FRAGMENTS, HOTSPOTS AND TARGET IDENTIFICATION: DECREASING COMPLEXITY IN DRUG DESIGN

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Abstract: Fragment-based drug discovery is a powerful and now widely used approach both in academia and industry to create novel high quality drug like molecules (Blundell *et al*., 2002; Murray & Rees (2009); Erlanson *et al*., 2016). This approach relies on screening a library consisting of small molecules (150-300 Da) against a target protein, using a variety of biochemical, biophysical and also structural biology methods. The low molecular weight of fragments represents a decrease of complexity and allows an efficient exploration of chemical space even when using small size libraries of around 1000 fragments. Although the fragments bind weakly, they tend to bind to hotspots forming well-defined interactions with the target protein. Thus, fragments can be subsequently elaborated into larger molecules with high affinity. An example of our approach to tuberculosis targets is found in Mendes and Blundell (2016)

The approach developed in our academic laboratories in Cambridge builds on that developed in Astex, the company I cofounded in 1999 with Harren Jhoti and Chris Abell. It involves two stages. First we screen to identify hits using differential scanning fluorimetry, ligand-based one-dimensional ^{1}H NMR spectroscopy and surface plasmon resonance (SPR). Second we determine the 3D-structures of protein-fragment complexes, followed by a study of thermodynamics using isothermal titration calorimetry (ITC) and kinetics of the binding process using again SPR

Due to their small size fragments interact weakly with the target protein, usually between 0.1-5 mM, but those interactions tend to bind to hotspots that make large contributions to binding affinity. Hotspots usually have the capacity to bind not only one, but a variety of fragment sized molecules. As fragments are elaborated into higher affinity compounds, analyzing the target protein hotspots can provide crucial insights on how to improve compounds. Several methods have been described in the literature to map protein hotspots. We have recently developed a method that identifies the hotspot and also the specific interactions that determine fragment binding (Radoux *et al.,* 2016)]. This method, beyond providing crucial insights about the fragment-binding mode, can also help generate constraints for docking experiments by ensuring the right interactions are made.

Elaboration of fragment hits exploits a continuous cycle of fragment-merging/linking/growing strategies supported by structural information from X-ray crystal and NMR structures and in silico methods, together with characterization of binding by ITC, SPR and functional biochemical assays.

In my talk I will illustrate the applications of these techniques to cancer and tuberculosis. I will also discuss current thoughts about the nature of fragment hotspot interactions.

References

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