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NTA Directed Protein Nanopatterning on DNA Origami Nanoconstructs

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Organizing functional proteins or enzymes in a well-designed pattern has great significance in biosensing and other applications. DNA self-assembled nanoconstructs can serve as ideal scaffolds for protein nanopatterning.[1] Several strategies have been used to produce protein nanoarrays using DNA nanoarrays as scaffolds, including biotin−streptavidin interaction;[2a,b] antibody−antigen interaction;[3] aptamer and aptamer-single chain antibody mediated assembly;[4a−d] RuvA-DNA Junction binding;[5] and covalent coupling of protein or peptides to DNA by heterobifunctional cross-linkers.[6] However, for these approaches, the active binding sites of the proteins are not necessarily optimally presented once they are organized. This limits the range of applications of these protein nanoarrays. Herein, we report a new method to direct protein nanopatterning, using the nitrilotriacetic acid (NTA) and Histidine-tag metal linked interaction.

His-tags are commonly used for protein purification via chromatography, and they have also been used recently for surface immobilization purposes.[7] The DNA nanoconstruct scaffold used here is a DNA Origami[8] structure which we designed to have a circular shape. Atomic force microscopy (AFM) images provided evidence of the well-defined protein nanopattern.

Figure 1a displays the chemical steps employed. The NTA group in Maleimido-C3-NTA can form chelate complexes with Histidine-tagged proteins in the presence of Ni^{2+} or other transition metal cations. The protein used in these experiments was enhanced green fluorescent protein (EGFP) with His-tags on both of its C and N termini (Biovision). EGFP has been widely used in bioimaging applications,[9] and its enhanced photostability makes it a protein of interest in optoelectronics.

EGFP has a cylindrical shape, with dimensions of 3 nm by 4 nm, in solution.[10,11] The procedure used in these experiments involved first conjugating the Maleimido-C3-NTA molecule to the sequence designed thio-modified DNA strands (strands I and II), and then dialysis was used to remove excess Maleimido-C3-NTA reagents. Next, the Maleimido-C3-NTA/DNA conjugates, together with over 200 other DNA staple strands (see Supporting Information for Origami design and sequences), were annealed with a single stranded M13mp18 DNA plasmid in a 100 to 1 ratio, after which the Origami constructs were deposited on a poly-L-lysine pretreated mica surface and visualized using AFM. Poly-L-lysine is used here to bind Origami molecules to the mica surface though the ensuing assembly process. The same Mica/Origami samples were then treated with Ni^{2+}, followed by incubating the assemblies, while on the mica, with EGFP. The resulting EGFP nanopattern forms by sequential self-assembly on the DNA Origami nanoconstructs.

Comparison of the AFM images of the blank DNA Origami and DNA Origami after treatment with the His-tagged EGFP protein (Figure 2a, 2b) indicates that EGFP proteins attached to the two designed positions on the DNA Origami structure. In most experiments (Figure 2b and S2), on the order of 50% to 60% of the Origami were observed to have two EGFP attached to them, some 20% to 30% of Origami only had one EGFP, and the remaining Origami had no EGFP. The distance between the two EGFP spots was measured, and the apparent separation ranged from 60 to 80 nm (Figure 3b), which is close to the designed separation of 81.6 nm (see Figure S5). Some of the variation in the distance between the two EGFP species might be attributed to mechanical tip effects, displacements caused during scanning. The height of the EGFP spots are between 3 and 4 nm (Figure 3b), which is in reasonable agreement with the expected value. Additionally, EGFP did not bind to Origami without Maleimido-C3-NTA modified DNA strands (Figure S3), suggesting that the attachment of EGFP to Origami is specifically through the NTA/Ni/His-tag interaction.

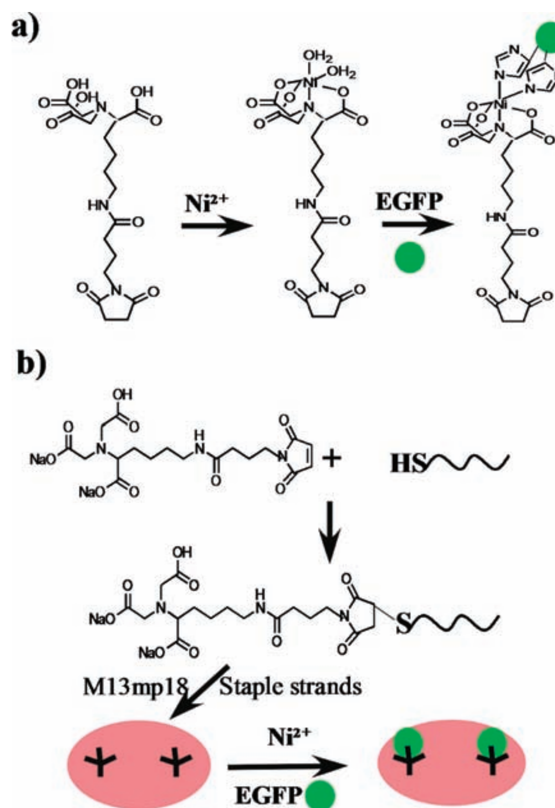

Figure 1. Schematic of the design of the experiment. (a) Sequential chemistry of forming the Ni^{2+} complex by chelation of the metal by the NTA functional group of the Maleimido-C3-NTA molecule, followed by capping of the complex with the His-tagged EGFP protein (green colored circle). (b) Three-stage directed assembly, which begins with the conjugation of Maleimido-C3-NTA to Thio modified DNA strands I and II, which is followed by Origami nanoconstruct formation, which is followed by the EGFP protein attachment process.

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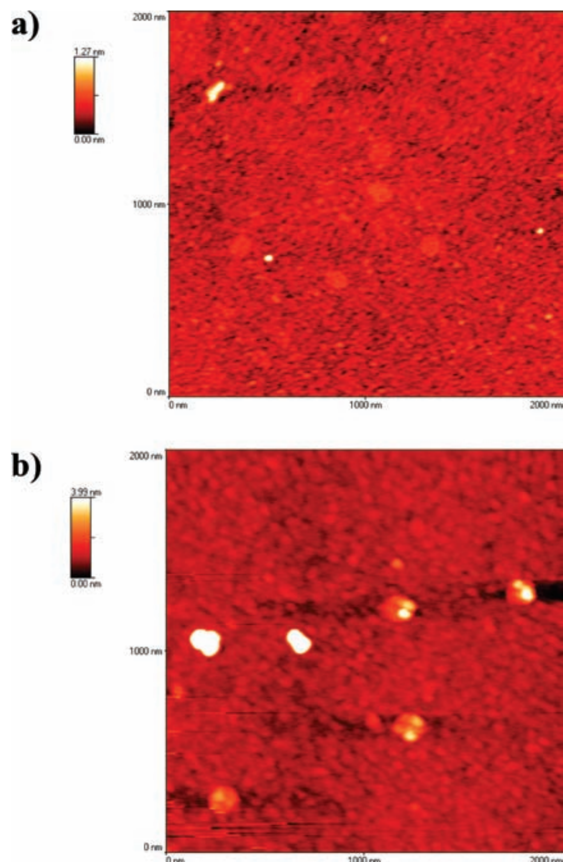

Figure 2. AFM images of DNA Origami nanoconstructs only and DNA Origami with EGFP protein attached to two designed positions. (a) DNA Origami only; (b) DNA Origami with EGFP attachment.

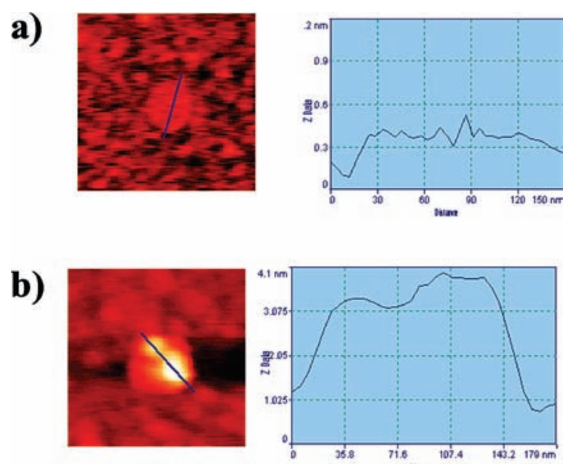

Figure 3. Zoom-in AFM images and corresponding height profiles of DNA Origami only (a) and DNA Origami with EGFP attachment (b).

There is a significant need for multiple, orthogonal mechanisms for protein surface immobilization. Our work demonstrates that the nitrilotriacetic acid (NTA)/Histidine-tag interaction can be used as a novel way to generate protein nanopatterns on DNA nanoscaffolds. However, the DNA and/or metal binding properties of a protein must be considered when evaluating the applicability of this approach. Because the proteins linked via this method are only connected to the scaffolds through their His-tag sites, which are amenable to design, bound proteins can be oriented to present open, active sites to optimum advantage. This will be of great significance for biosensing applications. Particularly this platform should enhance AFM visualization of the behavior of surface bound proteins and enable studies of movement in protein driven DNA nanodevices on DNA nanoconstructs.[12] This linker, if combined with a top-down approach,[13] represents an enabling technology for building nanostructured biosensors. Our continuing research is directed toward achieving positional and orientational control over DNA Origami structures.

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Supporting Information Available: Experimental methods, DNA Origami design and DNA sequences, Additional AFM images. This material is available free of charge via the Internet at http://pubs.acs.org.

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