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Supporting Information

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Probing Distance-Dependent Plasmon-Enhanced Near-Infrared Fluorescence Using Polyelectrolyte Multilayers as Dielectric Spacers**

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Experimental Section

Materials

All materials were used as received without any further purification. Polyallylamine Hydrochloride (MW: 56,000 g/mol) (PAH), Poly (sodium4-styrene sulfonate) (MW: 70,000 g/mol) (PSS), 3-(mercaptopropyl) triethoxysilane (MPTES), cetyltrimethylammonium bromide (CTAB), gold chloride ($HAuCl_4.4H_2O$), sodium borohydride ($NaBH_4$), ascorbic acid, sodium chloride (NaCl), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and pencillin-steptomycin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trypsin-EDTA (0.25% 1X) was purchased from Life Technologies Corp. McCoy's 5A medium and SKBR3 cells were purchased from American Type Culture Collection (ATCC). Hydrochloric acid (HCl) was obtained from EMD (Gibbstown, NJ). Cetyltrimethylammoniumchloride (CTAC) was obtained from TCI (Portland, OR, USA). The Formvar/carbon coated copper TEM grids were acquired from Ted pella (Redding, CA, USA). Nanopure water (>18.0 MΩ-cm) was used for all experiments.

Synthesis of shape-controlled gold nanostructures

Various shape-controlled gold nanostructures were synthesized using seed-mediated approach reported in literature with small modifications.[1] Gold nanorods were synthesized using a seed-mediated approach. Seed solution was prepared by adding 0.6 ml of an ice-cold sodium borohydride solution (10 mM) into 10 ml of 0.1 M cetyltrimethylammonium bromide (CTAB) and 2.5×10^{-4} M chloroauric acid ($HAuCl_4$) solution under vigorous stirring at room temperature. The color of the seed solution

changed from yellow to brown. Growth solution was prepared by mixing 95 ml of CTAB (0.1 M), 0.5 ml of silver nitrate (10 mM), 4.5 ml of $HAuCl_4$ (10 mM), and 0.55 ml of ascorbic acid (0.1 M) consecutively. The solution was homogenized by gentle stirring. To the resulting colorless solution, 0.12 ml of freshly prepared seed solution was added and set aside in the dark for 14 h prior to use, the AuNR solution was centrifuged twice at 10,000 rpm for 10 min to remove excess CTAB and redispersed in nanopure water (18.2 MΩ-cm). Details of other nanostructures synthesis are provided in the supporting information.

Goldnanorods adsorption on glass slides

Glass slides (1×2 cm) were cleaned using Piranha solution (3:1 sulfuric Acid to hydrogen peroxide) followed by extensive rinsing with nanopure water and drying under a stream of dry nitrogen. The Piranha-cleaned glass slides were exposed to 1% (3-mercaptopropyl) triethoxysilane in 100% pure ethanol solution for 30 minutes to render thiol functionality to glass slides. The glass slides were then rinsed with ethanol and dried under a stream of nitrogen. Subsequently, MPTES-functionalized glass slides were exposed to twice-centrifuged and acidified (pH 3) gold nanoparticle/nanorods solution for 15 min. Lowering the pH of the nanoparticle solution causes rapid adsorption of the nanoparticles onto thiol-functionalized surface. Subsequently, the glass slides were thoroughly rinsed with nanopure water to remove excess and weakly bound nanostructures. A noticeable color change of the glass slides indicates the successful adsorption of the nanostructures. UV-vis extinction spectra from glass slides exhibit distinct plasmon bands characteristic of the nanostructures adsorbed on the surface.

Plasmon Enhanced Fluorescence

Polyelectrolytes coating on slides

We used a custom-made Teflon glass slide holder to deposit polyelectrolyte multilayers on glass slides. The custom-made holder minimized the handling and consequent scratching of the glass surface. This experimental setup also ensures uniformity of layer-by-layer assembly across multiple samples, while making the coating process less tedious. LbL was performed using polystyrene sulfonate (PSS) (1mM) and poly allylamine hydrochloride (PAH) (1 mM) in 100mM sodium chloride (NaCl) solution. The molarity of the solutions is calculated from the molecular weight of the mer unit in each polymer. The weight to volume ratio for the PSS and PAH solution is 0.206 mg/mL and 0.093 mg/mL, respectively. Desired numbers of glass slides were placed in a Teflon holder and sequentially immersed in PSS, nanopure and PAH solution for 5 minutes. The process was repeated to attain desired number of polyelectrolyte multilayers. The multilayers were terminated with a PAH layer to adsorb the negatively charged NIR dye on the surface (Figure S4).

Polyelectrolytes coating of gold nanostructures in solution

The nanoparticles/nanorods solution was centrifuged at 8000 rpm for 12 min, which results in the formation of a pellet. The supernatant was removed and the pellet was redispersed in nanopure water. The procedure was repeated twice. To 500 μL of the nanoparticle solution, 25 μL of PSS (100 mM) was added and vortexed briefly. To the resulting mixture, 50 μL NaCl (1 mM) solution was added and vortexed briefly. The solution was subjected to magnetic stirring for 16-24 hours. Subsequently, the

polylectrolyte coated nanorods were washed by centrifugation and redispersion. The same procedure was repeated to coat the nanostructures with PAH. Desired numbers of bilayers were achieved by repeating PSS and PAH coating on the nanostructures.

Cell culture

Human epithelial breast cancer cells (SKBR3) were purchased from ATCC (Manassas, VA) and sub-cultured in Mc.Coy's 5A medium with 10% fetal bovine serum (FBS) and antibiotics (100 μg/ml penicillin and 100 μg/ml streptomycin) (Sigma, St. Louis, MO). Cells were grown in water jacket incubator at 37^0C with 5% CO_2-humidified atmosphere in 25 cm^2 tissue culture flasks. Once the cells reached to 90% confluence, they were washed with phosphate buffered saline (PBS) and detached with 1 mL of 0.25% trypsin-EDTA solution (Sigma). Cells were dispersed in 10 ml complete medium with 10% FBS and centrifuged. Cells were counted in trypan blue to confirm viability and plated at a density of 4×10^4 cells on poly(lysine) coated 0.5×0.5 cm^2 silicon substrates in a flat-bottom 24-well plates (Fisher Scientific, Pittsburgh, PA).

Fluorescence imaging

The cells were seeded on a 5 mm wide cover glass at a density of 5×10^4 per well in a 24-well culture plate and were incubated overnight at 37 °C with 5% CO_2. To demonstrate the *in vitro* bioimaging of ultra-bright bullets, three different kinds of PEF probes as indicated in Figure 4 were incubated with SKBR3 cells for 4 hrs at 37 ^{o}C in humidified incubator with 5% CO_2 for passive targeting. Then the cells were fixed in 4% formaldehyde and washed three times before passivation with 1% Triton- X. Finally Plasmon Enhanced Fluorescence

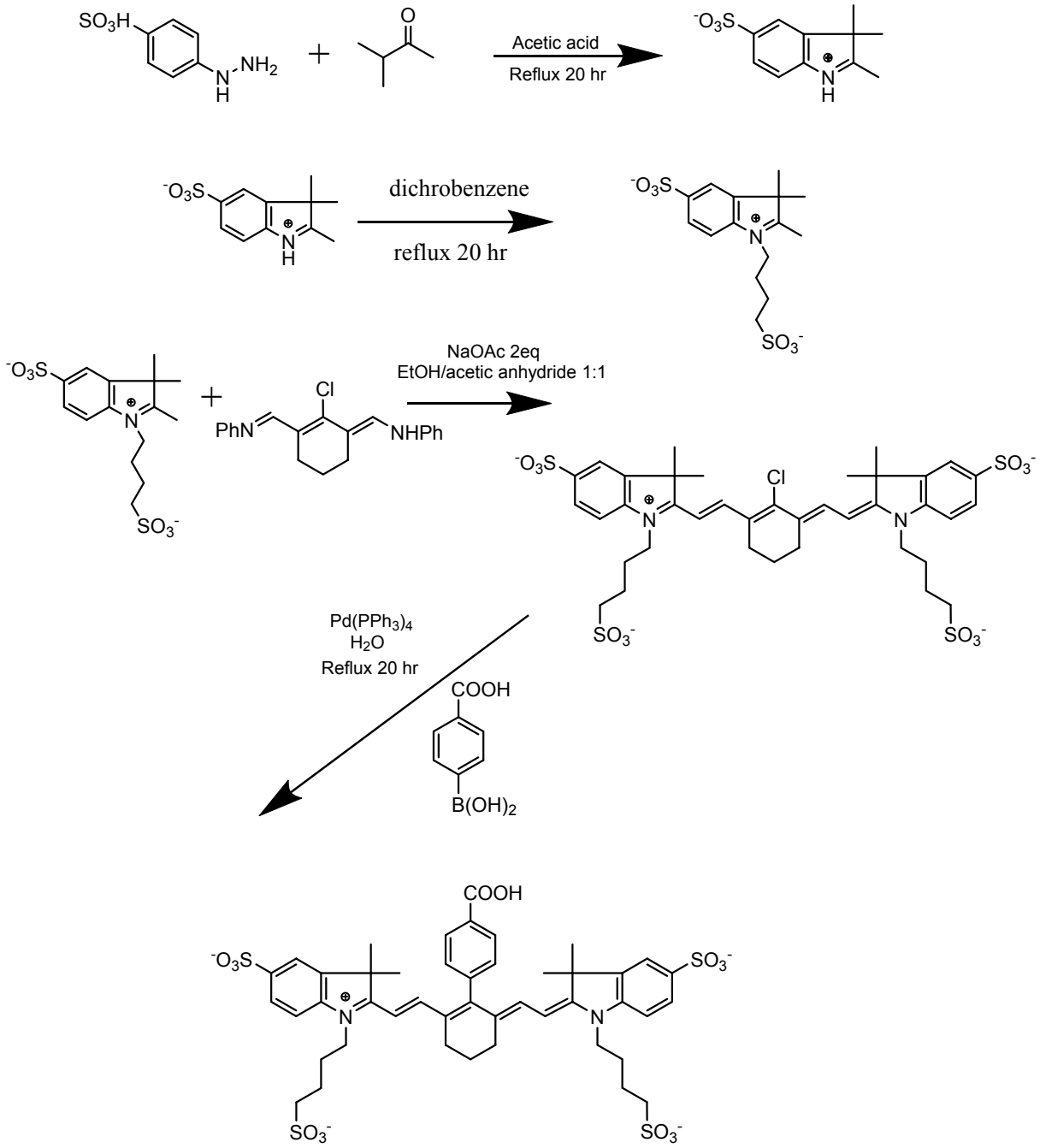
fixed cells were washed 3 more times in PBS buffer and image the cells with confocal fluorescence microscope at 785 nm excitation wavelength using 40X objective (Figure 6).

Instrumentation

Transmission electron microscopy (TEM) micrographs were recorded on a JEOL JEM-2100F field emission (FE) instrument. Samples were prepared by drying a drop of the solution on a carbon-coated grid, which had been previously made hydrophilic by glow discharge. The excess solution on the grid was blotted away after 30 s with filter paper. Atomic force microscopy (AFM) was performed using Dimension 3000 (Bruker) AFM in light tapping mode.[2] Triangular Si cantilevers with tip radius less than 10 nm (MikroMasch) were employed for AFM imaging. UV–vis extinction spectra were collected in air using a Shimadzu UV-1800 UV–vis spectrometer.

LS288 Synthesis

LS288 is synthesized by previously reported method as shown below.[3]



Scheme S 1. Different steps involved in the synthesis of LS288

Gold nanoparticles synthesis

Plasmon Enhanced Fluorescence

Gold nanoparticles were synthesized in three steps.[4] In the first step, seed solution was prepared by vigorous mixing of 5 ml of aqueous CTAC solution (0.2 M), 4.5 ml of nanopure water and 515 µl of $HAuCl_4$ (4.86 mM) with 450 µl of ice-cold $NaBH_4$ (0.02 M) solution. The seed solution was aged for 1 hr at 30°C in a hot bath. In the second step, the growth solution was prepared by mixing 4.5 ml of nanopure water, 5 ml of aqueous CTAC solution (0.2 M), 515 µl of $HAuCl_4$ (4.86 mM), and 75 µl of ascorbic acid (0.04 M). To this colorless solution, 5 µl of seed solution was added with vigorous stirring and kept undisturbed for two days to obtain highly uniform spherical nanoparticles with LSPR at 540 nm. The diameter of the nanoparticles obtained at this stage was ~50 nm.

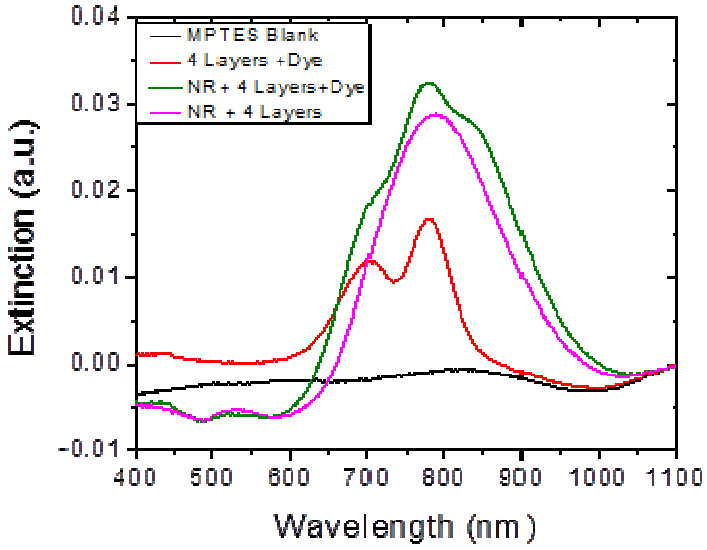


Figure S 1. Vis-NIR extinction spectra obtained from (i) MPTES coated blank glass slides (ii) Dye adsorbed on PAH (iii) AuNR coated with two bilayers and (iv) AuNR coated with two bilayers followed by dye adsorption.

Plasmon Enhanced Fluorescence

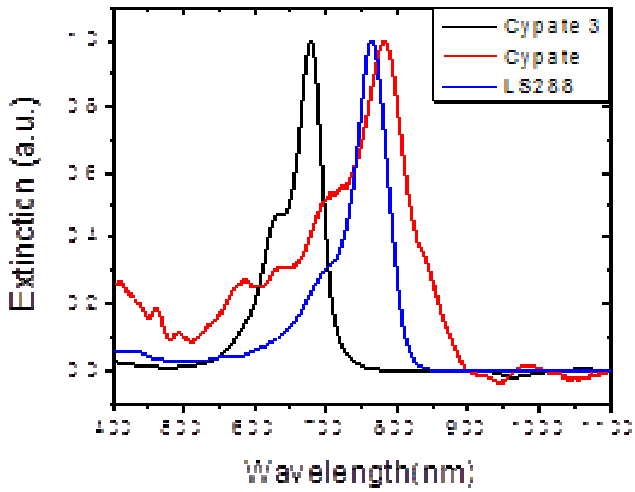


Figure S 2 Vis-NIR extinction spectra of three different dyes: LS288, Cypate and Cypate-3

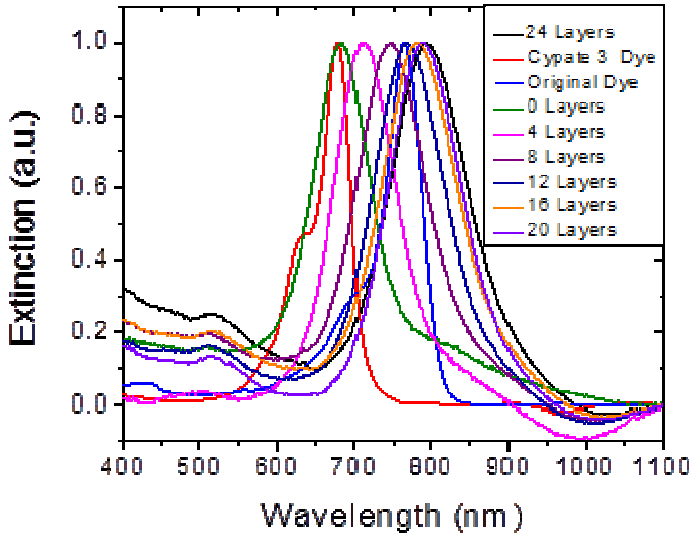


Figure S 3 Vis-NIR extinction spectra of AuNR coated glass slides with varying number of polyelectrolyte layers compared to the peak position of the LS288 and Cypate 3 dyes.

Plasmon Enhanced Fluorescence

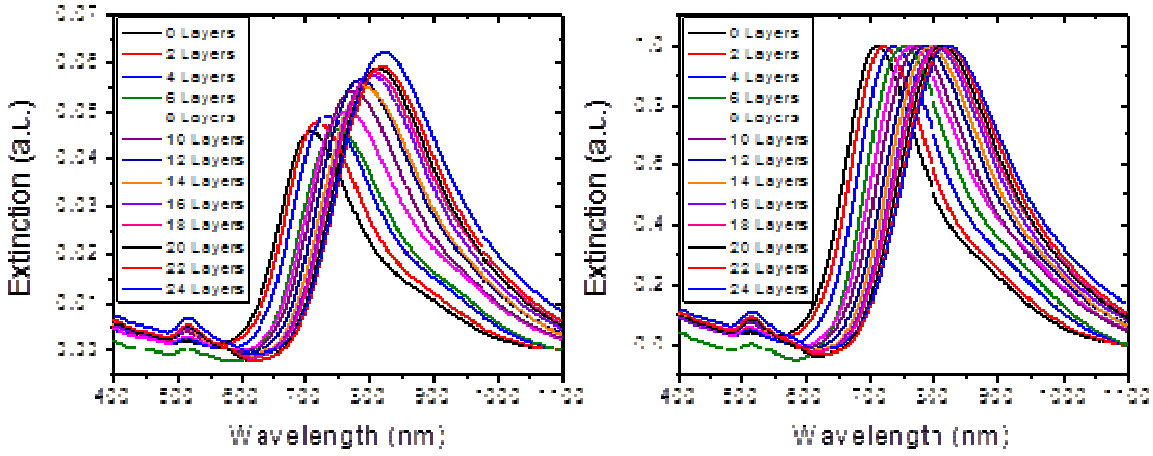


Figure S 4 Vis-NIR extinction spectra of AuNR with PEMs after each bilayer coating. Normalized extinction spectra exhibit progressive red-shift after every bilayer due to the increase in local refractive index with polyelectrolyte coating.

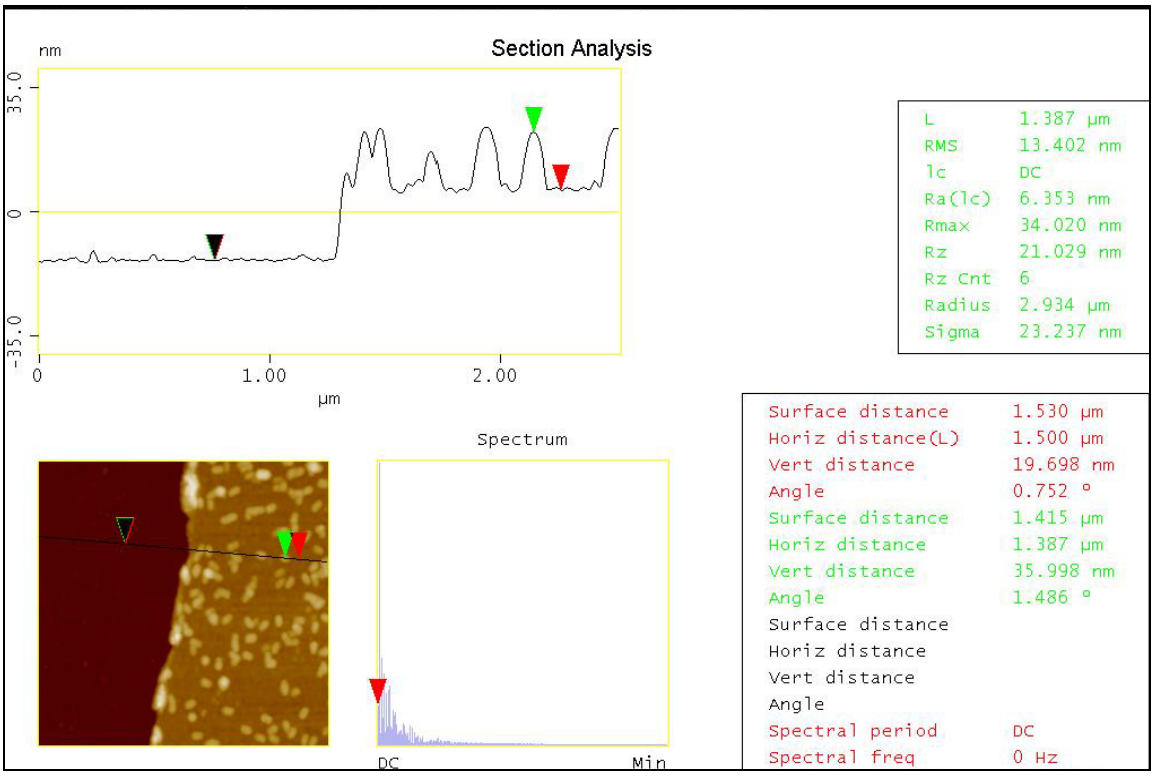


Figure S 5. AFM analysis of gold nanorods adsorbed glass slide coated with 20 layers of PEMs.

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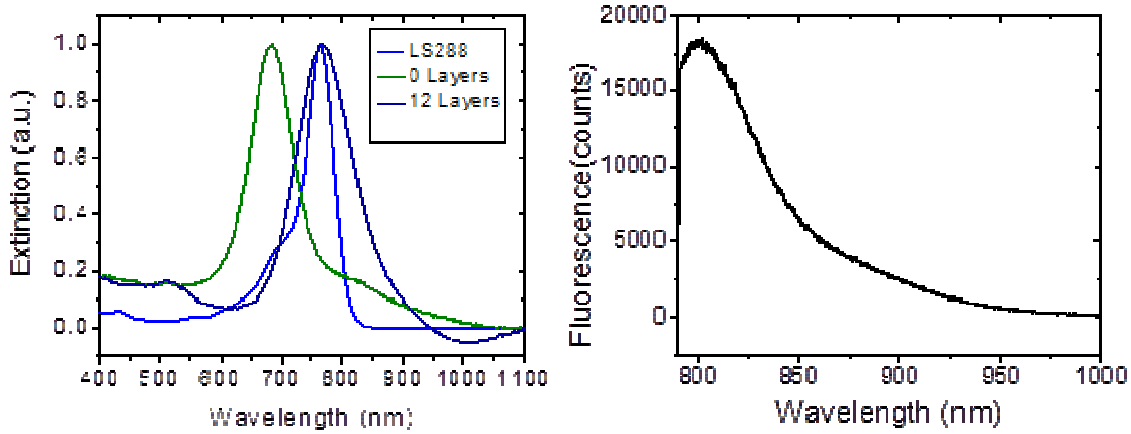

Figure S 6. Vis-NIR extinction spectra of AuNR with 0 layers and 12 layers compared with the absorption spectrum of LS288. After the deposition of 12 layers of polyelectrolytes on AuNR the LSPR of AuNR matches with the absorption of the dye. However, the increased distance from the surface of AuNR results in smaller (nearly 5 times) PEF compared to the best case (i.e., 4 layers separation and LSPR match with absorption).

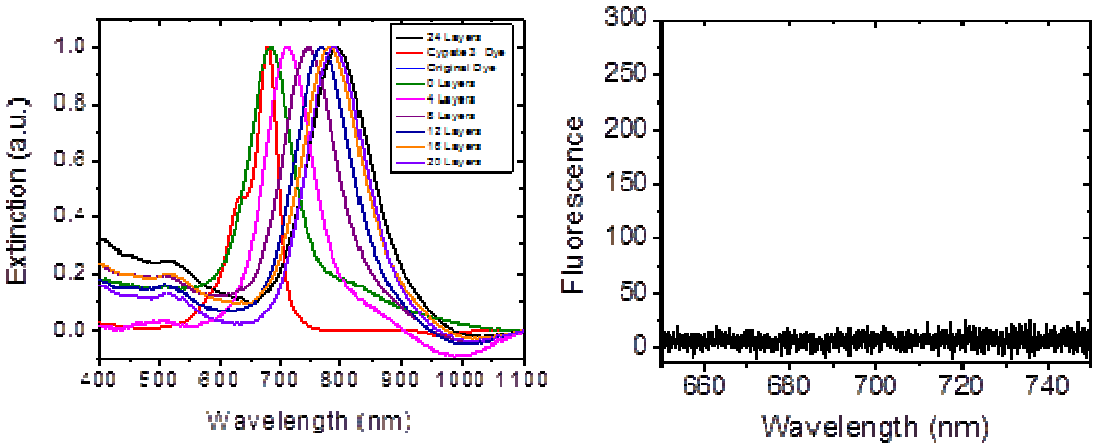

Figure S 7. Vis-NIR extinction spectra of a control dye Cypate-3 adsorbed on AuNR. Although the LSPR of AuNRs matches with the laser excitation, no fluorescence was observed due to the mismatched absorption maximum of the dye. This indicates the importance of the overlap of the dye absorption with LSPR of the nanostructures.

Plasmon Enhanced Fluorescence

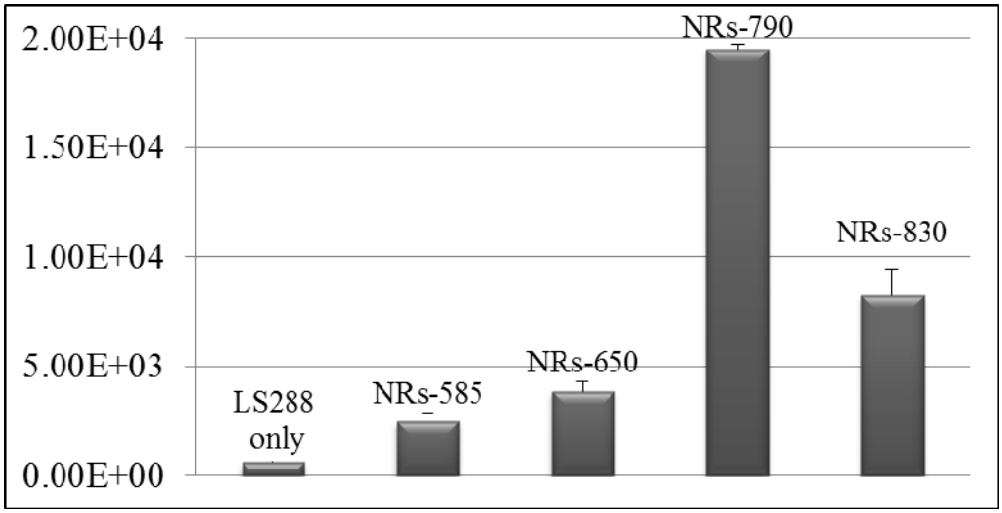

Figure S 8 PEF of dye adsorbed on AuNR of different aspect ratio. The maximum PEF was obtained in the case of NRs-790 due to strong overlap of the LSPR of AuNR and absorption of the dye.

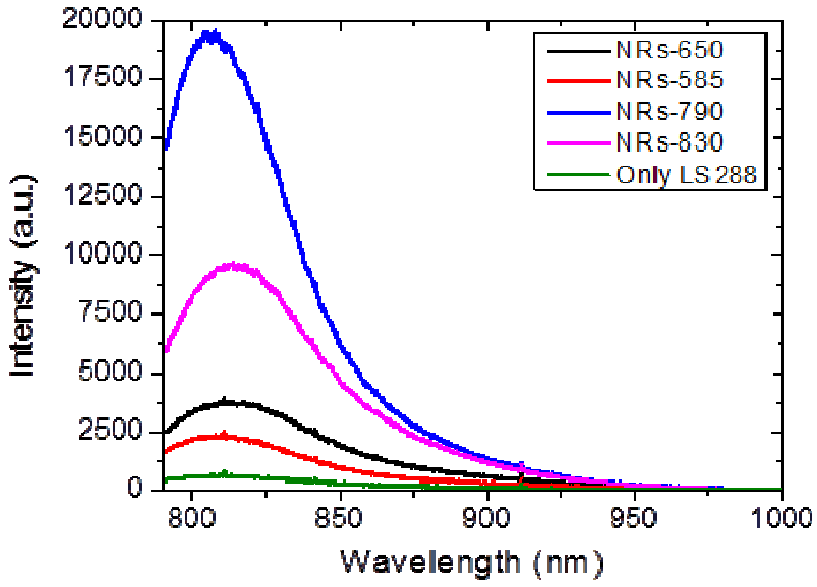

Figure S 9 Fluorescence spectra of LS288 adsorbed on AuNR of different aspect ratio. The LSPR wavelength of AuNR is indicated in the plot.

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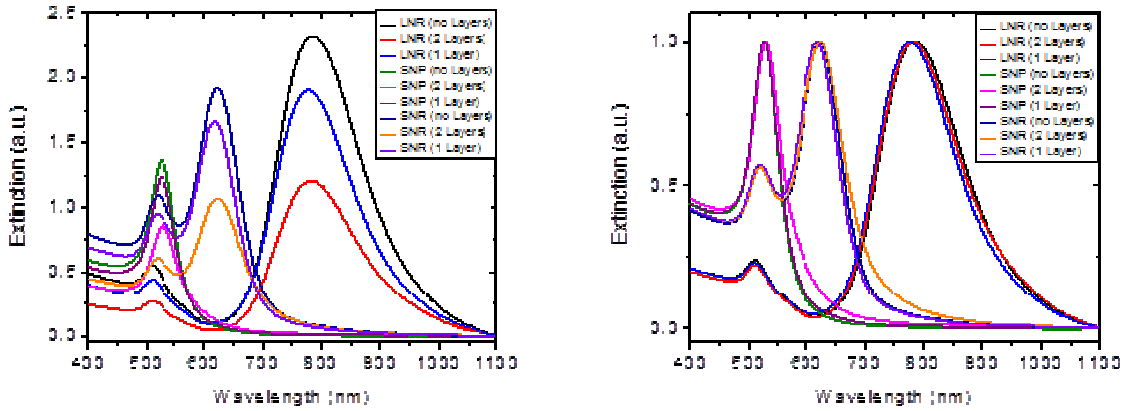

Figure S 10 Vis-NIR extinction spectra of AuNR-4.5, AuNR-2.3, and AuNR-1 in solution before (left) and after (right) polyelectrolyte coating.

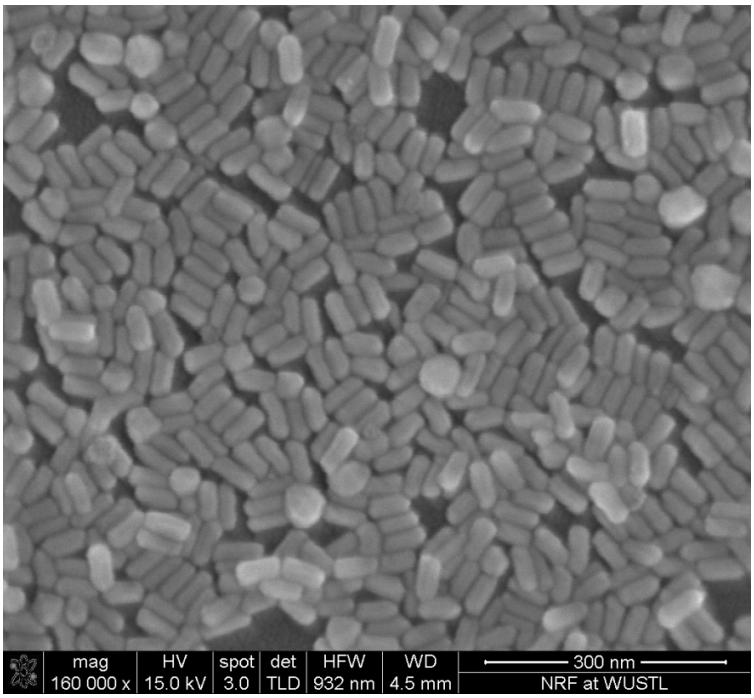

Figure S11 Scanning electron microscope (SEM) image of as synthesized AuNRs prior to polyelectrolyte coating.

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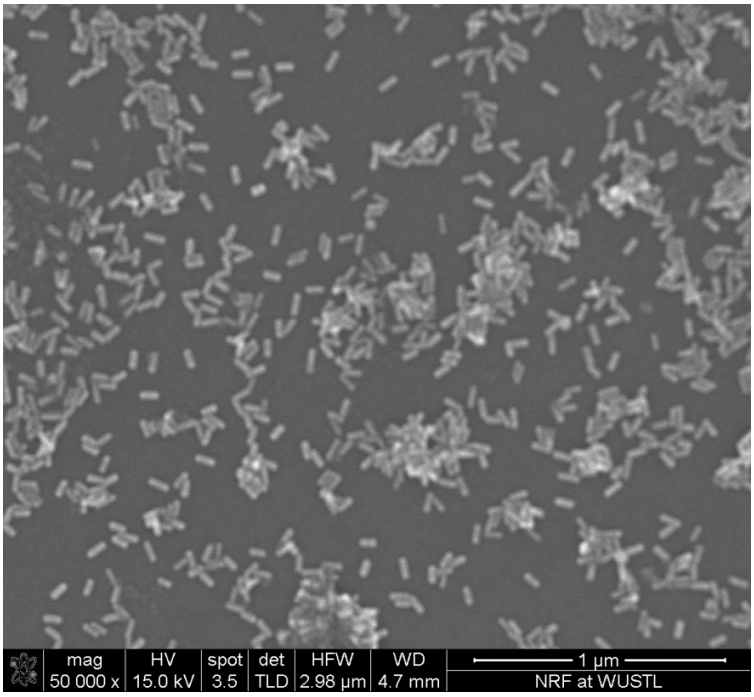

Figure S12 Scanning electron microscope (SEM) image of AuNRs coated with 2 bilayers of PSS and PAH in solution, which shows a thin layer of polymer on the surface of AuNR.

Dimension	**Average (nm)**	**Standard Deviation (nm)**
Planet Diameter	51.40	2.35
824NR Length	62.99	7.35
824NR Width	13.91	1.73
624NR Length	44.37	5.45
624NR Width	19.93	3.09

Table S 1 Size distribution of the nanorods and nanoparticle used in this article.

Plasmon Enhanced Fluorescence

Fluorescence mapping procedure

All fluorescence data were collected using confocal optical microscope using 785nm laser as excitation source. A 2D map of the fluorescence intensity at 815 nm (in the case of LS288) with a 1 μm step-size is shown in Figure S13. Fluorescence map confirms the uniform adsorption of LS288 on polyelectrolyte multilayer-coated glass slide.

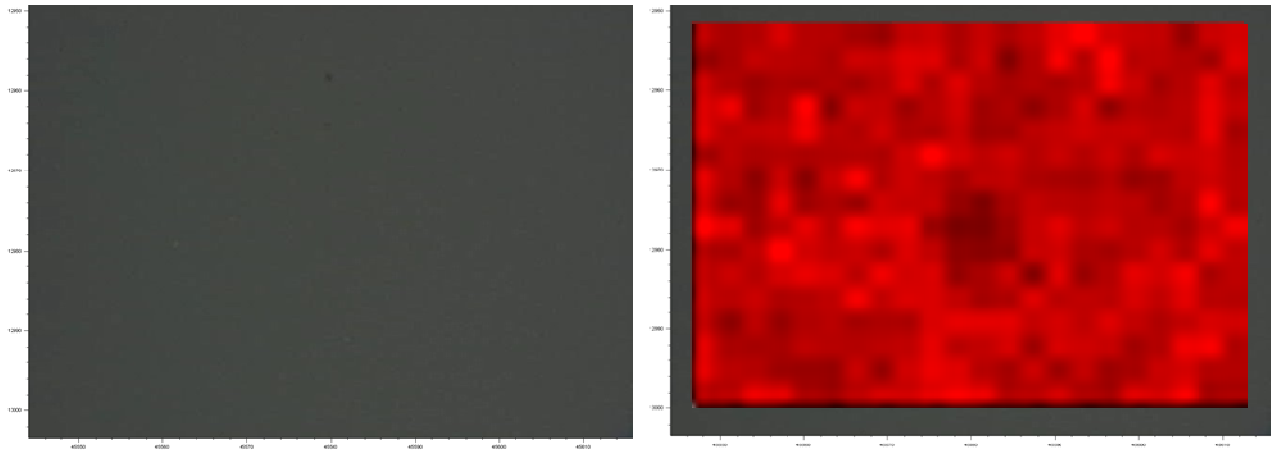

Figure S 13 Fluorescence mapping of LS288 on PAH coated glass slide.

Plasmon Enhanced Fluorescence

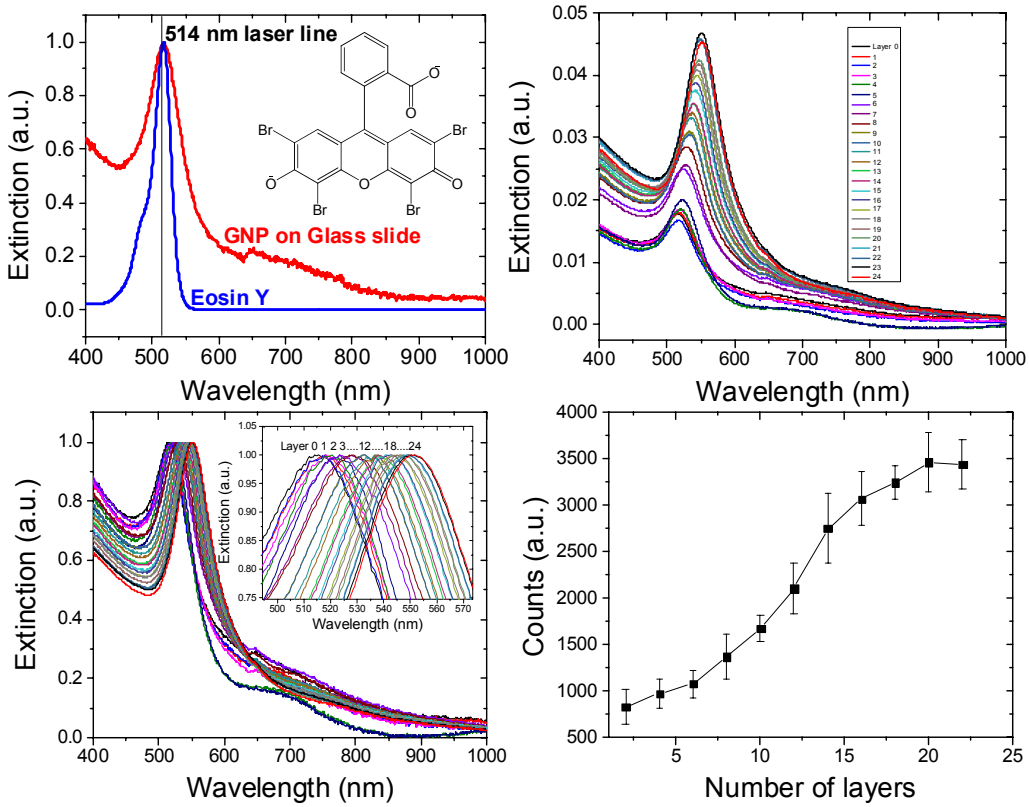

Figure S 14 (A) Absorption spectrum of Eosin Y (Inset) and extinction properties of GNPs adsorbed on glass slide. (B) Extinction spectra of GNPs obtained after the deposition of "n" bilayers indicated in the plot. (C) Progressive red shift after polyelectrolyte layers on GNPs confirms the successful deposition of polyelectrolytes. (D) Plot depicting the increase in fluorescence intensity with increasing spacing between the Eosin Y and GNPs.

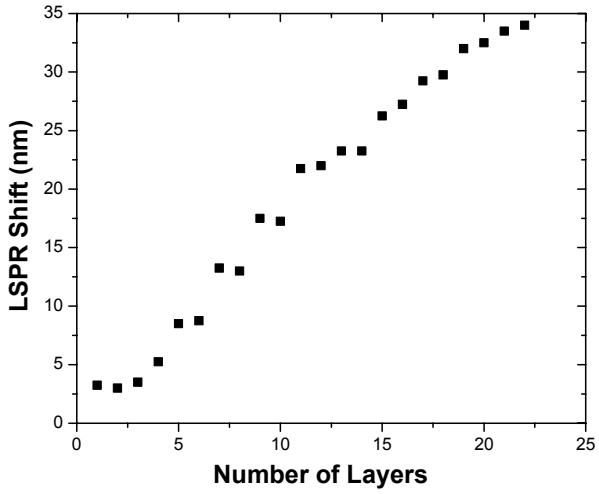

Figure S 15. Plot depicting the LSPR shift after deposition of polyelectrolyte layers on GNPs deposited glass slide.

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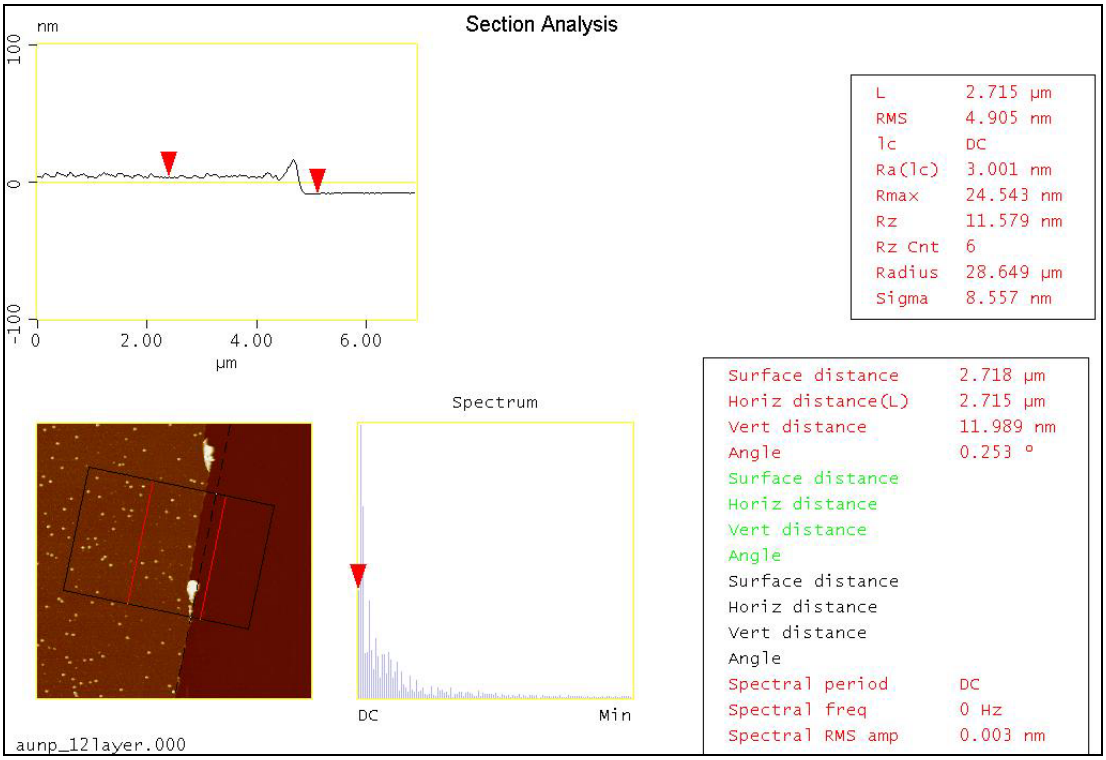

Figure S 16 Atomic Force Microscopy is employed to measure the thickness of polyelectrolyte layers after 12 layers on GNPs deposited glass slide. We confirmed that the average thickness of each layer is ~ 1 nm.

References

[1] H.-L. Wu, C.-H. Kuo, M. H. Huang, Langmuir 2010, 26, 12307; P. N. Sisco, C. J. Murphy, J. Phys. Chem. A 2009, 113, 3973.

[2] M. E. McConney, S. Singamaneni, V. V. Tsukruk, Polym. Rev. 2010, 50, 235; V. V. Tsukruk, S. Singamaneni, Scanning Probe Microscopy of Soft Matter: Fundamentals and Practice, Wiley-VCH Verlag GmbH & Co. KGaA, 2012.

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[4] N. Gandra, A. Abbas, L. Tian, S. Singamaneni, Nano Letters 2012, 12, 2645.