Site Selective Screening: Using Biophysics to Define a Mode of Action

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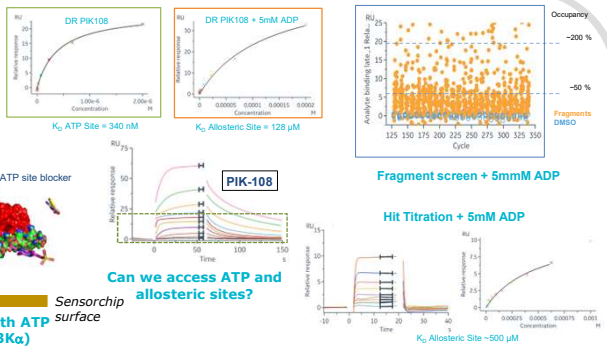
The mechanisms by which small molecules exert a pharmacologic effect have greatly diversified beyond active site inhibition in recent years. It is important to be able to direct screens towards or away from specific sites on the target. This ensures that hits for less ligandable sites can be found even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

Directing Screens Away from a Site

The goal of this project was H1047R mutant selective inhibition of PI3Kα

- Inhibition of WT PI3Kα has on target tox.
- A second crystal binding site of PIK-108 is proximal to H1047R. This could afford mutant selectivity.
- Solution: Screen for hits that bind outside ATP site using SPR.
- Proof of concept detected PIK-108 binding at both sites.
- Screen fragment library in the presence of 5 mM ADP. Titrate hits +/- ADP.
- Hits that bind independent of ADP assess for kinase inhibition at increasing concentration of ATP. If IC_{50} is invariant, the compound is an allosteric inhibitor.



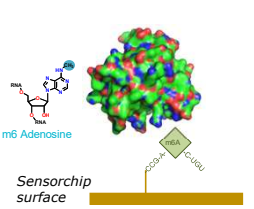
- 1995 fragments screened against PI3Kα H1047R in the presence of saturating ADP
- 326 fragments were classified as hit candidates
- 107 confirmed fragment hits on DR

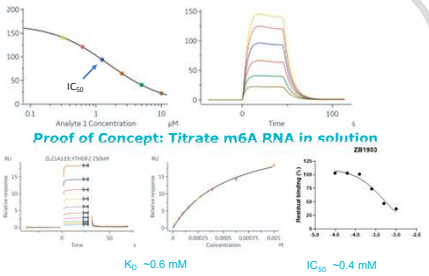
5 progressed non-ATP competitive, allosteric inhibitory chemotypes

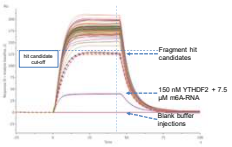
Directing Hits Towards a Biomolecular Interaction Site

The goal of this project was to inhibit binding of m6A RNA by YTHDF2

- An SPR screen of the 2k ZoBio fragment library yielded 250 confirmed hits, far too many for structural biology.
- As FRET-bases assays are prone to artifacts, a simple, sensitive, robust assay that detects binding at the desired site on YTHDF2 was needed.
- Solution: immobilize m6A containing RNA and assess binding of YTHDF2 from the mobile phase.
- Proof of concept with competition from soluble m6A RNA showed very clean data.
- Assessed all 250 confirmed screening hits and 82 showed substantial inhibition of m6A RNA binding.
- Prioritize most potent and diverse set of 82 inhibitors for structural biology.







Ligand binds as expected in the "W cage"

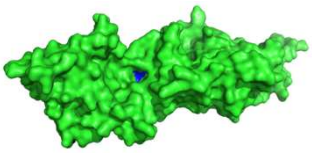
Summary screening results

- 1969 fragments screened for binding
- 250 confirmed hits
- 82 fragments showed ≥20% RNA binding inhibition

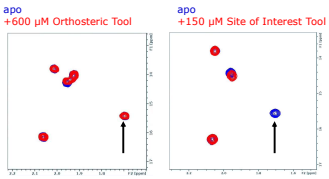
Directing Hits Towards an Arbitrary Site

In this project a secondary "cryptic" fragment binding site was discovered.

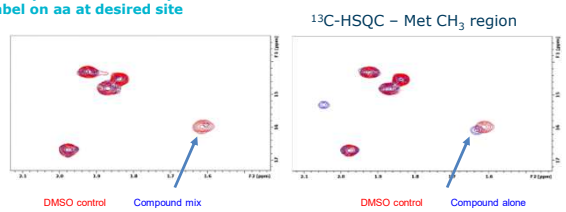
- Screened fragment library against apo protein. Many hits found with biological activity. Crystallography revealed all but 1 bound at expected site.
- 1 fragment had unexpected biological potency and bound a different site created by a conformational change. More hits for this site were desirable.
  - A Met residue lay at this site. We labeled only the ε-methyl group with ^{13}C.
  - 2D NMR spectra of the ~60 kDa protein contained 5 signals. Using tool compounds from the crystal structures, the identity of the proximal Met was discovered.
  - Nearly 1,100 selected fragments were assayed in pools of 5 compounds
  - 7 pools generated hits and could be readily deconvoluted as singletons.



Concept: Selective isotope label on aa at desired site



^{13}C-HSQC – Met CH_3 region



- Screened – 217 mixes with 1083 compounds
- 7 mixes and 7 confirmed deconvoluted hits
- 7 novel chemotypes found