AFM-assisted DNA origami patterning for lipid nanodisc studies

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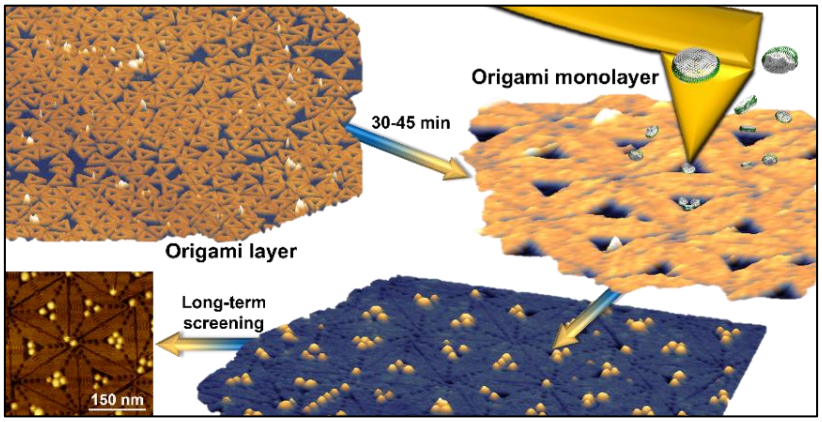

Fig. 1: Filling of NDs into cavities of a DNA origami monolayer forming large-scale 2D lattices.

DNA origami, as first presented by Rothemund [1], has become a versatile tool with great potential in many different research areas such as nanotechnology [2], biophysics and basic research at the single molecule level. By hybridizing a given single-stranded DNA scaffold with specially designed oligonucleotides one can achieve the self-assembly of almost arbitrary structures and offering the possibility of self-assemblies of regular patterns of chemical entities for various biomedical applications. The possibilities for novel approaches in membrane protein (MP) research is currently explored. Recent studies have immobilized MPs in lipid nanodiscs (NDs) on 2D streptavidin surfaces [3], without control over the lateral spatial arrangement of the NDs and the functionalization for supramolecular assembly into worm-like structures has been reported [4]. However, patterning of surfaces with NDs [5] by DNA origami may allow superior control of the final two or three-dimensional superstructures.

Here, we use DNA origami nanostructures as a substrate to create a defined arrangement of NDs in the origami lattice. We prepare highly hierarchical 2D lattices of triangular DNA origamis on solid support surfaces and analyze the filling of the cavities of the origami monolayers with NDs at different ND concentrations. Using Atomic Force Microscopy (AFM) over time, we find that the NDs fill and orient in the cavities without chemical or functional modifications. The AFM is not only used as an imaging method for the newly generated surface, it also serves at the same time as a tool for defined and controllable ND patterning. With increasing ND concentration, we find significant differences in the formation of the ordered surface structure of the newly generated ND origami monolayer system.

These proof-of-principle experiments demonstrate the applicability of the DNA origami structure as a highly hierarchically generated 2D lattice for the specific arrangement of large (several μm^2) ND-filled structured surfaces enabling quantitative AFM analysis of parallelized structural and functional studies of membrane proteins over long time scales.

[1] P. W. K. Rothemund, Nature 440 (2006) 297-302
[2] C. Kielar et al., ACS Appl. Mater. Interfaces 10 (2018) 44844-44853
[3] T. Haruyama et al., Structure 27 (2019) 152-160
[4] M. Subramanian et al., Molecules 26(6) (2021) 1647
[5] T. H. Bayburt et al., Nano Letters 2 (2002) 853-856