New cages to trap molecules push boundaries of protein design

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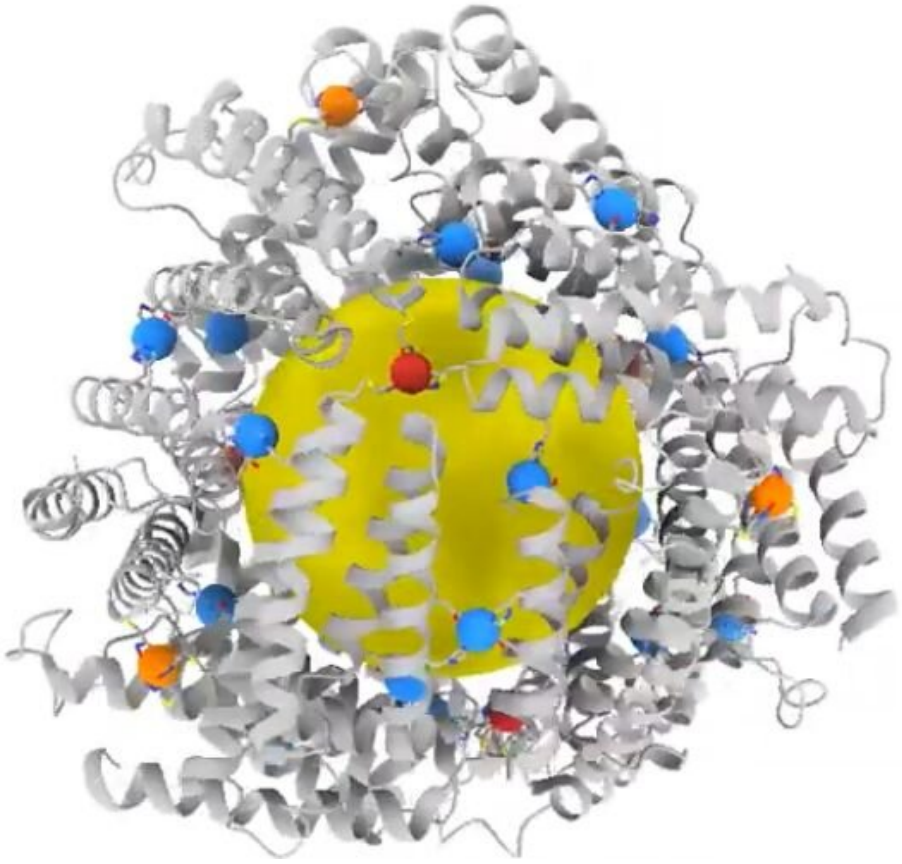

Protein design is a popular and rapidly growing field, with scientists engineering novel protein cages—capsule-like nanostructures for

purposes such as gene therapy and targeted drug delivery. Many of these structures fashioned in the lab, while perhaps aesthetically pleasing to chemists, have holes too big to trap a target molecule or don't open on command, limiting their functional scope.

But new research findings, by UC San Diego Professor of Chemistry and Biochemistry Akif Tezcan, offer a protein architecture with small holes—"pores" in chemistry jargon. The findings, published in *Nature*, push the boundaries of synthetic protein design past what is considered state-of-the-art.

"If molecules can freely go back and forth through these holes, you're not going to be able to store little things on the inside," explained Tezcan. "Protein cages that people have designed before have the right shape and symmetry, but they're mostly like Wiffle balls—they don't necessarily isolate the interior from the exterior."

By tailoring the surface of small protein building blocks with multiple metal-binding sites, Tezcan's team developed a new protein cage with small pores that trap molecules securely inside.

"This project is a significant addition to the field because it demonstrates that minimal design can be used to generate modular, stimuli-responsible protein cages that approach the complexity of naturally evolved systems," said co-author Rohit Subramanian, a graduate student in the Tezcan Lab.

Additionally, the new structure can be opened via chemical, thermal or redox (transfer of electrons between a set of atoms, molecules or ions with the same chemical formula) reactions. According to Tezcan, the UC San Diego research team was ideally situated to create the new protein cage design with its inorganic chemistry insights—specifically metal coordination chemistry, which made the difference.

The first author of the paper, titled "Constructing Protein Polyhedra via Orthogonal Chemical Interactions," is Eyal Golub, a former postdoctoral scholar in the Tezcan Lab who conceived the project and performed many of the experiments.

"In evaluating our designs, we discovered that one resulted in the formation of a six-protein cage instead of the 12-protein cage we were expecting," said Golub. "This result was especially important for the project because it demonstrated an adaptability that permitted different types of cage symmetries using the same design scaffold."

Because protein cages have tightly interconnected, polyhedral shapes—like a soccer ball—their construction from simpler building blocks must meet stringent symmetry requirements. Other designers have largely avoided this challenge by using protein building blocks with inherent symmetries, connecting them via relatively strong interactions. These strategies, however, lead to highly porous architectures which cannot open and close like natural protein cages do. Viruses, for example, are examples of protein cages in nature. They contain genetic cargo in their interior and deliver them to host cells they infect. The UC San Diego researchers' novel strategy allowed them to arrange the building blocks in precise orientations and proper symmetries for building protein cages while also controlling their dynamics via the metal ions.

The paper also includes detailed visualizations of the protein cage made possible through collaborations with Professor Tim Baker and his group in the UC San Diego Division of Biological Sciences, Section of Molecular Biology, with UC San Diego's Crystallography and Cryo-EM (cryo-electron microscopy) facilities.

"We knew that we needed different techniques to understand the structures of our protein cages," said Tezcan. "At UC San Diego, there's

always someone who has the expertise to help, somebody willing to collaborate and teach us how to do it."

As for the next step, Tezcan said there is more development to be done.

"Can we make larger cages, can we encapsulate bigger cargo, can we actually deliver it into the cells? But we are most excited about the fundamental and interdisciplinary aspect of this project, which shows the power of simple chemical intuition in addressing a complex biological puzzle," he said.

More information: Eyal Golub et al. Constructing protein polyhedra via orthogonal chemical interactions, *Nature* (2020). DOI: 10.1038/s41586-019-1928-2

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