Supplementary Information

We fabricated the PDM substrate as reported in our previous study.4a Briefly, the silver thin layer nanoparticles were deposited over 50 nm thick silica layer which was deposited on 300 nm thick silver film. Top Ag layer was converted into Ag nanoparticles using thermal annealing.

PDM substrates or cover glass slides were incubated in buffer solution of 10 mM biotinylated bovine serum albumin (BSA-bt, Sigma) for 20 h (5 1C). After being washed 3 times with PBS buffer, the PDM substrates and glass slides were incubated with buffer solution of 200 pM streptavidin (SA) conjugated Cy5 (GE Amersham) for 2 h at 5 1C. Thus, the individual SA–Cy5 molecules were randomly distributed on PDM or glass substrates. The substrates were washed multiple times with PBS buffer and used for subsequent single molecule measurements. Single molecule measurements were performed using a Microtime 200 scanning confocal microscope equipped time-correlated single photon counting (TCSPC) capabilities (PicoQuant, Germany). A pulsed laser diode (635 nm, 100 ps, 40 MHz) was used as the excitation source. The excitation laser was directed through a 10 nm bandpass excitation clean-up filter and reflected by a dichroic mirror into an inverted Microscope (Olympus, IX 71). A water immersion objective (Olympus 60_, 1.2 NA) was used for focusing the laser light onto the sample and for collecting the fluorescence emission from the sample. The fluorescence signal was passed through a dichroic mirror and a band-pass filter (HQ685/70, Chroma) and focused through a 75 mm pinhole to a single photon avalanche photodiode detector. Images were acquired by raster scanning the sample. Intensity–time trajectories and intensity decays were obtained by positioning the incident laser light over individual Cy5–SAmolecules. Lifetimes were estimated by multi-exponential fitting. For acquiring single molecule emission spectra, the Microtime 200 system was attached to an Acton spectrograph consisting of high reflectance mirrors and dispersion grating with efficient imaging in the 450–750 nm wavelength region. An electron-multiplied CCD (Princeton Instruments PhotonMax512) was used as the detector. Numerical calculations were performed based on the FEM using COMSOL Multiphysics as described in details.4b