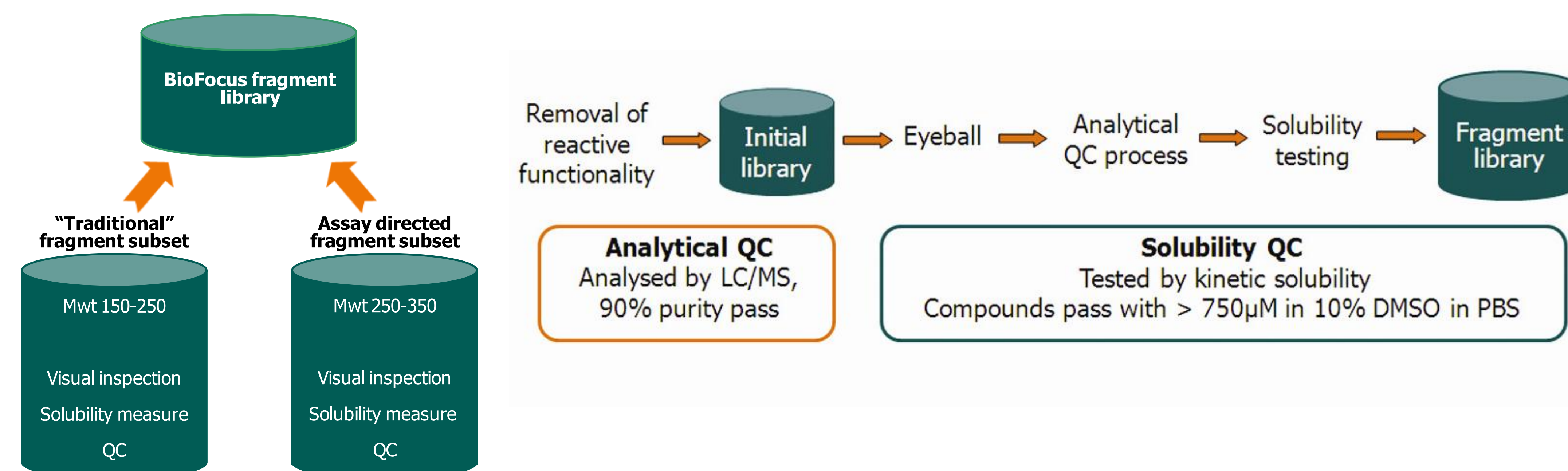


Abstract

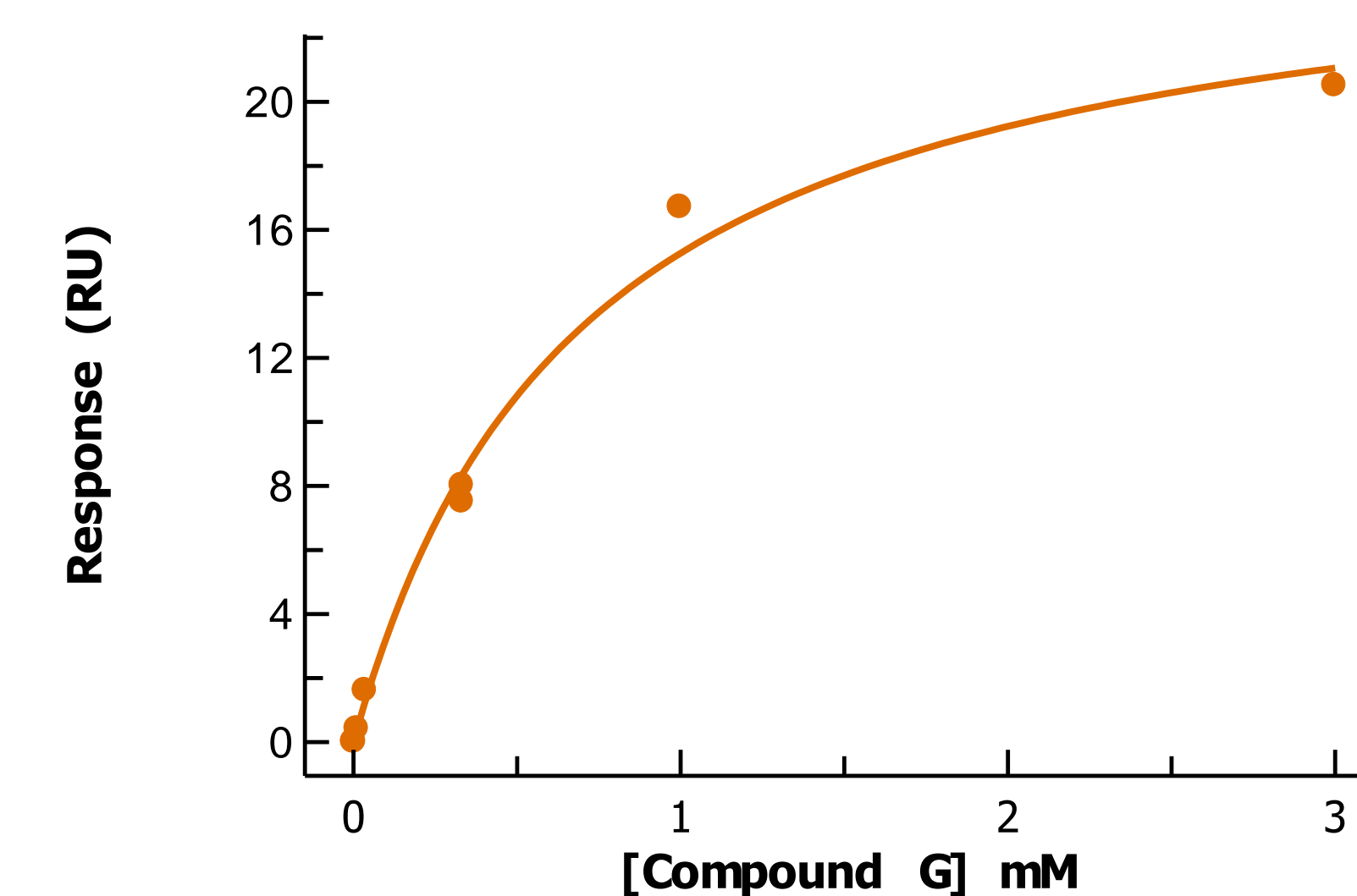
The stress-activated kinase p38 α was used to evaluate an approach to fragment-based drug discovery utilizing the BioFocus fragment library, surface plasmon resonance (SPR) technology and X-ray crystallography. The library, comprising approximately 900 structurally diverse compounds that have passed rigorous QC tests (>90% LC-MS purity; >750 μ M solubility in 10% DMSO) was screened by SPR on a Biacore T100™ against p38 α and two selectivity targets. 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, while three of the complexes involved binding in the ATP binding site, the fourth compound bound in a different region that offers potential as a novel drug target site.

BioFocus fragment library

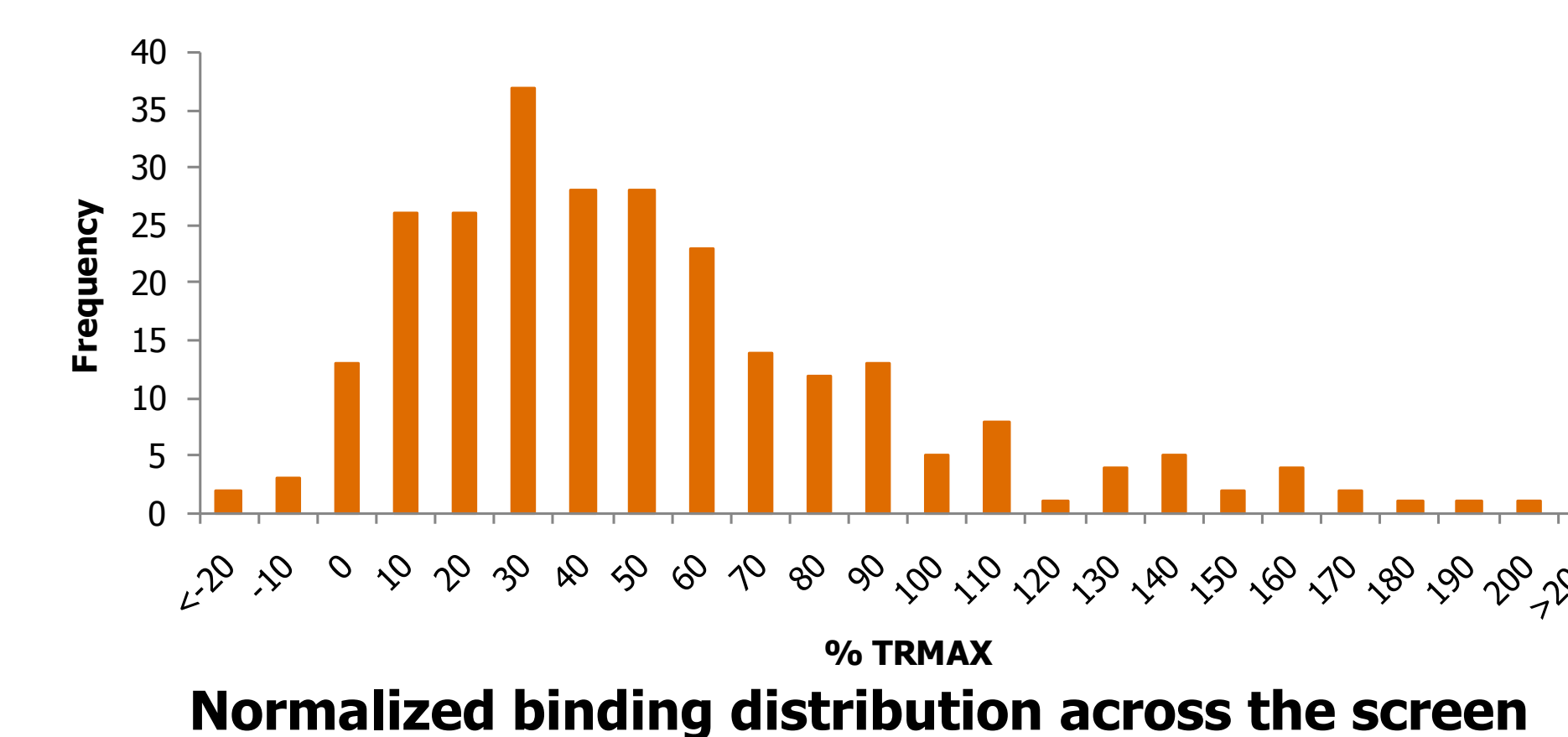


Hit selection criteria and affinity determination

- 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



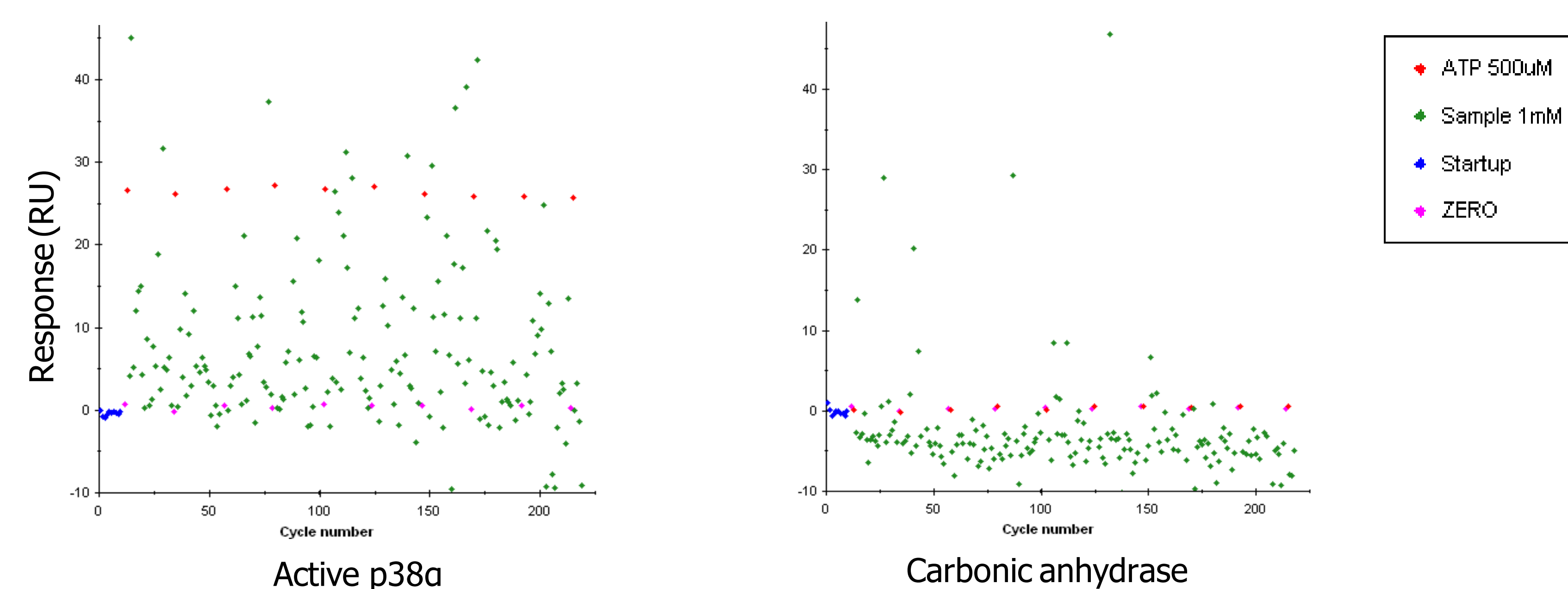
Normalized binding distribution across the screen

Compound	Molecular mass	Active p38 α K ₀ (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

SPR affinity data for the 12 confirmed hits selected for structure determination. Ligand efficiency is in units of kcal per mol per non-hydrogen atom

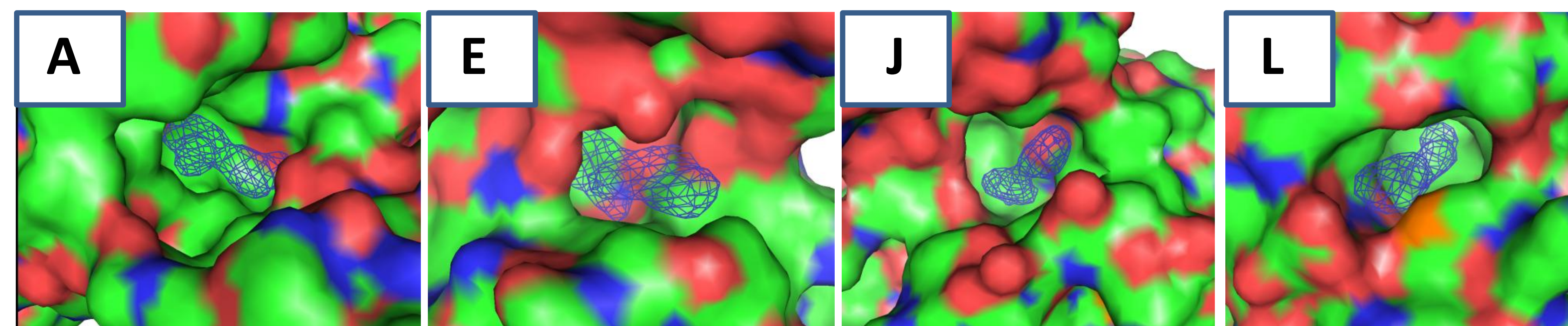
SPR screening the BioFocus fragment library against p38 α

- Biacore T100 SPR technology
- immobilization using NTA capture/mild amine coupling
- active and non-active p38 α target surfaces (flow cells 2 and 3)
 - > most hits bound both forms
 - > carbonic anhydrase specificity surface (flow cell 4)
- 891 fragments initially screened @ 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-p38 α complex structure determination

- structures obtained with 4 of 12 compounds selected from SPR screen (others in progress)
- 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



Competition experiments confirmed X-ray crystallography results

- 4 fragments with X-ray data
 - > Compounds A, E and J – ATP competitive binding mode
 - > Compound L – ATP non-competitive binding mode

Next step:

- fragment growth and/or linking to increase affinities

