Supporting Information

Plasmonic Nanorod Arrays for Enhancement of Single-Molecule Detection

Qing Huang*[a], Zhulin Huang[a], Guowen Meng[a], Joseph R. Lakowicz[b], and Yi Fu*[b]

S1.Fabrication of Ag-NR arrays

The arrays of Ag-NRs were fabricated by using AAO template-assisted electrochemical deposition (ECD) approach. First, the through-pore AAO templates were prepared via a two-step anodization of pure aluminum foil in oxalic acid (0.3 M) aqueous solution under 40 VDC at 10 ^{o}C for at least 14 h. The barrier layer was removed and the pores were widened subsequently via wet chemical etching in diluted H_3PO_4 (5%) aqueous solution at 40 ^{o}C for 30~50 min. A silver layer (50~100 nm) was sputtered onto one planar surface side of the through-pore AAO template to serve as working electrode. Then Ag-NRs were pulsed electrodeposited inside the template channels at a shifted potential of 0.4~0.8 V at 15 ^{o}C, using an electrolyte containing $AgNO_3$ (10g/L), ethylene diamine tetraacetic acid (5g/L), Na_2SO_3 (50g/L), and K_2HPO_4 (20g/L).Upon a typical pulsed ECD duration of 1~5 min, a strong copper base was electrodeposited onto the bottom surface of the AAO template embedded with Ag-NR arrays, using an electrolyte containing $CuSO_4\times5H_2O$ (160 g/L) and H_3PO_4 (30 g/L). Finally, the AAO template was completely removed by immersing in 5% H_3PO_4 to expose the arrays of Ag-NRs that supported on the copper base.

Finite-element method (FEM) modeling was conducted by using the RF module of Comsol Multiphysics V3.5a, parameters were based on the scanning electron microscopy (SEM) images of the Ag arrays.

S2. DNA immobilization

Complementary single stranded DNA oligomers used in the experiment:

Biotin-5'-TCC-ACA-CAC-CAC-TGG-CCA-TCT-TC-3'

3'-AGG-TGT-GTG-GTG-ACC-GGT-AGA-AG-5'-Cy5

All oligonucleotides were obtained from the Biopolymer Core Facility at the University of Maryland School of Medicine. Nanopure water, purified using Millipore Milli-Q gradient system, was used for all experiments. All other compounds were purchased from Sigma-Aldrich and used as received. The labeled oligomers with Cy5 and biotin on the 5' ends were obtained from Biopolymer Core facility of University of Maryland Baltimore (as illustrated above). Solutions of dsDNA samples were prepared by mixing complementary oligonucleotides in 5 mM Hepes (pH 7.5), 0.1 M KCl, and 0.25 mM EDTA buffer to a final concentration of 5 nM and cooling very slowly after incubation at 70 C for 2 min. Binding of the biotin disulfide to the nanorods was accomplished by incubating the nanoarray slides with biotin-disulfide solution (5 μM) for 1 hour, followed by tethering with streptavidin. Immobilization of DNA samples on streptavidin-biotin linked silver nanoarrays was performed by immersing the above slide in a solution of dsDNA (1 nM) for 48 h at 5 °C. A final washing step largely removed the unbound DNA probes from the substrate. The sample was dried and kept at 5 °C before use.

S3. Single-Molecule experiments

The single molecule studies were performed with a time-resolved confocal microscopy (Micro-Time 200, PicoQuant). A single mode pulsed laser diode (635 nm, 100 ps, 40 MHz) (PDL800, PicoQuant) was used as the excitation light. The collimated laser beam was spectrally filtered by an excitation filter (D637/10, Chroma) before directing into an inverted microscope (Olympus, IX 71). An air objective (Olympus, 100×, 0.95NA) was used both for focusing laser light onto sample and collecting the reflected fluorescence emission from the sample. The fluorescence that passed a dichroic mirror (Q655LP, Chroma) was focused onto a 75 μm pinhole for spatial filtering to reject out-of-focus signals and then reached the single photon avalanche diode (SPAD). To further isolated single-molecule fluorescence emission and reduce background, the desired spectral detection range was selected by placing a band-pass filter (HQ685/70, Chroma) in front of the single-photon avalanche diode (SPAD). Images were recorded by raster scanning (in a bidirectional fashion) the sample over the focused spot of the incident laser with a pixel integration of 0.6 ms. The excitation power into the microscope was maintained less than 200 nW. Time-dependent fluorescence data were collected with a dwell time of 50 ms. The fluorescence lifetime of single molecules was measured by time-correlated single photon counting with time-tagged-time-resolved (TTTR) mode (TimeHarp 200, PicoQuant). The reflectance images were recorded using the same optics setup after removing the emission band-pass filters. All measurements were performed in a dark compartment at room temperature.

S4. Additional SEM image