Supporting information

for

In Situ Modulation of Gold Nanorod's Surface Charge Drives End-to-End Assemblies from Dimers to Large Networks that Enhance Single-Molecule Fluorescence by 10000-fold

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1. Experimental details

Materials

Chloroauric acid trihydrate ($HAuCl_4.3H_2O$), cetyltrimethylammonium bromide (CTAB), ascorbic acid, silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), hydrochloric acid, polyacrylic acid (sodium salt, Mw = 8000), Sodium chloride (NaCl), Crystal violet, and glycerol were purchased from Sigma-Aldrich. Acetonitrile was purchased from Merck. Triethylamine (Et_3N) was purchased from Finar Limited. All chemicals were used as received without any further purification. Milli pore water (18.2 MΩ) was used for the purpose of nanorod synthesis and surface modification steps.

Synthesis of gold nanorods

Gold nanorods were synthesized by following seed mediated growth method developed by the groups of Murphy and El-Sayed.[1,2] First, a gold seed solution was prepared by reducing $HAuCl_4$ (0.25 mM, in 10 mL 0.1 M CTAB solution) with 0.6 mL of i freshly prepared 0.1M $NaBH_4$ under vigorous stirring. Solution colour changes from yellow to brown indicating the formation of seeds. The solution was then kept undisturbed for another 30 min at 30˚C.
Growth solution was prepared by mixing 25 μL of 0.1M $AgNO_3$ in 5 mL of 0.1M CTAB. The solution was kept undisturbed for 15 min. 25 μL 0.1M $HAuCl_4$ was added to the solution and was stirred for 5 min. Subsequently, 27.5 μL of 0.1M ascorbic and 6 uL of seed solution was added to the growth solution. The solution was then kept undisturbed for 12 hrs at 30 ˚C to form gold nanorods. Excess of the growth solution was removed by centrifugation and the particles were redispersed in 0.5 mM CTAB solution and stored for further use.

Polyelectrolyte overcoating

CTAB-coated gold nanorods were further functionalized with PAA through electrostatic adsorption developed by Murphy group.[3] 500 μL of CTAB-coated nanorods were centrifuged at 11,000 rpm for 5 minutes, and then redispersed in 200 μL water. Then it was added drop wise into 2mL of previously prepared PAA (10 mg/mL) aqueous solution containing 6 mM NaCl under stirring. After stirring for 20 min, excess unreacted polyelectrolytes were removed by centrifugation at 11000 rpm for 5 minutes and the PAA modified nanorod precipitate were redispersed in water.

Self-assembly formation

Aqueous colloidal solution of PAA overcoated nanorods was first centrifuged and the supernatant was replaced by acetonitrile (2 mL volume) followed by bath sonication for 30 seconds. The PE modified nanorods in acetonitrile were then incubated with 5 μM of 1,6-hexanedithiol solution in a quartz cuvette to monitor the temporal evolution of the nanorods. The solution was made slightly acidic by adding 0.5 μL of 1 M HCl prior to the addition of dithiol. The aggregation was allowed to progress until 30 – 40 min under this condition before adding 10 μL Et_3N. The reaction progress was monitored by SEM and absorption spectroscopy over 7 days' time period.

Characterizations

Extinction spectra were recorded in JASCO V750 UV-visible spectrophotometer using a 1 cm quartz cuvette. All spectra were recorded at room temperature. Zeta potential of samples was measured in water and acetonitrile using Zetasizer Nano series (Malvern) instrument. Scanning electron microscopy (SEM) micrographs were accrued using a SEM (JSM 7600FJEOL) at 10 kV. SEM samples were prepared by spin coating 10 μL of reaction solution on silicon substrate/ITO coverslip. The substrates were cleaned with mili-Q water, dried under N_2 flow, and UV-ozone cleaned for 30 minute before imaging. TEM analysis was done using Technai 20, Phillips instrument by drop-casting sample on copper grids at 200 kV.

Confocal microscopy

All fluorescence enhancement measurements were done on a home-built confocal microscope (schematic shown in figure S6). Sample was excited by a 633 nm laser. A spatially filtered 633 nm laser (Oxxius Lasers) was focused onto the sample using an oil immersion objective (NA: 1.25). The luminescence signal from the sample was collected by the same objective and separated from the excitation laser using a 633 nm notch filter. The luminescence light was directed to an avalanche photodiode (APD; Excelitus, SPCMAQRH-14). One photon luminescence image of the nanorods was acquired by scanning the sample across the diffraction-limited laser focus using an XYZ Piezo nanopositioning stage (Physik instruments).

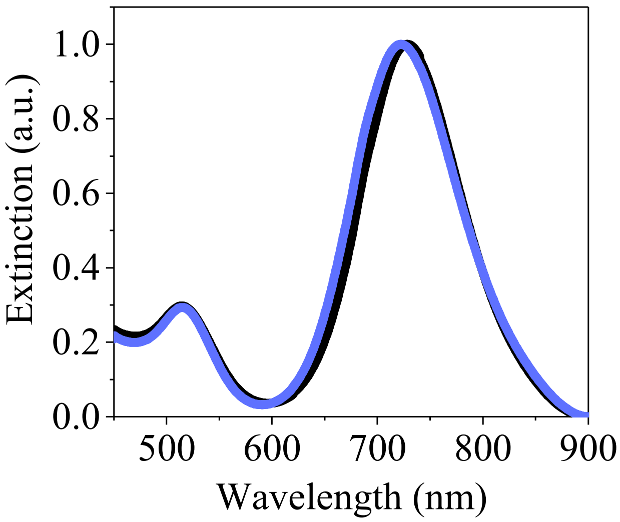

Figure S1: *Extinction spectra of CTAB coated nanorods (Black) and PAA8k coated nanorods (Blue) in water.*

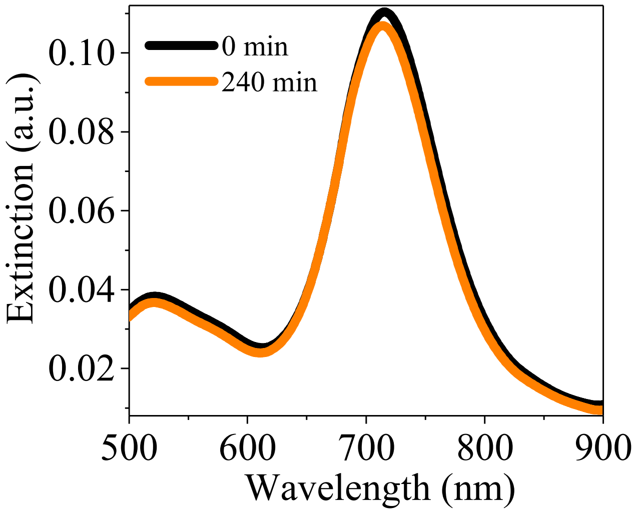

Figure S2*: Extinction spectra of PAA8k coated nanorods after transferring to acetonitrile (black) and after 240 min (orange).*

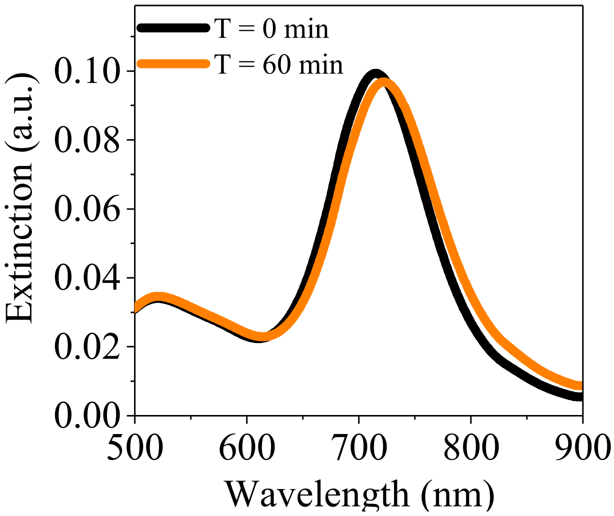

Figure S3: *Extinction spectra of PAA8k coated nanorods in acetonitrile immediately after the addition of 1,6-hexanedithiol (black, T=0min) and after 60 min (orange, T= 60 min)*

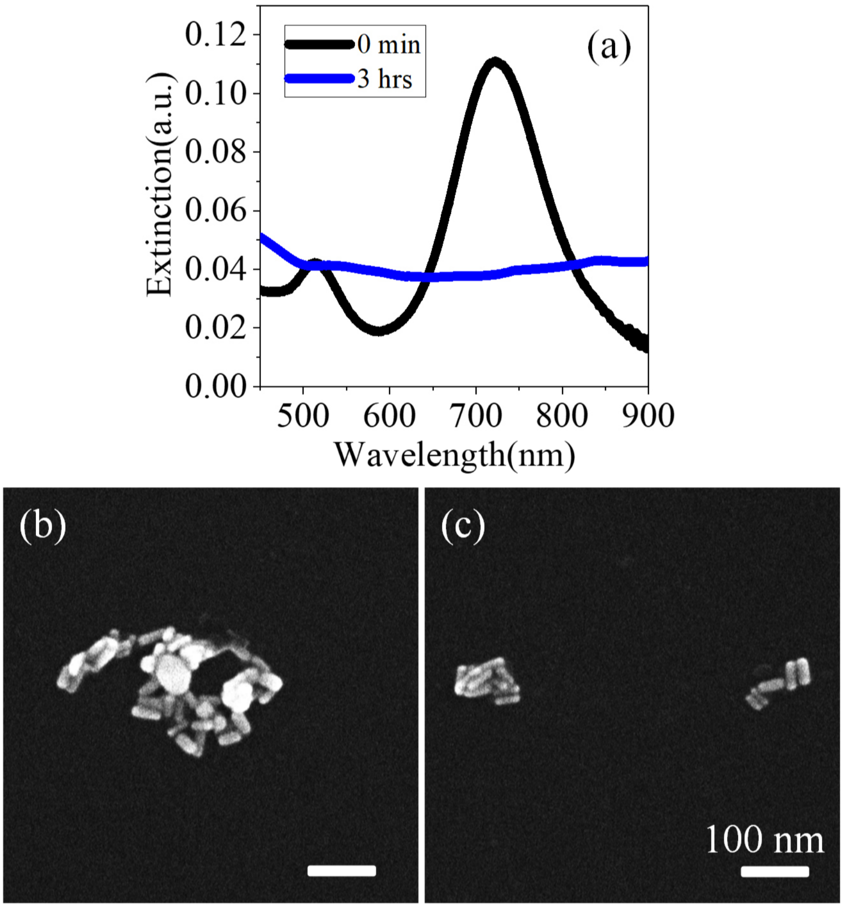

Figure S4: *(a) Extinction spectra of PAA8k coated nanorods in acetonitrile immediately after the addition of 1,6-hexanedithiol (black, T=0min) and after 3 hrs in absence of* Et_3N *(blue, T= 3 hrs). (b, c) SEM images of nanorod clusters formed in absence of* ET_3N *after 3 hours from dithiol addition.*

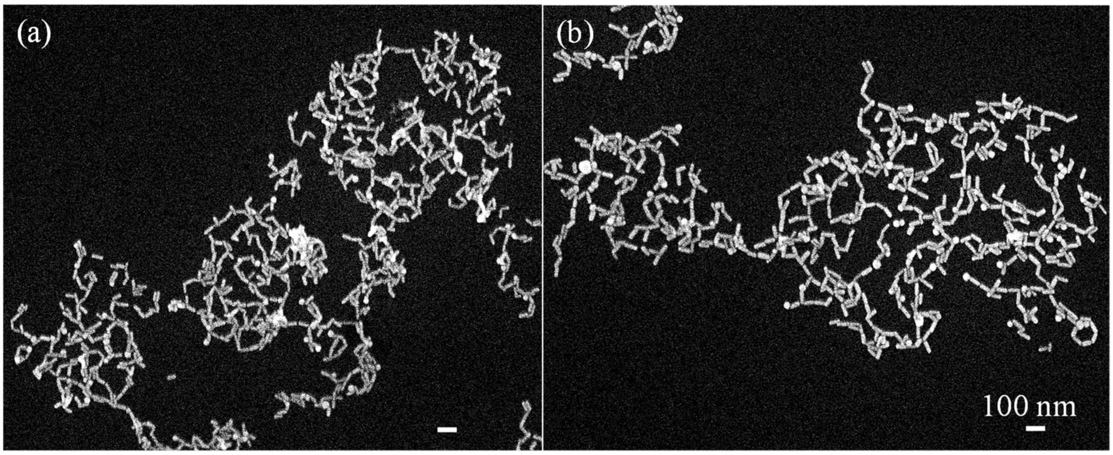

Figure S5: *SEM images showing presence of networks in sample stored for one month.*

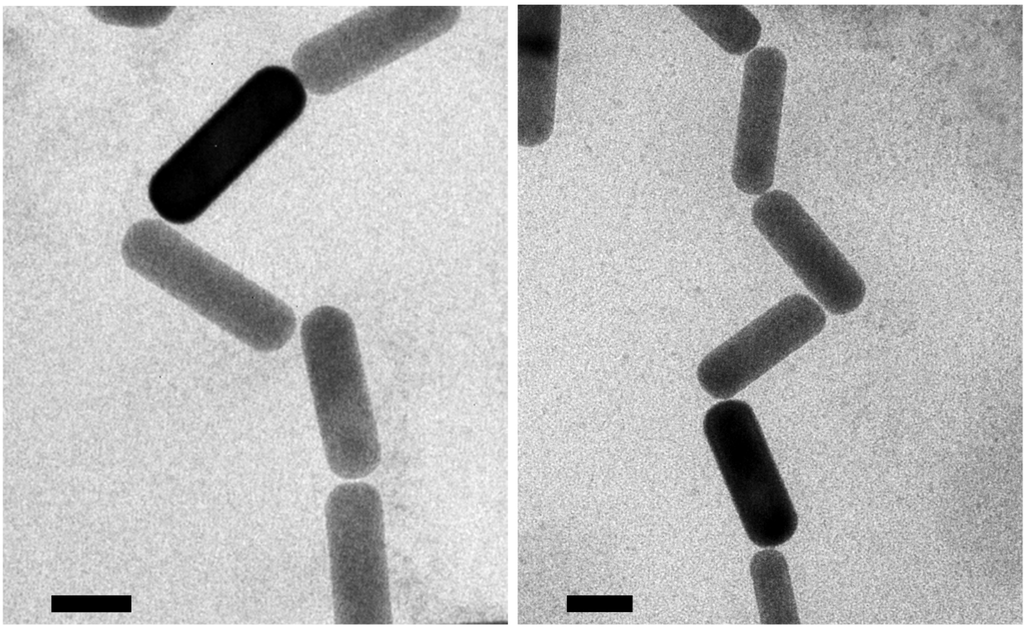

Figure S6: *TEM images of nanorod assembly. An average gap size of 0.9 ± 0.2 nm was obtained from fifteen nanogaps. (Scale: 20 nm)*

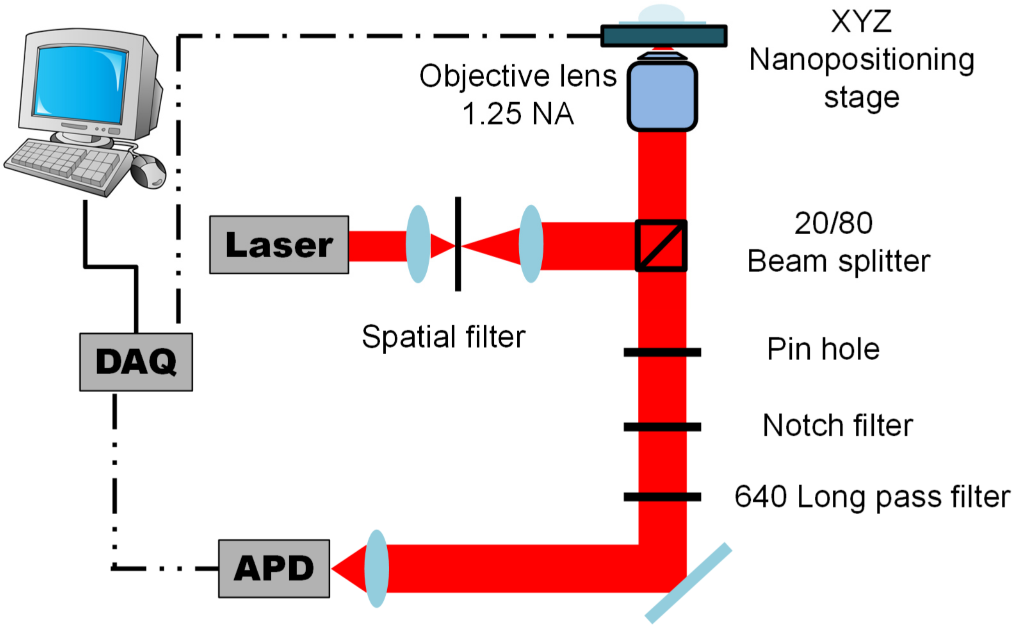

Figure S7*: Schematic of the home-built confocal microscope setup.*

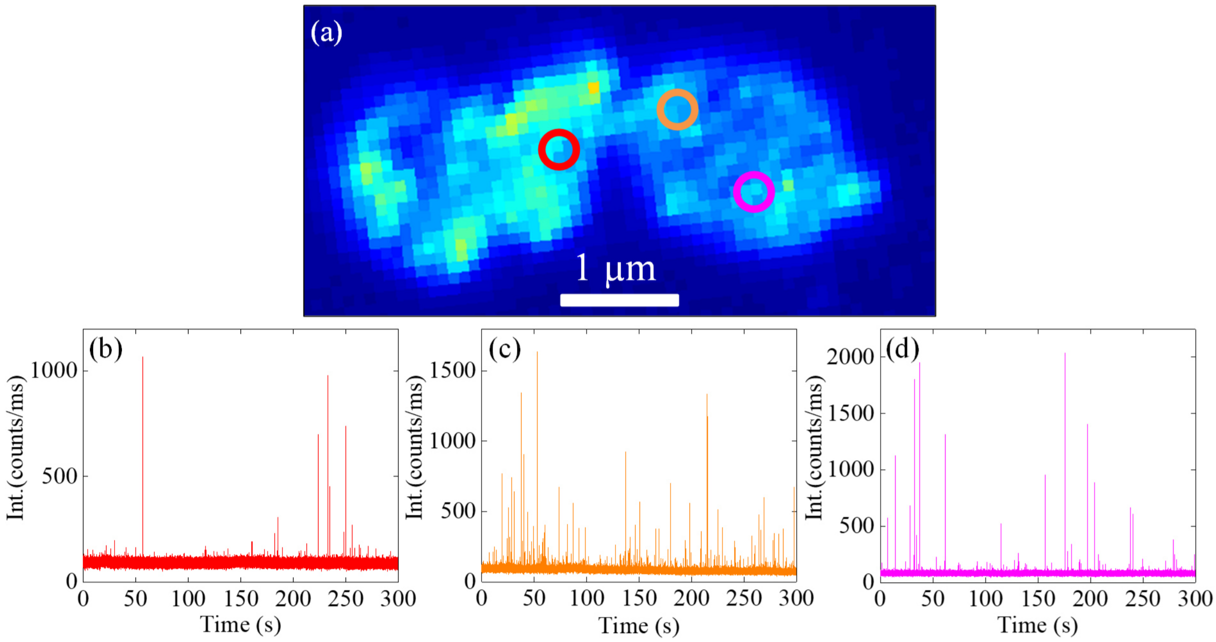

Figure S8: *(a) one-photon luminescence image of a gold nanorod assembly isolated on an ITO-coated glass substrate and covered with 100 nM CV in glycerol. (b-d) Fluorescence time traces recorded on a diffraction-limited spot at different places on nanorod assembly marked by red, orange and magenta circles. The enhancement factor calculated for b, c and d are respectively 9946, 9801 and 12217.*

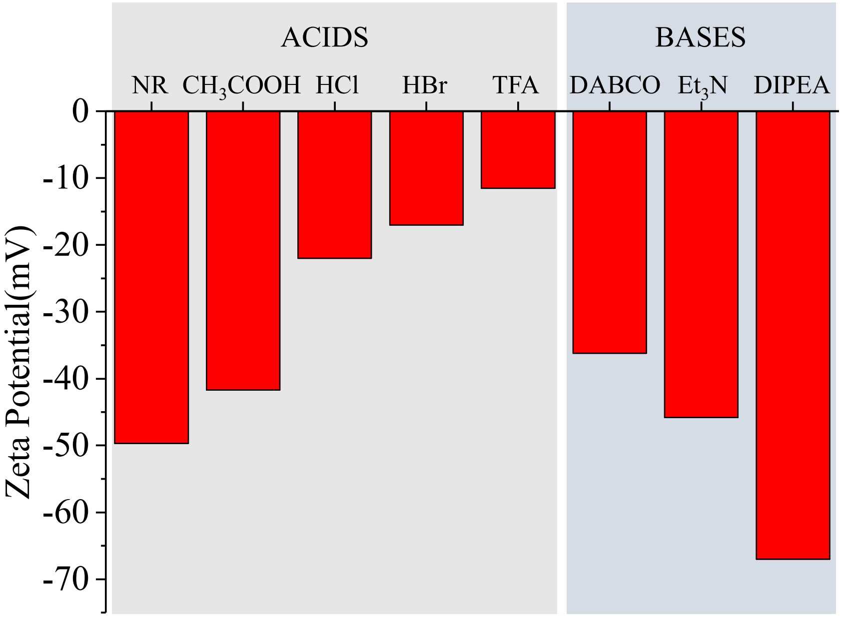

Figure S9: *Bar diagram showing the variation of nanorod's surface charge using different acids and bases in acetonitrile medium. 1st column (NR) represents as coated PAA nanorods. The different acids used are Acetic acid (CH3COOH), Hydrochloric acid (HCl), Hydrobromic acid (HBr) and Trifluoroacetic acid (TFA). The different bases used are 1,4-diazabicyclo[2.2.2]octane (DABCO), Thriethylamine (Et₃N), Diisopropylethylamine(DIPEA).*

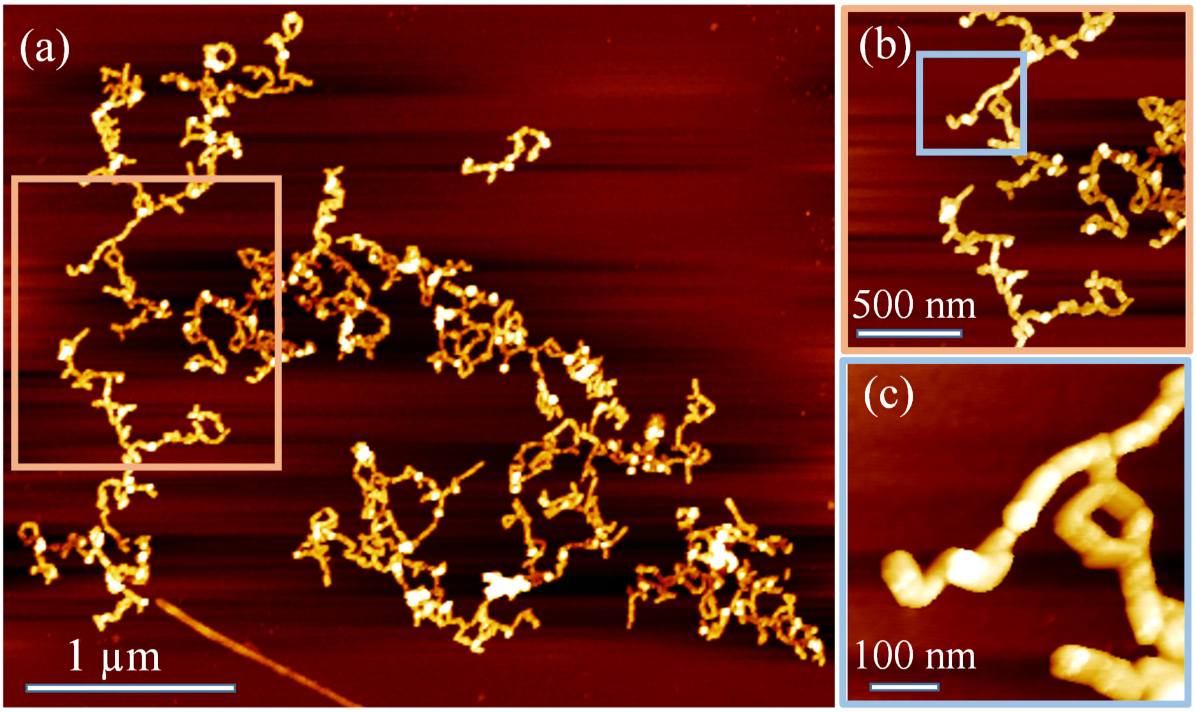

Figure S10:* *(a) *AFM images of network structure on silicon substrate obtained by spin coating 10 µL of reaction solution.* ***(b, c)*** *Zoomed in image on the areas marked by coloured box showing clear presence of tip-specificity of the assembled nanorods.*

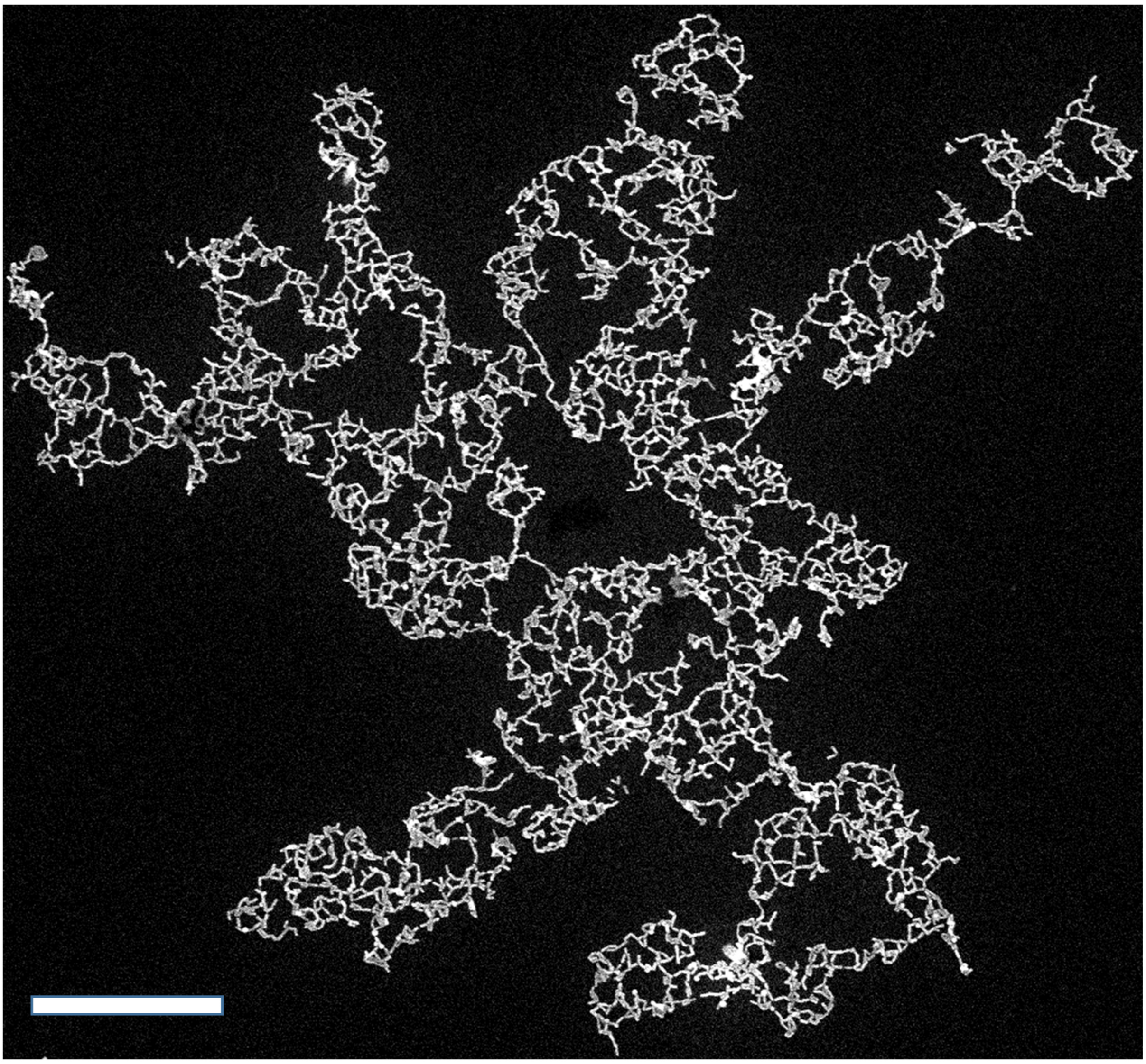

Figure S11: *Large area SEM image of a self-assembled network of gold nanorods. Scale: 1µm*

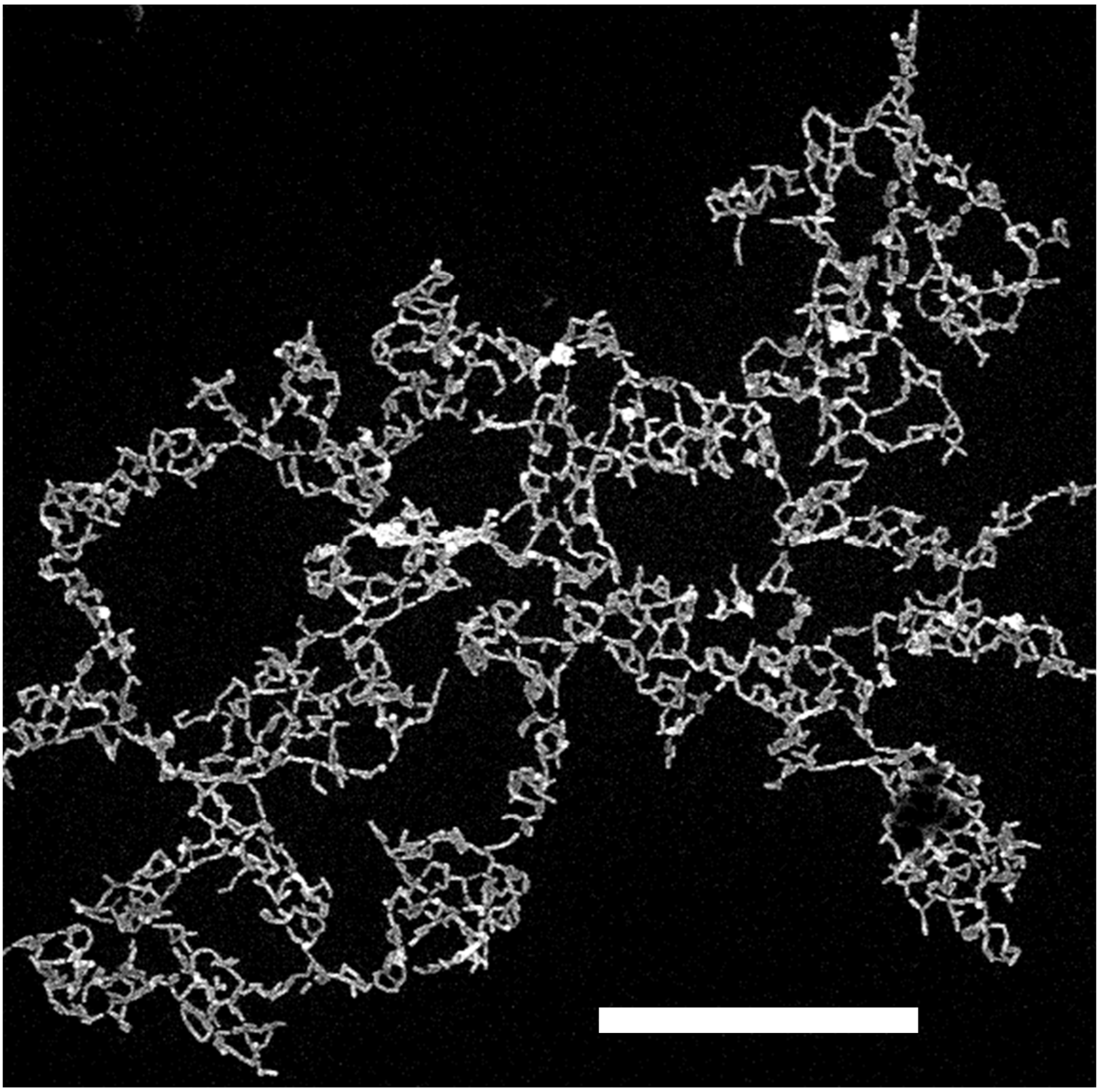

Figure S12: *Large area SEM image of a self-assembled network of gold nanorods. Scale: 1µm*

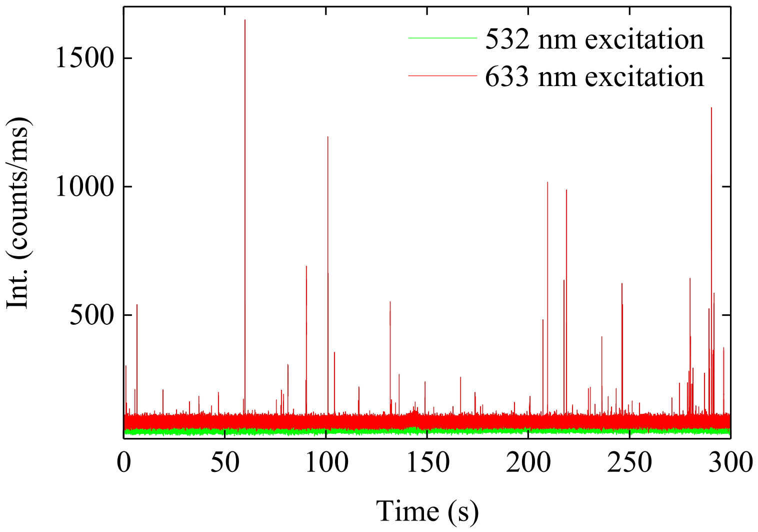

Figure S13: *Fluorescence time traces recorded on a diffraction-limited spot on nanorod assembly covered with 100 nM CV solution in glycerol using 532 nm (green) and 633 nm (red, same as shown in figure 4c of main text) laser excitation. Note that no strong fluorescence intensity burst was observed under 532 nm excitation.*

References:

(1) Sau, T. K.; Murphy, C. J.; Commun, M. L. H. C. Seeded High Yield Synthesis of Short Au Nanorods in Aqueous Solution. **2004**, No. 11, 6414–6420.

(2) Nikoobakht, B.; El-Sayed, M. A. Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method. *Chem. Mater.* **2003**, *15* (10), 1957–1962.

(3) Alkilany, A. M.; Thompson, L. B.; Murphy, C. J. Polyelectrolyte Coating Provides a Facile Route to Suspend Gold Nanorods in Polar Organic Solvents and Hydrophobic Polymers. *ACS Appl. Mater. Interfaces* **2010**, *2* (12), 3417–3421.