

Materials

Material needed for DNA origami (see preparation)
mica sheet (Ted Mela)
AC-240TSA-R3 tips (Oxford Instrument)
Asylum Research Cypher ES environmental AFM (Oxford Instruments)

Procedures

1. Loading helper strands
 - a. Prepare the M13, S-x, and S-PAM-cap-x mix in a microcentrifuge tube on ice by adding the components indicated below. The molar ratio of M13 (5nM) and helper strands (50 nM) is 1:10.

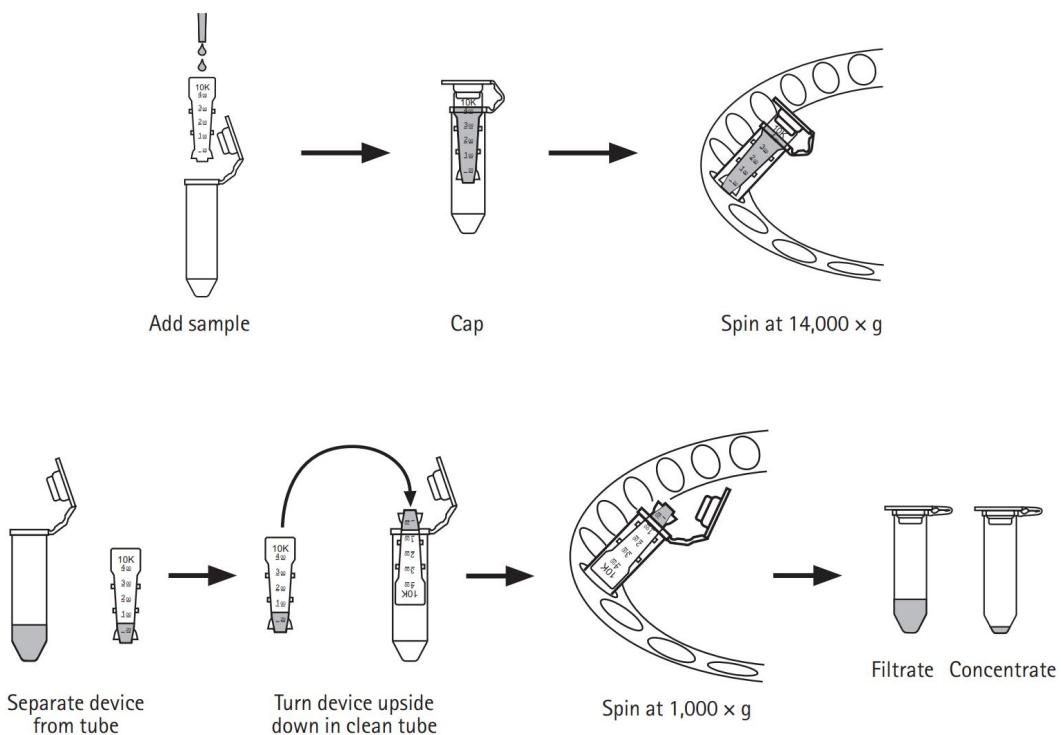
DNA origami (DO) (with S-PAM-cap-x)

Reagents	Volume
TAE/Mg ²⁺ buffer	47µl
S-x	0.25ul × 186 = 46.5µl
S-PAM-cap-x	0.25ul × 6 = 1.5µl
M13	5µl

S-13	S-53	S-93	S-139	S-179
S-14	S-54	S-94	S-140	S-180
S-15	S-55	S-95	S-141	S-181
S-16	S-56	S-96	S-142	S-182
S-17	S-57	S-97	S-143	S-183
S-18	S-58	S-98	S-144	S-184
S-19	S-59	S-99	S-145	S-185
S-20	S-60	S-100	S-146	S-186
S-21	S-61	S-101	S-147	S-187
S-22	S-62	S-105	S-148	S-188
S-23	S-63	S-106	S-149	S-189
S-24	S-64	S-107	S-150	S-190
S-25	S-65	S-108	S-151	S-191
S-26	S-66	S-109	S-152	S-192
S-27	S-67	S-110	S-153	S-193
S-28	S-68	S-111	S-154	S-194
S-29	S-69	S-112	S-155	S-195
S-30	S-70	S-116	S-156	S-196
S-31	S-71	S-117	S-157	S-197
S-32	S-72	S-118	S-158	S-198
S-33	S-73	S-119	S-159	S-199
S-34	S-74	S-120	S-160	S-200

S-35	S-75	S-121	S-161	S-201
S-36	S-76	S-122	S-162	S-202
S-37	S-77	S-123	S-163	S-203
S-38	S-78	S-124	S-164	S-204
S-39	S-79	S-125	S-165	S-PAM-Cap102
S-40	S-80	S-126	S-166	S-PAM-Cap103
S-41	S-81	S-127	S-167	S-PAM-Cap104
S-42	S-82	S-128	S-168	S-PAM-Cap113
S-43	S-83	S-129	S-169	S-PAM-Cap114
S-44	S-84	S-130	S-170	S-PAM-Cap115
S-45	S-85	S-131	S-171	
S-46	S-86	S-132	S-172	
S-47	S-87	S-133	S-173	
S-48	S-88	S-134	S-174	
S-49	S-89	S-135	S-175	
S-50	S-90	S-136	S-176	
S-51	S-91	S-137	S-177	
S-52	S-92	S-138	S-178	

- b. Mix the above reagents and briefly centrifuge them. The mix is first heated at 95 °C for 10 minutes. Subsequently, annealing is performed by slowly cooling the mixture from 95 °C to 20 °C at a rate of 1 °C/min.
- c. Pre-cool the centrifuge rotor to 4 °C.
- d. After pre-treating the Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa), add the solution obtained from step b to the filter device. Place the capped filter device into the centrifuge rotor, aligning the cap strap toward the centre of the rotor. Counterbalance with a similar device. Centrifuge at 14,000 × g for 10 minutes at 4 °C.
- e. Discard the filtrate collected in the collection tube. Add TAE/Mg²⁺buffer to the filter to restore the volume to 100 µL. Mix gently by pipetting up and down, ensuring that the filter membrane is not touched.
- f. Repeat steps d-e. Using the same centrifugal filter to remove excess staple strands.
- g. Centrifuge the centrifugal filter at 14,000 × g for 10 minutes at 4 °C and discard the filtrate in the collection tube.
- h. Invert the filter into a clean collection tube and centrifuge at 1,000 × g for 1 minute to obtain the concentrated DO solution.



2. Loading functional strands

- Add 1.5 μL of the PAM-rich stock solution to the concentrated solution. Then, restore the volume to 100 μL with TAE/Mg²⁺ buffer.
- Mix the above reagents and briefly centrifuge them. The mix is annealed from 45 °C to 25 °C at a rate of 5 min/°C for six cycles.
- As above, the Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa) are used to remove redundant PAM-rich three times. The concentrated DO_{PAM} solution is obtained.

3. AFM sample preparation

- After self-assembly and purification, the AFM samples are prepared by deposition of $\approx 20\mu\text{L}$ (after the DO_{PAM} solution is diluted into 5nM) onto a freshly cleaved mica surface (Ted Pella) and left for 10 min at room temperature to allow adsorption of the DNA nanotile structures.
- The samples are subsequently washed with ddH₂O three times and dried at room temperature before imaging in a vacuum dryer at room temperature (usually left to dry overnight).

Note: the concentration and volume of the DO_{PAM} solution during AFM sample preparation are determined through several preliminary experiments. By observing the dispersion density and area of the sample under AFM image, the most suitable value is obtained.

4. Imaging of nanostructures by AFM

Imaging is performed in Tapping Mode under air conditions on an Asylum Research Cypher ES environmental AFM (Oxford Instruments) with a

nanoAnalytics Q-control III (Asylum Research) and a vertical engage J-scanner, using the \approx 9.4 kHz resonance of the narrow 100 μ m, 0.38 N/m force constant cantilever of AC-240TSA-R3 tips ($f = 70$ kHz, $k = 2$ N/m, no tip coating, Oxford Instrument).

5. Image processing and data analysis
 - a. All images are analyzed by using the Gwyddion 2.69 and Origin 2025b software. The images obtained by AFM are imported into Gwyddion 2.69, conducting background correction by “leveling data by mean plane subtraction”, “correcting horizontal scars (strokes)”, and “aligning rows using various methods”.
 - b. Then under “extract profiles along arbitrary lines” mode, lines are drawn, and the given data is exported in a txt format. The images are exported in a tiff format, accompanied with the adding of scale bar and drawn line.
 - c. The detailed data is analyzed and plotted into diagram in a 2YS Y-Y point plot driven. Subsequently, a series of adjustments to the image parameters and aesthetic enhancements are carried out, resulting in the final height analysis image.