Isothermal self-assembly of multicomponent and evolutive DNA nanostructures

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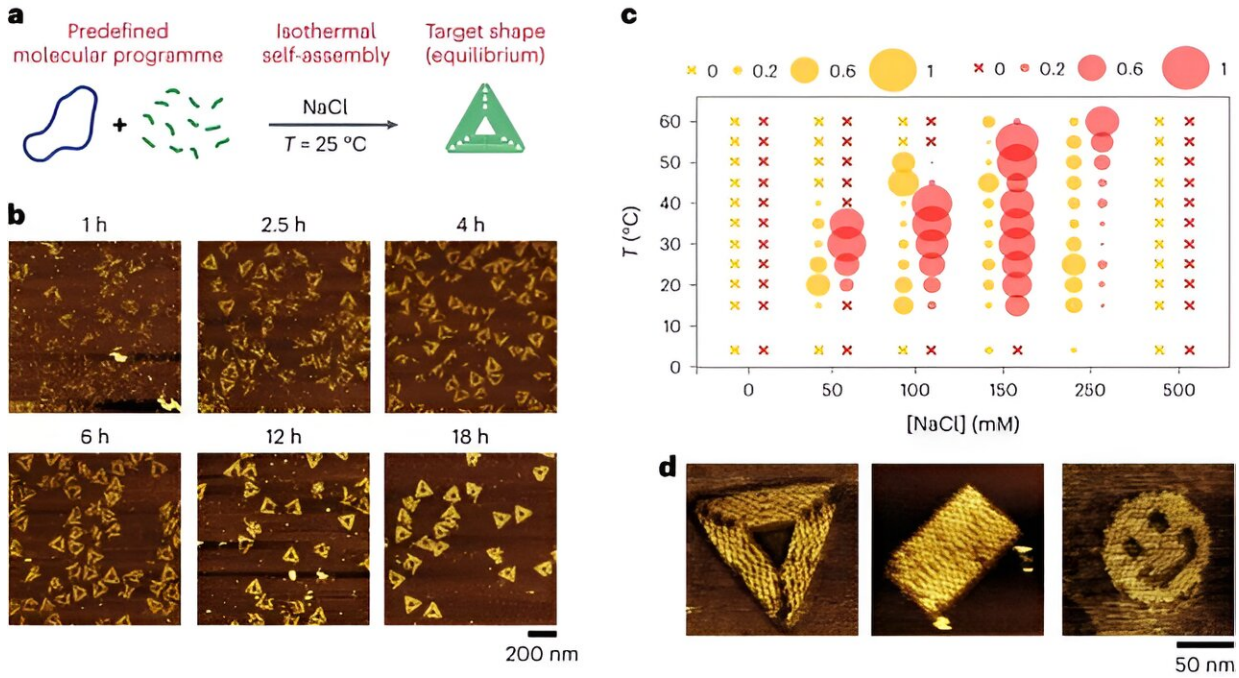


Isothermal self-assembly of user-defined DNA origamis in a magnesium-free NaCl buffer. (a), An origami mix (M13 scaffold plus a 40× excess of desired staples) can spontaneously self-assemble at constant temperature into the target equilibrium shape (for example, a triangle) in TANa buffer. (b), AFM observation of the isothermal origami formation at 25°C in TANa ([NaCl] = 100 mM), for a set of staples coding for sharp triangles, as a function of incubation time. (c), Fraction (bubble size) of partially folded (yellow) and fully folded (red) origamis after 24 h of isothermal self-assembly with a set of staples coding for sharp triangles, for various incubation temperatures (T) and NaCl concentrations. A cross symbol indicates a fraction of 0. For the sake of readability, the remaining fraction, which corresponds to non- or misfolded

origamis, is not plotted in this graph. All images used for these analyses are available in a citable public repository (doi: 10.5281/zenodo.7998757) and can be accessed directly at the following link: https://zenodo.org/record/7998757. d, Representative close-up AFM images of origamis obtained by isothermal assembly in TANa ([NaCl] = 100 mM) at 25°C for staples coding for sharp triangles (left), tall rectangles (middle) and smileys (right). For all experiments: [M13] = 1 nM; each staple concentration is 40 nM; no staple purification was performed before AFM imaging. Credit: *Nature Nanotechnology* (2023). DOI: https://doi.org/10.1038/s41565-023-01468-2

Multiple complementary DNA strands can be thermally annealed into desired entities to engineer DNA nanostructures. In a new study now published in *Nature Nanotechnology*, Caroline Rossi-Gendron and a team of researchers in chemistry, materials science and biology in France and Japan used a magnesium-free buffer containing sodium chloride, complex cocktails of DNA strands and proteins to self-assemble isothermally at room temperature or physiological temperature into user-defined nanostructures including nanogrids, DNA origami and single-stranded tile assemblies.

This self-assembly relied on thermodynamics, proceeding through multiple folding pathways to create highly configurable nanostructures. The method allowed the self-selection of the most stable shape in a large pool of competitive DNA strands. Interestingly, DNA origami can shift isothermally from an initially stable shape to a radically different one through an exchange of constitutive staple strands. This expanded the collection of shapes and functions obtained via isothermal self-assembly to create the foundation for adaptive nanomachines and facilitate evolutionary nanostructure discovery.

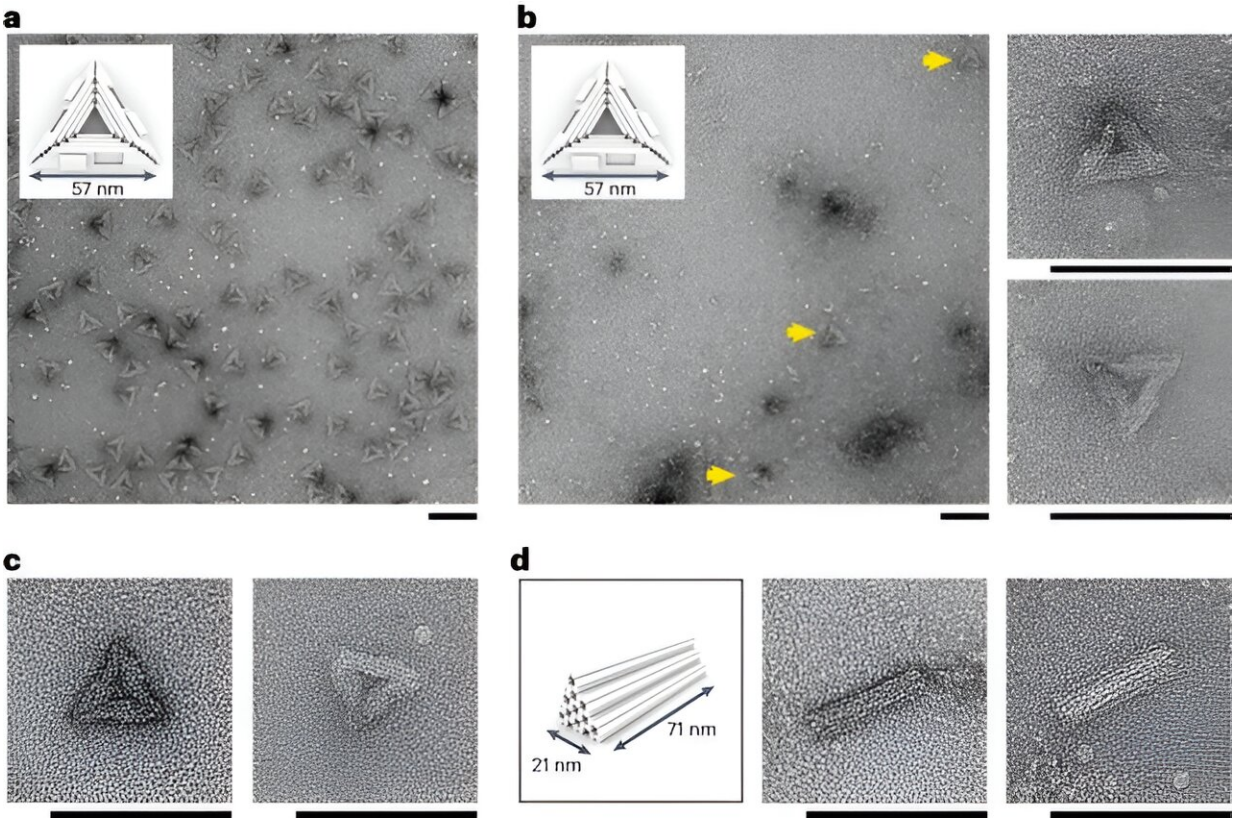
Self-assembly in nature and the lab

Self-assembly occurs when naturally occurring or rationally designed entities can embed necessary information to spontaneously interact and self-organize into functional superstructures of interest. Typically, synthetic self-assembled materials result from the organization of a repeating single component to create a stable supramolecular assembly containing micelles or colloidal crystals with a prescribed set of useful properties. Such constructs have limited reconfigurability, making it highly challenging to produce the desired structures.

Structural DNA nanotechnology explores the sequence-dependent base-pairing principle between synthetic DNA single strands to overcome this challenge, and assemble diverse and elaborate superstructures of an intended shape, size and functional specificity at large-scale with a range of applications. Multicomponent structures are typically derived from a thermal annealing process, where the DNA mixture is heated above its melting temperature at first and cooled down slowly to avoid kinetic traps and ensure sequence-specific DNA hybridization.

Structural DNA nanotechnology

Thermal annealing can hinder the possibility of spontaneous nanostructure formation under fixed conditions. In this work, Rossi-Gendron and colleagues therefore described that the major method of structural DNA nanotechnology depends on the same principle of generic isothermal DNA self-assembly to create user-defined elaborate DNA nanostructures such as DNA origami and DNA nanogrids. The research team studied the structural complexity of DNA origami designs and self-repeating nanogrids using atomic force microscopy to reveal the multiplicity of folding pathways in self-assembling 2D origami shapes.



Isothermal self-assembly of elaborate 3D structures at room or body temperature leads to well-shaped 3D origamis at low yield. a–d, Negative-stain TEM images of the structures obtained by thermal annealing (a) or isothermal assembly (b–d) and after removal of excess staples by gel electrophoresis. a, T1 triangular structures (scheme in inset) obtained by 41 h of thermal annealing in an optimized Mg buffer (5 mM Tris–HCl, pH 8.0, 1 mM EDTA, 18 mM $MgCl_2$). b–d, Structures obtained by isothermal self-assembly (no thermal pretreatment) in TANa buffer: T1 triangular structures (scheme in inset) indicated by yellow arrows and obtained with [NaCl] = 100 mM at 25°C for 48 h (b); T1 triangular structures obtained with [NaCl] = 200 mM at 25°C (left) and with [NaCl] = 100 mM at 37°C (right) for 72 h (c); Tb "Toblerone"-like structures (left, scheme) obtained with [NaCl] = 100 mM at 25°C for 48 h (middle) and with [NaCl] = 200 mM at 25°C for 48 h (d). Scale bars, 100 nm. Credit: *Nature Nanotechnology* (2023). DOI: https://doi.org/10.1038/s41565-023-01468-2

DNA origami via self-assembly in sodium chloride

The team completed a series of experiments in a thermodynamically regulated isothermal self-assembly environment to complete shape transformation. They accomplished this by assembling a DNA origami mixture without thermal pretreatment and incubated the constructs for several hours in a conventional buffer. As observed previously, regardless of the incubation time, the outcomes did not show the formation of properly shaped objects.

The team opted for an alternative buffer supplemented with monovalent salts to promote staple exchange and reconfiguration to note the remarkable formation of properly folded sharp triangles at room temperature within a few hours. These results were consistent across intermediate salt concentrations. The researchers showed how isothermal self-assembly in buffer could be electrostatically driven to generate a variety of custom nanostructures under a broad temperature window.

They explored the concept for the isothermal self-assembly of 3D origami to highlight the possibility of spontaneous self-assembly at room or body temperature without thermal pretreatment to create a variety of morphologies to exemplify the versatility of self-assembly. Nevertheless, the very low yield of the constructs highlighted its current limitation that can be overcome by optimizing the nanostructure design.

Multiplicity of folding pathways and shape-shifting

Rossi-Gendron and colleagues further studied the mechanisms of isothermal self-assembly by devising a method to follow the folding pathway of 2D DNA origami in real-time. The work showed that achieving the equilibrium structure for an individual origami did not depend on one specific folding pathway, instead relying on multiple paths, until it reached the target equilibrium shape.

Partially folded structures showed diverse initial folding states to imply that multiple folding paths did not rely on surface-assisted self-assembly. The outcomes conclude that isothermal origami formation is a thermodynamically regulated process whereby the structures reached an equilibrium state via self-assembly. Upon exposing the origami shapes to a set of competitive staples, the team noted how the self-assembly led to spontaneous evolution from origami shape to a dramatically different stable construct to create a thermodynamically favored shape-shifting outcome.

Outlook

In this way, Rossi-Gendron and colleagues used a generic saline buffer and a highly multicomponent mixture of DNA strands to spontaneously self-assemble at constant temperature across a range of temperatures to form properly shaped objects as origamis or DNA nanogrids. They achieved these outcomes at room temperature for step-wise thermodynamically driven self-assembly. The results indicated the possibility for dynamic functions in ambient environments and living systems with fixed temperatures for nanostructure discovery using large libraries of DNA components.

More information: Caroline Rossi-Gendron et al, Isothermal self-assembly of multicomponent and evolutive DNA nanostructures, *Nature Nanotechnology* (2023). DOI: 10.1038/s41565-023-01468-2

Paul W. K. Rothemund, Folding DNA to create nanoscale shapes and patterns, *Nature* (2006). DOI: 10.1038/nature04586