Supporting Information

Resolving Sub-Diffraction Limit Encounters in Nanoparticle Tracking Using Live Cell Plasmon Coupling Microscopy

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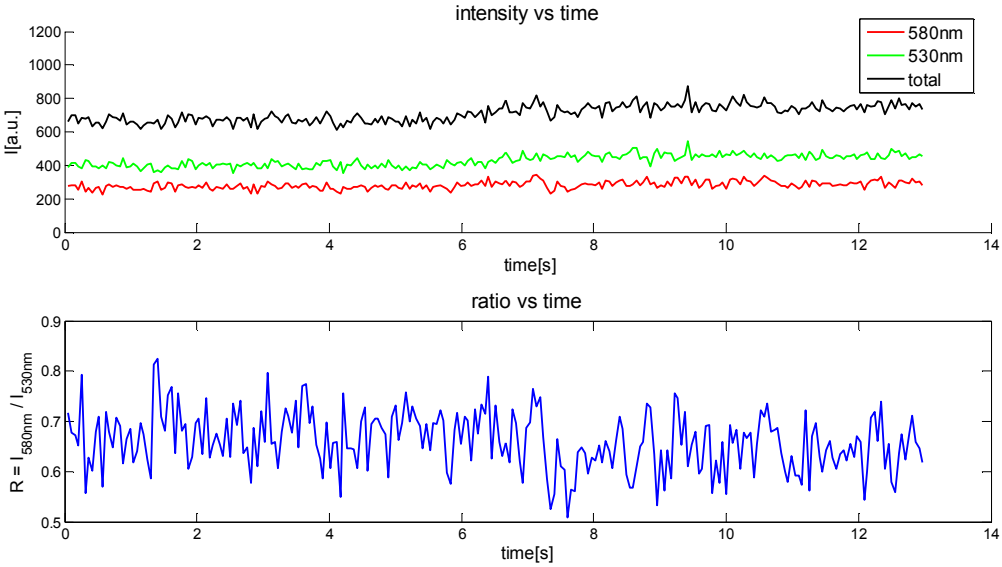

Figure S1: Intensities (top) and computed ratios *R* (bottom) as function of time for a pair of DNA linked gold nanoparticles that is not compacted upon addition of dendrimers. The intensity change caused by the change in the refractive index upon dendrimer addition is small. The resonance is not strongly shifted, consequently the intensity increase is detected on the 530 nm channel.

Materials and Methods

DNA linked Gold Nanoparticle Dimers

The dimers were obtained through a DNA programmed self-assembly process described in details elsewhere[1]. Commercial citrate stabilized particles (Ted Pella) were activated through reaction with bis(*p*-sulfonatophenyl)phenylphosphine (BSPP) dipotassium salt, at the concentration of 1 mg per 1 ml of colloids. The particles were washed by centrifugation and resuspended in T40 (40 mM NaCl/10 mM Tris·HCl, pH 8). The particles were split into two separate batches for the functionalization with complementary 50 nucleotides long single-stranded oligonucleotides (ssDNA) from Fidelity, Inc. (Gaithersburg, MD).

The sequence of the ssDNA (5' to 3') was :

Seq1:

Trithiol-GCA GTA ACG CTA TGT GAC CGA GAT ATC GGA GCA TTT GTA GTC TTG CAG AT

Seq2: complement to Seq1

The ssDNAs were added to the nanoparticle solutions at a ratio of ~25 ssDNAs per particle. Then the mixture was left to react for 8 h. Next the particles were split into two batches and pegylated. One batch was pegylated with thiolated carboxy-terminated PEG (7 EG units) at a ratio of 1:100,000. In the second batch, the thiolated carboxy-terminated PEGs were mixed with a thiolated biotin terminated PEG in a 25:1 ratio and reacted at the same total PEG concentration. An approximate thickness of the PEG brush around the particles can be obtained from worm-like-chain calculations.[2] The end-to-end distance of the used PEGs is ~4 nm, the persistence length ~2 nm yielding an average thickness of the PEG brush of 3 nm.

The ssDNA-particle mixtures were incubated with the PEG ligands for at least 8 h before cleaning. After two washing steps through centrifugation, the particles were resuspended in 160 mM NaCl/10 mM Tris·HCl (pH 8) and annealed. Dimers of DNA linked nanoparticles were purified from monomers and higher-order aggregates by gel electrophoresis. The annealed particle mixtures were mixed with 1/5 volume Ficoll 400 loading buffer solution and loaded into 1% agarose gels. The gels were run horizontally at a fixed potential of 175 V for 15 min with 0.5´ Tris·borate buffer as running buffer. After running the gels, the particle mixtures were found to be in discrete bands, with the second band from the top corresponding to the nanoparticle dimers. These bands were manually extracted from the gels and collected into an electroelution chamber. The dimers were then isolated from the gel through eletroelution.

The isolated dimers could then be bound to the surface of a neutravidin functionalized flowchamber. Only one of the two particles in the dimer was directly connected to the surface through the Biotin-PEG; the second DNA tethered particle was free to move in solution, so that the interparticle separation was variable.

Anti-Fibronectin Particles

Citrate stabilized gold nanoparticles with a nominal diameter of 40 nm were purchased from Ted Pella. To 1 ml of gold sol (9.0×10^{10} particles/mL)☐ 25 μL of anti-Fibronectin (Invitrogen, A 21316) and 10 μL of 10 mM phosphate buffer pH 6.0 were added. The particles were incubated overnight and then washed 1x by centrifugation (3500 rpms for 15 min) and resuspended in Hanks' buffered saline solution (Gibco, 24020) with 11 mM Mg^{2+}, 1mM Ca^{2+}, and containing 10 mM HEPES buffer pH7.2. The particles were prepared fresh before each experiment.

Dendrimers

4th generation Polyamidoamine (PAMAM) dendrimers were purchased from Sigma-Aldrich (4142449). In a typical experiment the dendrimers were diluted 1:1000 in T50, pH 7.

Enzymes

EcoRV was purchased from New England Biolabs (NEB, R0195S). For a typical experiment we diluted 2 μL of Enzyme solution in 200 μL NEB 3 buffer.

Cell Culture

HeLa cells (ATCC, CCL-2) were grown in the advanced Dulbecco's Modified Eagle Medium (Gibco,12491) supplemented with 10% fetal bovine serum (Gibco,16000), 50 units/ml penicillin (Gibco, 15140), 50 μg/ml streptomycin (Gibco, 15140) and 2mM L-glutamine (Gibco, 25030) at 37°C in a humidified, 5% CO2 atmosphere.

1. Reinhard, B.M., et al., *Use of plasmon coupling to reveal the dynamics of DNA bending and cleavage by single EcoRV restriction enzymes.* Proceedings of the National Academy of Sciences of the United States of America, 2007. 104(8): p. 2667-2672.
2. Kratky, O. and G. Porod, Recueil Des Travaux Chimiques Des Pays-Bas-Journal of the Royal Netherlands Chemical Society, 1949. 68: p. 1106-1122.