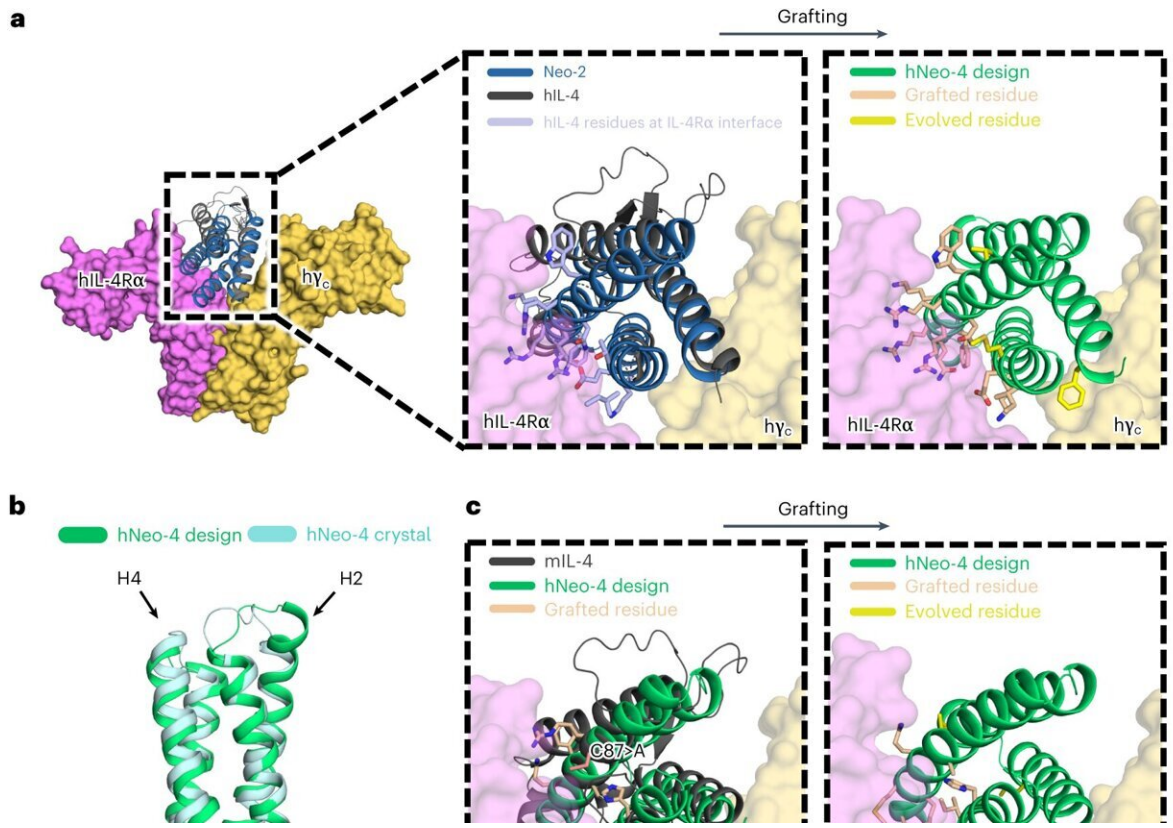
Researchers enhance the function of natural proteins using 'protein Legos'

September 14 2023, by Jonathan Deutschman



Computational design of IL-4 mimetics. a, Progression of the hNeo-4 evolution process. hIL-4 residues at the hIL-4–hIL-4Rα interface were grafted onto the Neo-2 structure, and the resulting molecule was subjected to directed evolution to improve its binding affinity toward hIL-4 receptors. b, Comparison of the computationally predicted hNeo-4 structure and the experimentally determined hNeo-4 crystal structure. c, Progression of the mNeo-4 evolution process. mIL-4 residues at the mIL-4–mIL-4Rα interface were grafted onto the designed hNeo-4 structure, and the resulting molecule was subjected to directed evolution

to improve its binding affinity toward mIL-4 receptors. Cys 87 in mIL-4 at the mIL-4–mIL-4Rα interface, depicted in salmon, was not grafted onto the hNeo-4 structure to avoid an unpaired cysteine bridge. Instead, alanine was substituted into the corresponding position 47 in hNeo-4 before directed evolution. d,e, BLI sensograms depicting the interactions between hIL-4 or hNeo-4 (threefold serial dilutions starting at 200 nM for both) and hIL-4Rα (d) and between mIL-4 or mNeo-4 (threefold serial dilutions starting at 67 nM for mIL-4 or starting at 260 nM for mNeo-4) and mIL-4Rα (e). K_d values derived from the kinetic parameters are indicated. Raw data were fitted using a 1:1 Langmuir binding model. Fitted curves are shown in gray. Credit: *Nature Chemical Biology* (2023). DOI: 10.1038/s41589-023-01313-6

Johns Hopkins engineers have helped develop and characterize an artificial protein that triggers the same response in the human body as its natural counterpart—a breakthrough that not only has the potential to facilitate the design of drugs to accelerate healing but also sheds light on the mechanisms behind various diseases.

The team's research was published in Nature Chemical Biology.

"It's protein Legos, essentially," said team leader Jamie Spangler, an assistant professor of chemical and biomolecular engineering and biomedical engineering. "We know what the different pieces look like, and we put them together in an arrangement that is predicted to look like the protein we're trying to mimic. As far as the body is concerned, this newly created protein is as genuine as the one that occurs in nature."

The synthetic protein, called Neo-4, mimics the function of the natural protein interleukin-4 (IL-4), a crucial player in immune system regulation. White blood cells release IL-4 in response to a range of immune triggers, from allergic inflammation to muscle injuries. IL-4 can then attach to various receptors on cells throughout the body. However,

when IL-4 is directly injected as a drug, it can bind to unintended cells, causing unwanted side effects.

"If you give someone IL-4 it just acts on everything," said Zachary Bernstein, team member and Ph.D. candidate in biomedical engineering. "But that makes it difficult for therapeutic use. Neo-4 is more specific and only activates immunologically relevant cells."

Neo-4 attaches to a narrower range of cells than IL-4, a characteristic that the researchers say could make it a promising candidate for future drug development. For instance, a torn anterior cruciate ligament (ACL) is a common season-ending sports injury. Cytokines like Neo-4 have the potential to speed up the healing of torn ACLs and other damaged ligaments and muscles.

"These are computationally designed proteins that behave like proteins in nature but have better properties," Spangler said. "That means we can build these robust, hyper-stable proteins to do whatever we want. The hope is that we can use this mimetic to deliver IL-4 in a way that is safer and more robust than the natural cytokine, which could help with its therapeutic advancement."

Huilin Yang, graduate of the doctoral program in chemical and biomolecular engineering and current postdoctoral fellow at ETH Zurich, contributed to this research.

More information: Huilin Yang et al, Design of cell-type-specific hyperstable IL-4 mimetics via modular de novo scaffolds, *Nature Chemical Biology* (2023). DOI: 10.1038/s41589-023-01313-6

Provided by Johns Hopkins University