Finding Key Differentiating Strands for Folding Multi-Scaffold DNA Origami

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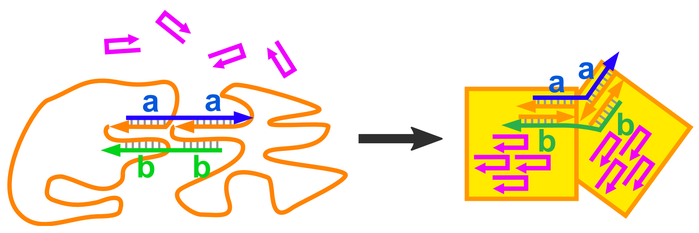
Introduction

Goal: Better understand how to build nanostructures composed of several DNA origamis bound together and based on identical scaffold strands.

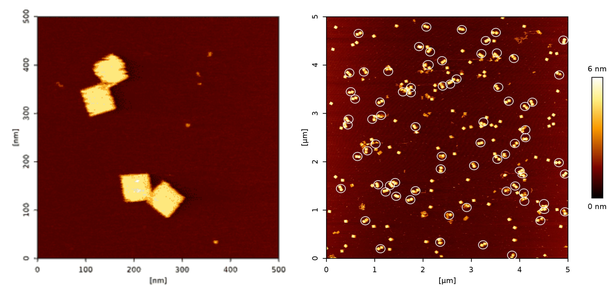
Motivations:

- finding a cost-effective method to create arbitrary objects of larger dimension than simple DNA origami,
- better understanding and controlling DNA origami folding pathways.

Preliminary Work: joining 2 identical origamis

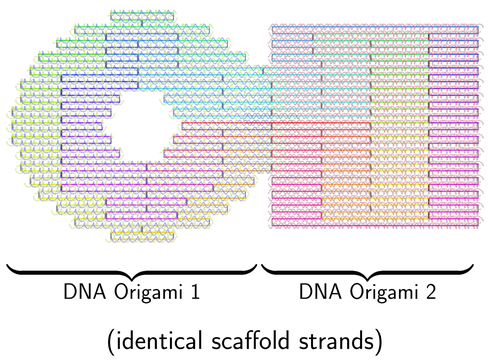


Stoichiometric amount of **aa** and **bb** (1 : 2 scaffold) results in a 60% yield.



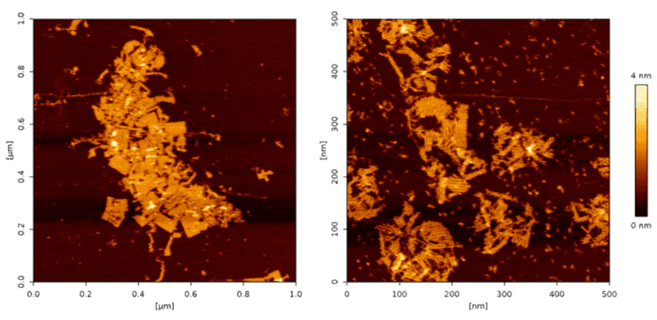
Our approach

Our test design using ENSnano:

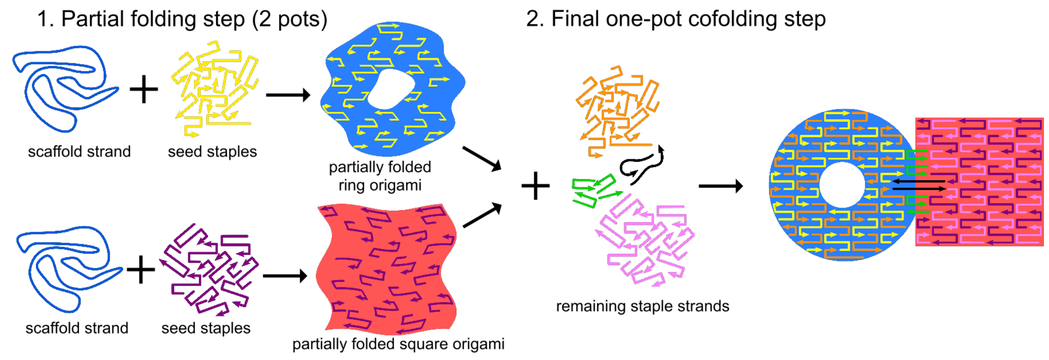


(identical scaffold strands)

Problem: The classical single annealing method produces chimeric structures.



Looking for key staples using a two-stages folding method.

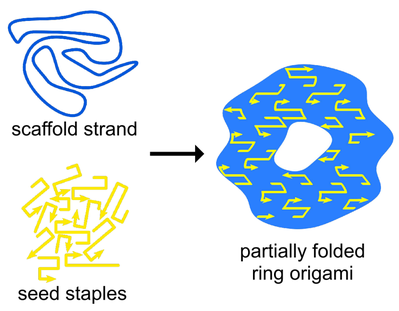


Key staples: looking for seed strands

A subset of staples that:

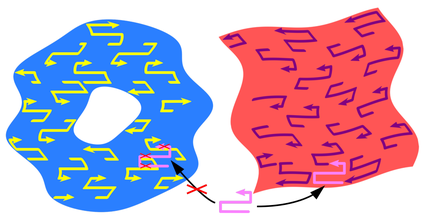
- Pre-folds each scaffold separately (step 1),
- Is as small as possible,
- Produces the desired structure at the final annealing step (step 2).

Checkerboard pattern doesn't work. (see results c-d).



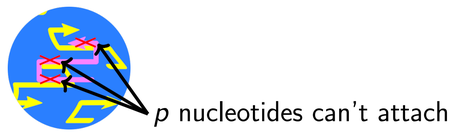
Seed strands selection method

Principle: Preventing staples from attaching to the wrong partially folded scaffold. We select **ring seed staples** so that **square staples** preferably won't attach to the partially folded **ring scaffold**.



Nucleotide counting greedy algorithm:

- **Constraint:** Uniform penalty for attaching a staple to the wrong scaffold (overlap $\geqslant p$ nucleotides).
- **Algorithm:** Greedily construct a small seed abiding by this constraint.



ΔG based LP algorithm:

- **Constraint:** $\forall$ staple, $\Delta G_{\text{bad}} \geqslant$ threshold.
- **Approximation:** ΔG is approximated as a linear function $\widetilde{\Delta G}$ of the binding domains:

$$\widetilde{\Delta G}\left(\text{⟨duplex⟩}\right) = \widetilde{\Delta G}\left(\text{⟨duplex⟩}\right) + \widetilde{\Delta G}\left(\text{⟨duplex⟩}\right) + \widetilde{\Delta G}\left(\text{⟨duplex⟩}\right)$$

- **Algorithm:** Solving the integer linear program with IBM Cplex.

Thermodynamics model

```
5′ C-G-T-T-G-A 3′
   * * * * * *
3′ G-C-A-A-C-T 5′
```

$\Delta G°_{37}(\text{pred.}) = \Delta G°(\text{CG/GC}) + \Delta G°(\text{GT/CA}) + \Delta G°(\text{TT/AA}) + \Delta G°(\text{TG/AC}) + \Delta G°(\text{GA/CT}) + \Delta G°(\text{init.})$

(SantaLucia 1998)

Conclusion

- We managed to guide multi-scaffold DNA Origami folding pathway using properly computed *small* seeds.
- Best working seed sets have intriguing random-like structures: *is it due to our test design*?
- Three driving forces are guiding folding pathways: topology (scaffold routing), thermodynamics (staple length and sequences), and geometry (the intermediate shapes of the partial assembly as it grows).
- Further analysis of obtained seed structures should help us design new experiments to better understand the relative roles of these driving forces.

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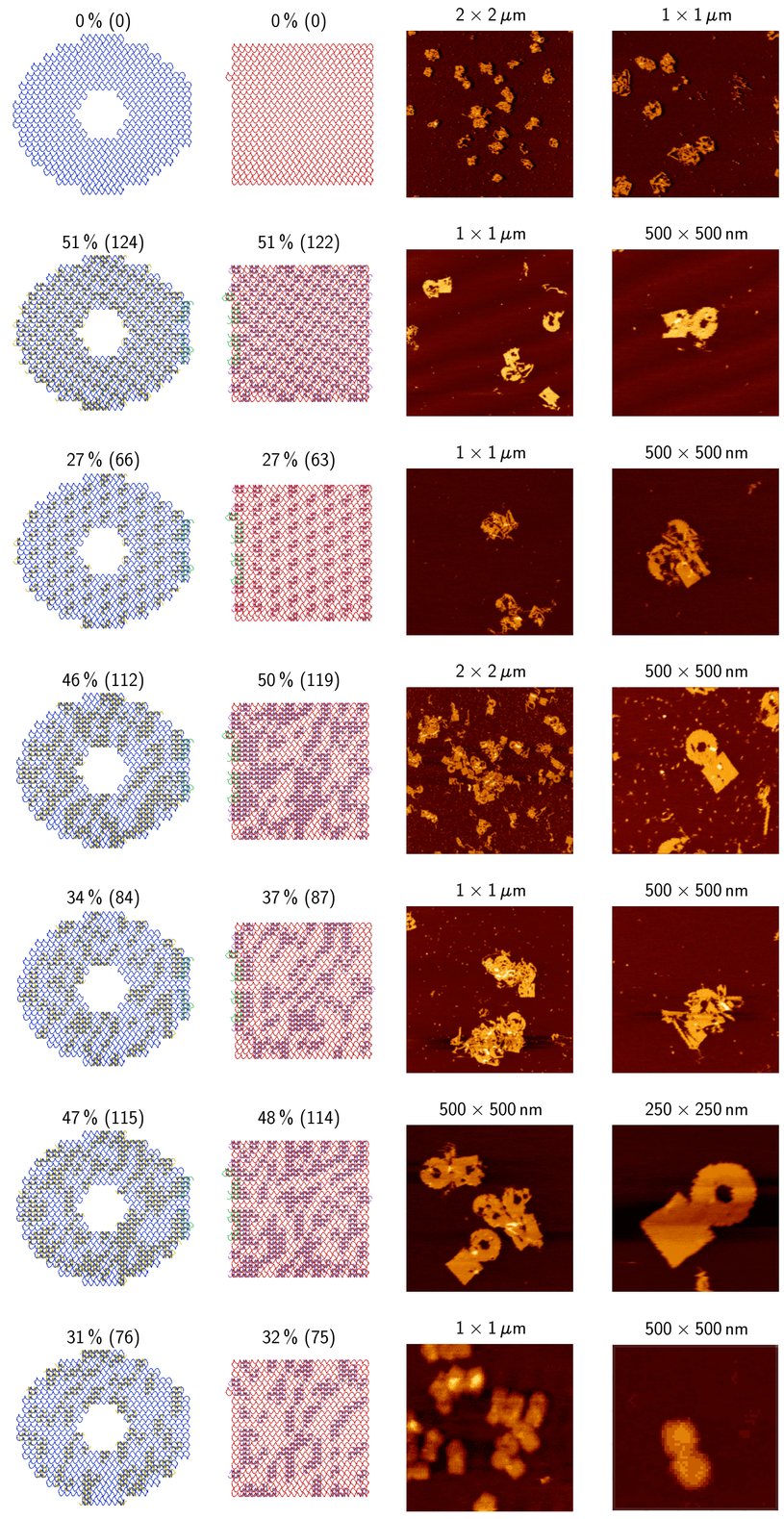
Computed seed structure and experimental results

	Seed algorithm	Ring seed	Square seed	2-steps co-folding AFM images	
(a)	Full seed	100 % (243)	100 % (237)	2 × 2 µm	500 × 500 nm
(b)	No seed	0 % (0)	0 % (0)	2 × 2 µm	1 × 1 µm
(c)	Checkerboard seed (51 %)	51 % (124)	51 % (122)	1 × 1 µm	500 × 500 nm
(d)	Checkerboard seed (27 %)	27 % (66)	27 % (63)	1 × 1 µm	500 × 500 nm
(e)	8nt-Greedy seed (48 %)	46 % (112)	50 % (119)	2 × 2 µm	500 × 500 nm
(f)	4nt-Greedy seed (36 %)	34 % (84)	37 % (87)	1 × 1 µm	500 × 500 nm
(g)	LP 85 % (48 %)	47 % (115)	48 % (114)	500 × 500 nm	250 × 250 nm
(h)	LP 70 % (31 %)	31 % (76)	32 % (75)	1 × 1 µm	500 × 500 nm



Typical folding conditions:

Step 1: 20 nM M13mp18 scaffold strand and 100 nM staple strands in 1× TAE buffer with 12.5 mM Mg^{2+}.
95°C for 15 min, 95 → 65°C at −1°C/2 min, 65 → 45°C at −0.5°C/15 min, 45 → 25°C at −1°C/2 min.

Step 2: Mixing tubes from step 1 and adding all staples (50 nM), 51 → 25°C at −0.5°C/ min.