'Mirror-image' molecules pave new path for cancer drug discovery

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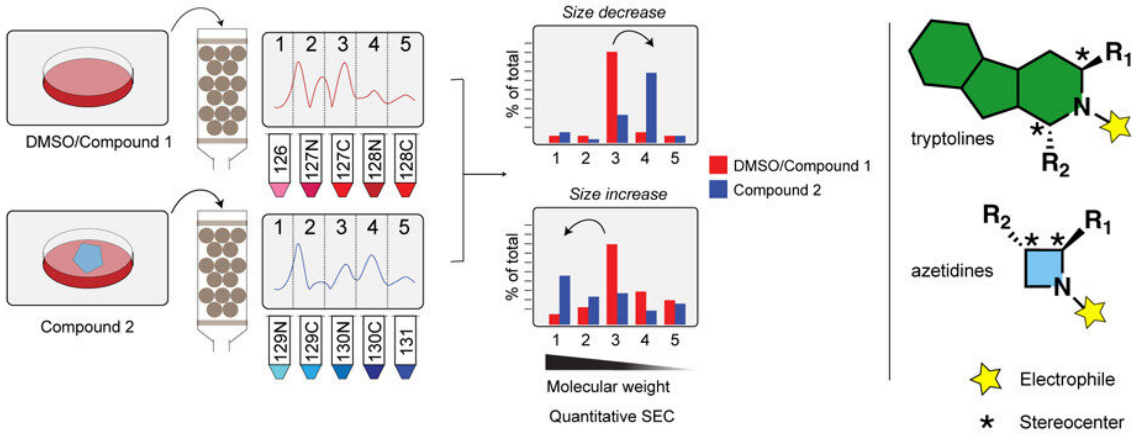

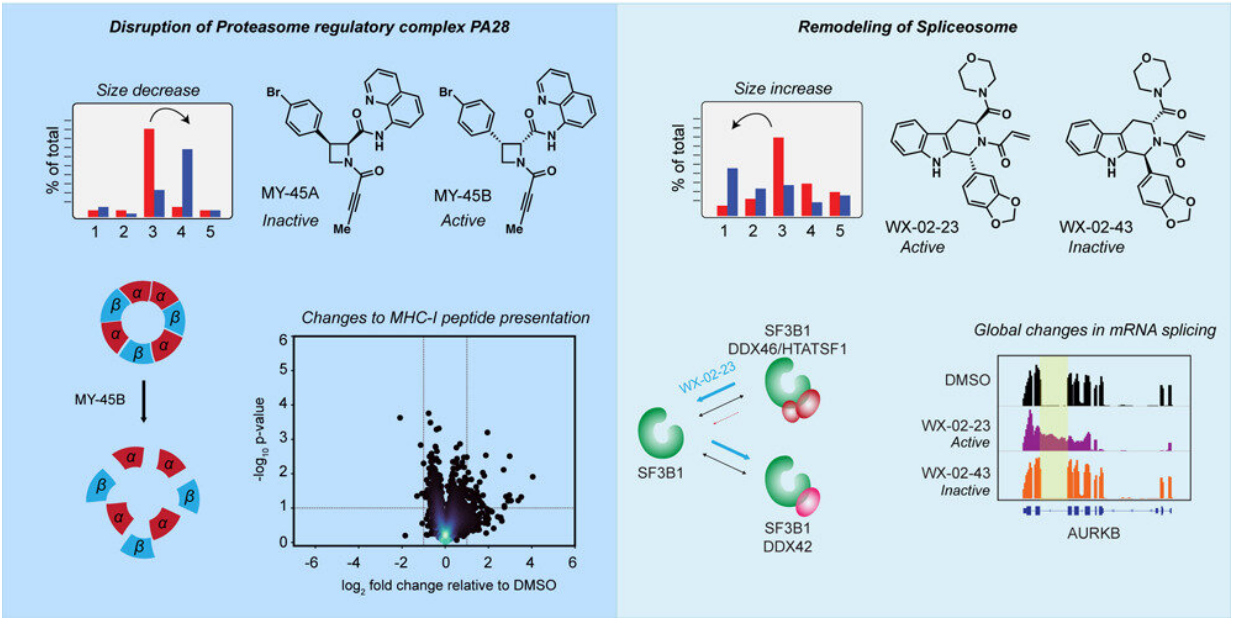

Graphical abstract. Credit: *Molecular Cell* (2023). DOI: 10.1016/j.molcel.2023.03.026

Scripps Research scientists have developed a new strategy for identifying small molecules that can change the function of proteins, offering a promising path for discovering targeted drugs. In collaboration with scientists at other institutions, the group used their new approach to find small molecules that can alter the activity of proteins involved in cancer.

The research, published in *Molecular Cell* on April 20, improves on previous methods that could screen for whether small molecules selectively attached to proteins, but not whether they affected the proteins' biological activities. The new method revolves around using two mirror-image versions of a small molecule and comparing how they change the size of protein complexes in cells.

"The ability of small molecules to specifically bind to a protein and cause a biological consequence is the fundamental basis for most drugs today," says senior author Benjamin Cravatt, Ph.D., Gilula Chair of Chemical Biology at Scripps Research. "With this assay, we're expanding our ability to discover these small molecules that not only bind proteins, but have functional impacts."

In recent years, Cravatt's lab has designed sets of small chemicals that can irreversibly bind to certain parts of proteins. However, screening these chemical libraries to discover their possible impact on protein function was generally a slow and tedious process. Since individual proteins have different roles in cell biology, researchers often have to develop specialized functional screens for each protein of interest. One screen, for instance, might determine whether the chemicals affected

cell growth, while another might determine whether the chemicals changed levels of a different molecule.

"Just because a small molecule engages a protein physically doesn't mean that it changes the protein's function in the cell," says co-first author Jarrett Remsberg, Ph.D., who carried out the work as an American Cancer Society postdoctoral research fellow in the Cravatt lab at Scripps Research. Former graduate student Michael Lazear, Ph.D. and postdoctoral fellow Martin Jaeger, Ph.D. were also first authors of the paper.

In the new work, Cravatt's group used the conglomeration of proteins into complexes as a proxy for their function. Proteins often work by binding to other proteins—if this binding doesn't happen or if it is induced to happen, it indicates a protein's function may have changed.

The research team designed pairs of "mirror image" molecules, called stereoisomers, that could each bind irreversibly to proteins in the same way that their previous chemical libraries had worked. The pairs of stereoisomers let them be sure that the impact of each small molecule was due to its unique structure (if only one version of a molecule changes the proteins' function, it is likely a specific and direct interaction).

Once they exposed cells to the pairs of stereoisomers, they tested whether a protein-of-interest was in a different size complex, using a technique called size exclusion chromatography in which proteins are sifted through beads with different sized pores.

To show the utility of this approach, the researchers screened the set of small molecules for their ability to change the sizes of protein complexes in prostate cancer cells. They pinpointed a molecule, MY-1B, which selectively disrupted a complex of proteins known as PA28, previously

found to play a role in degrading proteins in cancer. Further work in leukemia cells confirmed that, by specifically binding to the protein PMSE1, MY-1B or a related compound (but not their mirror images) could effectively inactivate the PA28 complex.

Cravatt and colleagues also followed up on an observation that a different chemical, EV-96, changed the size of a protein complex involved in splicing strands of RNA inside cells. The team discovered that EV-96 slowed the growth of cancer cells and pinpointed SF3B1 as the protein the chemical was binding to.

In both cases, the new chemicals represent the first time scientists have been able to target the protein complexes—PA28 and the so-called spliceosome— with small, simple synthetic chemicals.

"This means that researchers have new chemical tools in their arsenal that they didn't have before," says Remsberg. "It's an opportunity for better understanding these proteins as well as investigating potential therapeutic opportunities."

The team hopes their approach can be expanded to use other functional readouts than complex size, and they intend to use it to study different cell types in the future.

"The long-term idea is that we can use this approach to discover chemical compounds that impinge upon any readout," says Cravatt. "There are certainly other readouts that we hope to be able to look at in the future."

More information: Michael R. Lazear et al, Proteomic discovery of chemical probes that perturb protein complexes in human cells, *Molecular Cell* (2023). DOI: 10.1016/j.molcel.2023.03.026. www.cell.com/molecular-cell/pd … -2765(23)00239-3.pdf

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