Cooperativity in self-assembly – unlocking the magic of DNA origami

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Since its invention in 2006,[1] DNA origami has been the leading method for creating nucleic acid nanostructures and the most successful molecular self-assembly approach. Despite its widespread adoption, the mechanisms responsible for its remarkably robust performance have remained elusive. This is due to the complexity of the assembly process, typically involving 200 oligomers or staple strands, each with three binding domains, hybridizing with a scaffold strand in 600 reactions. The entropic and enthalpic contributions of each reaction to the whole depend on all the preceding reactions and affect all the subsequent ones, making origami formation a highly cooperative process. This inherent complexity creates three interconnected problems that must be solved. First, the sources of cooperativity must be identified, second, we must devise quantitative metrics to accurately capture those sources, and third, we must find ways to deconvolve their effects.

Fortunately, the solution lies in another inherently complex aspect of origami – design. Even for a simple origami structure, such as a rectangle of defined size, there are an almost infinite number of choices to made in terms of the combination of oligomer length, location, binding domain size and distribution, sequence, etc. Any given set of design choices leads to a different distribution of cooperative effects. The balance between positive and negative effects (Fig. 1) may be expected to affect the assembly defectivity, which can, in principle, be measured, thus providing some insight into the balance.

We have used a comprehensive set of 2D design variations to probe cooperative effects in DNA origami assembly. By means of a customized annealing protocol, we amplify the impact of design parameters that affect defectivity, which we quantify via atomic force microscopy. Careful analysis of the results indicates that, for non-pathological designs, defectivity is connected to the combination of thermal stability, as determined by the melting temperature, the distribution of folding distances, as measured by the skew in the fold entropy penalty distribution, and the probability that a fold is blocked, determined using the sum of all such probabilities (Fig. 2).

Given the high dimensionality of the combination of design space and cooperative effects, it is possible to devise any number of potential metrics. However, the ones we have developed are both physically intuitive and highly predictive. We have thus provided the first comprehensive quantitative insight into this complex self-assembly process.

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[1] Folding DNA to create nanoscale shapes and patterns, P.W.K. Rothemund, *Nature*, 297, **440**, (2006)

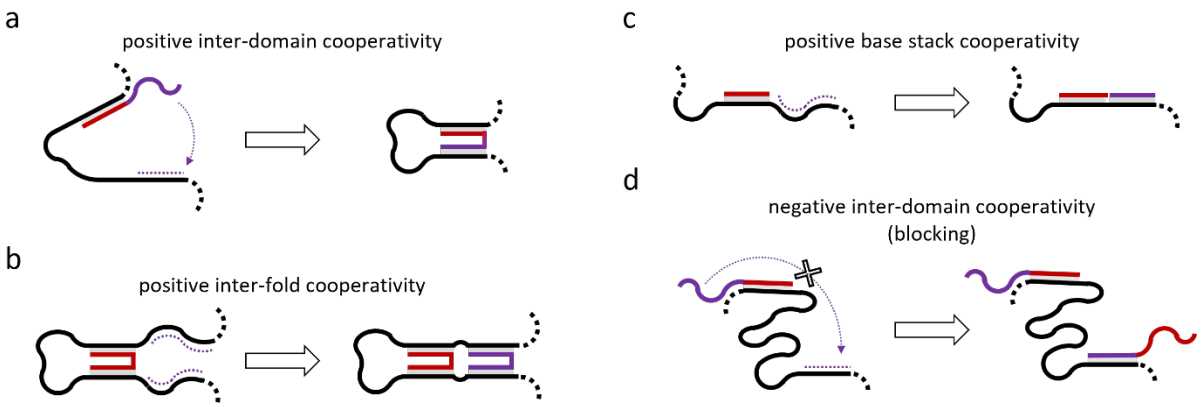

Fig 1. Schematic of the primary forms of cooperativity in DNA origami. a) positive inter-domain cooperativity: at short scaffold distances, when a single domain on a staple binds, neighboring domains on that staple experience positive cooperativity as their effective local concentration is higher than their competitors in solution. This is reduced by loop entropy at longer distances, b) inter-fold cooperativity: at any fold distance, the closing of one fold reduces the possible conformations available to the entire remaining scaffold, stabilizing all other non-competing folds but most heavily affecting close neighbors c) base stacking cooperativity: for immediate neighbors there is an energetic bonus for π-π stacking between helix ends d) negative inter-domain cooperativity, or blocking: at very long scaffold distances, the second domain on a staple can become less likely to bind than a competing domain on another copy.

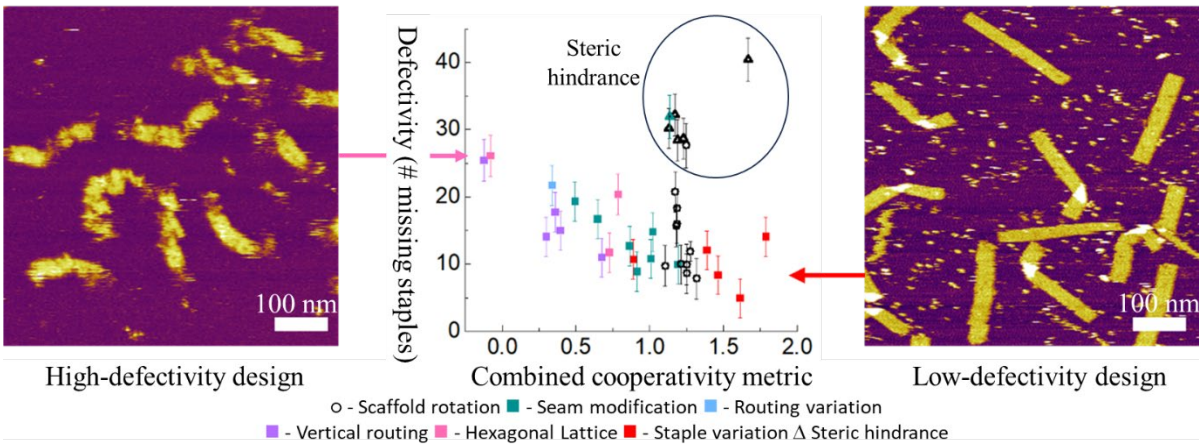

Fig. 2. AFM images of high- (left) and low-defectivity (right) DNA origami structures. Plot of defectivity as a function of a metric that combines melting temperature, entropy penalty distribution skew, and the sum of blocked fold probabilities (center).