Supporting Information

Surface Plasmon Enhanced Fluorescence of Cationic Conjugated Polymer on Periodic Nanoarrays

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Layer-by-layer assembly of PFDBT-N^+ on gold nanoarrays:

The layer-by-layer assembly begins with the self-assembly of biotinylated gold binding polypeptide (bio-*l*-AuBP2) onto the gold nanopillars. *L*-AuBP2 is a genetically engineered polypeptide that has specific biomolecular recognition for gold surfaces.[1] The gold nanoarray substrate is immersed in a 5 mL (100 ug/mL in PBS) solution of bio-*l*-AuBP2 for a reaction time of 2 hours. After 2 hours, the substrate is gently removed from the solution using tweezers and the surface is thoroughly rinsed with PBS for 2 min. followed by additional rinsing with deionized water (DI water) for 2 min. Then, the substrate is dried under a gentle stream of nitrogen (N_2) gas.

Next, the peptide functionalized substrate is immersed in a 5 mL (1 μM in DI water) solution of streptavidin (SA) for a reaction time of 35 min. Because *L*-AuBP2 has been biotinylated with a biotin group at the N-terminus of the peptide chain, SA can self-assembly and attach on the surface. The attachment of SA increases the distance of PFDBT-N^+ from the metal surface. After the reaction time 35 min., the substrate is thoroughly rinsed with DI water for 2 min. and dried under a gentle stream of N_2 gas.

Then, 1,4-dibiotinylbutane (dibiotin) is self-assembled onto the SA and peptide functionalized substrate thru biotin-streptavidin binding interactions.[2] The substrate is immersed in a 5 mL (1.0 mM in 200 proof ethanol) solution of dibiotin for a reaction time of 35 min.

Afterwards, the substrate is thoroughly rinsed using ethanol for 2 min. and gently dried under a stream of N_2 gas.

Another SA layer is self-assembled onto the functionalized substrate. The substrate is immersed in a 5 mL (1 μM in DI water) solution of streptavidin (SA) for a reaction time of 35 min. After the reaction time 35 min., the substrate is thoroughly rinsed with DI water for 2 min., and dried under a gentle stream of N_2 gas. The attachment of SA allows for the self-assembly and attachment of bio-*l*-AuBP2 thru biotin-SA binding.

Another layer of bio-*l*-AuBP2 is self-assembled onto the functionalized substrate for a reaction time of 35 min. thru biotin-streptavidin interactions. After the reaction time of 35 min., the substrate is thoroughly rinsed using PBS for 2 min., then DI water for 2 min., and lastly dried under a gentle stream of N_2 gas. The second self-assembly of bio-*l*-AuBP2 allows for the self-assembly of DNA thru electrostatic interactions.

The functionalized substrate which has the second self-assembly of bio-*l*-AuBP2 is then immersed in a 5 mL (1 mg/mL in PBS) solution of salmon DNA for a reaction time of 30 mins. Afterwards, the substrate is thoroughly rinsed using PBS for 2 min., then DI water for 2 min., and then dried under a gently stream of N_2 gas. DNA can self-assemble with the second bio-*l*-AuBP2 layer because the *l*-AuBP2 peptide sequence contains four arginine residues which give *l*-AuBP2 a +4 charge and DNA carries a negative charge because of the phosphate backbone. Thus *l*-AuBP2 can interact and self-assemble DNA through electrostatic interactions.

Lastly, the DNA functionalized substrate is immersed in a 5 mL (1 mg/mL DI water) solution of PFDBT-N^+ for a reaction time of 30 min. and then thoroughly rinsed with DI water, and dried using N_2 gas. PFDBT-N^+ can self-assemble and interact with DNA via electrostatic interactions because of its cationic properties.

Assembly of PFDBT-N^+ on flat gold substrates for linker height measurements:

Instead of using EBL-fabricated gold nanopillars, micro-contact printing (μCP) a nonfouling molecule such as OEG-thiol (purchased from Aldrich) on a flat gold substrate was used because of the resolution of the 50 nm nanopillars to the infinitely small bio-*l*-AuBP2 resulted in error. A typical μCP stamp was made by casting a 10:1 (v/v) mixture of polydimethylsiloxane (PDMS) and curing agent (Sylgard 184, Dow Corning, Midland, MI) against a silanized master for 2 days at room temperature in ambient conditions. The silanized

master contained negative relief structures of the stamp and a square array of 2 μm circles with interspacing of 2 μm was fabricated by photolithography (Microfabrication Lab, Washington Technology Center). Stamps were used as cast and the surface of the stamp was not modified. Freshly prepared stamps were washed several times with ethanol and dried with nitrogen gas before using. Inking was performed by covering the patterned side of the stamp with OEG-thiol (1 mM in 200 proof ethanol) solution for 1 min. and dried under nitrogen gas. The inked stamp was brought into conformal contact with the flat gold substrate by hand for ~30 seconds and carefully lifted-off. Then, the gold substrate was rinsed thoroughly with ethanol and dried with nitrogen gas. After each cycle of inking and printing, stamps were cleaned by ultra-sonication in a 2:1 solution of water and ethanol for 5 min.

Characterization of peptide and biotin-SA linkers:

The height of OEG that was micro-contact printed onto the flat gold surface was measured using AFM. The height was determined to be ~2.90 ± 0.21 nm. Then bio-ℓ-AuBP2 was backfilled into the holes of the OEG regions. AFM was used to measure the change in height profile of OEG. The height of OEG after backfilling with bio-ℓ-AuBP2 was measured to be ~1.75 ± 0.30 nm. The measured height difference between OEG (~2.90 ± 0.21 nm) and bio-ℓ-AuBP2 with OEG gave the calculated height of bio-ℓ-AuBP2. Thus the height of bio-ℓ-AuBP2 was calculated to be ~1.15 nm. Then solutions of SA and dibiotin were backfilled and allowed to self-assembly onto bio-ℓ-AuBP2. The height difference between OEG and the bio-ℓ-AuBP2 with SA and dibiotin was measured. The height of OEG was increased by ~5.60 ± 0.35 nm. A second run of solutions of SA and dibiotin solutions was backfilled and allowed to self-assembly onto bio-ℓ-AuBP2 with SA and dibiotin. The height difference between OEG and the 2nd layer of SA and dibiotin was measured. The height of OEG increased by ~9.85 ± 0.30 nm. DNA is approximately 2 nm in diameter and increased the linker heights by 2 nm.[3]

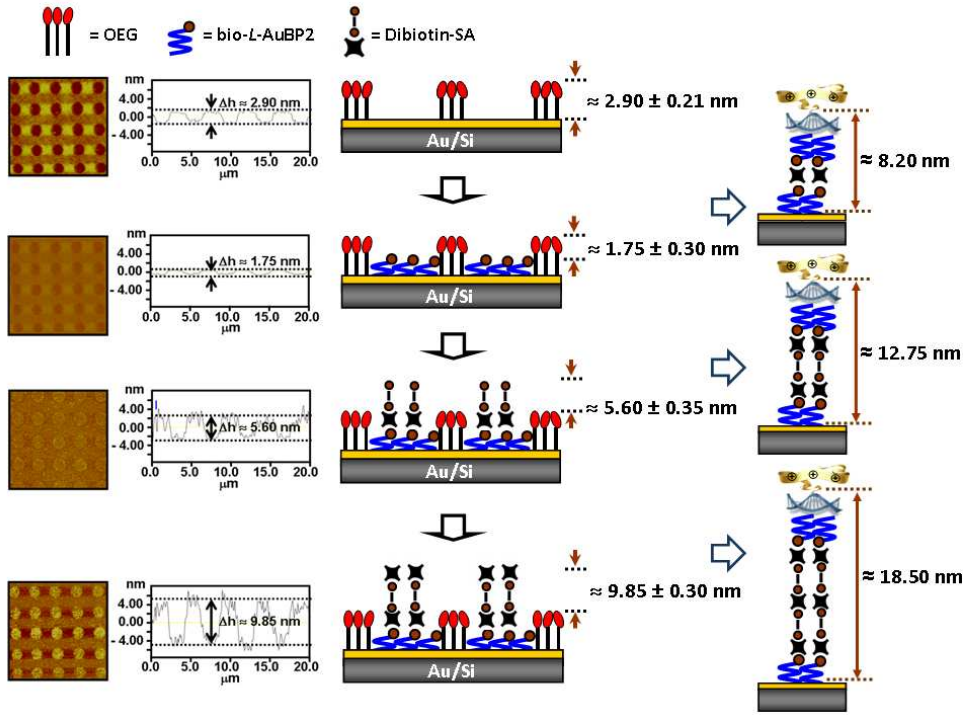

Figure S1: AFM measurement of linker lengths. The heights of molecular linkers were experimentally measured relative to the height of OEG self-assembled monolayers. The measurements were accomplished by backfilling the linkers into the 2-μm hole arrays generated by μCP OEG molecules followed by cross-sectional profile analysis to measure the relative differences in heights between patterned OEG regions and linker regions.

References:

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