Mechanism of action: selective screen of small molecules by SPR

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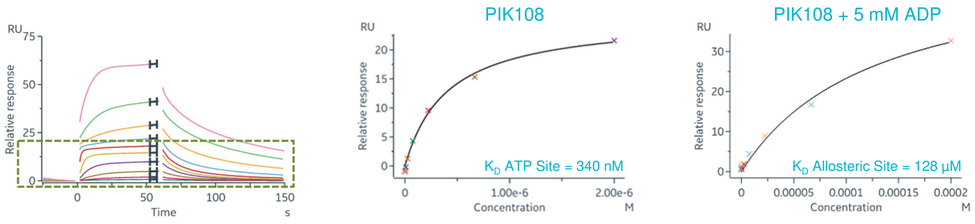
The mechanisms by which small molecules exert a pharmacologic effect have greatly diversified beyond active site inhibition in recent years. It is important to be able to direct screens towards or away from specific sites on the target. This ensures that hits for less ligandable sites can be found even in the presence of highly ligandable ones and that the hits will have the desired Mechanism of Action. Here we show three setups to selectively screen for small molecules by SPR.

Directing Screens Away from a Site

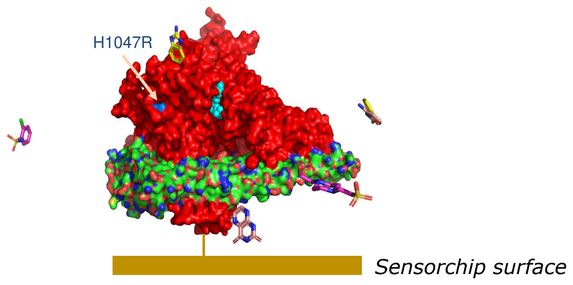
Non-ATP competitive allosteric inhibition of PI3Kα

- PI3Kα H1047R mutant is a strong driver of tumor development

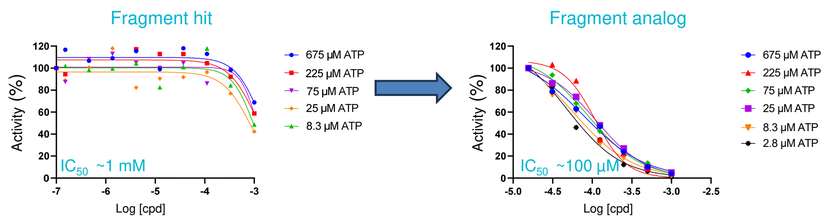
1. Proof of concept detected PIK-108 binding at the ATP binding site and to a site proximal to the H1047R mutation as identified by crystallography
2. Screened ZoBio fragment library for hits that bind outside the ATP site of the PI3Kα H1047R mutant using SPR in the presence of ADP
3. Titrated identified binders to the PI3Kα H1047R mutant +/- ADP
4. Assessed if hits allosterically (non-ATP competitive) modulated the activity of PI3Kα H1047R mutant in ADP-Glo kinase assay (Promega)



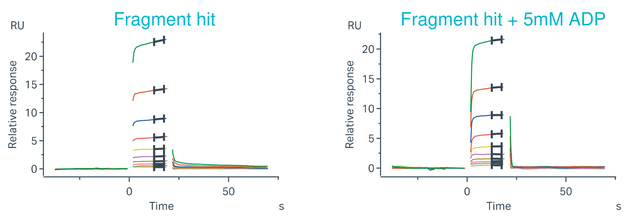
Proof of concept: determine orthosteric and allosteric affinities for PIK108



Concept: Screen with ATP site blocked (PI3Kα)



Inhibition determination and allosteric assessment: titrate fragments in ADP-Glo assay



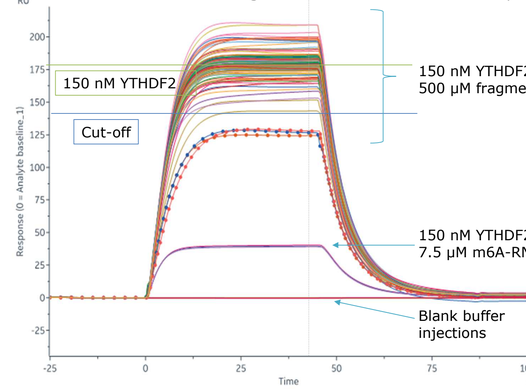
Affinity determination: Titrate fragments

Identifying Hits towards a Biomolecular Interaction Site

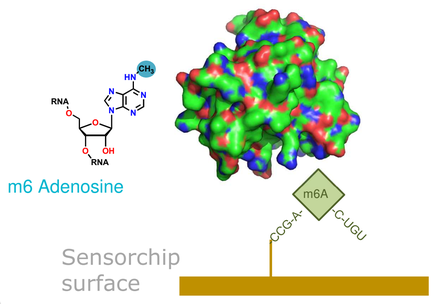
Inhibit binding of m6A RNA to YTHDF2

- YTHDF2 has an important role in tumor immune evasion and is therefore a promising target

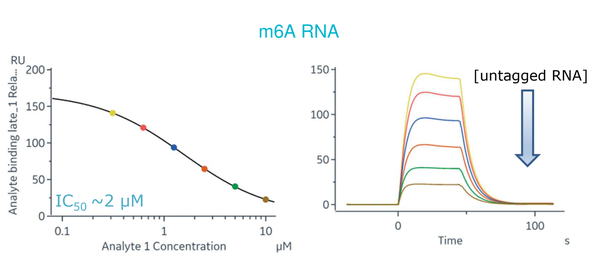
1. Immobilized the m6A containing RNA and assessed binding of YTHDF2 from the mobile phase and performed proof of concept with competition from soluble m6A RNA, good correlation of K_D of RNA to YTHDF2
2. Screened fragments for binding inhibition of YTHDF2 to immobilized m6A-RNA
3. Determined IC_{50} and affinity to YTHDF2 of those showing ≥20% RNA binding inhibition in the screen
4. Prioritized the most potent and diverse set of inhibitors for structural biology



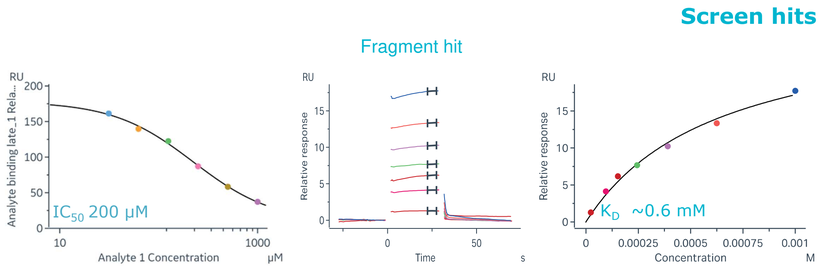
Screen hits for inhibitory activity



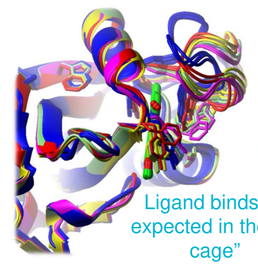
Concept: Competition study



Proof of Concept: Titrate m6A RNA in solution



Affinity determination: Titrate fragments



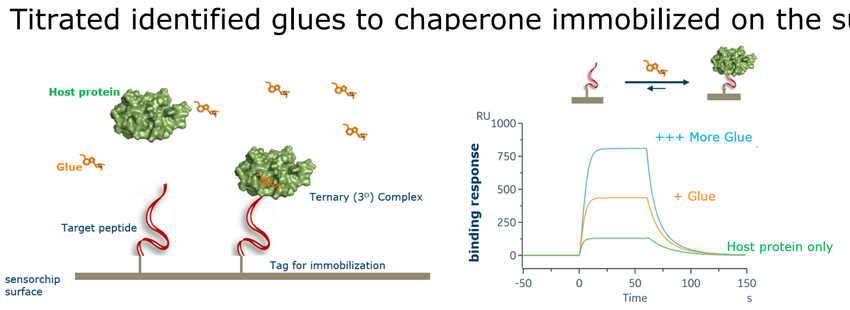
Ligand binds as expected in the "W cage"

Focusing On the Detection of Molecular Glues

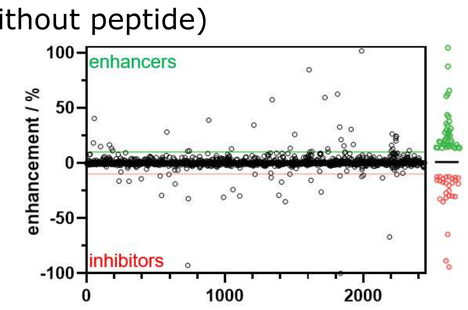
Identification for molecular glues that enhance binding of a peptide to a chaperone

- Molecular glues can enhance interactions of protein-protein interactions and can be very selective because they target an interface rather than a single protein

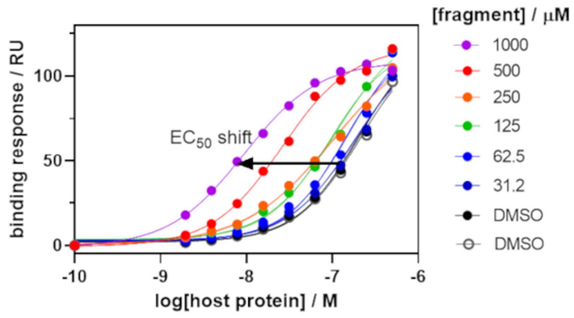
1. Immobilized target peptide on the surface and screened for glues by co-injecting chaperone and fragments
2. Co-injected titration of identified enhancers, which are possible glues with chaperone
3. Co-injected titration of chaperone with increasing concentrations of the glue
4. Titrated identified glues to chaperone immobilized on the surface (without peptide)



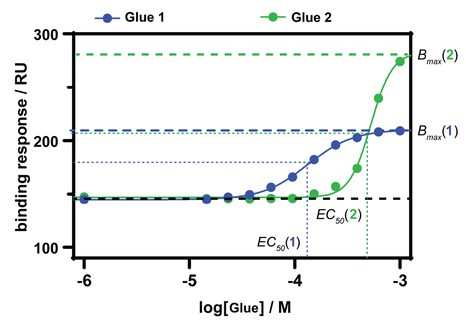
Concept: Molecular glue screening



SPR screen with fragment library



Determine potency: titration of host protein at various concentrations of glue



Enhancement determination: Titrate fragments