Electronic Supplementary Information for

Self-assembly of PEGylated Gold Nanoparticles with Satellite Structures as Seeds

Marie Bachelet and Rongjun Chen*

Department of Chemical Engineering, Imperial College London, South Kensington Campus, London SW7 2AZ, United Kingdom

*Corresponding author: Rongjun Chen
Email: rongjun.chen@imperial.ac.uk

Contents

1 Materials

Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4$), poly(ethylene glycol) methyl ether thiol Mn 800 g mol^{-1} (PEG0.8k), Mn 2000 g mol^{-1} (PEG2k) and Mn 6000 g mol^{-1} (PEG6k), 2-(N-morpholino)ethanesulfonic acid (MES) monohydrate and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma Aldrich (Dorset, UK). Nitric acid, sodium chloride, sodium phosphate dibasic ($Na_2HPO_4•7H_2O$) and monobasic (NaH_2PO_4), potassium dihydrogen orthophosphate (KH_2PO_4), trisodium citrate dihydrate, and paraffin oil were purchased from Fisher Scientific (Loughborough, UK). Ethylenediaminetetraacetic acid (EDTA), hydrochloric acid 37% and sodium hydroxide were acquired from VWR (Lutterworth, UK). Hydrochloric acid 37% was acquired from VWR (Lutterworth, UK).

2 Methods

2.1 Synthesis of gold nanoparticles (AuNPs)

Prior to the synthesis, the glassware and the utensils were soaked in aqua regia and rinsed with MilliQ water. HAuCl4 (200 mL, 1 mM) was heated under reflux while stirring, followed by addition of trisodium citrate (20 mL, 39 mM). After 15 s the color of the solution changed from yellow to deep red and the solution was stirred for a further 50 min. The solution was then allowed to cool under stirring and the final AuNPs were stored at 4˚C until further use. The particle size and concentration, 14.1 nm and 10.0 nM respectively, were calculated via UV-vis spectroscopy using the Haiss method.

2.2 PEGylation of AuNPs

Aqueous PEG-SH solution (60 µL) at various concentrations was added to the as-synthesized AuNPs (1.2 mL) and stirred for 2 h. The final mixture was then centrifuged at 15,000 G for 30

min, and after removal of the supernatant and 5 min sonication, the particles were redispersed in the required media (dH_2O, PBS buffer or MES buffer) and sonicated for a further 15 min.

2.3 Characterization

Transmission Electron Microscopy (TEM) at 200 kV was performed on a JEOL FX2000 (Tokyo, Japan) after deposition of a droplet of AuNPs or PEGylated AuNPs (AuNPs@PEG) solution on a 300-mesh, carbon-coated copper grid. The grid was left at room temperature overnight before being imaged. The particle sizes were analyzed with ImageJ software. UV-vis spectrophotometry spectra were recorded on a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific, UK) between 400 and 800 nm with 0.5 nm increments. Hydrodynamic size (D_H) and zeta potential (ζ) measurements with dynamic light scattering (DLS) were carried out on a Brookhaven instrument with ZetaPALS software (Holtsville, USA) at 20 °C with an angle of 90° and a wavelength of 659 nm.

2.4 Determination of the minimum amount of PEG for stabilization of the AuNPs@PEG in acidic media containing salts

AuNPs@PEG were redispersed in 50 mM MES buffer at pH 5.5 (0.15 M NaCl), sonicated for 15 min and then left under stirring. After 4 h, the UV-vis scanning of the samples was carried out with a Genesys 10S UV-vis spectrophotometer (Thermo Fisher, UK) between 400 and 800 nm with 0.5 nm increments. Each sample was prepared in triplicate.

2.5 Determination of the PEG grafting density via the Ellman's assay

A calibration curve was prepared with PEG-SH solutions at different concentrations (500 µL) mixed with PB buffer (190 µL of 0.1 M PB buffer at pH 8.0 with 1 M EDTA) and DTNB (Ellman's reagent, 10 µL, 3 mM). In presence of a thiol group the DTNB reagent reacts and forms TNB^{2-} which can be further quantified via absorbance. The solutions were mixed and

incubated for 15 min and the absorbance was then read at 412 nm. The same procedure was applied to the supernatants of the PEGylated AuNPs after being centrifuged at 15,000 G for 1 h to separate residual AuNPs. Each sample was prepared in triplicate.

2.6 Study of the colloidal stability of the AuNPs@PEG in various media

In order to study the colloidal stability of the AuNPs solutions at different pHs and ionic strengths, the concentrated solutions were redispersed in various media: deionized water (dH_2O) (pH~6); PBS buffer (50 mM, 0.15 M NaCl, pH 7.4); MES buffer (50 mM, 0.15 M NaCl, pH 5.5). The absorbance was recorded over time. Each sample was prepared in triplicate.

3 AuNPs characterization

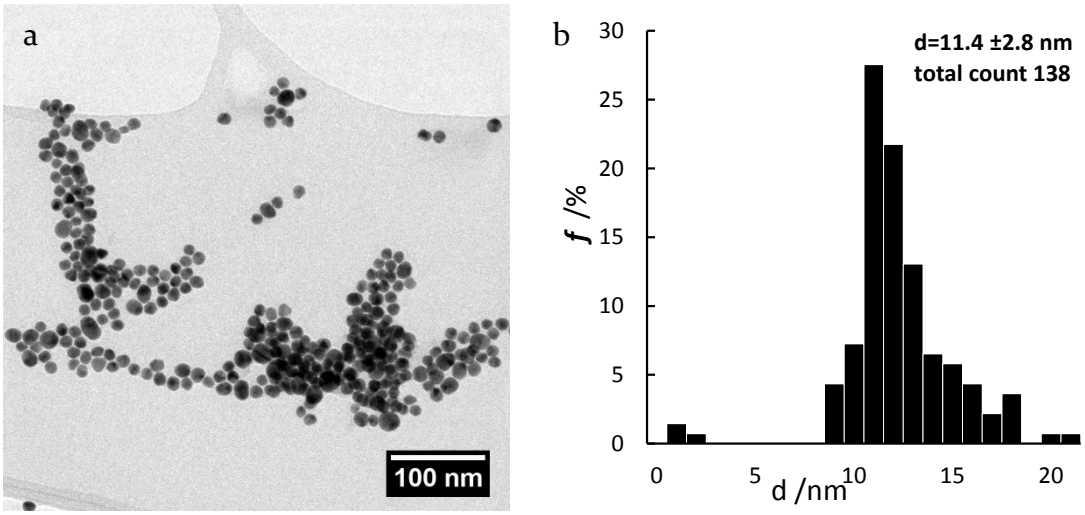

Figure S1. (a) TEM image of the citrate-coated AuNPs and (b) their particle size distribution via TEM image analysis.

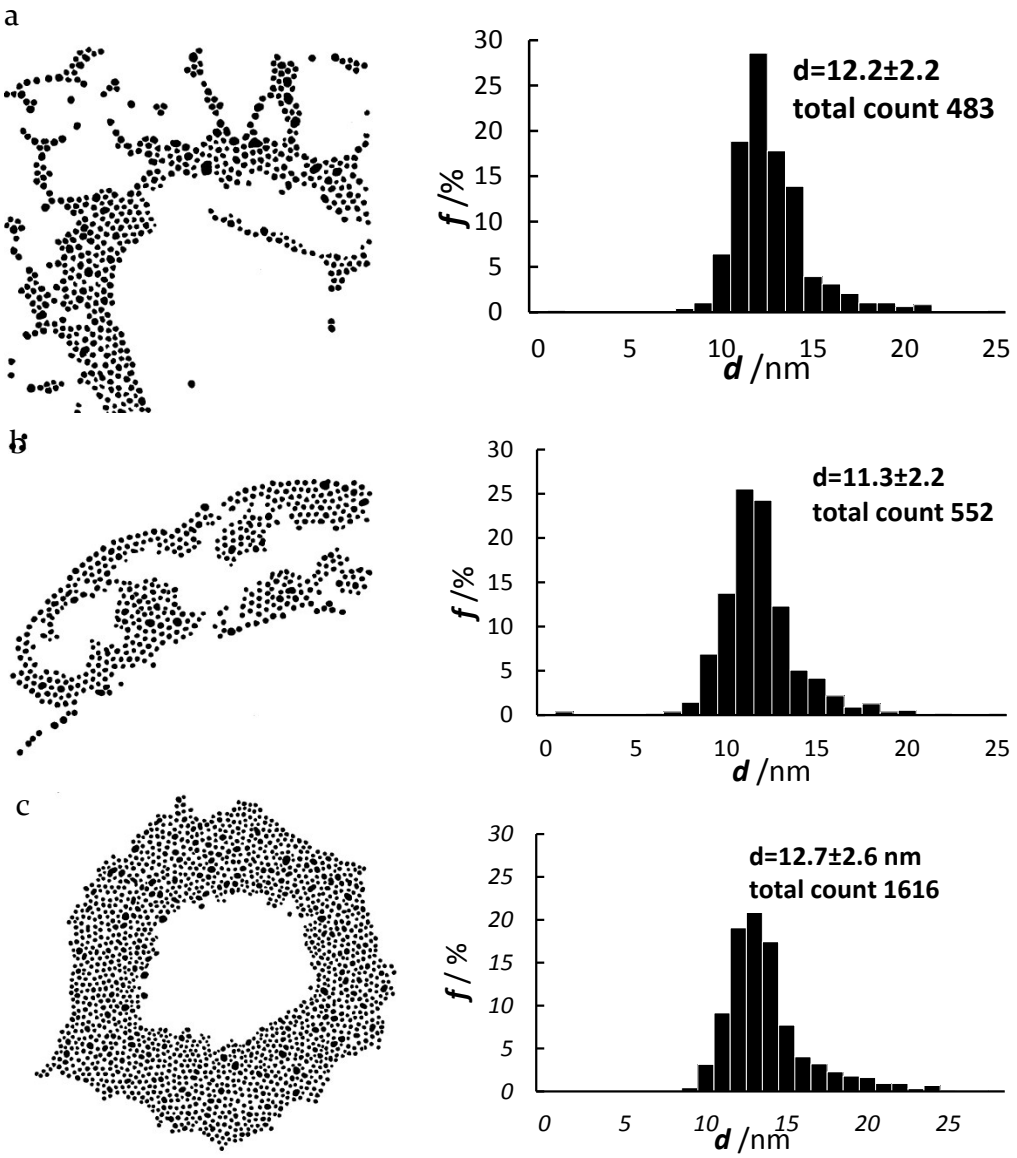

Figure S2. Particle size distributions in (a) AuNPs@PEG0.8k, (b) AuNPs@PEG2k and (c) AuNPs@PEG6k via TEM image analysis.

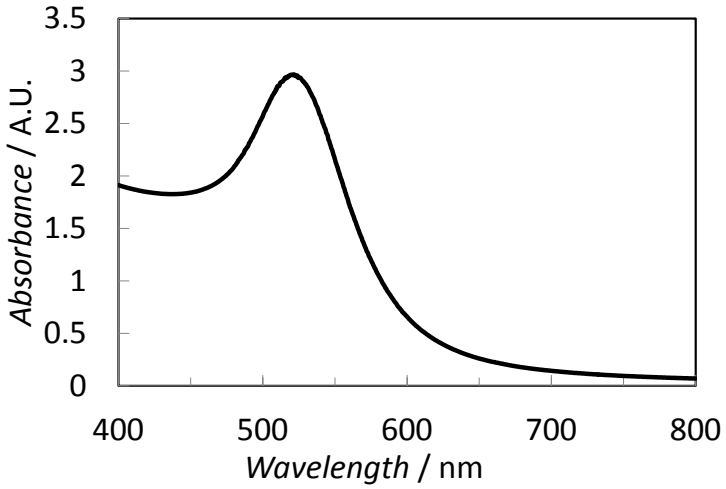

Figure S3. The UV-vis scan of the citrate-coated AuNPs for calculation of the diameter *d* and the concentration *c* via the Haiss method (SPR (521 nm; 2.967), d=14.1 nm, c=10.0 nmol L^{-1}).

4 Satellite structures characterization

%Satellite structures. The percentage of the number of satellite structures is calculated as the ratio of the number of nanoparticles present in the satellite structures and the total number of nanoparticles present in the self-assembly.

$N_{layer}/N_{core.}$ The ratio of the number of layer nanoparticles and the number of core nanoparticles is calculated using the total number of layer nanoparticles divided by the total number of core nanoparticles. Both layer and core nanoparticles belong to independent satellite structures (e.g. no sharing of nanoparticles between two satellites), as identified in Fig. S5.

$d_{layer}/d_{core.}$ The ratio of the diameter of the layer nanoparticles and the diameter of the core nanoparticles is calculated as the average diameter of the layer nanoparticles divided by the average diameter of the core nanoparticles. Both layer and core nanoparticles belong to independent satellite structures, as identified in Fig. S5.

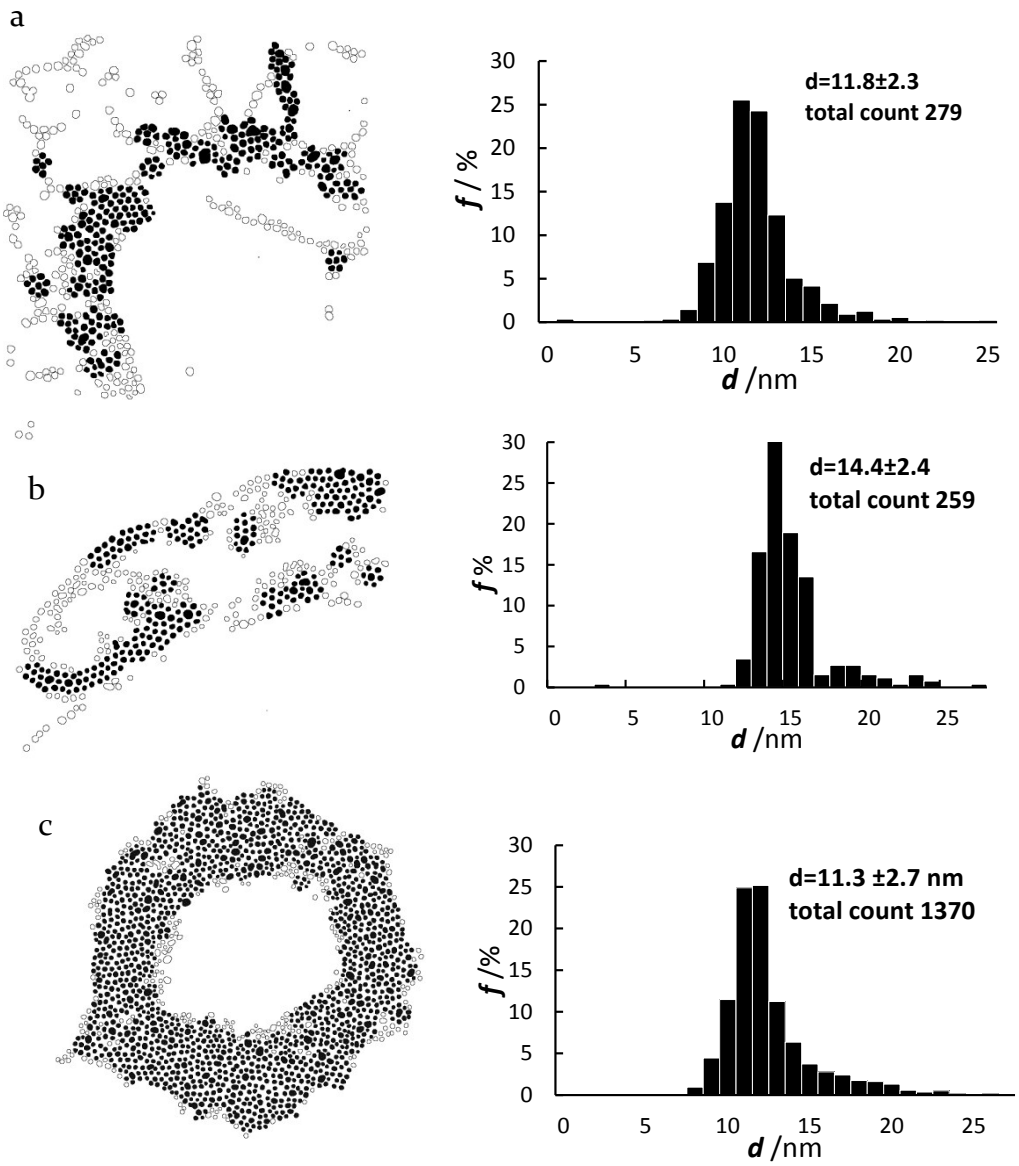

Figure S4. Particle size distributions of the satellite structures in (a) AuNPs@PEG0.8k, (b) AuNPs@PEG2k and (c) AuNPs@PEG6k via TEM image analysis.

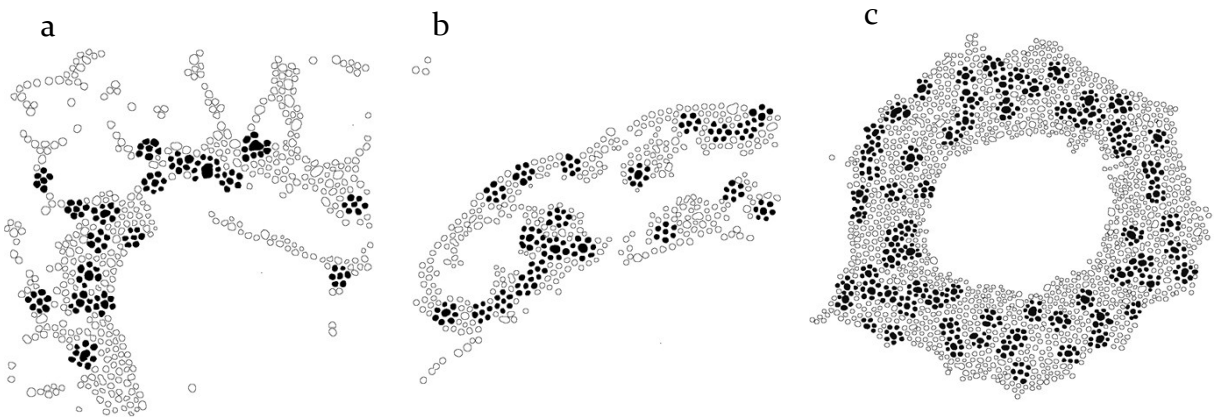

Figure S5. Typical independent satellite structures in (a) AuNPs@PEG0.8k, (b) AuNPs@PEG2k and (c) AuNPs@PEG6k via TEM image analysis.

Detailed calculations for the AuNPs coated with PEG of different chain lengths

1) AuNPs@PEG0.8k

   % satellite structures: 279/552 = 51%

   Results for the independent satellite structures:

   - Core nanoparticle size = 14.9±4.0 nm (N=18)
   - Layer nanoparticle size = 12.4±1.6nm (N=116)
   - N_{layer}/N_{core} = 116/18 = 6.4. As shown in the TEM images, the satellites have 6-8 layer nanoparticles surrounding every single core nanoparticle.
   - d_{layer}/d_{core} = 12.4/14.9 = 0.8±0.4

2) AuNPs@PEG2k

   % satellite structures: 259/483 = 54%

   Results for the independent satellite structures:

   - Core nanoparticle size = 15.5±4.2 nm (N=24)
   - Layer nanoparticle size = 14.9 ±1.9 nm (N=143)

- N_{layer}/N_{core}= 143/2 4 = 6.0. As shown in the TEM images, the satellites have 6-8 layer nanoparticles surrounding every single core nanoparticle.
- d_{layer}/d_{core} = 14.9/15.5 = 1.0±0.4

3) AuNPs@PEG6k

% satellite structures: 1370/1616= 85%

Results for the independent satellite structures:

- Core nanoparticle size = 16.7±2.9 nm (N=67)
- Layer nanoparticle size = 9.6±2.1 nm (N=456)
- N_{layer}/N_{core} = 456/67 = 6.8. As shown in the TEM images, the satellites have 6-8 layer nanoparticles surrounding every single core nanoparticles.
- d_{layer}/d_{core} = 9.6/16.7 = 0.6 ±0.2.

5 Polymer layer characterization

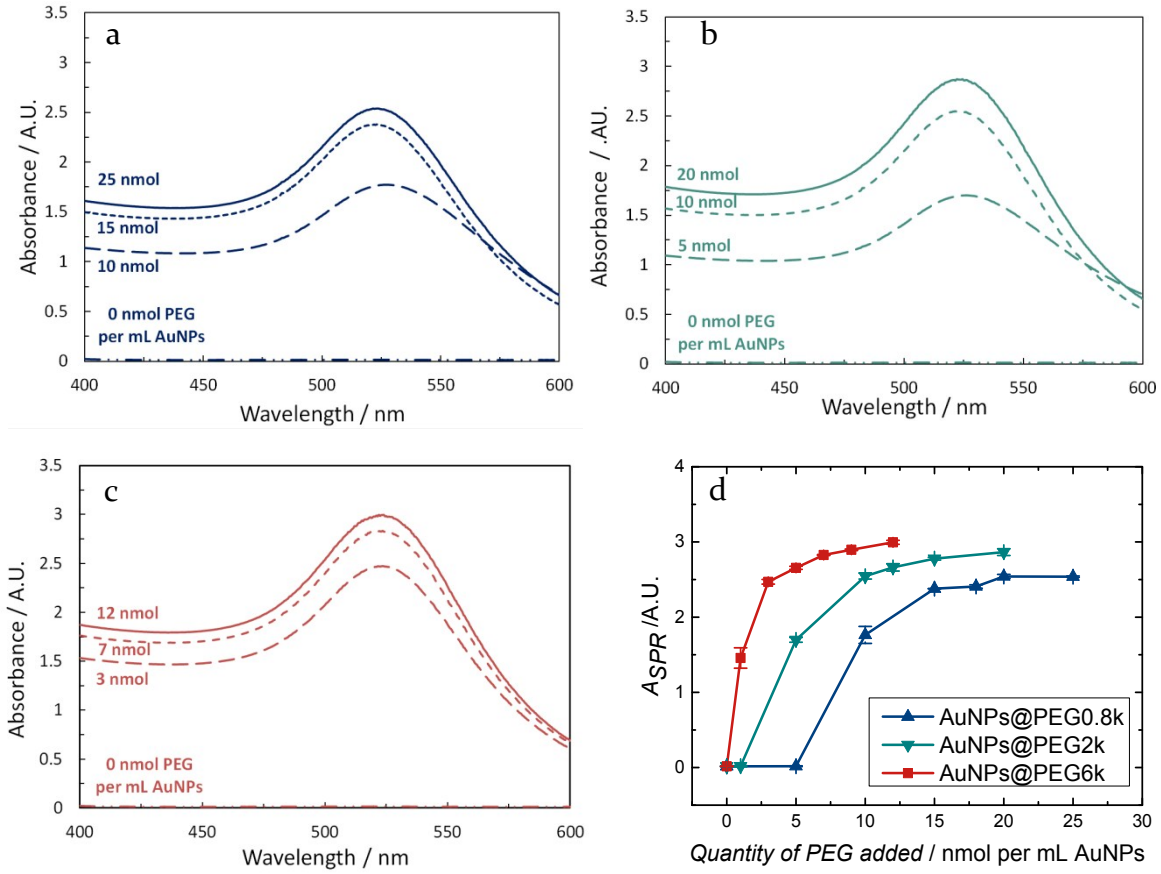

Figure S6. Determination of the minimum amount of PEG for stabilization of AuNPs in 50 mM MES buffer at pH 5.5 (0.15 M NaCl). Absorbance profiles of (a) AuNPs@PEG0.8k, (b) AuNPs@PEG2k and(c) AuNPs@PEG6k at 6 h after redispersion in the MES buffer with different amounts of PEG added. (d) Maximal absorbance of these samples redispersed in the MES as a function of the quantity PEG added. Errors bars represent the standard deviations of triplicates.

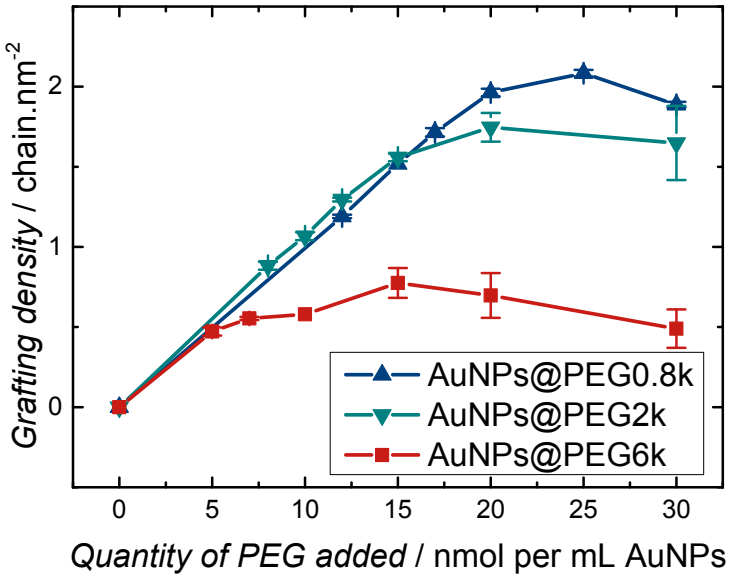

Figure S7. Grafting density of the PEGylated AuNPs determined via the Ellman's assay. Errors bars represent the standard deviation between triplicates.

6 Determination of the polymer conformation

1) Determine the grafting density via the Ellman assay: σ_{Ellman} [chain nm^{-2}] (see Section 2 Methods in this Supporting Information for details)

2) Calculate the the distance between PEG attachments on the AuNP surface:

$$D(nm) = 2\sqrt{\frac{FP}{\pi}} = 2\sqrt{\frac{1}{\sigma\pi}} \quad (1)$$

Where FP is the footprint of the polymer (nm^2 per chain) and σ the grafting density (chain nm^{-2})

3) Calculate the Flory radius, R_F, which is the radius of the volume occupied by an extended polymer chain in a good solvent:

$$R_F = aN^{\frac{3}{5}} \quad (2)$$

- a is the monomer size (3.5 Å for PEG)
- N is the number of monomers in a PEG chain (18 for PEG0.8k, 44 for PEG2k and 133 for PEG6K).

4) Conformation determination:

- If $D > 2R_F$: Mushroom regime (and the polymer layer thickness is $L = R_F$ and $FP = \pi R_F^2$)
- If $D < 2R_F$: Brush regime

Table S1. Characteristics of the polymer layer

PEG Mw [g mol^{-1}]	R_F [nm][a]	D_H [nm][b]	L_{DLS} [nm][c]	σ_{trans} [chain nm^{-2}][d]	σ_{Ellman} [chain nm^{-2}][e]	D [nm][f]	L [nm][g]
0.8k	2.0	38.3±0.8	13.5±2.8	0.079	1.54±0.01	0.31±0.43	3.32±0.00
2k	3.4	44.1±5.0	16.3±3.9	0.022	1.07±0.02	1.09±0.00	7.21±0.06
6k	6.6	45.2±1.2	16.9±1.8	0.073	0.55±0.01	1.52±0.01	17.51±0.10

[a] Flory radius.

[b] Hydrodynamic size measured via DLS.

[c] Polymer brush thickness L_{DLS}, calculated according to:

$$L_{DLS} = \frac{D_H - d_{TEM}}{2} \quad (3)$$

[d] Grafting density at which the mushroom-to-brush conformational transition occurs:

$$\sigma_{trans}\left(\frac{PEG}{nm^2}\right) = \frac{1}{FP} = \frac{1}{\pi R_F^2} \quad (4)$$

[e] PEG grafting density calculated via the Ellman's assay.

[f] Calculated distance between PEG attachments on the AuNP surface.

[g] Height of the brush thickness:

$$L = a\,N\left(\frac{a}{D}\right)^{\frac{2}{3}} \quad (5)$$

Note: Comparing directly the grafting density determined via the Ellman's assay, σ_{Ellman}, and the grafting density at which the mushroom-to-brush conformational transition occurs, σ_{trans}, can also be used to determine whether the conformation is brush or mushroom.

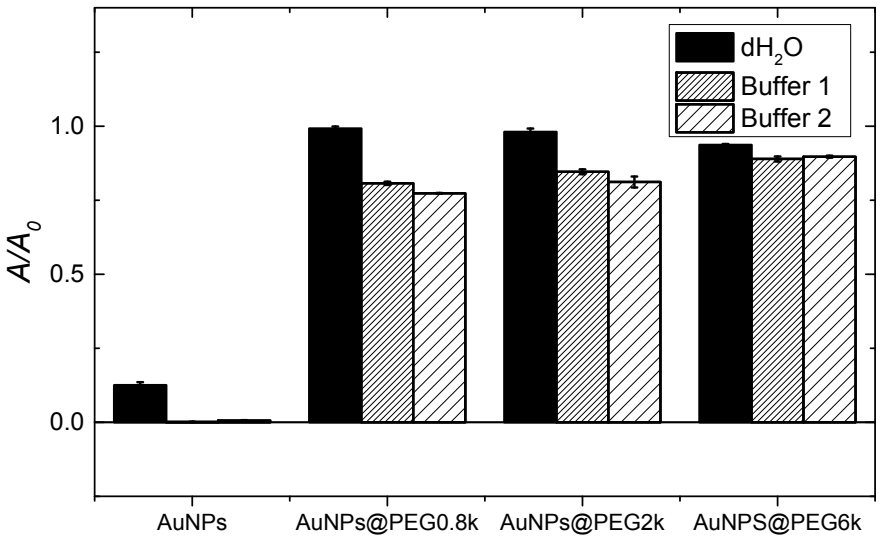

Figure S8. Relative absorbance (ratio of the SPR absorbance after and before redispersion) of the AuNPs and the PEGylated AuNPs in different media: dH_2O (pH~6); Buffer 1 (50 mM

PBS at pH 7.4, 0.15 M NaCl) and Buffer 2 (50 mM MES at pH 5.5, 0.15 M NaCl). Errors bars represent the standard deviation between triplicates.

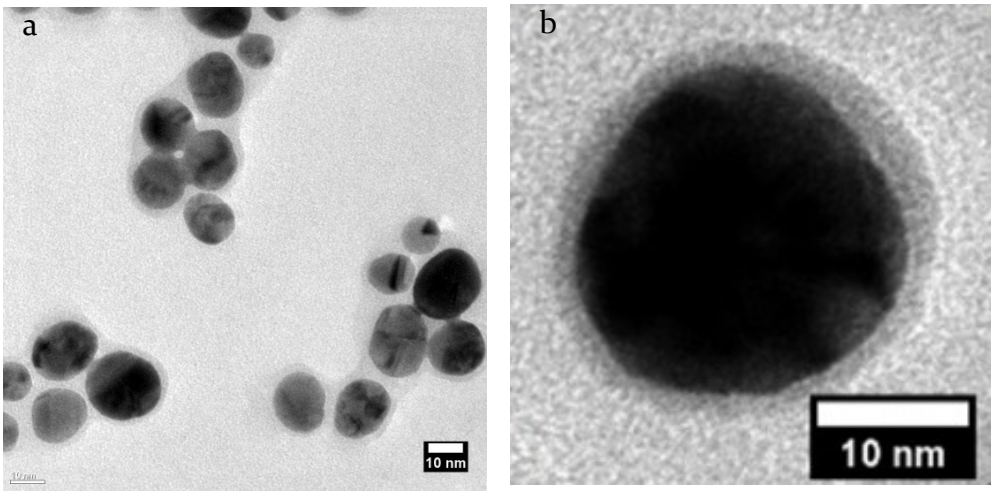

Figure S9. TEM images of (a) the AuNPs@PEG0.8k showing the visible polymer layer, and (b) the representative single nanoparticle in the AuNPs@PEG0.8k.

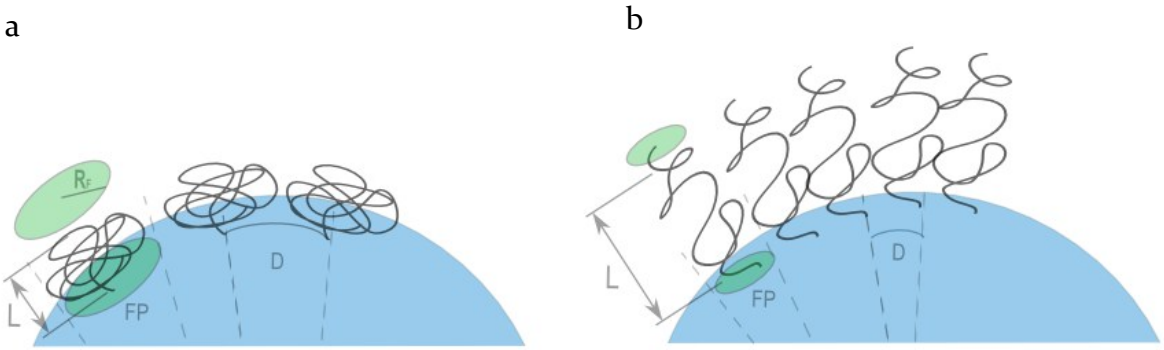

Figure S10. Schematic of the PEG conformation (a) in a mushroom regime and (b) in a brush regime. *D* is the distance between PEG attachments, *L* is the height of the brush domain, R_F is the Flory radius and *FP* is the footprint of the polymer (nm^2 per chain).