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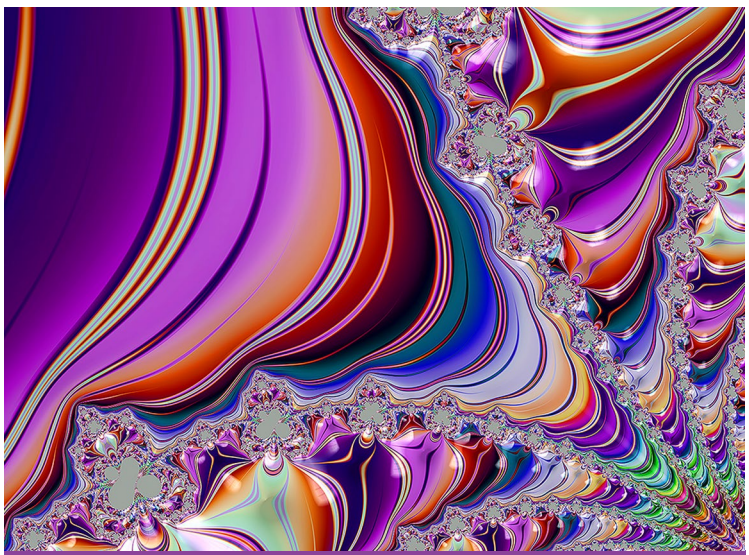

Credit: Tim Bird/GettyImages

BIOMATERIALS

Enzymes fraternize with fractals

Fractal objects, such as trees or lungs, have the same shapes but at smaller and smaller dimensions. These self-similar arrangements are characterized by a high surface area-to-volume ratio, enabling them to efficiently exchange matter and energy. For example, human lungs have the surface area of the size of a tennis court in a volume of only 6 litres. Now, writing in *Nature Chemistry*, Sagar Khare and colleagues use a multiscale modelling approach to design proteins that self-assemble into various fractal morphologies in response to a chemical stimulus. These protein-based structures are self-similar over a large length scale, and their large pore size and high surface area make them ideal biomaterials for the capturing and release of nanometre-sized cargo.

Khare and colleagues chose two oligomeric enzymes (AtzA and AtzC) with dihedral symmetry to recreate fractal structures at the supramolecular level. AtzC was engineered to contain Src homology (SH) 2 domains, which can bind to phosphorylated tyrosine residues of AtzA. Thus, the interaction of the two enzymes can be reversibly controlled by adding Src kinase, which phosphorylates tyrosine, or a phosphatase, which dephosphorylates tyrosine.

To identify the large-scale self-assembly behaviour of the two enzymes and thus emergent higher-order structures, the researchers applied atomic-resolution computational modelling. "We designed the proteins to have high interaction affinity and limited (but not zero) conformational flexibility," explains Khare. "Based on the energy distribution of interactions, we could then derive a probability distribution of the different binding modes." Subsequent sampling of the binding orientations enables the calculation of protein assemblies and their fractal dimensions.

Based on this modelling approach, Khare and colleagues designed different fractal structures, for example, rod-like and tree-like morphologies, by altering the stoichiometry of the two proteins, and the affinity of the SH2–phosphopeptide interaction in the simulation. These fractal protein assemblies can be experimentally realized by mixing the recombinantly expressed protein variants with ATP and Src kinase, with the reaction rate and size depending on the concentration of ATP and the stoichiometry, respectively.

The resulting fractal assemblies have a diameter of 1–10 μm and a substantially higher protein density than similar-sized 2D or 3D crystalline lattices. The high effective surface area in combination with large pores allows the binding of a high concentration of molecules, compared with globular structures. By adding an excess of phosphorylated AtzA, SH2-functionalized molecules can be tethered to the entire volume of the fractal material. Subsequent incubation with phosphatase then triggers release of the captured molecules through dephosphorylation, which can be explored, for example, for phosphorylation-dependent antibody purification.

Khare and colleagues are testing their approach for different proteins and chemical and physical stimuli. "We have some exciting preliminary results for light-controlled assemblies," says Khare. "Light should allow greater control over the assembly kinetics than phosphorylation, resulting in more interesting shapes." Moreover, they want to explore fractal protein assemblies as components of extracellular matrix-like materials to facilitate controlled and reversible spatial modification with growth factors, antibodies or aptamers.

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ORIGINAL ARTICLE Hernández, N. E. et al. Stimulus-responsive self-assembly of protein-based fractals by computational design. *Nat. Chem.* **11**, 605–614 (2019)