Electronic Supplementary Information

Complement Activation by Gold Nanoparticles Passivated with Polyelectrolyte Ligands

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1. Experimental section

a. Materials

SC5b-9 ELISA kit was purchased from Cusabio Life Science (College Park, MD, USA). All other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) unless specified otherwise. Milli-Q water with a resistivity of 18.2 MΩ.cm at 25 °C was used for throughout experiments.

b. Synthesis of citrate-stabilized gold nanoparticles (AuNPs)

Spherical AuNPs of average diameter of 20 nm (Au20) and 40 nm (Au40) with citrate-capping were synthesized by seed-mediated method.[1] The AuNP colloidal was washed by centrifugation at 6,500 g (Au20) and 2,500 g (Au40) for 30 min, re-dispersed in water, and stored at 4 ^{o}C for further experiments.

Rod-like shape gold nanorods (AuNR) of around 40×10 nm were prepared by seedless method using CTAB as capping agent.[2] After synthesis, CTAB moieties on the surface of AuNR were exchanged with poly (styrenesulfonate) (PSS), then sodium citrate tribasic dihydrate to obtain citrate-capped AuNRs, following previously published protocol.[3] The AuNR colloidal was washed by centrifugation at 12,000 g for 30 min, re-dispersed in water, and stored at 4 ^{o}C.

The optical properties of AuNPs were characterized by UV-Vis spectroscopy (MultiSkan GO, Thermo Fisher Scientific Inc., Waltham, MA, USA). Their zeta potentials (ζ) and hydrodynamic diameters (D_h) were measured by dynamic light scattering (DLS) technique at 25 °C using a Zetasizer (Nano ZS, Malvern, UK). Concentrations of AuNPs were calculated based on their UV-Vis absorbance, following previously reported method.[4] Size and size distribution of synthesized AuNPs were determined from transmission electron microscopy (TEM) (JEOL 2200FS Cryo TEM, JEOL USA, Inc. Peabody, MA, USA) images using free ImageJ software.

c. Preparation of polymer-stabilized AuNPs

PVA-passivated AuNPs: poly (vinyl alcohol) (PVA, $M_w \approx 67$ kDa) was used to prepare AuNPs-PVA following previously published method.[5] AuNPs-PVA were washed 3 times by repeated centrifugations and re-dispersed in water for further experiments.

PAA-passivated AuNPs: AuNPs passivated with poly (acrylic acid) (PAA, $M_w \approx 1.8$ kDa) were prepared from citrate-capped AuNPs, following reported protocol.[6] AuNPs-PAA were washed 3 times by repeated centrifugations and re-dispersed in water for further experiments.

PSS-passivated AuNPs: Au20 and Au40 passivated with poly (styrenesulfonate) **(**PSS, $M_w \approx 70$ kDa) were prepared by using layer-by-layer method.[7] PSS-passivated AuNR was obtained from intermediate step of the preparation of citrate-capped AuNR as described above. AuNPs-PSS were washed 3 times by repeated centrifugations and re-dispersed in water for further experiments.

PEI-passivated AuNPs: AuNPs passivated with branched poly (ethylene imine) (PEI, $M_w \approx 25$ kDa) were prepared from PSS-passivated AuNPs by using layer-by-layer method.[7] AuNPs-PEI were washed 3 times by repeated centrifugations and re-dispersed in water for further experiments.

PAMAM-passivated AuNPs: AuNPs passivated with polyamidoamine (PAMAM) dendrimer (generation 2.0, aminoethanol surface) were prepared by adding PAMAM solution into citrate-capped AuNPs dropwise under stirring. The mixture was further stirred for 3 hours. Then, AuNPs-PAMAM were washed 3 times by repeated centrifugations and re-dispersed in water for further use.

Heparin-passivated AuNPs: AuNPs passivated with heparin were prepared by adding 2% heparin solution into citrate-capped AuNPs dropwise under stirring. The mixture was further stirred for 3 hours. Then, AuNPs-heparin were washed 3 times by repeated centrifugations and re-dispersed in water for further use.

d. Measurement of hydrophilicity of polymer-stabilized AuNPs

Relative hydrophilicity of all polyelectrolyte-passivated AuNPs was determined by the absorption of Nile Blue (NB) as hydrophilic dye.[8] Briefly, increasing concentrations of AuNPs were incubated with fix concentration of NB (40 μg/ml) in the dark at room temperature for 3 hours. Then, AuNPs were isolated by centrifugation and the amount of unbound NB in supernatant was determined by measuring UV-Vis absorbance at 597 nm. The amount of NB bound on the surface of AuNPs was calculated by subtracting the amount of unbound NB in supernatant from the amount of NB added (40 μg/ml). Finally, partitioning quotient (PQ) was determined as the ratio of NB bound onto the surface of particles to free NB in supernatant, i.e., PQ = NB_{bound}/NB_{free}.

A plot of PQ versus total surface area of AuNPs was made. The slope of this linear regression line represented the relative hydrophilicity of AuNPs with different polyelectrolyte ligands, where increasing slope correlates with increasing hydrophilicity.

e. Complement activation in human serum

AuNPs passivated with different polyelectrolyte ligands (0.5 nM, 100 μl) were incubated in commercially available normal human serum (100 μl) (Sigma Aldrich, St. Louis, MO, USA) for 1 h at 37 °C. PBS (10 mM, 100 μl) and zymosan (10 mg/ml, 100 μl) were used as negative and positive control, respectively. The mixture was then centrifuged to isolate AuNPs and the serum-containing supernatant (100 μl) was used to analyze the concentration of final product of complement activation, SC5b-9, induced by AuNPs of different configurations using ELISA kit, following the procedure provided by the kit.

2. Supplementary figures

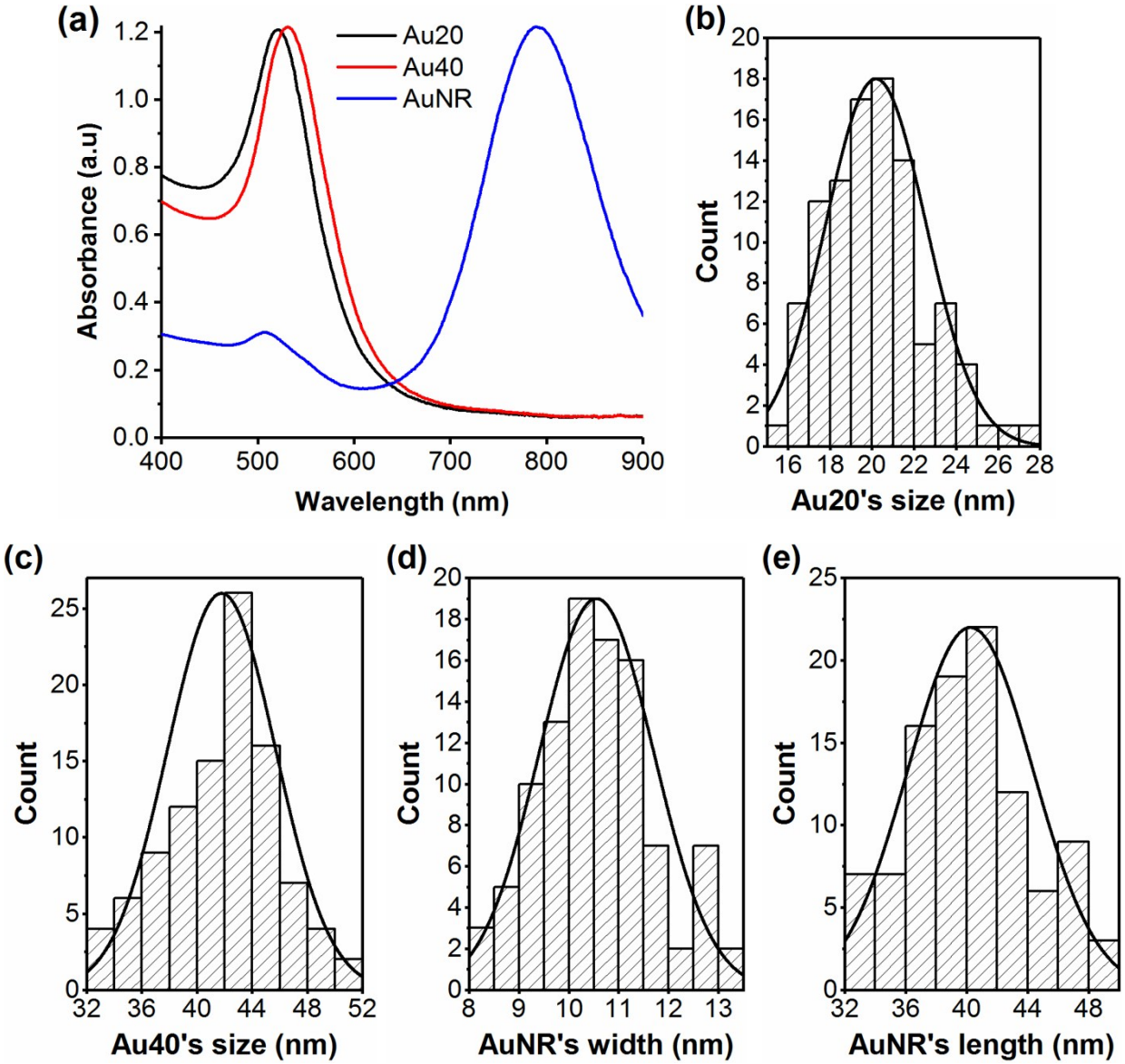

Figure S1. Physical properties of synthesized core AuNPs. (a) UV-Vis absorption spectra of core AuNPs. Size distribution of (b) Au20, (c) Au40, (d) AuNR (width), and (e) AuNR (length) was determined from 100 nanoparticles under TEM images using ImageJ software.

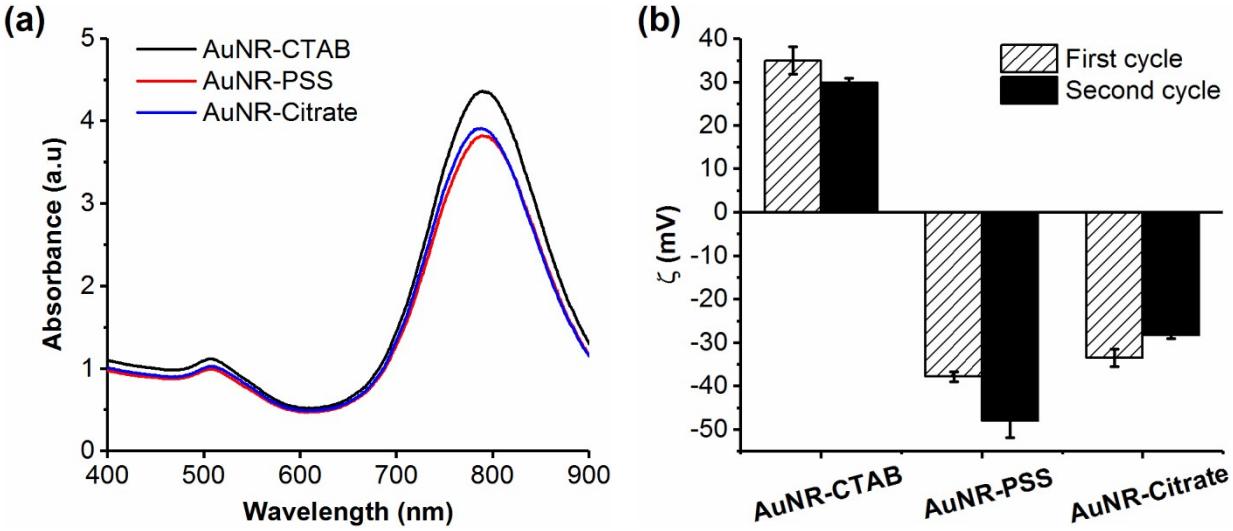

Figure S2. Synthesis of citrate-stabilized gold nanorods. (a) Normalized (at 450 nm) absorbance spectra of CTAB-stabilized gold nanorods (AuNR-CTAB) dispersed in water after second cycle of washing, PSS-stabilized gold nanorods (AuNR-PSS) after second cycle of washing/re-dispersion, and citrate-stabilized gold nanorods (AuNR-citrate) dispersed in water after second cycle of washing/re-dispersion. (b) Zeta potentials, ζ, of AuNR-CTAB, AuNR-PSS, and AuNR-citrate after first and second cycle of washing and re-dispersion. Each data point represents the mean ± standard deviation (SD) of triplicate experiments.

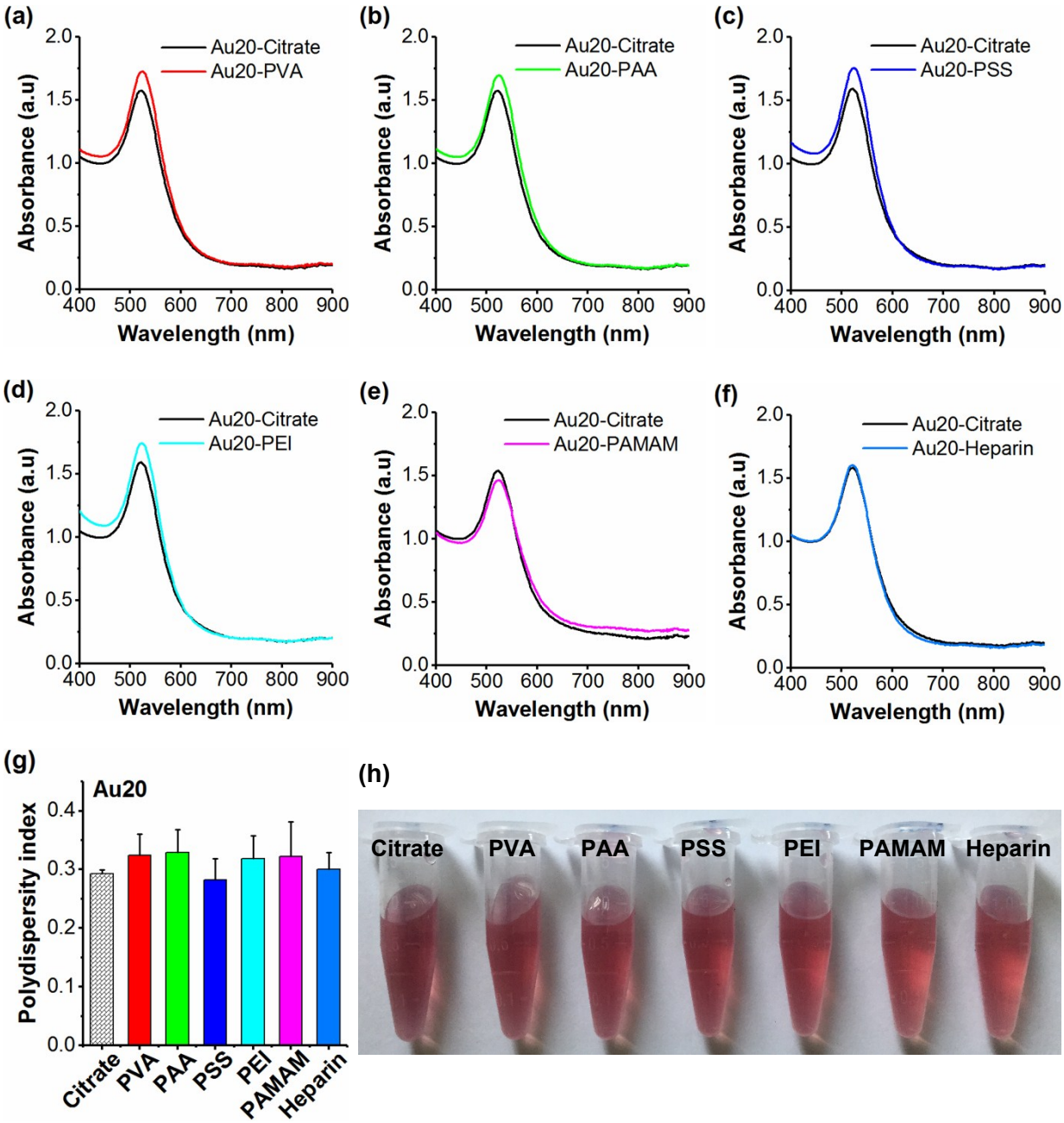

Figure S3. Coating 20 nm spherical gold nanoparticles (Au20) with polyelectrolytes. Normalized (at 450 nm) absorbance spectra of citrate-stabilized Au20 (Au20-citrate) and Au20 coated with (a) PVA, (b) PAA, (c) PSS, (d) PEI, (e) PAMAM, and (f) Heparin. (g) Polydispersity index of Au20 with different ligands. Each data point represents the mean ± standard deviation (SD) of triplicate experiments. (h) Photograph of Au20 passivated with different polyelectrolyte ligands dispersed in water.

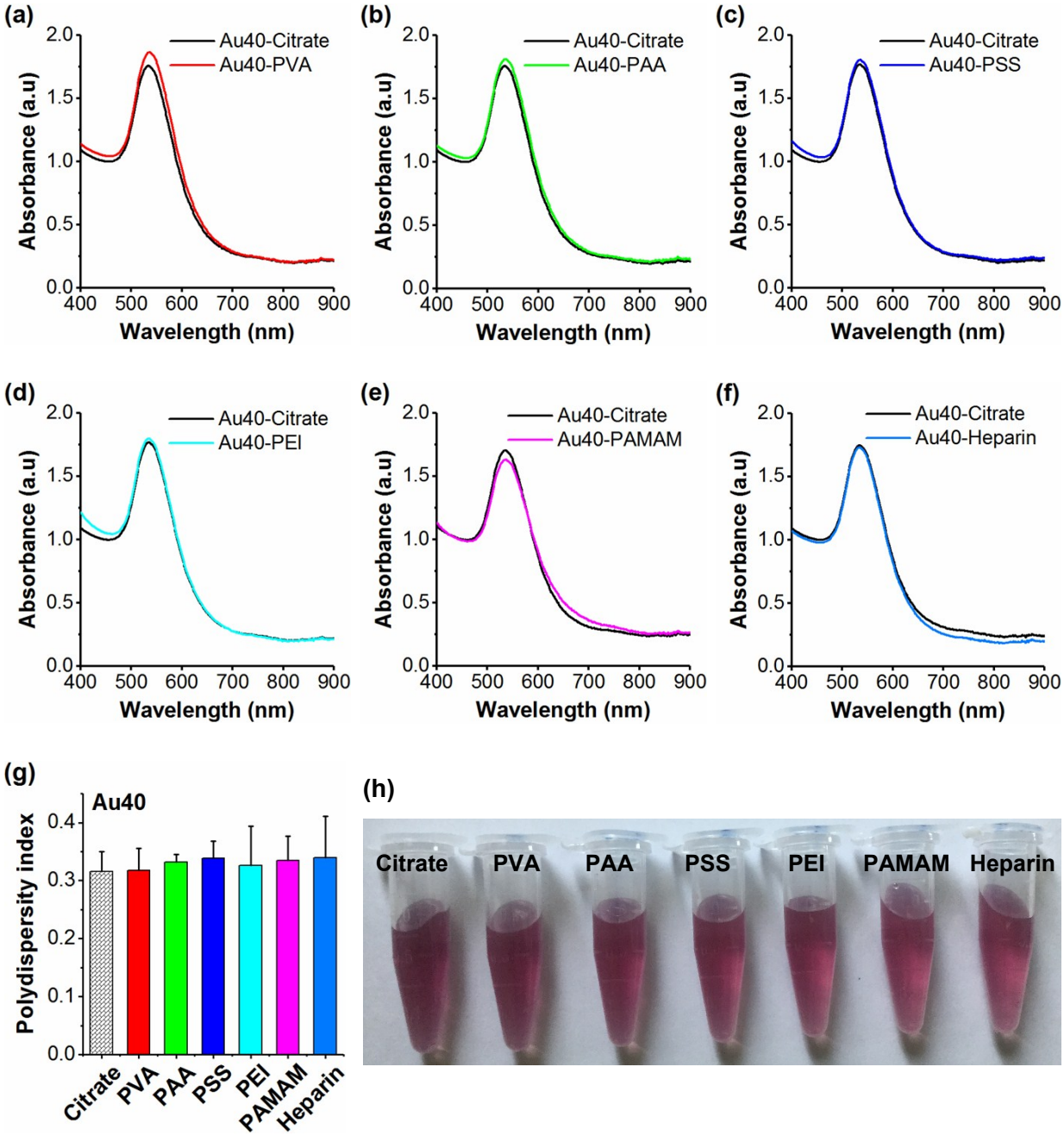

Figure S4. Coating 40 nm spherical gold nanoparticles (Au40) with polyelectrolytes. Normalized (at 450 nm) absorbance spectra of citrate-stabilized Au40 (Au40-citrate) and Au40 coated with (a) PVA, (b) PAA, (c) PSS, (d) PEI, (e) PAMAM, and (f) Heparin. (g) Polydispersity index of Au40 with different ligands. Each data point represents the mean ± standard deviation (SD) of triplicate experiments. (h) Photograph of Au40 passivated with different polyelectrolyte ligands dispersed in water.

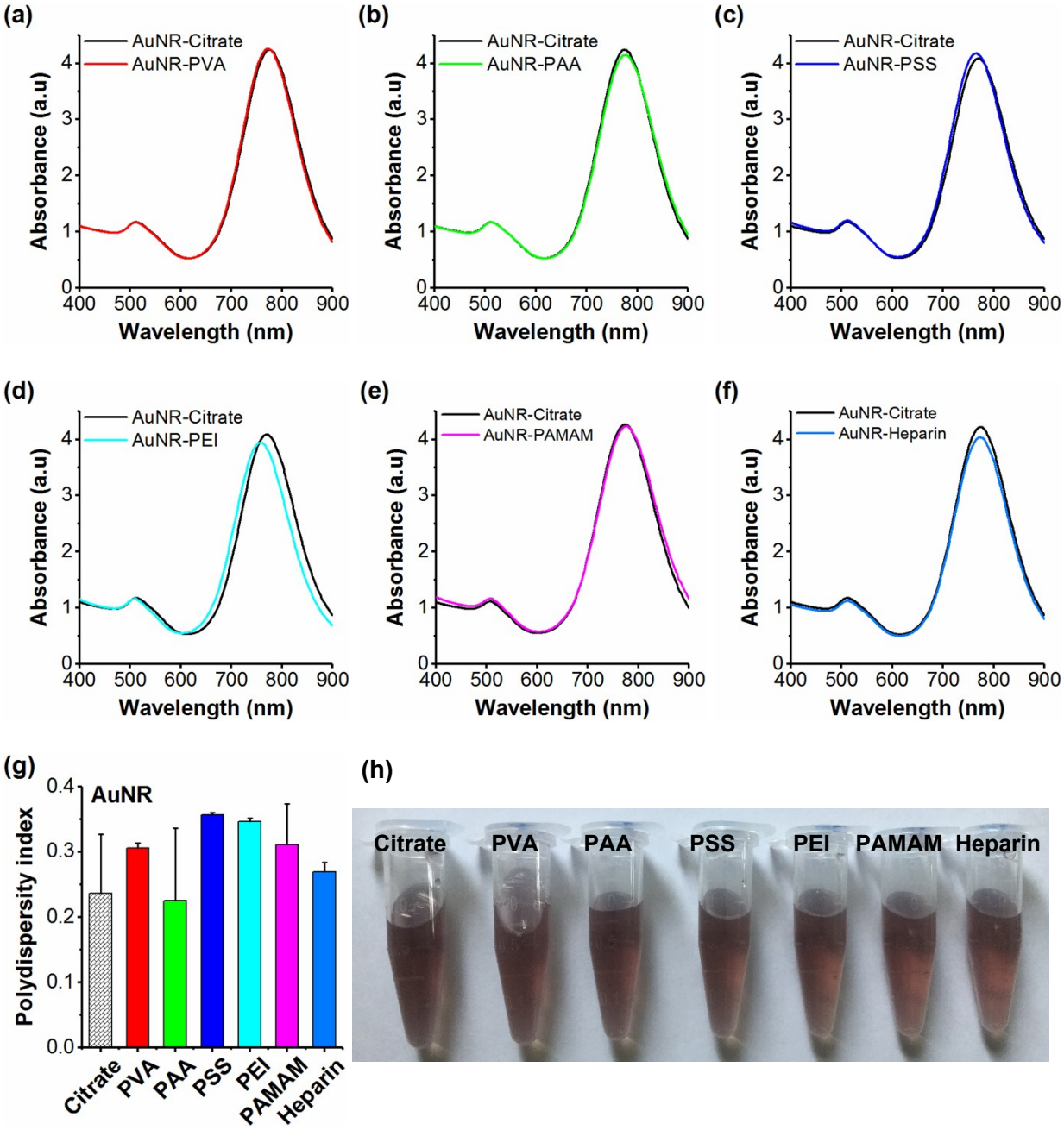

Figure S5. Coating rod-like shape gold nanorods (AuNR) with polyelectrolytes. Normalized (at 450 nm) absorbance spectra of citrate-stabilized AuNR (AuNR-citrate) and AuNR coated with (a) PVA, (b) PAA, (c) PSS, (d) PEI (e) PAMAM and (f) Heparin. (g) Polydispersity index of AuNR with different ligands. Each data point represents the mean ± standard deviation (SD) of triplicate experiments. (h) Photograph of AuNR passivated with different coatings dispersed in water.

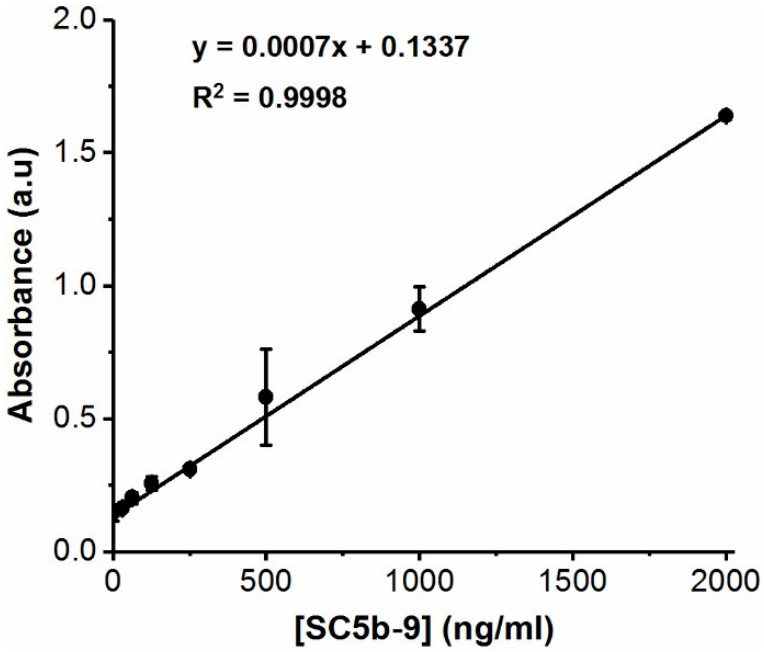

Figure S6. Calibration curve for the determination of SC5b-9. Standard SC5b-9 powder (provided by manufacturer) was reconstituted in sample diluent buffer, serially diluted, and used to construct the calibration curve, following the instruction provided. Absorbance was measured at 450 nm. Each data point represents the mean ± standard deviation (SD) of triplicate experiments.

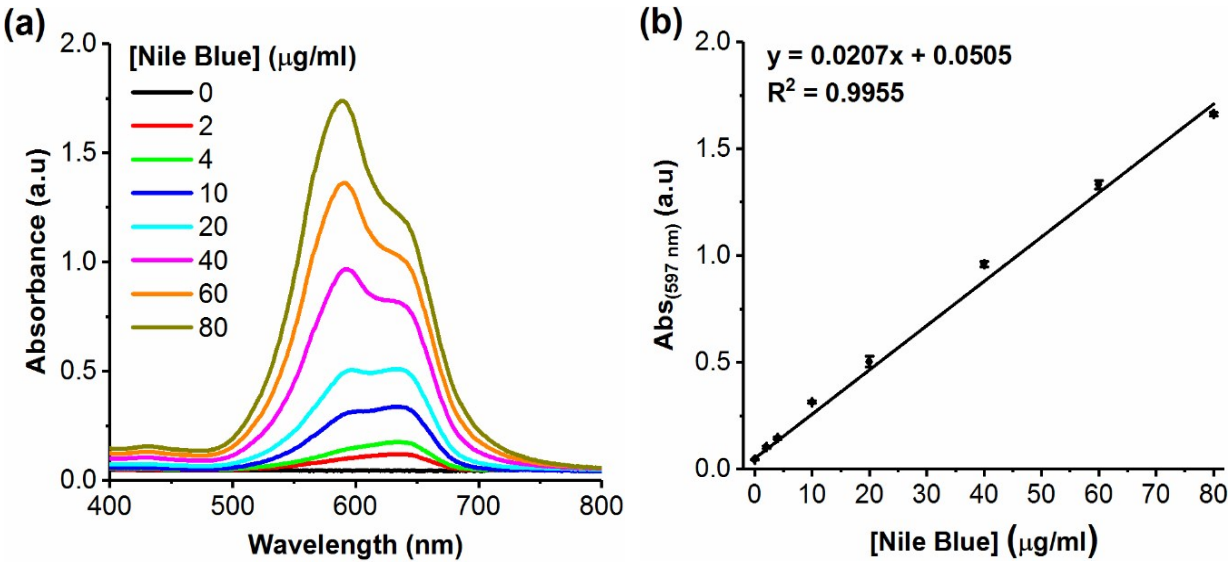

Figure S7. Calibration curve for the determination of Nile Blue concentration. (a) UV-Vis absorption spectrum of Nile Blue at various concentrations (as indicated). (b) Absorbance at 597 nm of Nile Blue as a function of its concentration. A stock solution (400 μg/ml) of Nile Blue was prepared in phosphate buffer (10 mM) and serially diluted (as indicated in Figure a) in phosphate buffer (10 mM in final concentration). Each data point in Figure b represents the mean ± standard deviation (SD) of triplicate experiments.

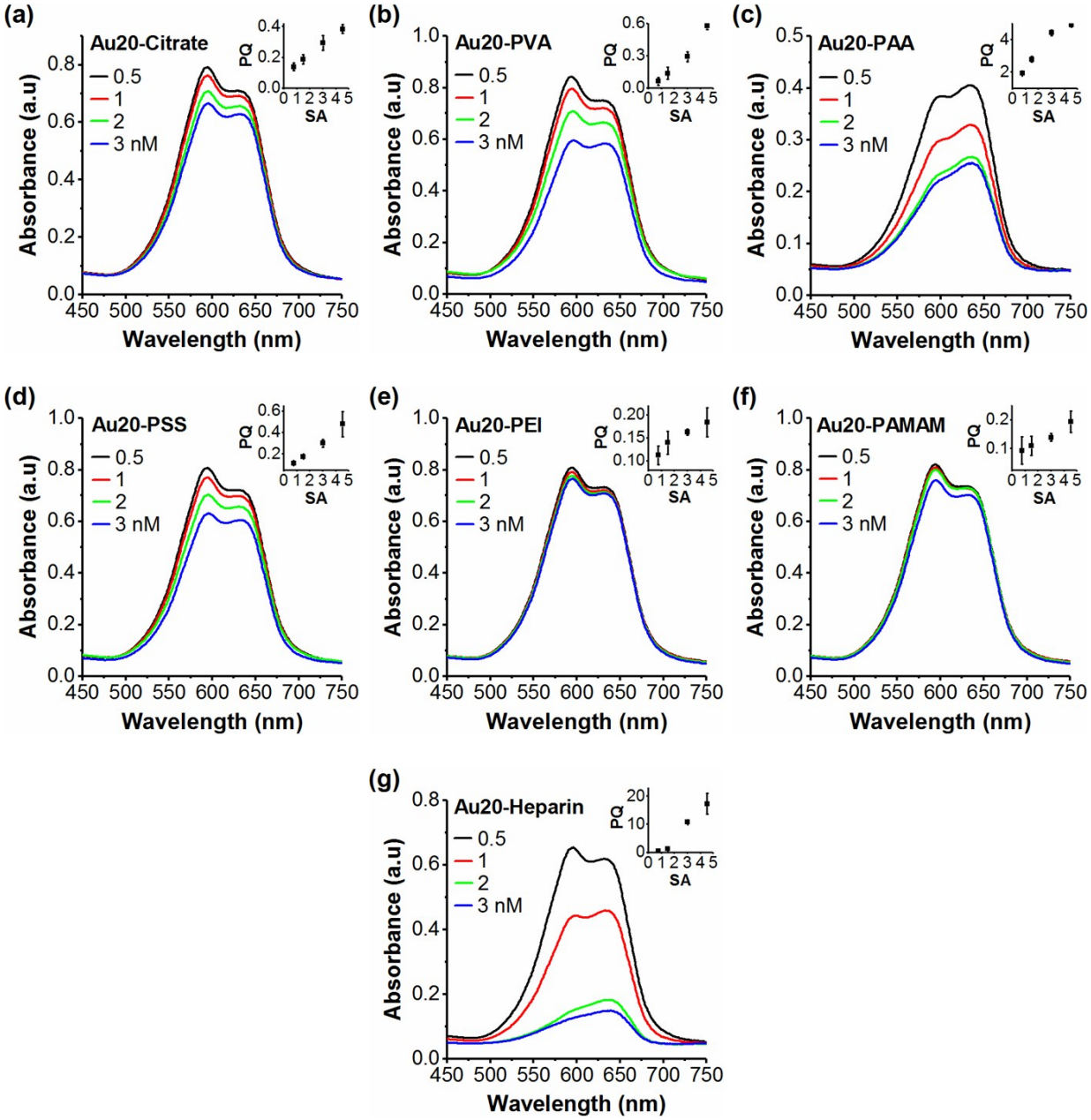

Figure S8. UV-Vis absorption spectra of Nile Blue dye in supernatant after 3h incubation with polyelectrolyte-passivated Au20. After incubation, unbound dye was isolated in supernatant phase after centrifugation at 6,500 g and its UV-Vis absorbance was measured. While the concentration of Nile Blue was fixed at 40 µg/ml (final concentration), the concentration of polyelectrolyte-passivated Au20 was varied from 0.5 to 3 nM in total volume of 400 µl. The inset in each figure was a plot of partitioning quotient (PQ) versus surface area (SA) of nanoparticles (in $\times 10^{11}$ μm^2/ml). Each data point in the insets represents the mean ± standard deviation (SD) of triplicate experiments.

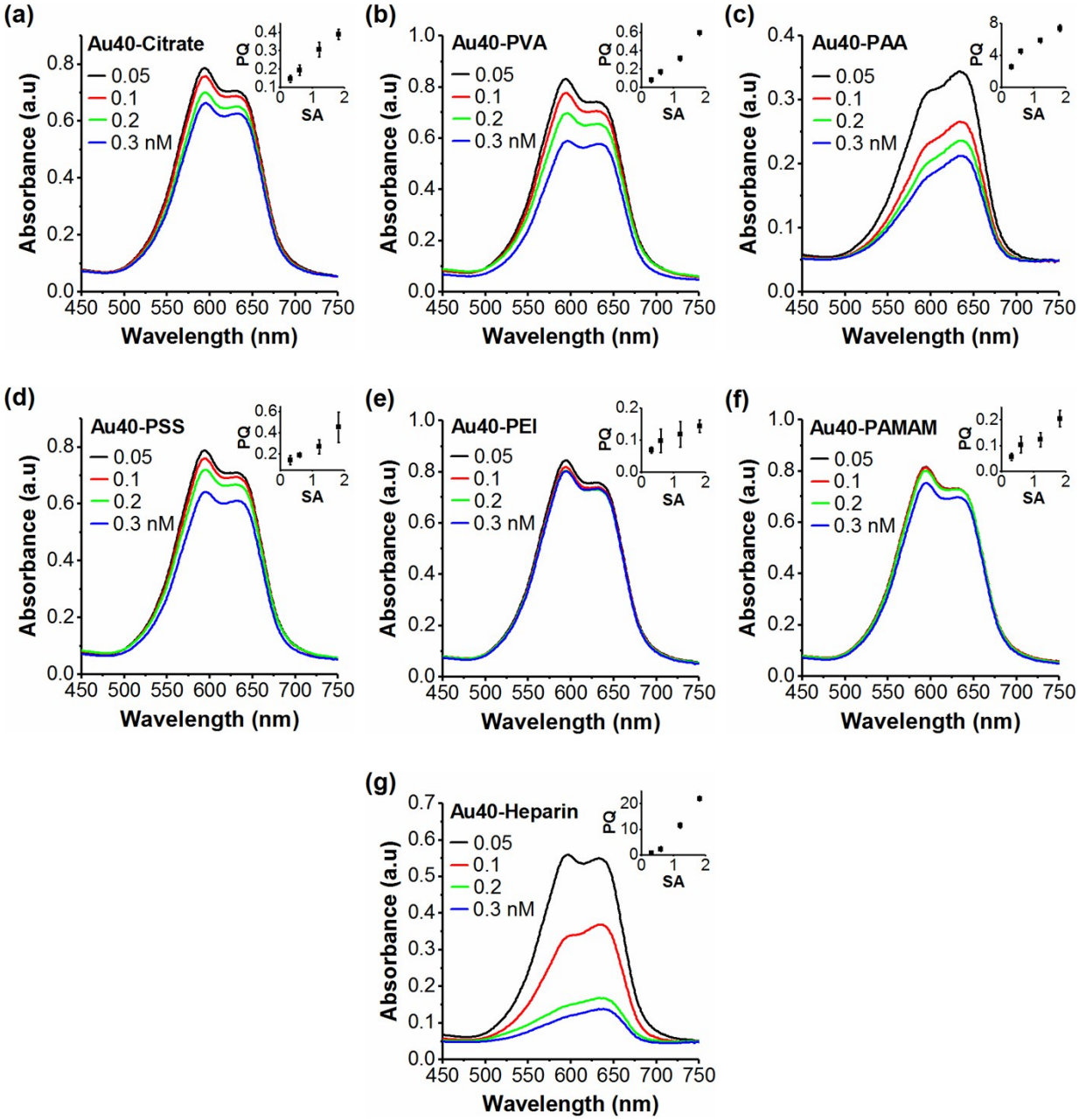

Figure S9. UV-Vis absorption spectra of Nile Blue dye in supernatant after 3h incubation with polyelectrolyte-passivated Au40. After incubation, unbound dye was isolated in supernatant phase after centrifugation at 2,500 g and its UV-Vis absorbance was measured. While the concentration of Nile Blue was fixed at 40 µg/ml (final concentration), the concentration of polyelectrolyte-passivated Au40 was varied from 0.05 to 0.3 nM in total volume of 400 µl. The inset in each figure was a plot of partitioning quotient (PQ) versus surface area (SA) of nanoparticles (in $\times 10^{11}$ μm^2/ml). Each data point in the insets represents the mean ± standard deviation (SD) of triplicate experiments.

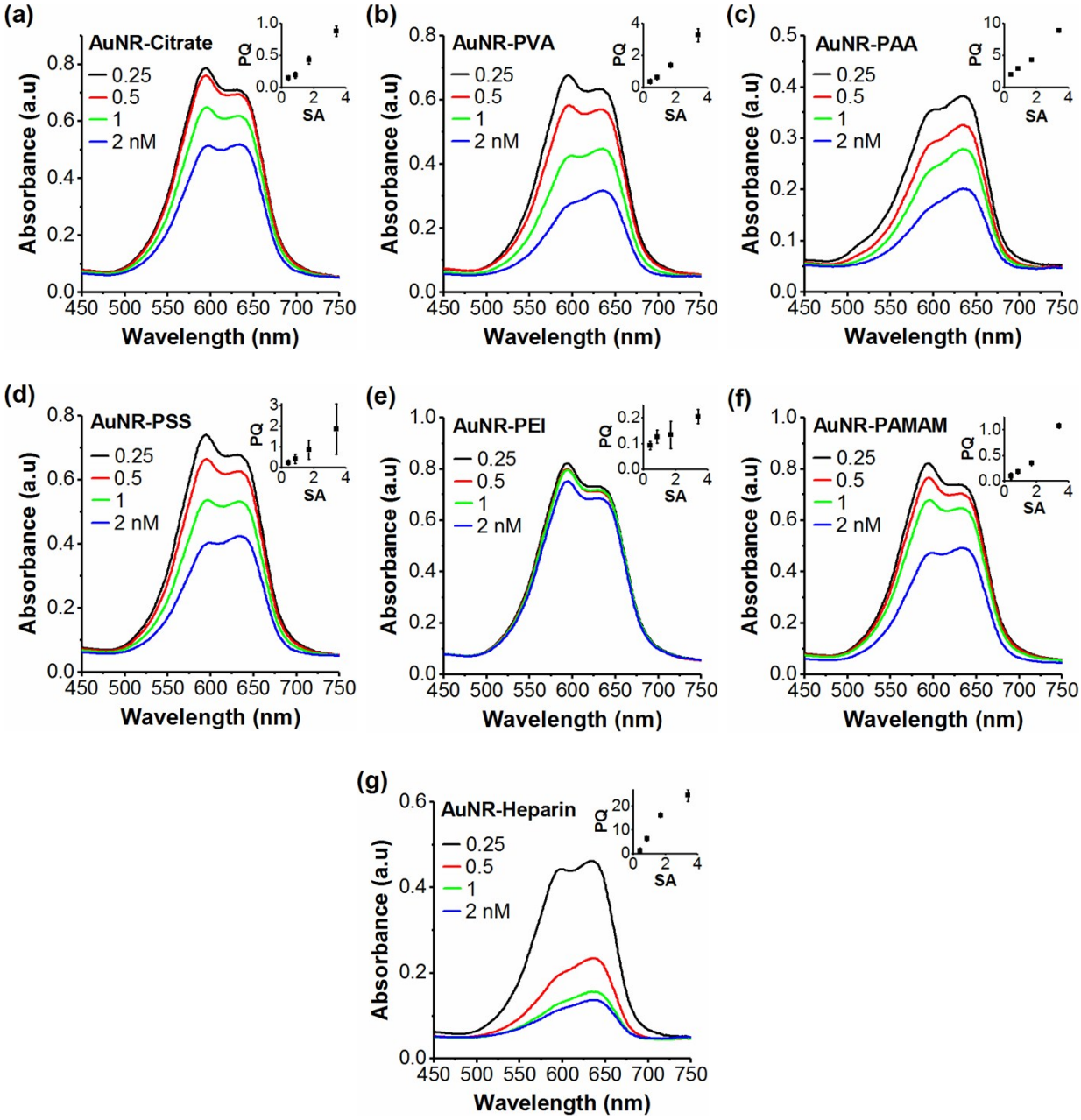

Figure S10. UV-Vis absorption spectra of Nile Blue dye in supernatant after 3h incubation with polyelectrolyte-passivated AuNR. After incubation, unbound dye was isolated in supernatant phase after centrifugation at 12,000 g and its UV-Vis absorbance was measured. While the concentration of Nile Blue was fixed at 40 µg/ml (final concentration), the concentration of polyelectrolyte-passivated Au40 was varied from 0.25 to 2 nM in total volume of 400 µl. The inset in each figure was a plot of partitioning quotient (PQ) versus surface area (SA) of nanoparticles (in $\times 10^{11}$ µm^2/ml). Each data point in the insets represents the mean ± standard deviation (SD) of triplicate experiments.

Table S1. The slope (represent relative hydrophilicity) of linear regression line of partitioning quotient (PQ) and total surface area of nanoparticles.

Surface coating	Au20	Au40	AuNR
Citrate	0.0655	0.1640	0.2527
PVA	0.1335	0.3330	0.9893
PAA	0.8108	2.9761	2.2820
PSS	0.0955	0.1974	0.5493
PEI	0.0179	0.0460	0.0352
PAMAM	0.0260	0.0899	0.3341
Heparin	4.7578	14.465	3.6636

References

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