Electronic Supporting information

Site-Selective Immobilization of Functionalized DNA origami on Nanopatterned Teflon AF

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SI 1 Chemical structure of Teflon AF and fabrication procedure

Teflon AF is the main product of the copolymerization of PDD (2, 2-bis-trifluoromethyl-

4, 5-difluoro-1, 3-dioxole) with TFE (tetrafluoroethylene)



Figure S1 a) Chemical structure of "Teflon AF" amorphous fluoropolymer, b) Complete process sequence and the response of Teflon to e-beam treatment and development

SI 2 DNA preparation

DNA origami was prepared with a similar procedure as previously described. ¹ The DNA origami rectangle with a seam in the middle from Rothemund's original paper was chosen, as it has been thoroughly studied. A standard Tris-acetate buffer with added Mg²⁺ ions was used for origami preparation (40 mM TRIS, 11.5 mM magnesium acetate, pH set to 8 using acetic acid). Briefly, a single stranded M13mp18 scaffold strand (New England Biolabs, USA) at a concentration of 4 nM was annealed with a 10-100 times excess of unmodified staple strands (DNA Technology, Denmark), fluorescently modified staple strands (ATTO 647N, AtdBio, UK), and porphyrin modified staple strands by cooling from 85 °C to 5 °C over 3.5 hours, following a rapid heating ramp. The suspension was subsequently filtered 3 times through centrifugal filters (100K Amicon Ultra, Millipore, US) to remove excess staple strands.

The design of staple strand modifications was assisted by use of the SARSE program, developed by Andersen et. al.² All modified staple strands are shown in Table 1.

Strand [†]	Sequence (5' - 3')	Modification
r-7t18f	TAGATGGGGGGGTAACGCCAGGGTTGTGCCAAG*	ATTO-647N
r-7t20f	CTTGCATGCATTAATGAAT*CGGCCCGCCAGGG	Porphyrin
r-5t8e	TTTGCCAGATCAGTTGAGATTTAGTGGTTTAA*	ATTO-647N
r-5t6e	TTTCAACTATAGGCTGGCT*GACCTTGTATCAT	Porphyrin
r-3t12f	CGAGTAGAACTAATAGTAGTAGCAAACCCTCA*	ATTO-647N
<mark>r-3t14f</mark>	TATATTTTAGCTGATAAAT*TAATGTTGTATAA	Porphyrin
r5t8e	ATCAGAGAAAGAACTGGCATGATTTTATTTTG*	ATTO-647N
r5t6e	TCACAATCGTAGCACCATT*ACCATCGTTTTCA	Porphyrin
r5t18e	AACCTACCGCGAATTAT*TCATTTCCAGTACAT	ATTO-647N
r5t20e	CTAAAATAGAACAAAGAAACCACCAGGGT*TAG	Porphyrin

Table S1. Staple strand modifications.

[†]Strand names are identical to those from Rothemund's original paper.¹ Equal color signifies positions which are in close proximity on the origami. * shows the position of a modification, for example: T* means that this thymine base is modified.

The porphyrin is connected to a thymine base on the staple strand by a triphenylethynylene linker. Only a certain number of thymine nucleobases exist on the origami for porphyrin modification and 5 were chosen which had the same orientation, thereby allowing all the anchors to point in the same direction perpendicular to the origami surface. Furthermore, the placement of porphyrin anchors on the origami was chosen to help maximize the structural stability of the construct when attached to the Teflon AF surface, with one near each corner and one near the middle. The fluorophores were coupled to the DNA using a standard procedure. Porphyrin modified staple strands were prepared using a previously published synthetic route, ³⁻⁴ and used as described for the other staple strands. Figure S2 shows an assembled DNA origami imaged by AFM.



Figure S2 AFM image of a single DNA origami on an atomically flat mica substrate.

SI 3 Intensity distribution

Differences in intensity were observed in the TIRF images, Figure S3 shows the intensity distribution of 300 pillars.



Figure S3 Histogram of the intensity distribution for 300 pillars.

References

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