PROTEIN ASSEMBLY

Biomaterials in non-integer dimensions

Nature harnesses fractal geometry to create structures with unusual surface-to-volume ratios. Now, a new design approach enables the reversible assembly of functional enzymes into arboreal patterns with fractal geometry.

Iris D. Young and James S. Fraser

Biomolecules can be repurposed to do useful reactions, but it is often an uphill battle to get them to perform efficiently at scale. Studying natural systems, which have the advantage of millions or even billions of years of optimization, offers a route to develop more efficient, more modular and more evolvable biomolecular catalysts. Directed evolution studies have shown that it is possible to repurpose the chemical principles encoded in amino acid residues, and synthetic biology is revealing how biological modules can be "remixed" to produce new outcomes. A final trick that nature often performs is to increase the reactive surface area, and thereby the overall efficiency, by self-assembling biomaterials into fractals.

Observed in nature long before they were described in mathematics, fractal structures — such as coastlines, snowflakes, mountains and river tributaries, but also various surfaces and networks in plants, fungi and animals — exhibit self-similarity over multiple length scales (Figs. 1a, 1b). Each self-similar pattern has a dimension describing how it scales, which may be fractional: if three smaller copies of the same pattern may be found in the original, each half as large, its dimension would be log3/log2 ≈ 1.58. Non-integer dimensionality gives rise to counterintuitive surface-to-volume ratios, helping explain the many cases of convergent evolution of these features in structures for signalling, breathing, filtering, transport of materials and other core biological functions[1].

The principles of fractal assembly have recently been encoded into DNA[2], and getting proteins to self-assemble with fractal geometry and then do something useful was an obvious next challenge. Now, writing in *Nature Chemistry*, Sagar D. Khare and co-workers report the self-assembly of protein structures with fractal geometry[3]. The team set out to generate fractal assemblies of proteins with tunable properties, including molecular cargo capture and release. High surface-to-volume ratios are critical for efficient cargo capture in synthetic materials, and the added advantage of tunability of several properties of the bulk material makes such engineered fractals particularly appealing targets. Another key aspect of this work is the choice of phosphorylation as the trigger for reversible assembly and cargo capture. The selection of a ubiquitous biological signalling mechanism for this purpose anticipates the materials' potential use in, or interfacing with, living biological systems — a very exciting and imminent possibility!

In order to promote formation of a fractal pattern during assembly, Khare and co-workers selected phosphorylation-activated enzymes and engineered the interfaces and linkers to obtain the requisite stability, symmetry and flexibility. Their target fractal was an arboreal (branching), stochastic, directional pattern. To design the interfaces, they developed a procedure using the Rosetta macromolecular modelling program to generate loops at the protein–protein interfaces. This approach allowed them to design custom building blocks to match the desired fractal properties. The computational approaches involved in the design of these structures are well-justified, including any necessary simplifications and assumptions, such as the choice of coarse-grained model for large-scale simulations. Furthermore, the measured fractional dimensionalities of the synthesized structures are in close agreement with the predictions based on simulations.

The team selected two enzymes — hexameric AtzA and tetrameric AtzC from the atrazine biodegradation pathway — as the components that would enable multiple branching structures. To add

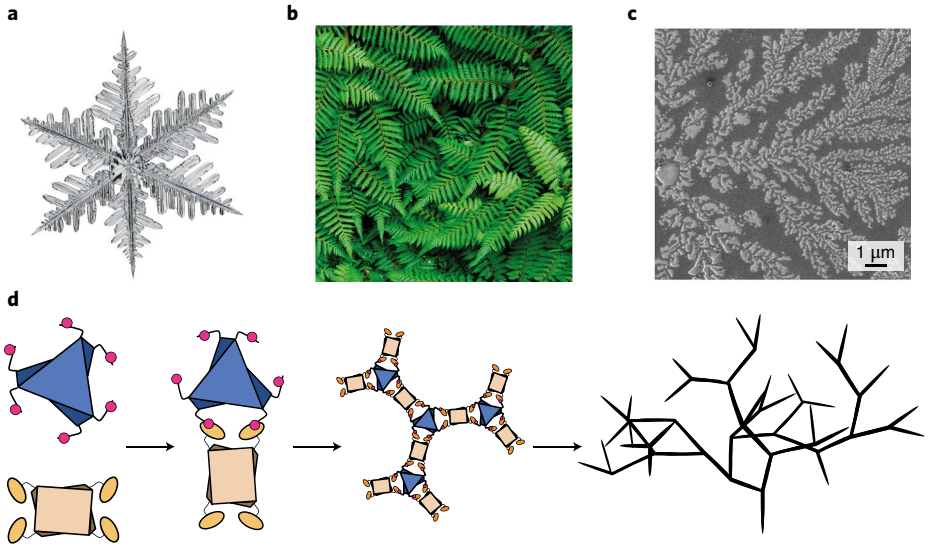

Fig. 1 | Natural and synthetic patterns showing self-similarity over multiple length scales. **a**,**b**, Fractal properties of a snowflake (**a**) and fern leaves (**b**). **c**, Helium ion microscopy reveals the fractal-like assembly of the protein structure. **d**, Schematic diagram showing how the enzymes AtzA (blue triangular) and AtzC (tan rectangular) are functionalized with phosphopeptides (pink circles) and SH2 domains (yellow ovals) and the subsequent assembly into a fractal topology. Credit: Razvan Cornel Constantin / Alamy Stock Photo (**a**); Top-Pics TBK / Alamy Stock Photo (**b**); adapted from ref. [3], Springer Nature Ltd (**c**,**d**).

a response to phosphorylation and the potential for oligomerization, they fused a phosphopeptide pY tag to AtzA; and a high-affinity Src homology 2 (SH2) domain, which acts as a binding module for pY, to AtzC. By substituting a longer linker between modules, they were also able to switch from fractal to globular assemblies. These control assemblies exhibited lower cargo loading: cargo localized uniformly throughout the fractal assemblies, but only to the surface of the globular ones. However, both topologies produced equivalent enzymatic activity. The team attribute this to the small size of the atrazine substrate molecule, which allows it to diffuse readily throughout both. In designs where the scaffold components and substrates are comparable sizes, morphology and functional efficiency can be anticipated to be more strongly linked. Dynamic designs where assembly is not triggered once, but where the structures exist in equilibrium, may also exhibit different properties than these static designs. Synthetic globular formations assembling and dissolving continuously may more closely resemble biomolecular liquid droplets, membrane-less compartments in cells which are organized by a combination of strong/specific/multivalent and weak/nonspecific interactions[4]. The ease with which linker alterations in designed assemblies promote formation of either globular or fractal geometries suggests that fractal geometries may soon be discovered in natural multivalent assemblies.

The thorough testing of the morphology of the resulting structures and the dynamics of assembly and disassembly stand out as a highlight of this work. For example, the team verify bulk morphology and self-similarity across three orders of magnitude in length by cryo-electron tomography, helium ion microscopy and atomic force microscopy. They quantify particle assembly by biolayer interferometry and dynamic light scattering, and they track cargo capture with GFP fluorescence and enzymatic activity. The kinetics of assembly and disassembly appear to be highly cooperative. Although the micrographs reveal beautiful "snowflake"-like and dendritic patterns (Fig. 1B), the team are also careful to implement objective computational assessments of geometric properties of the assemblies.

This study constitutes a synthesis of advancements in several areas: Khare and co-workers have successfully generated a self-assembling fractal that traps a cargo molecule, using readily-available, biocompatible building blocks to do so, ensuring it is triggered by a common and well-studied biological signalling mechanism, documented the dependence of the fractal morphology on component concentrations, and described computational techniques that may be used to design similar systems using other proteins. One exciting future direction will be the extension of this technique to cargo capture and release independent from fractal assembly — components in a static fractal scaffold may be functionalized for other purposes, responding to other signals. Extension of this general design concept to fractal morphologies beyond arboreal is another promising possibility.

Materials with fractal geometry promise to be particularly useful at the interfaces between synthetic and biological systems, where systems will benefit from the precision and control of the fabrication process while being natively compatible with the biological system of interest. More immediate applications may be in filters or environmental remediation, where the surface area to volume ratios of these materials will offer advantages. The next challenge is to integrate the lessons of fractal assembly demonstrated here into a system that is currently limited by the bounds of conventional, non-fractal, geometry. To accomplish this goal will likely require using additional biomolecular building blocks and testing whether they can be assembled using the design principles employed here. Success in these endeavours could enable development of a wide range of fabricated materials with interesting geometric and functional properties. ❐

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COLD CHEMISTRY

Too slow to be activated

Light is often used to trigger reactions, energetically exciting the reactant(s) to kick them over the intrinsic reaction barrier. Now, however, the reaction between an excited atom and a charged molecule at very low temperatures has been shown not to adhere to this paradigm, instead undergoing a reaction blockading effect.

Roland Wester

Initiating chemical reactions with laser photons instead of thermal energy has been driving research in chemical reaction dynamics for almost as many years as lasers have been around. Lasers can trigger reactions and modify reaction rate coefficients through single or multiple vibrational transitions, or by selective electronic excitation of one of the reactants. For vibrational transitions, the well-known Polanyi rules tell us that reactions with a late barrier (for which the transition state resembles the products more than the reactants) can be strongly enhanced by exciting vibrational motion in the bond to be broken[1]. Vibrational-mode-specific dynamics is also at work when partially deuterated water (HDO) is dissociated preferentially along the OH or the OD bond using selective overtone excitation[2]. In other cases, vibrational excitation is found to be a spectator to a reaction[3] and does not affect the reactivity appreciably.

Not only vibrationally, but also electronically exciting reactants can substantially enhance a chemical reaction. This effect has become an important tool