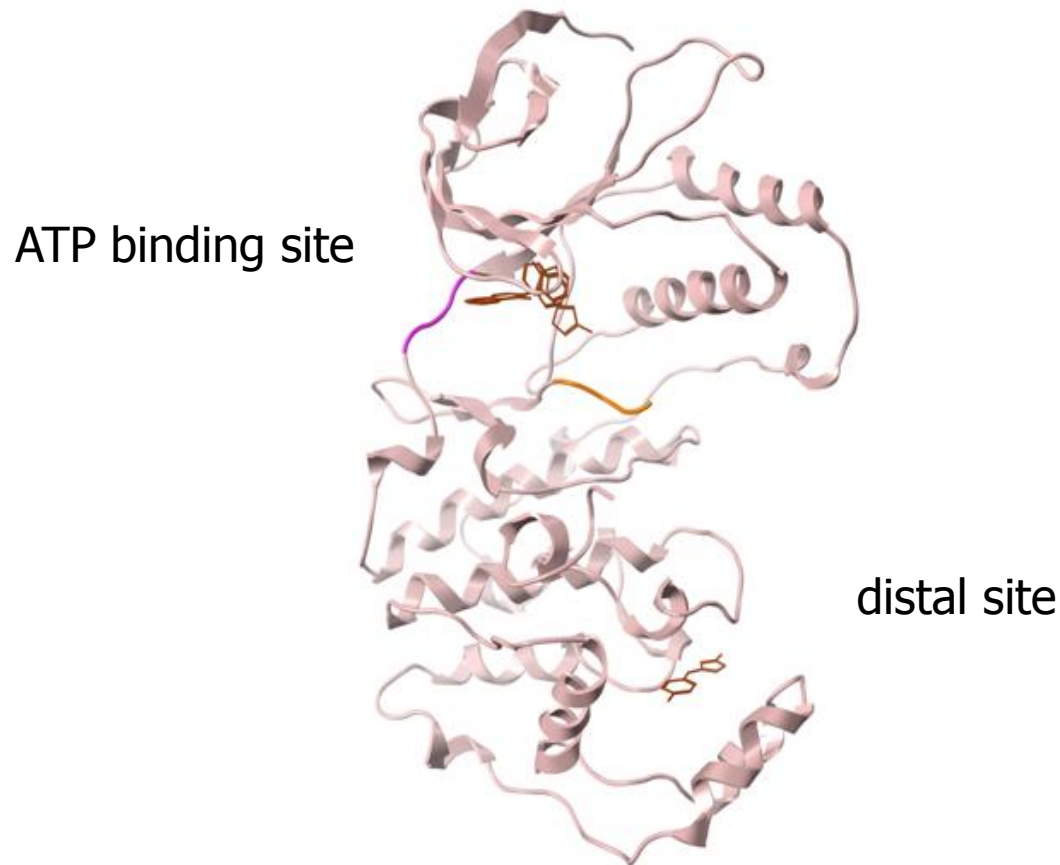


- 12 compounds selected after SPR screen
- Co-crystal structures to ~ 2.3 Å resolution obtained via soaking
- Active site binding mode of three compounds identified (A,E,J)
- Compound L binds away from the active site



Case study – binding sites of p38 α

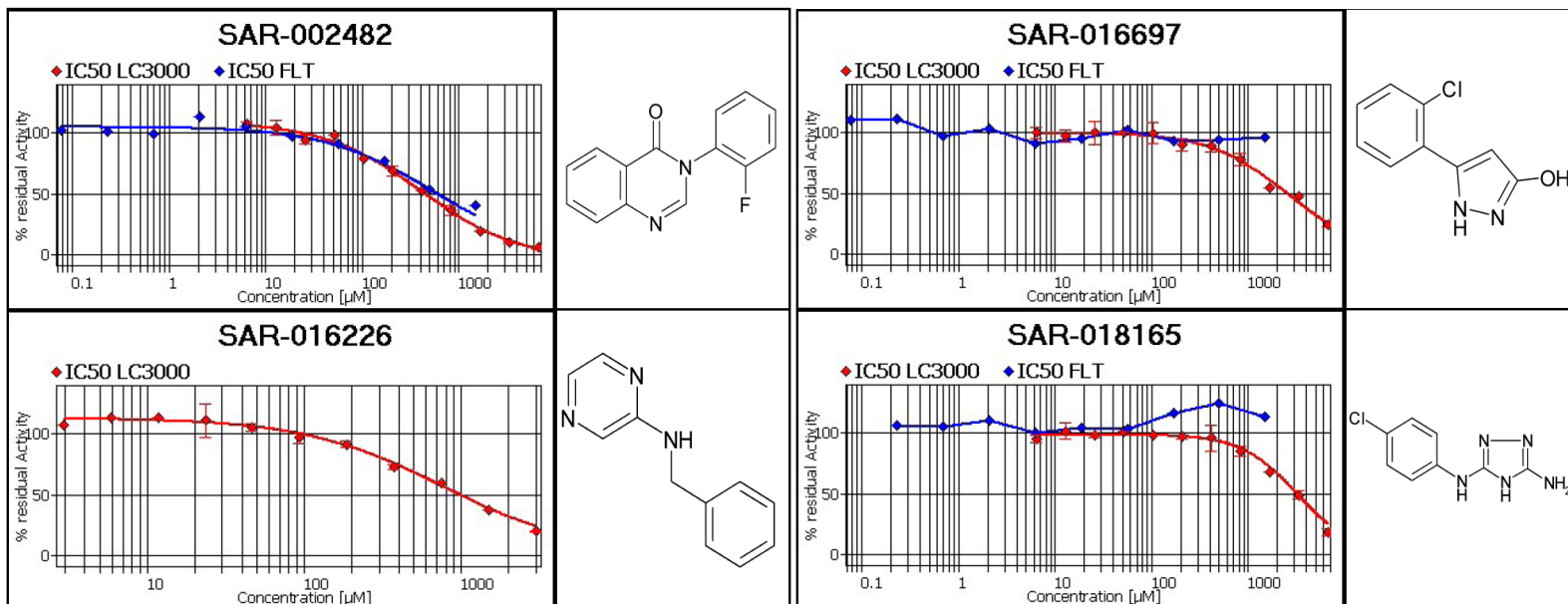
- Ligands with various binding modes and binding sites



IC50

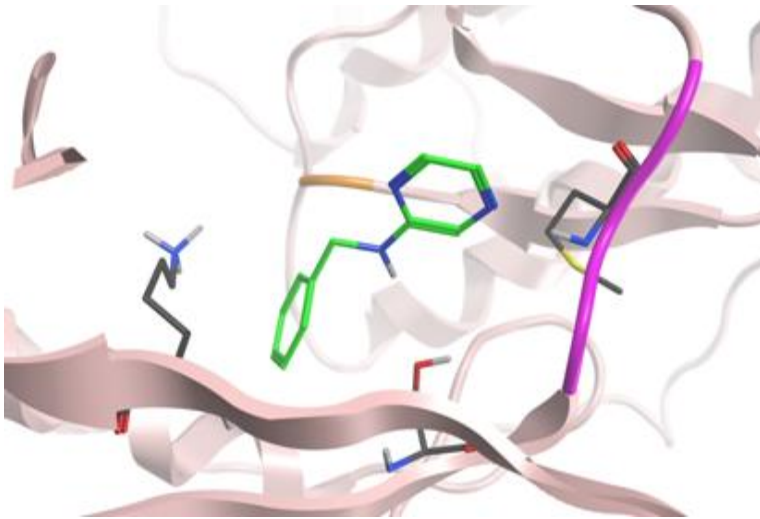
- Comparison K_d Biacore and IC50 in LC3000 and FLT for the four fragments with resolved X-ray structure
 - good correlation of IC50 and K_d s obtained in SPR.
 - no problem to generate dose response curves at very high fragment concentrations

Cpd	fragment	K_d active p38a (mM)	K_d unactive p38a (mM)	LC3000 IC50 [mM]	FLT IC50 [mM]
SAR-002482	E	0.777	0.455	0.411	0.524
SAR-016226	J	1.27	0.946	0.778	n.d.
SAR-016697	A	1.59	1.77	2.6	no fit
SAR-018165	L	1.38	1.27	3.247	no fit

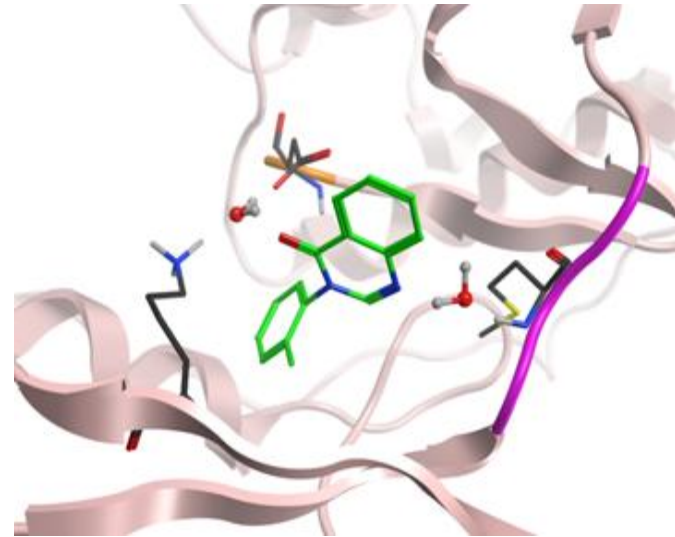


ATP site bound fragments

Hinge binding



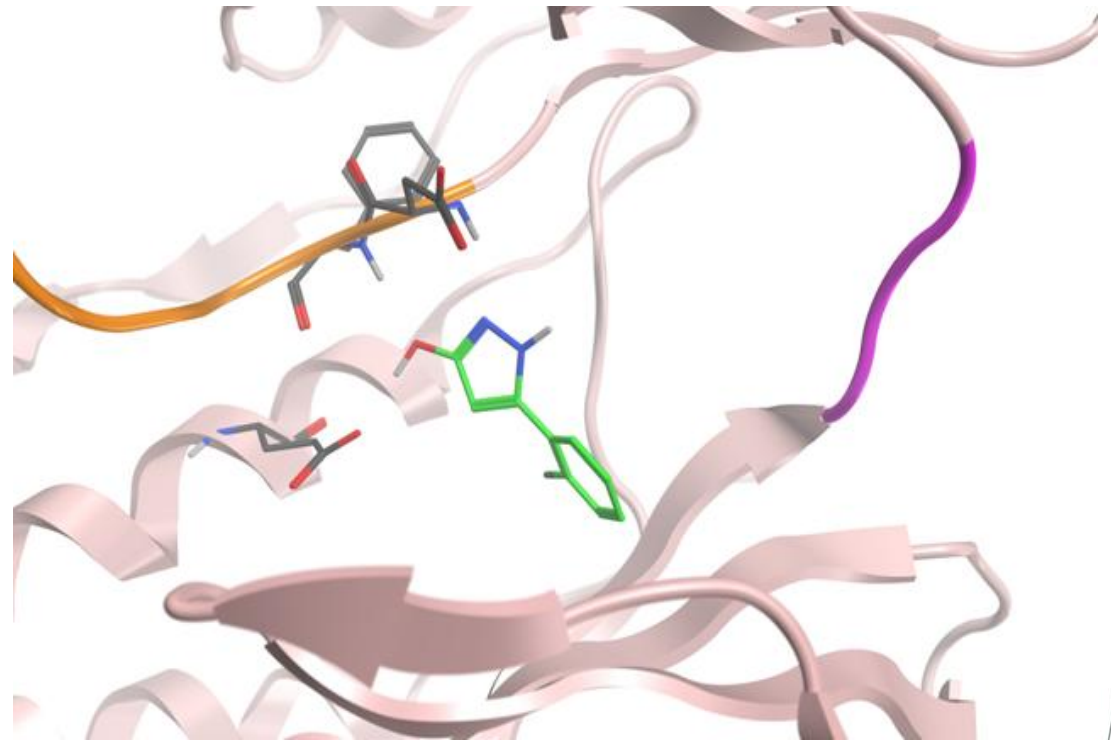
LE = 0.28



LE = 0.24

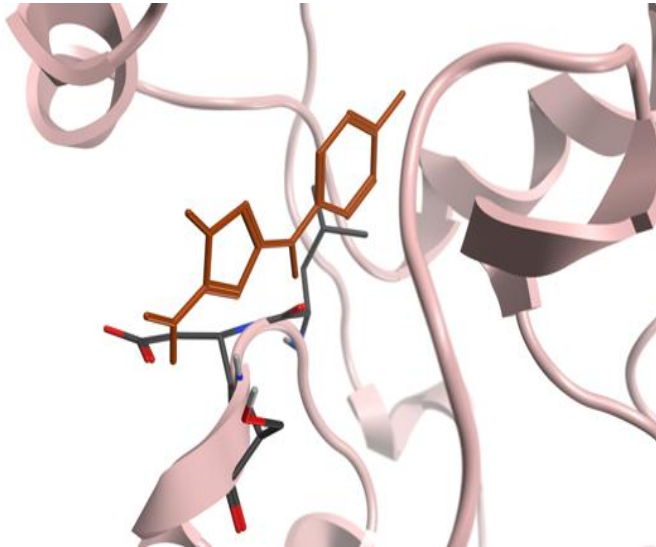
ATP site bound fragments

Activation loop binding

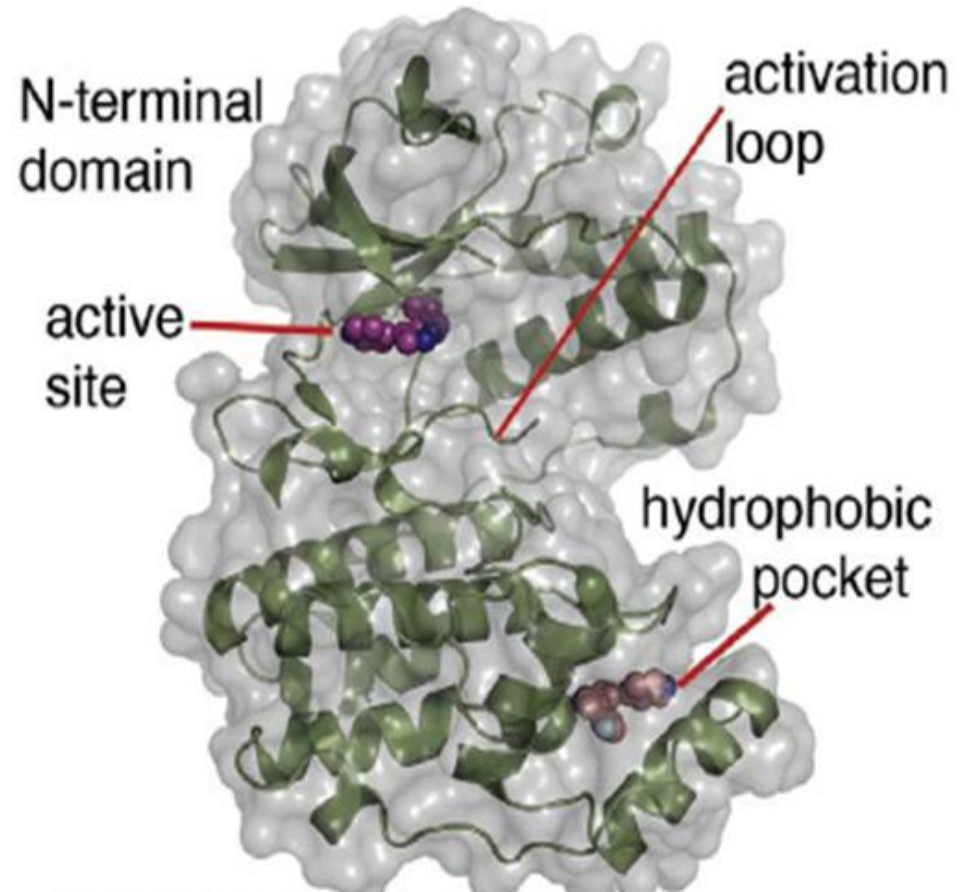


LE = 0.23

The distal hydrophobic pocket site



LE = 0.3



Perry et al. J. Mol. Biol. 2009, , 391, 1.
Diskin et al. J. Mol. Biol. 2008, , 375, 70-79.



The next steps

- Strategy from hit to lead
 - rational hypothesis: synthesize close analogs to validate hypotheses/confirm hit
 - several hypotheses formed: growing to optimize hydrophobic interaction, increasing basicity of H-bond acceptor function
 - pragmatic approach: similarity search followed by 'SAR by catalogue'
 - tested 704 cpds similar to 4 cpds with X-ray crystal structure in LC3000
 - tested cpds similar to fragment L in SPR
 - hits with higher potency found in both approaches
 - confirm mode of binding through structure elucidation and SPR competition experiments



Summary case study p38 α

- Fragment library screened against p38 α
 - Methods used for screening
 - SPR
 - LC3000
 - FLT (subset)
 - X-ray structure resolved to date for 4 fragments
- Results
 - SPR also detects binding at non-functional sites
 - two biochemical assays set up LC3000 and FLT that allow for screening at high concentration
 - similar results for both assays observed
 - active site binders detected in both systems
 - X-ray crystallography:
 - fragments with several binding modes at ATP site identified
 - fragment binding at lipid binding site identified
 - optimization of the fragment hits by testing analogues under way



Conclusion

Fragment-based screening cascade at BioFocus
validated with p38 α

➔ dynamic interaction among SPR, structural,
biochemical, computational and synthetic chemistry
studies



Thank you!

- Roland Bürli (chemistry)
- Daniel Todd (SPR)
- Scott Pollack (SPR)
- Alison Ritchie (X-ray)
- Ilka Mueller (X-ray)
- Markus Sanner (Mobility shift)
- Manuel Baader (Thermophoresis) -> see also poster P140
- Thomas Ahrens (FLT) -> see also poster P254
- David Sheppard (Computational Chemistry)
- Andreas Bergner (Computational Chemistry)
- Stephan Fasler
- Doris Hafenbradl

- NanoTemper (Thermophoresis)
- Almac (*FLEXYTE*™), Edinburgh instruments (NanoTaurus reader)