Chemical Fragments in Protein Purification, Process Optimization, and Target Validation

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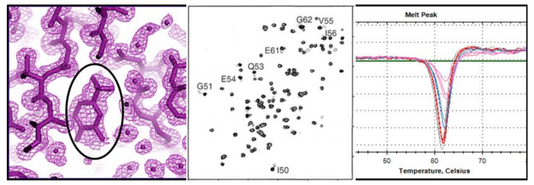
Background

There are many successful methods for identifying fragment binding to a target.

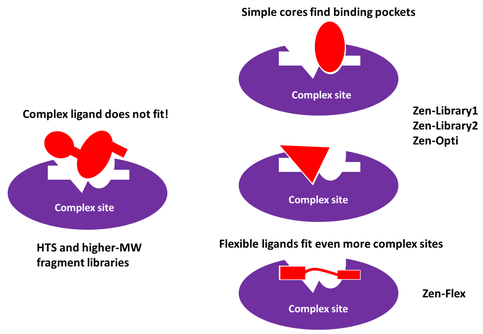
Fragment-based lead discovery (FBLD) is a proven method for drug discovery that has been broadly implemented around the world, yielding multiple clinical candidates and marketed drugs. It was invented at Abbott Laboratories in the mid-1990 using NMR (Shuker et al., 1996) and x-ray crystallography (Nienaber et al., 2000). Since then, multiple biophysical techniques have been used to identify fragment hits including SPR, DSF, and enzyme assay.



Small simple compounds have a higher probability of binding to targets than larger molecules.

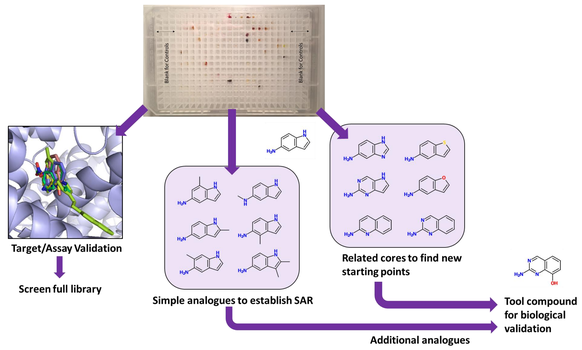
Hann et al., (2001) demonstrated computationally that small simple compounds have a higher probability of binding to complex binding sites. This is the basic premise of screening fragments for complex targets like proteins. Larger molecules are less likely to fit!

We follow the guideline of two for fragment library design to keep the molecules very simple. Diversity is measured at the chemical core level. These cores can find binding pockets on proteins and in some cases create their own pockets. These libraries are available commercially from Zenobia. Zenobia also sells a "flexible" ligand library for small or very complex binding pockets.



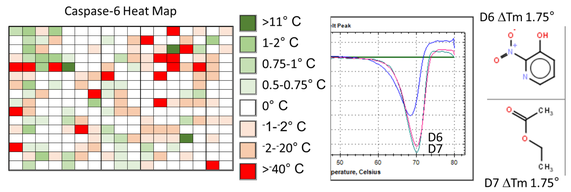
Express-Zen-CORE-288™

Express-Zen-Core-288™ is a rapid screen sampling 288 compounds of Zenobia's 960 fragment library compound collection. Enough compound for one screen (1µl at 200mM) is supplied in 384 well format with the outer columns left blank for internal controls supplied by the consumer. *Express*-Zen-Core-288™ is an ideal first-screen to validate the drugability of a target and provide rapid information about its binding properties. Dry powder is provided upon request for follow-on studies.



Hits from *Express*-Zen-CORE-288™ may move directly into fragment optimization through purchase of analogues through internal resources (SAR-by-Catalogue). Zenobia provides such follow-on libraries upon request. Validation of the target and screening platform may also justify screening Zenobia's full fragment library or libraries designed based upon the initial screening hits (fragment-hopping).

Zenobia is currently developing a "Heat-Map" algorithm to provide quick-readout and categorization of active sites based upon results from the *Express*-Zen-CORE-288™ screen. A Heat-Map for Caspase-6 DSF thermal melt screen is shown below.



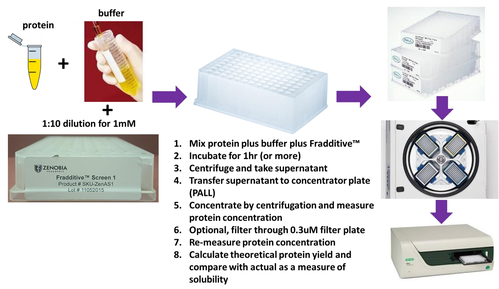
Hits with ΔTm > 1.0 are considered significant. Very high ΔTm may be due to binding at the dimer interface allosteric site. Binding sites have not been verified by x-ray analysis. Negative ΔTm are destabilizers and have been shown by SAXS to result in a general disorder of structure for other targets. They are generally not pursued for further studies.

Fradditive™

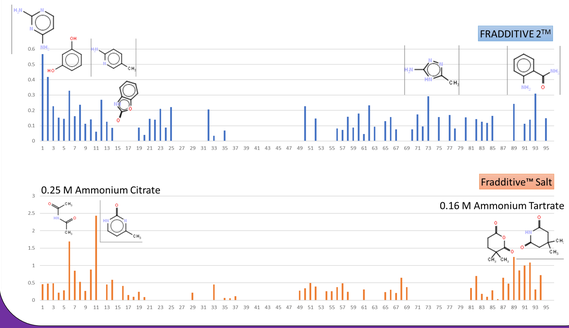
Protein stabilization by small molecules is a well-known phenomenon. Ligands may be added to proteins for crystallizations or fortuitously observed in crystal structures. These are often either natural ligands, buffer components or salts. The concept of using additives like salts, buffers and detergents to stabilize proteins in solution is also a common practice.

We have shown that very small fragments (Fradditives™) may serve as general additives for protein solubility and stabilization through specific or non-specific binding. This builds upon the field of osmolytes which are naturally occurring small molecule protein stabilizers and includes weakly binding ligands that can stabilize proteins in solution.

Fradditive™ screens are provided in 96 well plates. Fradditive™ 1 and 2 screens are unique small molecules. The Fradditive™ Salt and Fradditive™ pH screens vary the fragment and the salt or pH, respectively.



Results from two Fradditive™ stability screens are shown below. Fradditive™ 2 is 96 unique compounds tested at low salt and neutral pH to identify fragments that solubilize the protein. Fradditive™ Salt identifies stabilizing salts and fragments to be tested further. Fradditive™ stability screens may also be carried out by DSF or added directly to crystallization drops. The protein shown below is insoluble without these additives.



Conclusions

Application of fragments in discovery research is no longer limited to traditional hit-to-lead.

Target/Process Validation:

- A coarse sampling of Zenobia's 960 compound collection has been prepared as a pre-screen for new targets.
- Compounds are provided in a single-use format to minimize the initial investment allowing routine application for:
  - Testing the drugability of a target
  - Validating screening processes
  - Identification of starting points towards development of tool compounds for target validation.

Protein Stability and Solubility Screen:

- Because small simple fragments not only find binding sites on proteins at the active site, but also in other un-related sites, they can be very useful in protein stabilization.
- Certain fragments such as osmolytes have been shown to stabilize protein in solution without interacting at a specific binding site. Fragments may also stabilize non-specifically.
- Zenobia has created four unique protein solubility screening kits by pairing very low molecular weight fragments with salts and buffers and identified stabilization conditions for protein purification.

Bibliography

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