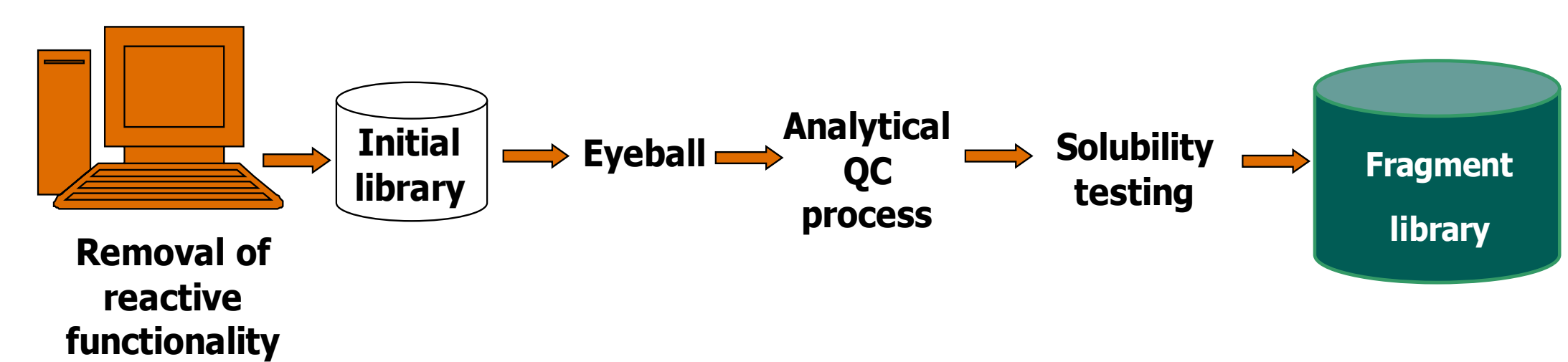


Abstract

The stress-activated kinase p38 α was used as a test case for evaluating a fragment-based drug discovery approach using the BioFocus fragment library. This collection comprises approximately 900 structurally diverse compounds that have passed rigorous QC tests (>90% LC-MS purity; >750 μ M solubility in 10% DMSO). The fragment library was screened by SPR on a Biacore™ T100 against p38 α and two selectivity targets. A segment of the library (262 fragments) was the focus of a detailed follow-up analysis that included hit confirmation at a lower concentration, potency determinations of the 24 confirmed hits and determination of competition of these hits with respect to known ATP binding site inhibitors. In addition, functional activity against p38 α was assessed in a biochemical assay using a mobility shift platform (LC3000, Caliper LifeSciences). A high correlation between the data of the different assays was found; virtually all fragments leading to inhibition in the biochemical assay were also identified by SPR. Furthermore, SPR was able to identify a collection of assay unique hits, highlighting the advantage of using a biophysical approach when fragment screening. 12 compounds that exhibited selective and measurable binding affinities for p38 α were evaluated by X-ray crystallography. X-ray structures were solved for four of the small molecule-p38 α complexes. Interestingly, as determined both by X-ray crystallographic analysis and SPR competition experiments, three of the complexes involved binding in the ATP binding site, while the fourth compound bound in a different region that offers potential as a novel drug target site. Currently, these four fragments form the basis for developing novel and active p38 α inhibitors. Moreover, this approach is readily extendable to the discovery of fragments that facilitate the development of novel modulators of other kinase and non-kinase drug targets.

BioFocus fragment library



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

Solubility QC
Tested by kinetic solubility
Compounds pass with >750 μ M
in 10% DMSO in PBS

SPR screening BioFocus fragment library against p38 α

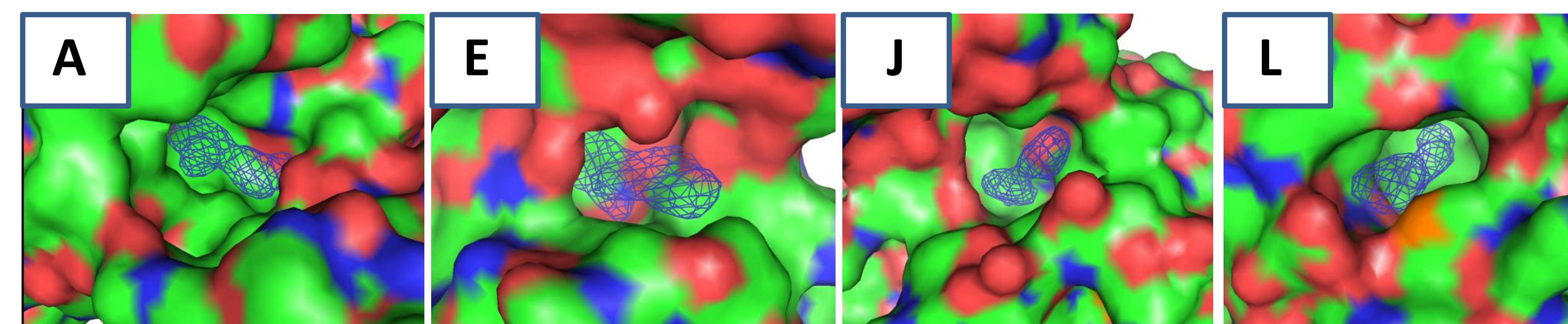
- Biacore T100 SPR technology
- Immobilization using NTA capture/mild amine coupling
- Active and non-active p38 α target surfaces in parallel
 - most hits bound both forms
 - carbonic anhydrase specificity on remaining surface
- 890 fragments initially screened at 1 mM (5% DMSO)
 - follow-up focus on 262 fragment subset
- High hit rate vs. p38 α (126 of 262 fragments bound with >60% TR_{max})
- Robust assay performance
 - Z' = 0.78-0.92; %CV = 1-4; Avg. S:B = 23
- Hits re-tested at 200 μ M with MKK6 as a specificity control

Fragment hits analyzed for binding affinity (SPR), functional inhibition (mobility shift assay) and X-ray structure elucidation in p38 α complex

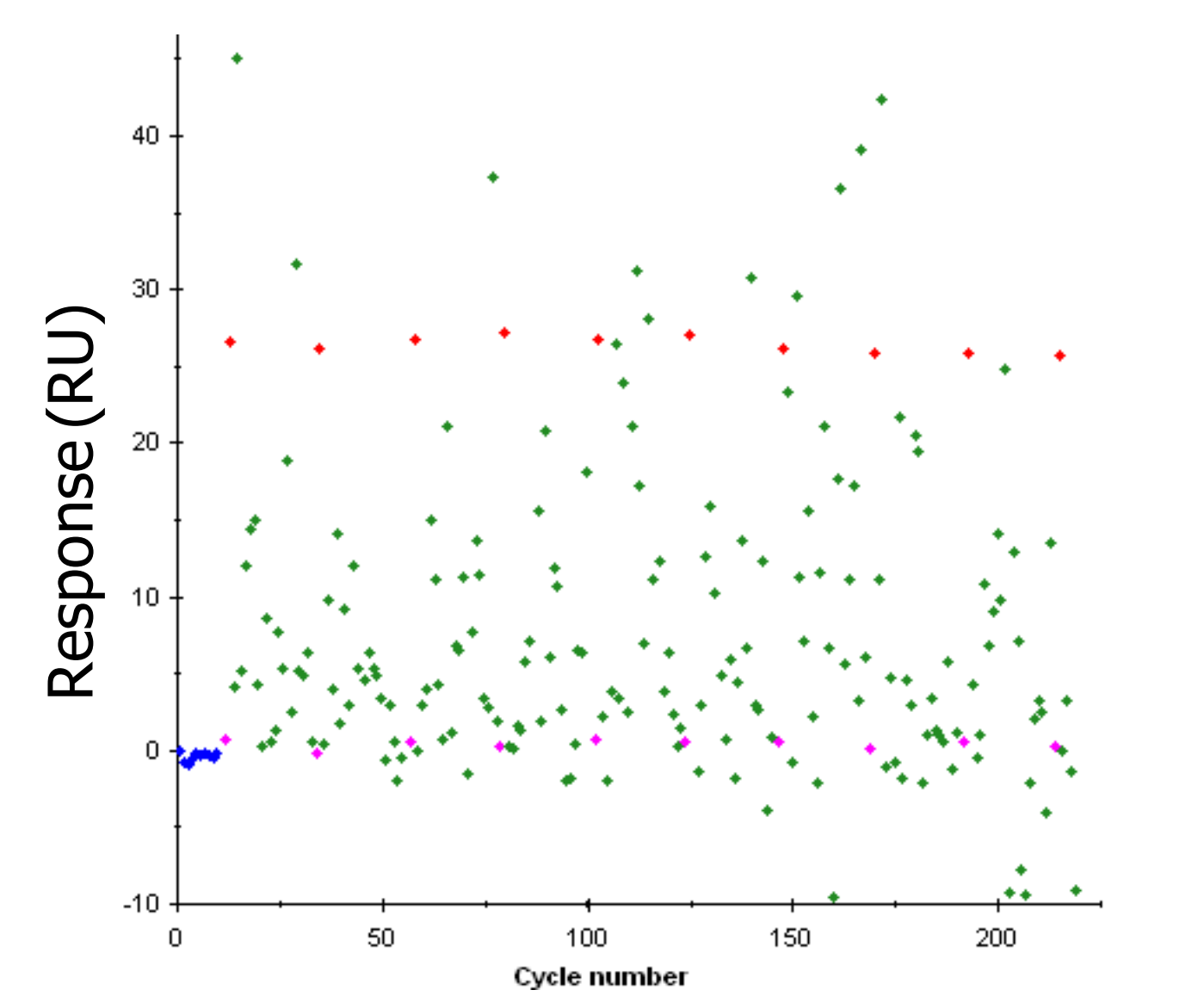
Compound	Molecular mass	Active p38 α K ₀ (mM)	Ligand efficiency	Caliper % INH (1 mM)	Caliper K ₀ (mM)
A	195	1.59	0.29	19.4	2.60
B	214	2.21	0.23	28.7	
C	234	1.97	0.23	19.4	
D	235	1.84	0.25	70.9	
E	240	0.78	0.24	67.6	0.41
F	208	0.22	0.33	0.7	
G	218	0.79	0.26	35.2	
H	236	1.54	0.23	28.0	
I	212	2.34	0.22	11.5	
J	185	1.27	0.28	55.4	0.78
K	245	3.19	0.19	12.9	
L	210	1.38	0.30	7.9	3.25

SPR affinity data for the 12 confirmed hits selected for structure determination. Data are also shown for inhibition in the mobility shift assay (Caliper LabChip 3000). Ligand efficiency (LE) is in units of kcal per mol per non-hydrogen atom

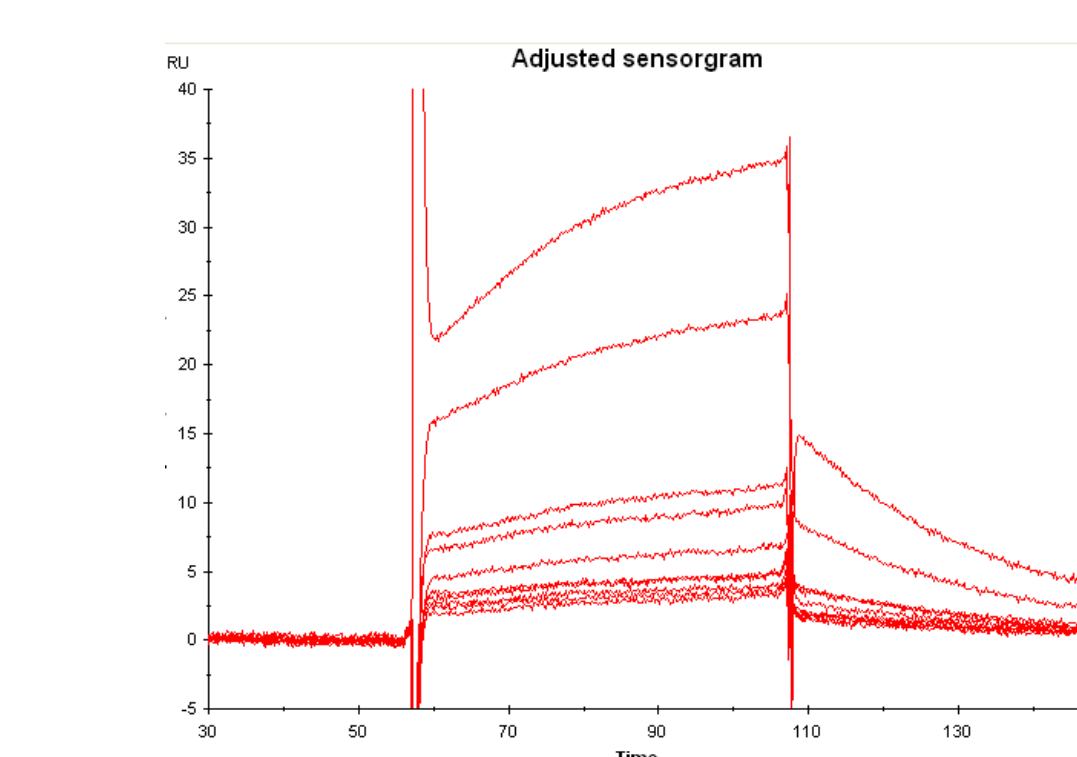
- Structures obtained with 4 of 12 compounds selected from SPR screen
- Co-crystal structures to ~2.3 Å resolution via soaking
- Active site binding mode of 3 compounds identified; 4th compound (L) binds outside ATP site
 - confirmed in SPR competition study
- Correlation: binding vs. functional effects
 - nearly all functional inhibitors bound by SPR
 - SPR identified fragment hits that were not always detected in the functional screen



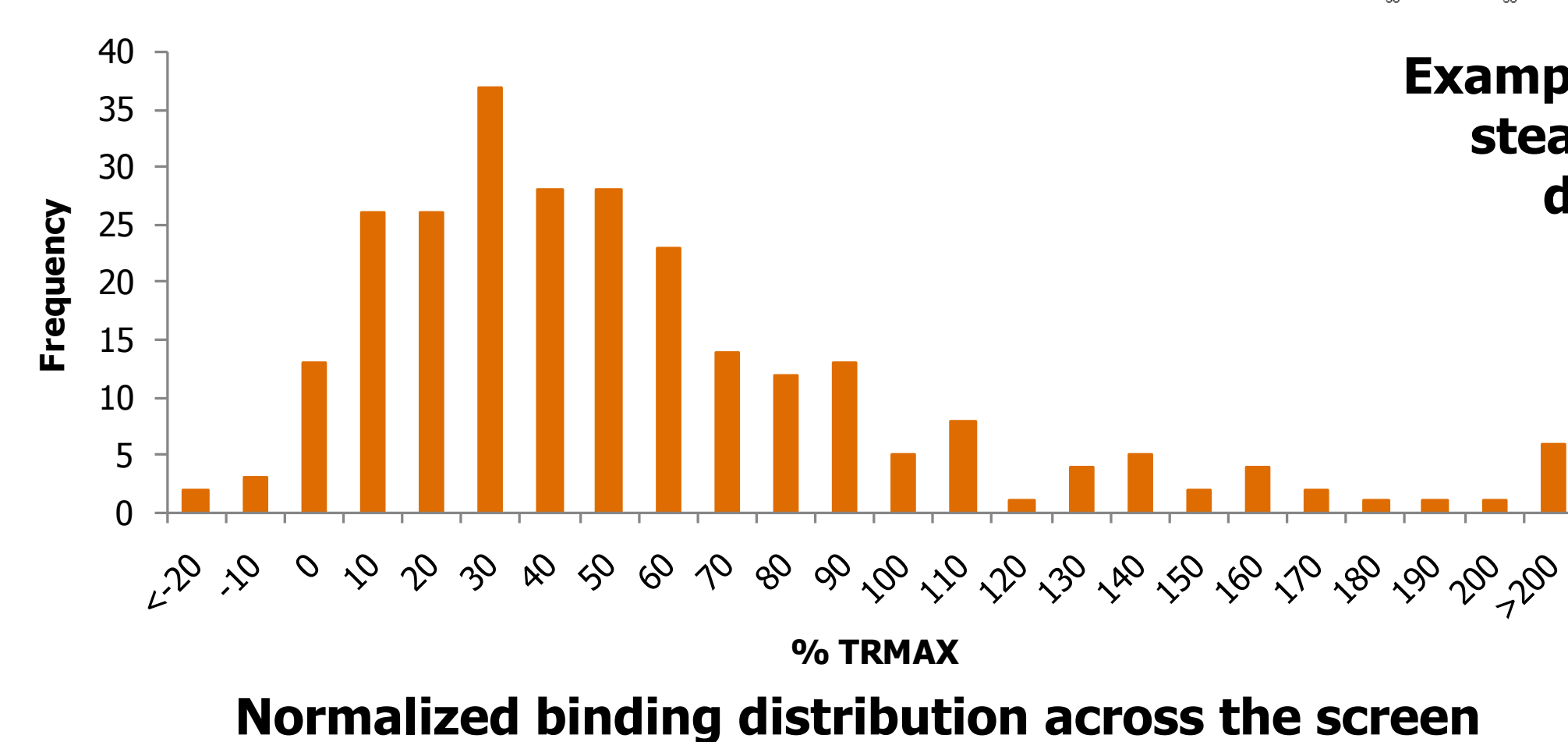
Hit identification and affinity determination



Active p38 α
Fragment binding levels during screen with calibration standard levels indicated

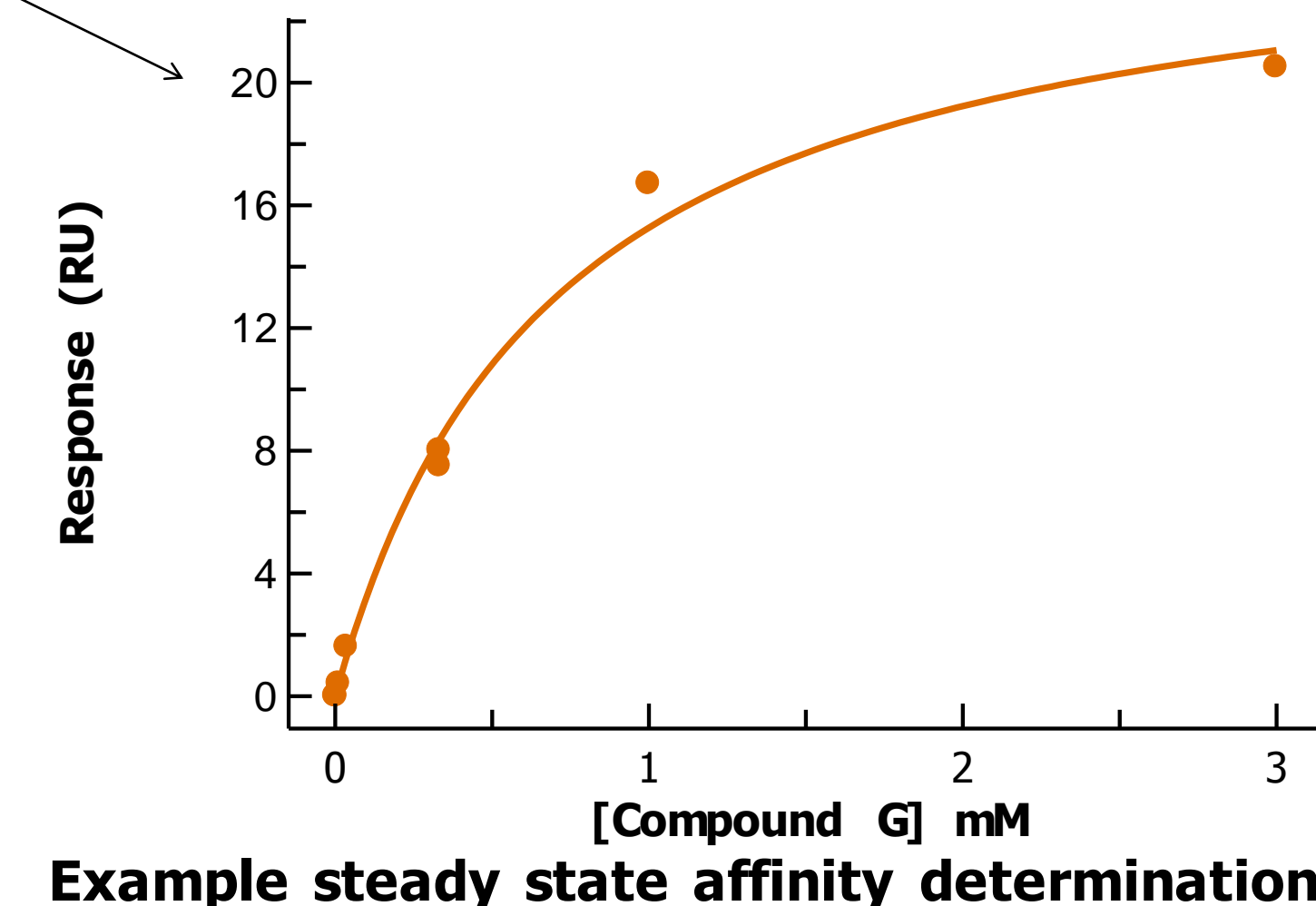


Example sensorgrams for steady state affinity determination



Normalized binding distribution across the screen

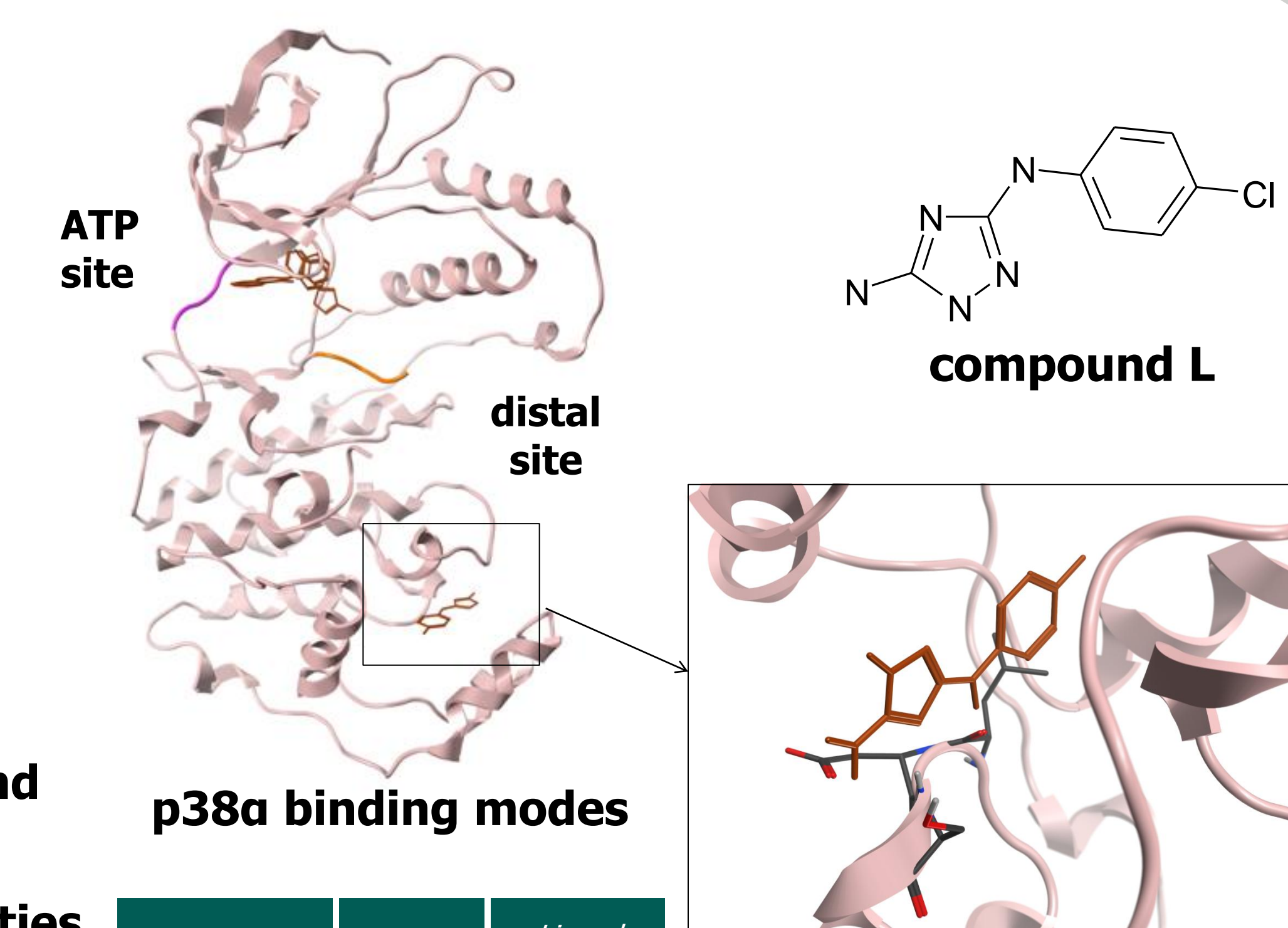
- Hit criteria:
 - activity >50% TR_{max} @ 200 μ M
 - specificity vs. reference surfaces
 - sensorgram shape
- 24 fragments selected for affinity, binding stoichiometry & competition determinations
 - affinities ranging from 0.2 to >10 mM
- 12 confirmed hits selected for structure determination
 - including an ATP non-competitive fragment



Example steady state affinity determination

Optimization of the fragment hits

- 704 analogues of fragment hits analyzed directly in functional assays
- 11 triazole analogues synthesized based around the structure of compound L (novel binding mode)
 - improved potencies obtained while ligand efficiencies maintained
 - SAR picture emerging
- Next steps:
 - confirm mode of binding through structure elucidation and SPR competition experiments
 - further fragment growth and/or linking to increase affinities



p38 α binding modes

Structure	K ₀ (mM)	Ligand efficiency
R=H	11.0	0.28
R=4-F	6.9	0.28
R=4-Br	0.61	0.37
R=4-Me	10.0	0.27
R=4-Et	7.6	0.26
R=C=CH	1.1	0.33
R=3-F	7.8	0.28
R=3-Cl	7.5	0.28
R=3-Me	7.9	0.28
R=3-Ph	0.31	0.30
R=4-Br	0.68	0.35

compound L analogues

Conclusions

- Fragment-based screening cascade at BioFocus validated with p38 α
 - dynamic interaction among SPR, structural, biochemical, computational and synthetic chemistry studies
- Novel mode of binding identified
- This approach can be applied to a range of targets from different classes