DRUG DISCOVERY

To affinity and beyond

A quick, sensitive screen for identifying the binding preferences of protein kinases for various compounds has made it possible to assemble detailed interaction maps, with initial data revealing surprising and important ramifications for drug design.

Protein kinases are important potential targets of drugs for the treatment of cancer and other diseases. But the human genome encodes over 500 protein kinases, and it is of paramount importance to develop drugs that specifically bind only a narrow range of targets. These drugs generally work by binding the ATP site, a motif common to all kinases, and although many have made it to clinical trials or even the pharmacy, surprisingly little is known about their overall specificity.

To address this problem, high-throughput screening pioneers David Lockhart and Patrick Zarrinkar and their colleagues at Ambit Biosciences developed a quick and accurate strategy for obtaining detailed data on drug-target interactions (Fabian *et al.*, 2005). For each assay, a kinase is expressed on the surface of the T7 bacteriophage. The phage is combined with a test compound of interest and beads conjugated with a bait ligand for which the kinase is known to have a strong affinity. If the test compound also binds the kinase, then competition will reduce binding to the beads. Plating out of bound phage or quantitative PCR permits highly sensitive measurement of a kinase's affinity for a given compound.

To start, Lockhart and Zarrinkar's group have assembled detailed interaction maps (**Fig. 1**) for 119 protein kinases. Beyond the goldmine of information this work has produced—available as supplemental information to their article—these studies have also challenged basic assumptions about many drug-target interactions, with several clinical compounds previously considered to be quite specific instead revealed to be victims of overly narrow screening.

Another surprise was the extent to which structurally similiar compound can have startlingly different binding preferences. One compound, GW-2016, had extremely tight specificity for a handful of kinases (**Fig. 1a**), whereas EKB-569, a compound with similar structure, showed more promiscuity, binding numerous targets from a variety of different kinase classes (**Fig. 1b**). Lockhart and Zarrinkar were equally surprised to find that Gleevec (imatinib), an effective kinase inhibitor that has seen widespread clinical use, interacts tightly with the lymphocyte-specific kinase LCK,but not with the closely related kinase SRC. "That was a big surprise," says Zarrinkar, "[because] oftentimes when people talk about [Gleevec's binding properties], they use the structure of LCK as a surrogate for the structure of SRC to explain why Gleevec doesn't bind to SRC."

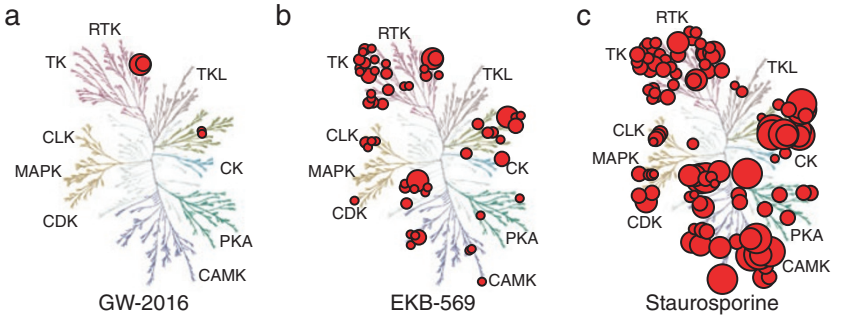

Figure 1 | Profiles of three kinase inhibitors. (**a**–**c**) A dendrogram-style diagram (adapted from Manning *et al.*, 2002) illustrates the binding affinity of several protein kinases for inhibitors with narrow specificity (**a**), broad specificity (**b**) and a promiscuous interaction profile (**c**). Circle size is proportional to binding affinity on a $\log_{10}$ scale. Reprinted with permission from *Nature Biotechnology*.

These findings could ultimately aid the design of compounds capable of better exploiting the fine structural details of their target protein. "Even though they're all binding to the ATP site or very near the ATP site in one way or another, they are still able to exploit subtle differences between ATP sites," says Lockhart. "[but] it's not predictable..., and that's why we felt it was so important to develop a direct experimental measure of the interaction between any given molecule and as many kinases as possible."

The Ambit team is applying their interaction data to ongoing drug discovery and development projects, says Zarrinkar, but public exposure might also encourage other structural and computational biologists to take the bait. "We don't have a big computational effort here..., and we thought it would be valuable to put these data out there so that people can look at them and start answering, or at least asking, some of these questions that people have talked about a lot over the last several years." Developing cocrystal structures for interacting pairs is also a priority, and the team is now involved in a collaboration to study some of these interactions in greater detail.

Above all, both men hope that their publication will inspire additional research. "The greater life of the data, you hope as an author, is when people will take [it] and go beyond what you were able to do," says Lockhart, and then adds, "and of course, when you develop a cool new approach to something, you want to show it to the rest of the world, show what's possible."

Michael Eisenstein

RESEARCH PAPERS

Fabian, M.A. *et al.* A small molecule–kinase interaction map for clinical kinase inhibitors. *Nat. Biotechnol.* **23**, 329–336 (2005).

Manning, G. *et al.* The protein kinase complement of the human genome. *Science* **298**, 1912–1934 (2002).