Materials and methods for DNA origami

Content

1- Preparation of staple mixtures and folding buffers

The 5 types of staples: core (118 staples), edge (39 staples), no hang (12 staples), helper (12 staples) and anchor (12 staples) were ordered in Biomers, already purified by reverse-phase cartridge.

1. 194 staple sample tubes were dissolved with MilliQ water according to information provided by company. (ensured that the concentration is 100 µM, but need Nanodrop to see if the concentration is right).

2. Five mixtures of staple types were made: for each type, 5µL of each sample were added and mixed well by vortexing.

3. Dilutions of core, edge, no hang, anchor and helper staples to the final concentration of 500, 2000, 4000, 4000 µM respectively were made by adding appropriate amount of MilliQ water.

staple original concentration (µM)	**Water volume (µl)**	**staple stock concentration (nM)**	**final volume (µl)**
0.847457627	410	500	590
2.564102564	55	2000	195
8.333333333	65	4000	60
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4. Folding buffers containing different concentrations of Magnesium Chloride were prepared: 80, 100, 120, 140, 160, 180 and 200 mM from 1M magnesium chloride stock in 10X TE buffer (50mM Tris, 10mM EDTA).

MgCl2 stock volume (µl)	50xTE volume (µl)	water volume (µl)	final volume (µl)	10X MgCl2 concentration (mM)
400	1000	3600	5000	80
500	1000	3500	5000	100
600	1000	3400	5000	120
700	1000	3300	5000	140
800	1000	3200	5000	160
900	1000	3100	5000	180
1000	1000	3000	5000	200

2- Long time folding reaction

The mixture of scaffolds, staples and folding buffer were prepared, for each folding buffer.

	concentration (nM)	Volume (µl)	final concentration (nM)
scaffold	100	15	15
core	500	20	100
edge	2000	5	100
No-hang/anchor	4000	2.5	100
helper	4000	2.5	100
10x folding buffer		10	1x
MilliQ H_2O		45	
Total		100	

For the incorporation of the hydrophobically modified oligos, a final concentration of 400 nm was used, and their incorporation was tried during the thermal annealing ramp, and once it was already assembled.

The mixture was subjected to a thermal annealing ramp, following the schedule adapted from “A logic gated nanorobot for targeted transport of molecular payloads” by Douglas et al. Science 2012:

- 80°C to 61°C at 2 min/°C
- 60°C to 24°C at 60 min/°C

3- Filtration of the origami

Amicon ultra 0,5 mL filters of 100 KDa cut-off were used, from Millipore.

1. Filter was placed to the special eppendorf and the sample was transferred (100 µL).
2. Another 200 µL of folding buffer 1x were added.
3. Centrifugation 10' 6000 rpm at table centrifuge.
4. 300 µL folding buffer 1X were added and centrifugation again 10' 6000 rpm.
5. The lower eppendorf was discarded, and the filter was placed to a new one, but this time the filter inverted.
6. Centrifugation 7' 7000 rpm.
7. Transference to a new tube.

The protocol was provided by Bezuayehu Teshome, Helmholtz-Zentrum Dresden Rossendorf.

4- AFM imaging

Sample preparation

A negatively charged mica substrate was used to absorb the DNA origami samples.

1. The substrate was cleaned with isopropanol and a scotch tape to remove the impurities.
2. The mica layer was rinsed with MilliQ H_2O, and immediately dried with a nitrogen gun.
3. 2 µl of the DNA origami sample at a concentration of about 0,5 nM were placed on the substrate and incubated for 2-5'.
4. The substrate was washed with Milli Q H_2O to get rid of the salts.

The protocol was provided by Bezuayehu Teshome, Helmholtz-Zentrum Dresden Rossendorf.

Device characteristics

AFM Probe Type: Tap150AI-G, from BudgedSensors. Rotated tip shape, height of 15-19 µm and radius of <10 nm.

5- TEM imaging

Grid preparation

Grids were prepared by Susanne Kretschmar, working in the EM facility in CRTD.

- Plasma cleaning to hydrophilize the surface
- Grids were placed on a glass slide wrapped in parafilm, and freshly glow discharged for 60s at 240V

Sample preparation and staining

A DNA origami sample concentration of approx. 5 mM was prepared.

1. 5 µl of the samples were place on the grid and incubated for 2'. The excess liquid was dried with a filter paper.

2. A dilution of uranyl acetate 2% in H_2O was centrifugued at 14500g during 3' to precipitate the big granules.
3. A drop of this solution was placed on the samples and incubated for 20'', then it was removed with a filter paper.
4. A water drop was placed to clean the excess and removed with a filter paper.
5. The grid was allowed to air-dry before imaging

Device characteristics

Tension of 80kV.

6- cryo-TEM imaging

1. For frozen hydrated specimen preparation, 2µl of sample was deposited on a holey carbon support film attached to a copper grid.
2. The liquid film was thinned by blotting for 0.5 sec and frozen rapidly in liquid ethane.
3. After rapid freezing, the sample was loaded to a Gatan cryo transfer holder keeping temperature below -130°C in order to prevent ice crystallization.
4. TEM inspection was done in ZEISS LIBRA 120, energy filtered images were recorded under low dose electron irradiation conditions.
5. Samples were kept below -130°C during the inspection.

7- Electrophoresis protocol for best $MgCl_2$ concentration determination

Gel preparation

1. An agarose 1% solution was prepared with TAE 1X buffer.
2. The solution was heated up until boiling and let cool down until 55ºC
3. A magnesium chloride solution was added to reach a concentration of 12mM.

Sample loading

Samples were mixed with the orange G-based loading dye in a ratio 1:5.

Gel running and staining

Gels were runned for 1h 30' at 60V, and then stained with Ethidium bromide for 20' for further UV visualization.