A NEW FLAVOR OF ALLOSTERIC MODULATORS: SYNTHETIC PROTEINS
BY ALLISON JOHNSON, STAFF WRITER

Relay co-founder and Brandeis Professor Dorothee Kern is turning to synthetic proteins rather than small molecules to drug targets at allosteric sites, a strategy she thinks will shorten the discovery process and provide added selectivity.

On Monday, Kern's Brandeis team and collaborators at New York University published a proof-of-concept study in *Proceedings of the National Academy of Sciences* describing a screen to discover protein-based allosteric modulators of aurora kinase A (AURKA, Aurora-A) kinase.

Most therapeutic molecules bind to an active site to directly inhibit a target's activity, but allosteric modulators offer an alternative approach by binding to pockets elsewhere on the protein that can regulate the protein's function by changing its shape, protein-protein interactions or cellular location.

The approach has some advantages over active site inhibitors such as selectivity that enables targeting some of industry's most intractable targets. A handful of companies including Relay Therapeutics Inc. are developing platforms to find allosteric modulators (see "Moving Beyond the Active Site").

But these companies all develop small molecules, and Kern told BioCentury she wanted to push the envelope on just how selective an allosteric agent could be by turning to another modality.

“With a proteogenic ligand, we have much more chance to get high selectivity than a small molecule,” she said.

This is because a protein-based ligand will bind a larger surface area than a small molecule would, giving it more points of contact at the target site that could help differentiate closely related family members, Kern explained.

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*Dorothee Kern, Brandeis University*

NYU Langone Health's Shohei Koide had the protein-based library Kern wanted to screen. Koide is a professor in the Department of Biochemistry and Molecular Pharmacology at NYU and director of cancer biologics at NYU's Perlmutter Cancer Center.

Koide has built libraries of small proteins dubbed monobodies over the past two decades that comprise an unchanging scaffold domain, plus domains with residues that can be swapped out to create a diverse set of proteins with different sequences and binding properties.

The synthetic proteins have been used as tools to help crystallize proteins or as active site inhibitors, but had never been used in a screen to identify a binder to a specific allosteric pocket prior to the *PNAS* study, Koide told BioCentury.

In fact, Koide and Kern had to devise a clever screen because the monobodies in the library are designed to bind all surfaces, not a specific site.

The collaborators described a two-step screen in the *PNAS* paper: first, they separated the monobodies that bound to AURKA from those that didn’t. Then, among the AURKA-binding monobodies, they identified which ones did and did not bind a version of AURKA mutated at the allosteric pocket of interest. Those that didn't bind to the mutated version were likely binding to the allosteric pocket on the unmutated version.

The screen yielded both inhibitory and activating monobodies targeting the AURKA pocket, validated via *in vitro* activity assays and co-crystal structures.

By using monobodies, the partners said the screen was faster and cheaper than one that used small molecules thanks to the library's diversity and speedy generation. The monobody library has many more variations than a
small molecule library, Kern added, providing a higher probability of identifying a highly selective binder in a screen.

Koide explained, “We can generate high affinity molecules much faster: monobody generation takes 3-6 months depending on how challenging the target is while small molecule development can take 1-2 years.”

While the group used AURKA in its proof-of-concept screen presented in PNAS, Koide and Kern said the screen is generalizable to other targets, as long as researchers know which pocket(s) they want to drug. AURKA was chosen initially because it’s a well-studied cancer target whose allosteric regulatory mechanisms are understood.

At least four companies are developing small molecule AURKA inhibitors, all of which target the active site.

Koide said the group’s next steps are to genetically encode the monobodies and express them in cells to validate their function in a cell-based system. Following that validation, the group can focus on making the molecules more drug-like to ensure adequate cell permeability, which is a big challenge with biologics. But because the monobodies don’t contain disulfide bonds, which prevents intracellular delivery of antibodies, they aren’t starting off with such a disadvantage.

Kern highlighted an immediate application of the study is for any researcher to use the solved crystal structures of AURKA presented in the paper in an activated and inhibited state to design their own allosteric modulators against them.

Patents are pending for the screening platform described in the PNAS study. The collaborators are open to partnerships.