

## Supporting Information

## Fabrication of Defined Polydopamine Nanostructures by DNA Origami-Templated Polymerization

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#### **Experimental Procedures**

#### **Materials and instruments**

All solvents and chemicals were purchased from commercial sources and were used without further purification. DNA staple strands and G-quadruplex containing staple strands (G4 staple) were either synthesized by 12-Column DNA Synthesizer from POLYGEN GmbH and purified by Agilent 1260 Infinity HPLC system with Agilent Eclipse XDB-C18 column or purchased from Sigma-Aldrich. Agarose gel electrophoresis was performed using Bio-Rad Mini-Sub Cell GT horizontal electrophoresis system. Bio-Rad MyCycler™ Thermal Cycler was used for annealing of MP13mp18 phage DNA and DNA staple strands to form DNA nanotile. Concentration of DNA nanotile was determined by Spark ® 20M with Nanoquant plate™.

#### **Fabrication of DNA nanotile with G-quadruplex staple**

DNA nanotile with G4 staples at different positions were prepared respectively by mixing M13MP18 phage DNA of 7k nt with desired staple strands and G4 staple strands in folding buffer (5 mM Tris, 1 mM EDTA, 5 mM NaCl, and 12 mM MgCl<sub>2</sub>, pH 8.0) and annealing from 65 °C to 20 °C over 2 h, followed by a purification with polyethylene glycol (PEG) precipitation method<sup>[1]</sup>. Briefly, the reaction solution containing DNA nanotile and excess of staple strands were mixed with 15% PEG8000 (w/v), 5 mM Tris, 1 mM EDTA, and 505 mM NaCl and centrifuged at 12000 rpm, at room temperature (RT) for 25 min. The supernatant was removed and the pellet was dissolved in 1  $\times$  TAE / Mg buffer (20 mM Tris, 1 mM EDTA, 12 mM MgCl<sub>2</sub>, pH 7.8).

#### **Hemin binding assay**

G4 DNA nanotile with square domain (50 nM) was added to a 384 well UV transparent plate. Hemin (1 μM) was mixed with G4 DNA nanotile solution. Immediately after mixing, the absorbance spectrum was measured every minute for a duration of 2 hours using a Tecan Spark® 20M plate reader. The absorbance spectrum of Hemin (1 μM) alone was also measured.

#### **The ABTS assay of G4/hemin DNAzyme on DNA nanotile**

G4 DNA nanotile with square domain (3.5 nM) in different buffer composition (20 mM Tris, 1 mM EDTA, 12 mM MgCl<sub>2</sub>, pH adjusted to 7.8 or pH 5.3 by addition of acetic acid) was mixed with hemin (70 nM) for 30 min at rt. 97.9 μL of the solution was added to a 384 well UV transparent plate. To G4/hemin DNA nanotile solution was added 1.1 uL of a freshly prepared 50 mg/ml ABTS solution and 1 uL of 0.1M H<sub>2</sub>O<sub>2</sub>. Immediately after H<sub>2</sub>O<sub>2</sub> addition, the absorbance spectrum was measured every minute for a duration of 1 hours using a Tecan Spark® 20M plate reader. The same procedure was used each for G4 DNA nanotile only (3.5 nM), G4/hemin (70 nM), and hemin (70 nM) only. For the low ionic strength buffer, (0.3 mM Tris, 0.2 mM acetic acid, 0.06 mM EDTA, 0.6 mM MgCl<sub>2</sub>, 10 mM KCl, pH 5.3) was used.

#### **Preparation of polydopamine on G4/hemin DNA nanotile**

G4 DNA nanotile with square domain (3.5 nM) was mixed with hemin (100 nM) in TAE / Mg / K (0.3 mM Tris, 0.2 mM acetic acid, 0.06 mM EDTA, 0.6 mM MgCl<sub>2</sub>, 10 mM KCl, pH 5.3) for 30 min at room temperature before polymerization of polydopamine. To G4/hemin DNA nanotile solution was added 1 uL of freshly prepared dopamine (1 M) and  $H_2O_2$  (1M), and the reaction solution was incubated at rt for 3h. The samples were purified by size exclusion chromatography (800 μL volume of Sephacryl S-400, GE Healthcare) equilibrated with a reaction buffer in Ultrafree-MC-DV (Millipore).

#### **Transformation of DNA nanotile to tube**

To DNA nanotile with stripy G4/hemin pattern solution (0.5 pmol) was added a set of folding DNA strands (250 pmol each) and the mixture was incubated at 32 degrees for overnight. The obtained DNA tube was purified again with PEG precipitation method.

#### **Kinetics of polydopamine formation on G4/hemin DNA nanotile**

G4 DNA nanotile with square domain (3.5 nM) or G4 staple (70 nM) in the decreased ionic strength buffer (pH 5.3) was mixed with hemin (70 nM) for 30 min at rt. 98 µL of the solution was added to a 384 well UV transparent plate. To G4/hemin DNA nanotile solution was added 1 uL of a freshly prepared 1M dopamine solution and 1 uL of 1M  $H_2O_2$ . Immediately after  $H_2O_2$  addition, the absorbance spectrum was measured every 5 minutes for a duration of 12 hours using a Tecan Spark® 20M plate reader.

#### **Extraction of polydopamine nanostructure via acid treatment**

The sample solution of purified polydopamine grown DNA nanotile was added to freshly cleaved mica surface and incubated for 5 min at room temperature to deposit DNA nanotile structure on mica surface. After imaging the polydopamine / DNA nanotile by AFM, the remaining solution on mica was removed and 1 M HCl solution was added to the mica surface. After 10 seconds, HCl solution was removed and the buffer was added again. The AFM measurement was performed at the same area as before acid treatment.

#### **Atomic force Microscopy (AFM)**

Imaging was performed with a Bruker Dimension FastScan Bio AFM equipped with the ScanAsyst mode. The sample solution was deposited onto freshly cleaved mica surface, and left for 5 min at room temperature to allow adsorption of the DNA nanotile structures. After addition of 70 μL of 1 x TAE / Mg buffer, the sample was scanned with the scan rates between 1 and 3 Hz. Several AFM images were acquired at different areas of the mica surface to ensure the reproducibility of the results. All images were analyzed by using the NanoScope Analysis 1.50 and Gwyddion 2.38 software.

#### **Agarose gel electrophoresis**

5 uL of sample (3 nM) was mixed with 6 x Loading buffer and run with 0.8 % agarose gel in 0.5 x TBE / Mg for 150 min in ice bath. After running, the gel was stained by SYBR Gold for 30 min and the image was taken by G: Box Chemi (Syngene).

#### **Transmission electron microscopy (TEM)**

5 uL of sample (1 nM) was applied on carbon coated copper grid with hydrophilic treatment. After 10 minutes incubation, the remaining solution was removed and the sample grid was stained with 2 % uranyl formate solution for 20 seconds. The stained grid SUPPORTING INFORMATION **WILEY-VCH** 

was washed with filtered water for three times and dried in air. Imaging was done with JEOL 1400 instrument and obtained images were analyzed by ImageJ software.

### **Results and Discussion**



**Figure S1. Proposed mechanism of polydopamine formation.** Through the oxidation, isomerization, oligomerization and self-assembly polydopamine is formed via covalent bond, charge transfer, π-interactions and so on<sup>[2]</sup>.



**Figure S2. Design of G4/hemin DNA nanotile.** Schematic of DNA nanotile[3] and the orange circles at the bottom figures indicate the positions of G4 staple DNA sequences.

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Figure S3. G4/hemin DNA nanotile. (a) Schematic illustration of DNA nanotile with 20 nm square G4/hemin domain and (b) agarose gel electrophoresis image of 1: M13MP18, 2: G4 DNA nanotile before purification, and 3: G4 DNA nanotile after purification. The staple DNA sequences were sufficiently removed after the purification. (c) AFM images before and after hemin incorporation. There was no clear difference observed before and after DNAzyme activation. Scale bar: 100 nm.



**Figure S4. UV-Vis spectrum of hemin incorporation to G4 DNA nanotile.** In the presence of G4 DNA nanotile, the hemin Soret band was immediately shifted<br>from 400 nm to 405 nm due to hemin binding to G4 domain for activatin







**Figure S6. No polydopamine formation on DNA nanotile under the high ionic strength buffer condition.**



**Figure S7. Nanomechanical property mapping of polydopamine DNA nanotile and G4 DNA nanotile.**



Figure S8 Proposed mechanism of polydopamine formation on DNA nanotile. Under high ionic strength condition, cations (Tris, Mg<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>) shielded negative charges of the DNA backbone so that after dopamine molecules were oxidized to dopaminochrome on DNA origami, it diffused back into solution and spontaneously self-polymerized in solution. On the other hand, under low ionic strength condition, the locally concentrated dopamine, dopaminochrome, and formed oligomers facilitate the formation of polydopamine on DNA nanotile.



**Figure S9. Tracking of polydopamine formation with free DNAzyme.** (a) Absorbance spectra of dopamine polymerization on G4/ hemin DNA nanotile and G4/hemin over 3.5 hours (inset: absorbance spectra of polydopamine. dopamine [2 mg/ml] polymerized in 10 mM Tris buffer for 3.5 hr). Initial increase around 320 nm and 480 nm attributed to dopaminochrome and oligmers, respectively. Later increase in baseline (broadband absorbance) attributed to polydopamine formation. Representative wavelength at 700 nm chosen to avoid contributions from early oxidation products. (b) Dopaminochrome, oligomers, and polydopamine formation by G4/hemin DNA nanotile (3.5 nM containing 70 nM G4/hemin or free G4/hemin (70 nM) are tracked by UV-Vis spectroscopy (the solid lines: G4/hemin DNA nanotile, dashed lines: free G4/hemin). In case of free G4/hemin molecules, the production of dopaminochrome and oligomers were lower than G4/hemin DNA nanotile. Additionally, the peak corresponding to polydopamine (700 nm, green line) didn't increase over the time (c) The schematic illustration of the reaction with free G4/hemin. Free DNAzyme can oxidize dopamine molecules to dopaminochrome. After further isomerization and oxidization, oligomers are formed, however polydopamine is not formed due to the limited production of oligomers.

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**Figure S10. Polymerization of L-dopa on G4/hemin DNA nanotile.** L-dopa is a derivative of dopamine having carboxylic acid group (left). Under pH 5.3 condition, it shows neutral charge which hinder its interaction with negatively charged DNAzyme domain resulting in no polymerization (right).



**Figure S11. Polymerization of dopamine on G4 DNA nanotile (without hemin).** Without hemin, no polydopamine was formed due to a lack of DNAzyme activity.



Figure S12. The effects of H<sub>2</sub>O<sub>2</sub> concentration and reaction time to the growth of polydopamine on G4/hemin DNA nanotile. Means + S.E.. n = 28



**Figure S13. Cross patterning of polydopamine nanostructure on DNA nanotile.** Scale bar: 100 nm.



**Figure S14. Polydopamine DNA tube with striped G4/hemin.** (Top) Schematic Illustration of tube formation and subsequent polydopamine formation. (Bottom) AFM height image and cross section of polydopamine DNA nanotube with stripy G4/hemin before (left) and after polydopamine formation (right).



**Figure S15. AFM images of polydopamine DNA nanotile with horizontal line.** (Upper) AFM image after 90 min reaction time (left) and 180 minutes reaction time (right). Tile structure, partially folded structure, and folded structure were indicated by T, P, and F, respectively. The percentages of different conformation after 1.5 h and 3 h reaction time was calculated from counting 92 structures. (Bottom) Dimension and cross section of (partially) folded polydopamine DNA nanotile.





**Figure S16. TEM images of polydopamine DNA nanotile with horizontal line before and after 180 minutes reaction.** Scale bar: 100 nm. In TEM image, the folding was more enhanced possibly due to the drying effect.



**Figure S17. Polydopamine DNA nanotile with vertical line.** There was no folding observed after 180 minutes reaction time.



**Figure S18. AFM image of extracted polydopamine nanostructures after HCl treatment.** The polydopamine nanostructures were indicated by red arrows.

#### **Table S1. The list of staple DNA sequences**



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