Scientists create new way to screen libraries of 10 million or more compounds

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The search for new drug compounds is probably worse than looking for a needle in a haystack because scientists are limited in the size of the haystacks they can rummage through—time and money make it virtually impossible to screen or search through super-large libraries of potential compounds. This is a serious problem, because there is enormous interest in identifying synthetic molecules that bind to proteins for applications in drug discovery, biology, and proteomics, and larger libraries should mean higher odds of success.

But large libraries come with large problems. Because even compounds with only modest affinity (binding to the target protein receptor with less force than those with high affinity) are usually marked as hits, researchers often end up with several hundred of them and, because of practical constraints involving time and money, no easy way to determine which might be the highest affinity or best compound to serve as a starting point to design a drug. These limitations and others have drastically blunted the use of very large libraries—monster libraries—in binding assays.

Now, in research published in the most recent issue of the journal Chemistry *& Biology,* Tom Kodadek, a professor at The Scripps Research Institute's Florida campus, and his colleagues at Scripps Florida and the University of Texas Southwestern Medical Center have devised an innovative new way to solve this longstanding problem.

"Current methods severely limit the size of the libraries you can screen,"

said Kodadek. "If you get 20 hits out of a 100,000 compound library, it's feasible to re-synthesize each of those hits to test which are the most effective. But what if you want to screen 10 million compounds? It takes an impossible amount of time to re-synthesize promising compounds for further study. To find the most potent ligands, our new method stands head and shoulders over what is available to researchers today."

Ligands are compounds that attach to proteins and alter their expression, potentially affecting a particular biomolecular activity, say, a protein pathway involved in a disease.

The new method displays millions of compounds on the surface of resin-based beads, each type of compound on a different bead. The hits are culled from the beads using a unique magnetic signature and then transferred to a microarray format—glass slides or silicon chips that can hold large numbers of compounds on their surface. The microarray format allows quantitative comparison of binding affinity that can be carried out without the need for tedious re-synthesis of many different compounds.

In the study, the team used mixed peptide/peptoid libraries—peptides make up proteins; peptoids are molecules closely related to, but more stable than peptides, making them more convenient for testing—but the method could be applied to any class of compound, according to Kodadek.

Changing the Paradigm

The Kodadek group's method combines several different technical advances to enable this convenient and efficient screening.

These days, most active molecules are discovered through screening of two basic types. There are functional screens, in which small molecules

are introduced into the wells of microtiter plates—flat plates with multiple wells that can reach as high as 9,600—and tested individually for their ability to alter the activity of an enzyme. Alternatively, there are binding assays, an approach first developed for bead-displayed peptide libraries, where each bead displays many copies of a single molecule.

"Our new method for screening synthetic libraries and characterizing the resultant hits combines many of the features of bead library screening and microarray-based analysis in a seamless fashion," Kodadek said. "The new technique uses several million beads, each of which displays a unique ligand—theoretically as many as 64 million compounds. The target protein has an antibody attached to it that is covered with iron oxide particles—magnetic dust. If the peptoid ligand is a legitimate ligand, and attaches to the protein, we can pull it from the mass by using a magnetized centrifuge."

The selected compounds are then removed from the beads through a unique cleaving process and attached to glass microarray slides. These arrays are mixed with different concentrations of the target protein, allowing the affinity strength of each compound on the array to be determined quickly and efficiently.

"This technology is relevant to custom libraries that are produced on beads," Kodadek said. "Right now, that probably constitutes five percent of screening going on. My guess, however, is that ratio will change once researchers begin to adopt this new method."

Adoption of this new technique will take time and something of a paradigm shift, Kodadek notes. The new screening technology monitors binding of the bead-immobilized molecule to the target protein; currently, the most widely used high-throughput screens monitor function of the compound. In addition, not all laboratories currently have the equipment and expertise necessary to make microarrays of small

molecules.

"I think our method can revolutionize medicinal chemistry," said Kodadek, "but this is only the first step."

More information: www.cell.com/chemistry-biology … 1074-5521(10)00005-0

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