Title

Biomaterials in non-integer dimensions

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Biomolecules can be exploited to do useful reactions, but it is often an uphill battle to get them to perform at high levels. How can we learn from natural systems, which have the advantage of millions or billions of years of optimization, to develop more efficient, more modular, and more future-proof biomolecular catalysts? Directed evolution studies have shown us how to repurpose the chemical principles encoded in amino acid residues and synthetic biology is revealing how modules can be “remixed” to produce new outcomes. A final trick that nature often performs to increase reactive surface area and overall efficiency is the self-assembly of biomaterials into fractals.

Observed in Nature long before they were described in mathematics, fractal structures — such as coastlines, mountains and river tributaries, but also various surfaces and networks in plants, fungi and animals — exhibit self-similarity over multiple length scales (**Figure 1A**). 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 signaling, 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. Khare and coworkers set out to generate fractal assemblies of proteins with tunable properties, including molecular cargo capture and release (Current paper citation). 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 these 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 signaling 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 coworkers 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 modeling 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 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 authors selected the hexameric AtzA and tetrameric AtzC enzymes of the atrazine biodegradation pathway as the multiply branching components. To add a response to phosphorylation and potential for oligomerization, they fused a phosphopeptide pY tag and a

high-affinity Src homology 2 (SH2) domain, which acts as a binding module for pY. 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 authors 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 at equilibrium may also exhibit different properties than these static designs. Globular formations assembling and dissolving continuously may more closely resemble biomolecular liquid droplets, membraneless compartments in cells organized by a combination of strong/specific/multivalent and weak/nonspecific interactions [3].

The incredibly thorough testing of the morphology of the resulting structures and the dynamics of assembly/disassembly stands out as a highlight of this work. For example, the authors 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 (**Figure 1B**), they 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 coworkers have successfully generated a self-assembling fractal that traps a cargo molecule, used readily-available, biocompatible building blocks to do so, ensured it is triggered by a common and well-studied biological signaling 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 remediators, where the surface area to volume ratios of these materials will offer advantages. The next challenge is to integrate the lessons of fractal assembly 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 exploited here. Success in these endeavours could enable development of a wide range of fabricated materials with interesting geometric and functional properties.

1. Mandelbrot, B. B. *The Fractal Geometry of Nature: Updated and Augmented*. (W.H. Freeman, 1983).
2. Tikhomirov, G., Petersen, P. & Qian, L. Fractal assembly of micrometre-scale DNA origami arrays with arbitrary patterns. *Nature* **552**, 67–71 (2017).
3. Banani, S. F., Lee, H. O., Hyman, A. A. & Rosen, M. K. Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* **18**, 285–298 (2017).

Figure 1: A. showing fractal properties of an actual snowflake or other natural system. B. bkgd: one of the fractal patterns in the SI (panel from Fig S17) overlaid with mockup zooming in to individual components (based on Fig S31 panel B).