Supplementary for TAM Nanocluster Formation Paper:

Calculation of ligand coverage:

The saturation coverage of citrate ligands on ~4 nm gold particles was compared with the experimentally determined citrate/gold ratio obtained by TGA. The saturation coverage of a gold particle by citrate was reported to be 4.6×10^{-10} mol/cm^2[1], which corresponds to a footprint of 0.36 nm^2/ citrate ligand. The surface area of the particle was divided by the footprint of the ligand to determine the maximum number of citrate ligands that could cover the gold particle was ~140. This amount of citrate coverage on the gold nanoparticle corresponds to 6.3% citrate by weight, which agrees well with the experimentally determined value of 7% obtained by TGA. Therefore, the gold surface was saturated by citrate ligands.

Calculation of polydispersity index (PDI):

The PDI was calculated as M_w/M_n, where M_w and M_n are the weight averaged and number averaged masses, respectively and may be calculated using[2]

$$M_w = \frac{\sum M_i^2 N_i}{\sum M_i N_i} \qquad \text{(S1)}$$

$$M_n = \frac{\sum M_i N_i}{\sum N_i} \qquad \text{(S2)}$$

where M is mass and N is the number of particles. PDI determinations were obtained using number weighted particle size distributions obtained using DLS.

Calculation of displacement of citrate ligands by lysine:

The displacement of citrate ligands by lysine on gold nanoparticles was estimated using the difference in zeta potentials measured for the citrate-only-capped nanoparticles, -44 mV, versus the citrate/lysine-capped nanoparticles, -30 mV. The charge on the particles, Q, was related to the surface potential, ψ_0, using

$$Q = \varepsilon_r \varepsilon_0 \Psi_0 a(1+\kappa a) \qquad \text{(S3)}$$

where ε_r, the dielectric constant of the aqueous medium, is 80, ε_0 is the permittivity of free space, a is the particle radius, and κ is the inverse Debye length, which is defined as

$$\kappa = e\sqrt{\frac{2N_A I}{\varepsilon_r \varepsilon_0 k_b T}} \qquad \text{(S4)}$$

where e is the elementary charge of an electron, N_A is Avagadro's number, and I is the ionic strength. I was calculated to be 0.0053M, shown in detail below, using ligand/gold ratios determined from TGA using the following equation:

$$I = 0.5\sum_{i=1}^{n} c_i z_i^2 \qquad \text{(S5)}$$

where c_i is the molar concentration of ion i and z_i is the charge of an ion. In this system, sodium ions are assumed to be the only free ions in solution. Surface potential was estimated using the experimentally measured zeta potential for this calculation. The ratio of charge for the citrate-only capped nanoparticles to that for the citrate/lysine capped nanoparticles was used to estimate the displacement of citrate by lysine.

Calculation of size distribution moments:

Size distribution moments, μ_1 and μ_3, were used to determine whether cluster formation was dominated by condensation or coagulation.

$$\mu_1 = \frac{r_3}{r_h} = \frac{\sqrt[3]{\sum r_i^3 / N_{Tot}}}{N_{Tot} / \sum \frac{1}{r_i}} \qquad \text{(S6)}$$

$$\mu_3 = \frac{r_1}{r_3} = \frac{\sum r_i / N_{Tot}}{\sqrt[3]{\sum r_i^3 / N_{Tot}}} \qquad \text{(S7)}$$

where r_1 is the arithmetic mean radius, r_3 is the cube mean radius, r_h is the harmonic mean radius, and N_{Tot} is the total number of particles in the system. When $\mu_1 = \mu_3 = 1$, the size distribution moments reflect a monodisperse system where aggregates have been formed by condensation. Size distribution moments where $\mu_1 > 1.25$ and $\mu_3 < 0.905$ suggest that aggregates were formed by coagulation[3].

Calculation of nanocluster extinction cross section:

For a dilute solution, absorbance, A, follows Beer–Lambert's Law:

$$A = \varepsilon(\lambda)\, C\ell \qquad \text{(S8)}$$

where ε is the extinction coefficient, C is the dispersion concentration, and ℓ is the path length through which light travels. For a path length of 1 cm, and a gold concentration of 56 μg/mL, the maximum absorbance, found at a wavelength of 700 nm, was 1.10. Using Eq. S8, we determined that the average $\varepsilon_{700\text{ nm}}$ was 0.017 ± 0.003 cm^2/μg for three dispersions. Assuming that the nanoclusters are spherical, 100 nm in diameter, and in a closest-packed state, the particle extinction cross section was calculated by, $\varepsilon_{700\text{ nm}}$ x M_{Au}, where $M_{Au}$is the mass of gold per nanocluster, and was estimated to be 1.0×10^{-5} ng/cluster ($M_{Au} = V_p\times\rho_p\times0.72$, where V_p and ρ_p are the particle volume and density, respectively). The average particle extinction cross section was calculated to be ~9.0×10^{-15} m^2.

Calculation of total interaction potentials and theoretical stability ratios:

Total interaction potentials were calculated by summing van der Waals (VDW), electrostatic, and depletion contributions. The VDW attractive component is described by [2]:

$$V_{VDW} = \frac{-A}{6}\left[\frac{2a^2}{H(4a+H)} + \frac{2a^2}{(2a+H)^2} + \ln\left(\frac{H(4a+H)}{(2a+H)^2}\right)\right] \qquad \text{(S9)}$$

where *A* is the Hamaker constant of particles interacting in an aqueous medium and *H* is the surface to surface separation distance between particles. The electrostatic interaction contribution is described by[2]:

$$V_{electrostatic} = \frac{64\pi a k_b T \Gamma_0^2 n_\infty}{\kappa^2}\exp[-\kappa H] \qquad \text{(S10)}$$

where n_∞ is the bulk ion concentration, and Γ_0 is a ratio of constants related to the surface potential, defined as[2]:

$$\Gamma_0 = \frac{\exp\left[\frac{ze\psi_0}{2k_bT}\right] - 1}{\exp\left[\frac{ze\psi_0}{2k_bT}\right] + 1} \qquad \text{(S11)}$$

where *z* is the valence of the electrolyte, taken to be 1 in this model, *e* is the elementary charge unit of an electron, and ψ_0 is the surface potential of the gold nanoparticle.

To construct the interaction potential curves, the necessary input parameters were calculated as described below. In order to determine the bulk ion concentration, n_∞, the number of citrate ligands per gold particle was estimated based on an experimentally observed *W* of ~7×10^9 for a colloidal gold suspension with no added polymer (Table 2). Using Eq. 6, the stability ratio *W* may be related to the interaction potential, V_{total}, and thus surface potential, ψ_0. Therefore, values for ψ_0.were obtained by matching experimentally determined *W* values to an interaction potential. The corresponding ψ_0 was calculated to be -95 mV. This value was then used to calculate the charge on the particle using Eq. S3[4]. The calculated particle charge was used to determine the number of charged citrate ligands per particle, which resulted in an estimated value of 11 charged citrate ligands per gold nanoparticle. By using an initial gold concentration of approximately 3 mg/ml, the number density of gold particles can be determined. From this value and the estimated charged citrate ligand coverage, the initial n_∞ was approximately 5.1×10^{22} charged citrate molecules per cubic meter. In order to determine κ, electro-neutrality was assumed, such that the ionic strength of the solution balances the charge on the gold particles. Therefore, κ is defined as[5]

$$\kappa = e\sqrt{\frac{z_{particle} n_{particle}}{\varepsilon_r \varepsilon_0 k_b T}} \qquad \text{(S12)}$$

where $z_{particle}$ is the valence of the gold nanoparticle, equivalent to the number of citrate ligands per particle, $n_{particle}$ is the number density of gold particles, and ε_r is 80 in this case.

The depletion contribution to the total potential is described in the text (Eq. 5). The number density of micelles, required for the depletion calculation, was dependent on the polymer concentration in the system[6].

$$\rho_\infty = \frac{(C_p - CMC)N_A}{N_{agg}} \qquad \text{(S13)}$$

where C_p is the polymer concentration, CMC is the critical micelle concentration for the PLA(2k)-PEG(10k)-PLA(2k) triblock copolymer, which was reported as 7 µg/ml[7], and N_{agg} is the aggregation number for the polymer, reported as 165 [8]. The summation of the potentials determines the total interaction potential (V_{total}), which was normalized by k_bT and then plotted as a function of surface to surface separation distance for various evaporation extents in Fig. 9. In order to calculate the potential for a dispersion of nanoclusters, the reduction in surface potential due to ion pairing caused by polymer adsorption was regressed from experimentally determined W values using Eq. 6, taking into account the electrostatic, VDW and depletion terms for V_{total}. Since it was not possible to perform a regression for evaporation extents greater than 50% because of the excessive turbidity of the solutions, the experimental W value corresponding to 50% solvent evaporation, obtained from the observed time required to elicit a change in the dispersion color after 50% evaporation, was chosen. The surface potential reduction from ion pairing was thus determined to be approximately 1.6. This value was then used to calculate the interaction potential and *W* values at other evaporation extents.

References for supplementary section

1. Kunze, J.; Burgess, I.; Nichols, R.; Buess-Herman, C.; Lipkowski, J., Electrochemical evaluation of citrate adsorption on Au(111) and the stability of citrate-reduced gold colloids. *J. Electroanal. Chem.* **2007,** *599*, 147-159.
2. Hiemenz, P. C.; Rajagopalan, R.; Editors, *Principles of Colloid and Surface Chemistry, Third Edition, Revised and Expanded*. 1997; p 688 pp.
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5. Smith, P. G.; Patel, M. N.; Kim, J.; Milner, T. E.; Johnston, K. P., Effect of Surface Hydrophilicity on Charging Mechanism of Colloids in Low-Permittivity Solvents. *J. Phys. Chem. C* **2007,** *111*, 840-848.
6. Mondain-Monval, O.; Leal-Calderon, F.; Phillip, J.; Bibette, J., Depletion Forces in the Presence of Electrostatic Double Layer Repulsion. *Phys. Rev. Lett.* **1995,** *75*, 3364-3367.
7. Venkatraman, S. S.; Jie, P.; Min, F.; Freddy, B. Y. C.; Leong-Huat, G., Micelle-like nanoparticles of PLA-PEG-PLA triblock copolymer as chemotherapeutic carrier. *Int. J. Pharm.* **2005,** *298*, 219-232.
8. Agrawal, S. K.; Sanabria-DeLong, N.; Tew, G. N.; Bhatia, S. R., Structural Characterization of PLA-PEO-PLA Solutions and Hydrogels: Crystalline vs Amorphous PLA Domains. *Macromolecules* **2008,** *41*, 1774-1784.

Supplementary Information

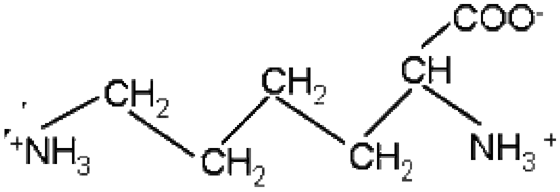

Figure S1: Schematic of lysine ligand

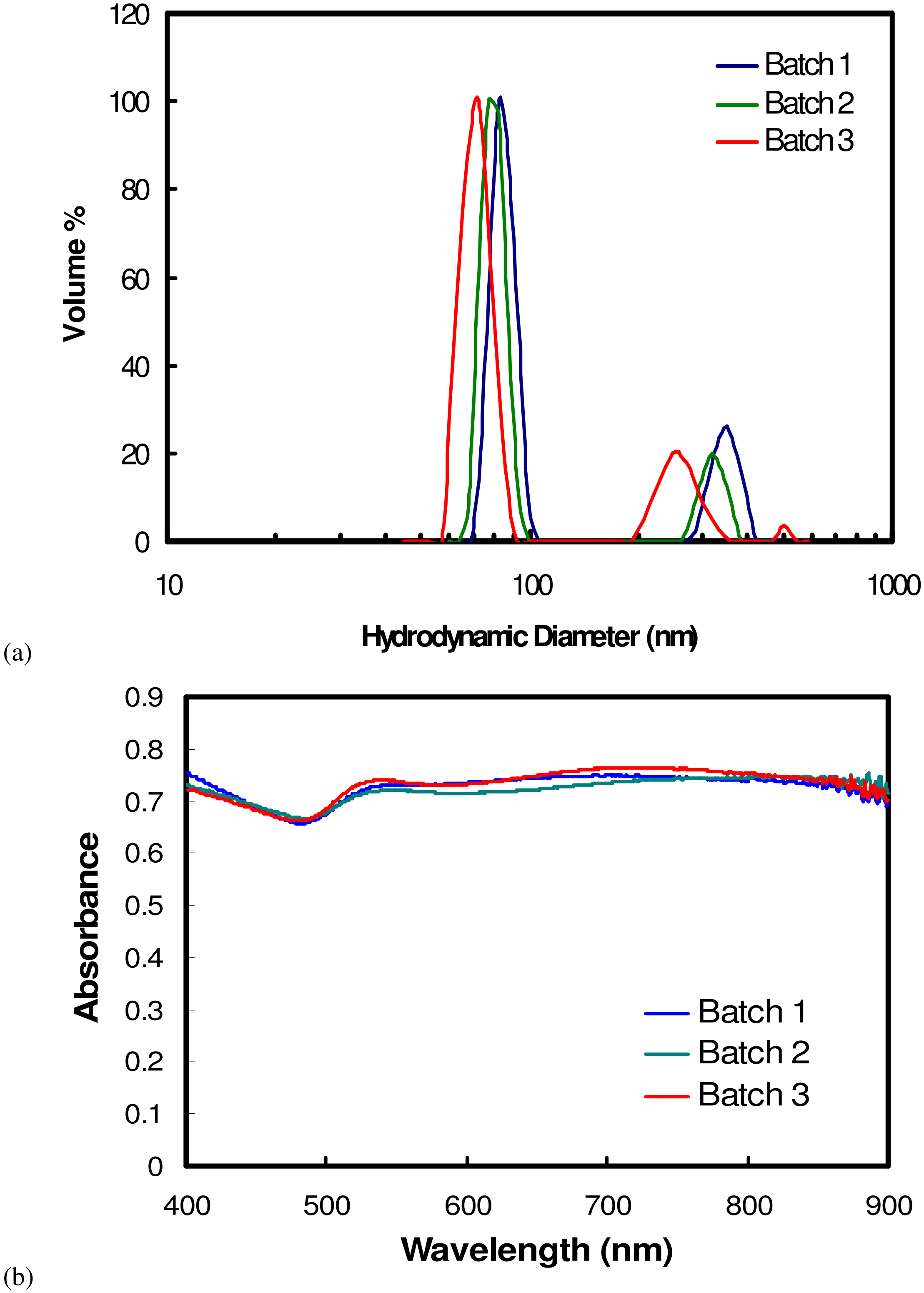

Figure S2: Reproducibility of nanoclusters of citrate/lysine-capped gold nanoparticles in terms of (a) size and (b) optical properties. Starting gold and PLA-*b*-PEG-*b*-PLA concentrations were 3 and 50 mg/mL, respectively. Nanoclusters were produced after 100% solvent evaporation.

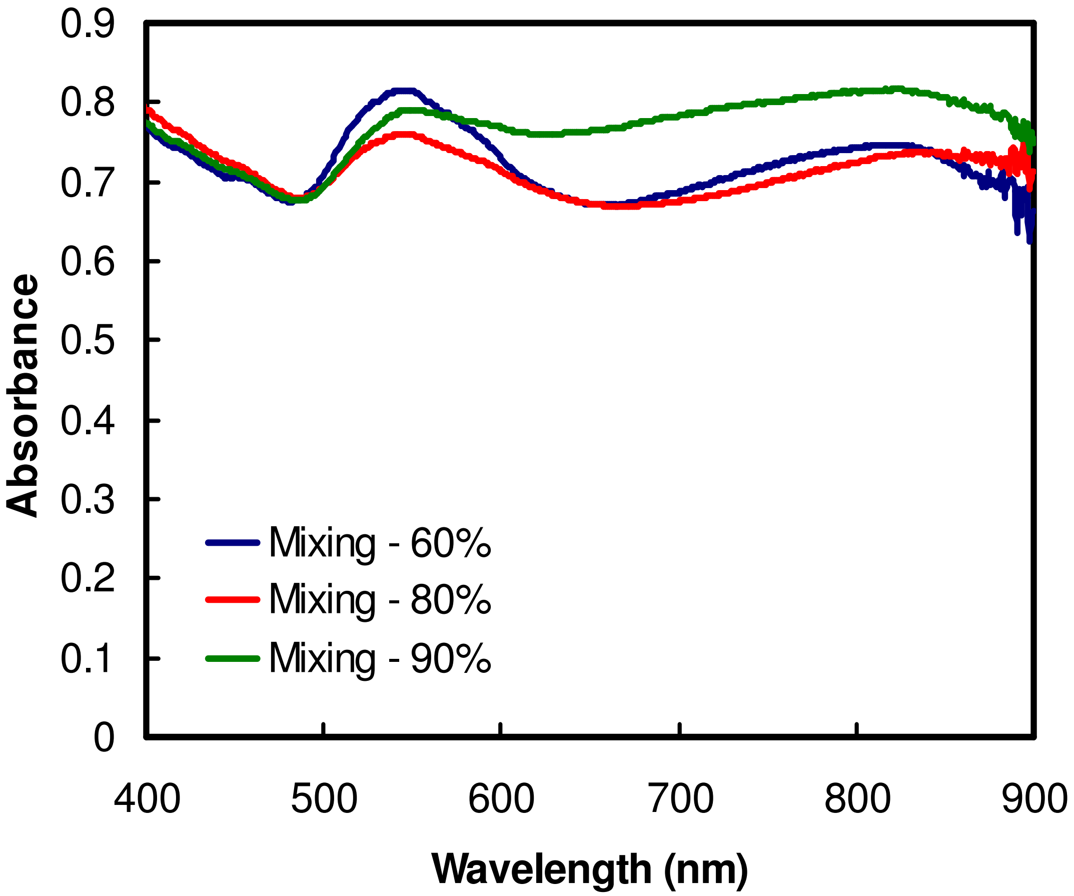

Figure S3: UV-vis spectra of clusters of citrate-capped nanoparticles made with the mixing protocol. The starting gold concentration was 3 mg/mL and the PLA-*b*-PEG-*b*-PLA /Au ratio was 16/1.

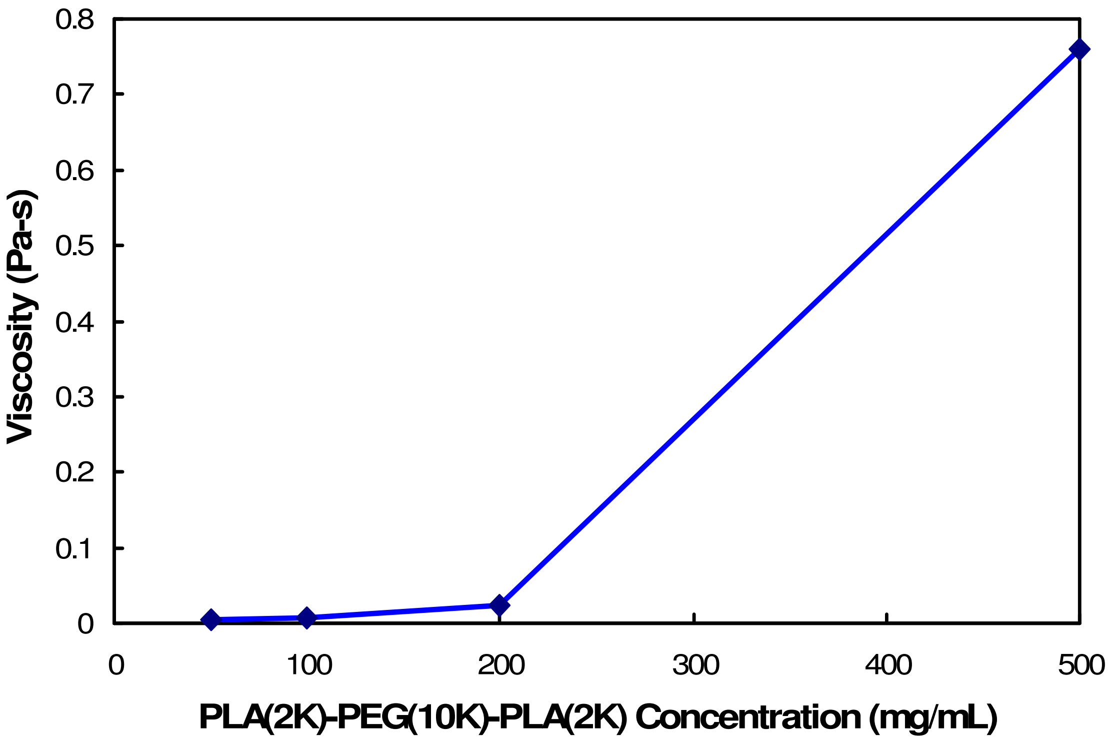

Figure S4: Viscosity of PLA-*b*-PEG-*b*-PLA as a function of concentration. Viscosity measurements were performed using a cone and plate viscometer (TA Instruments AR 2000ex with a Peltier plate base and aluminum cone, with a diameter of 40 mm, angle of 1° 59 minutes and 56 seconds and a truncation distance of 55 µm).

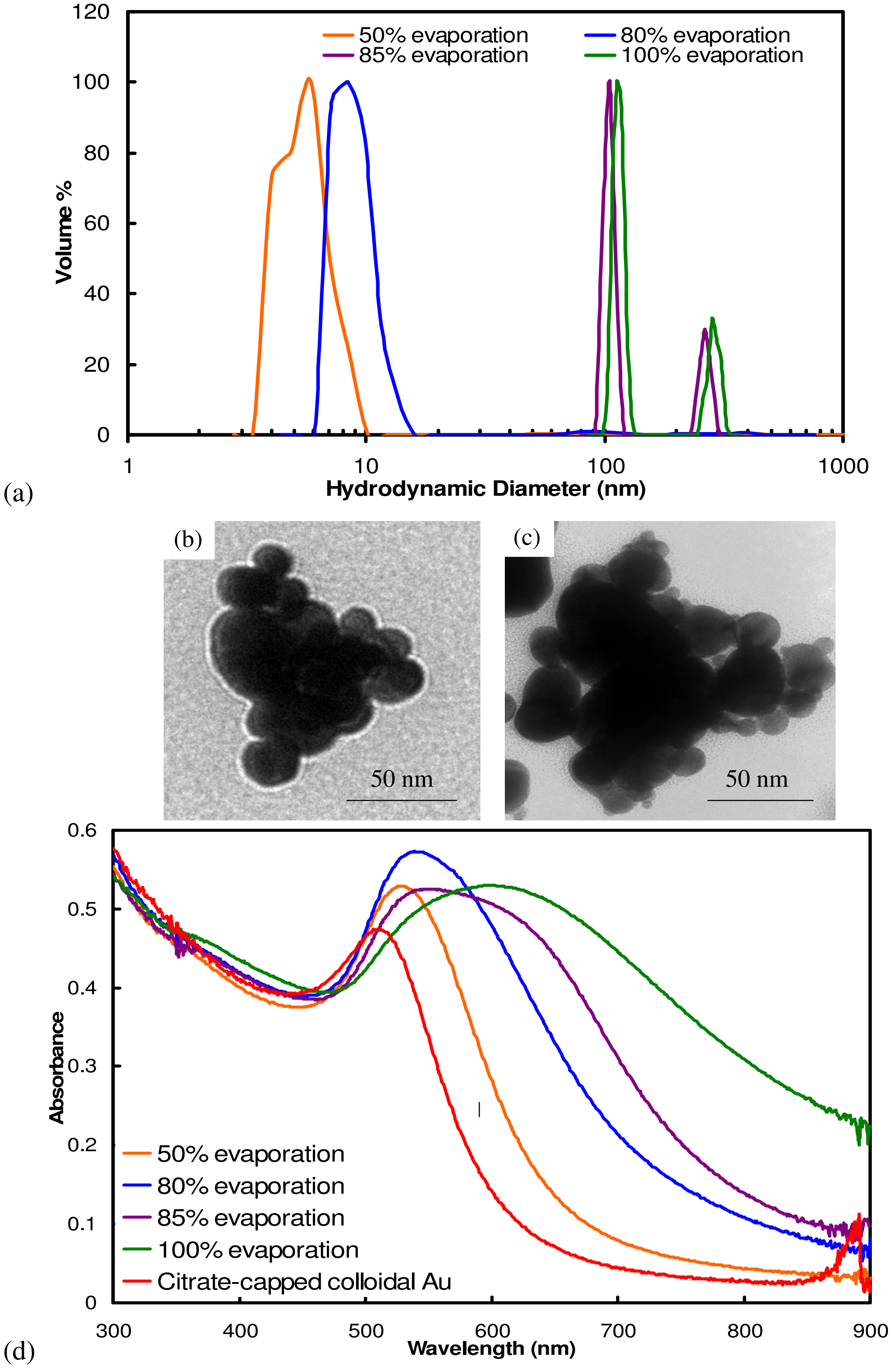

Figure S5: (a) DLS measurements, TEM images after (b) 85% and (c) 100% solvent evaporation, respectively, and (d) UV-vis, absorbance spectra for nanoclusters composed of citrate-capped gold nanoparticles produced after different extents of evaporation with a starting gold concentration of 3 mg/mL and a PLA-*b*-PEG-*b*-PLA/gold ratio of 16/1.

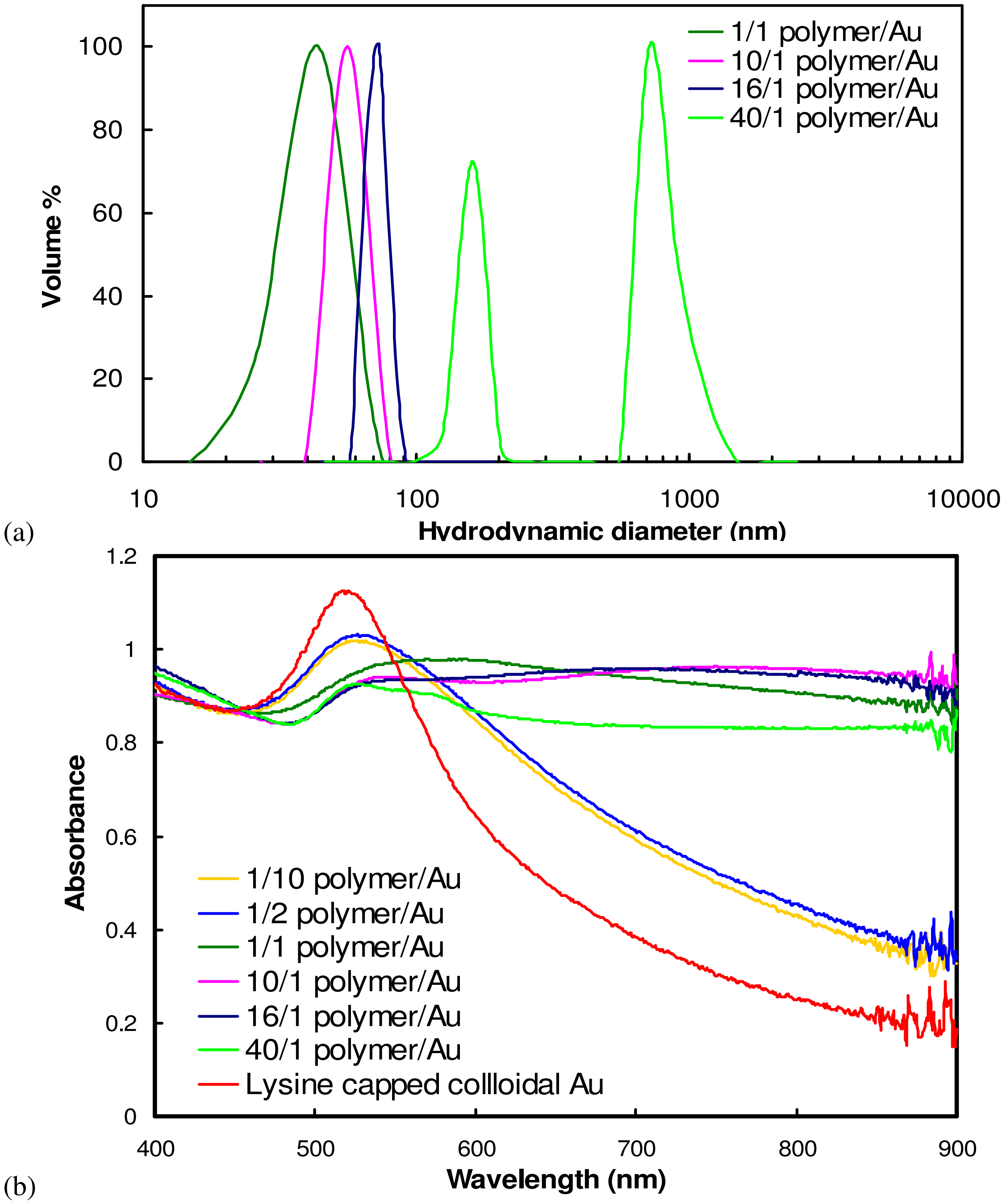

Figure S6: (a) Particle size measurements by DLS and (b) UV-vis absorbance spectra of clusters of citrate/lysine-capped nanoparticles formed when varying the PLA-*b*-PEG-*b*-PLA /Au ratio. The starting gold concentration was 3 mg/mL and the clusters were formed under 100% solvent evaporation.

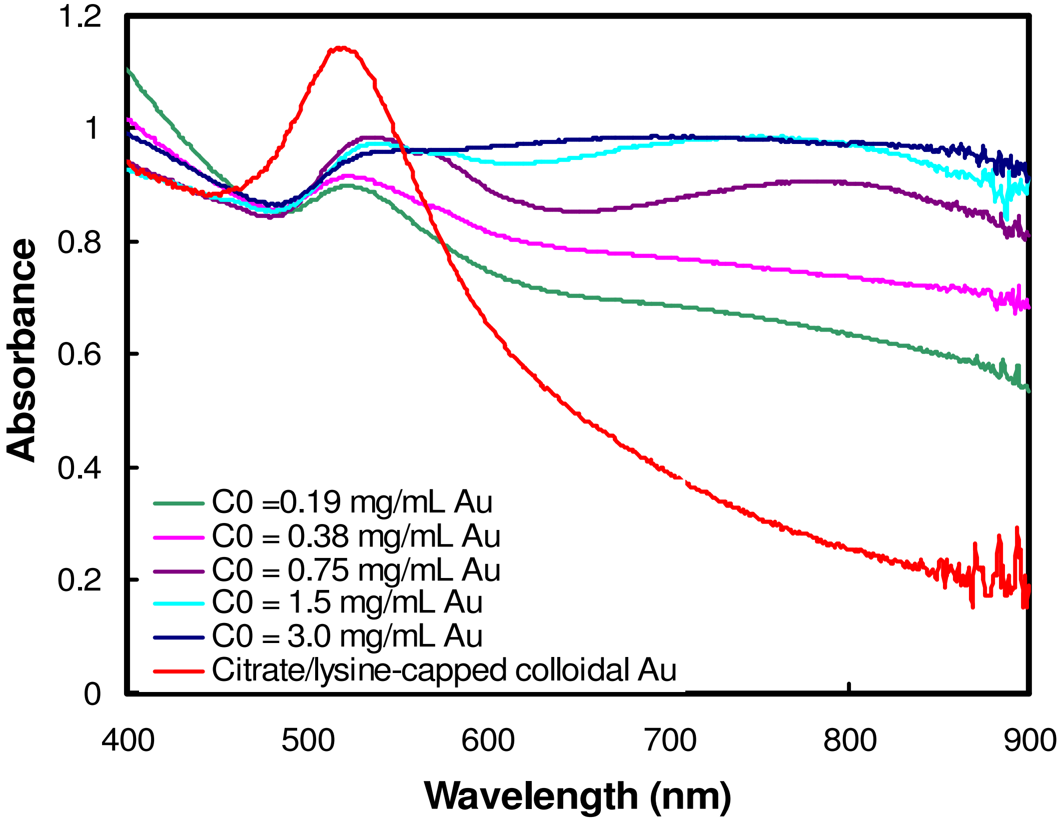

Figure S7: UV-vis absorbance spectra of citrate/lysine-capped nanoclusters formed when varying the starting concentration of the colloidal gold solution. The starting PLA-*b*-PEG-*b*-PLA concentration was 50 mg/mL.

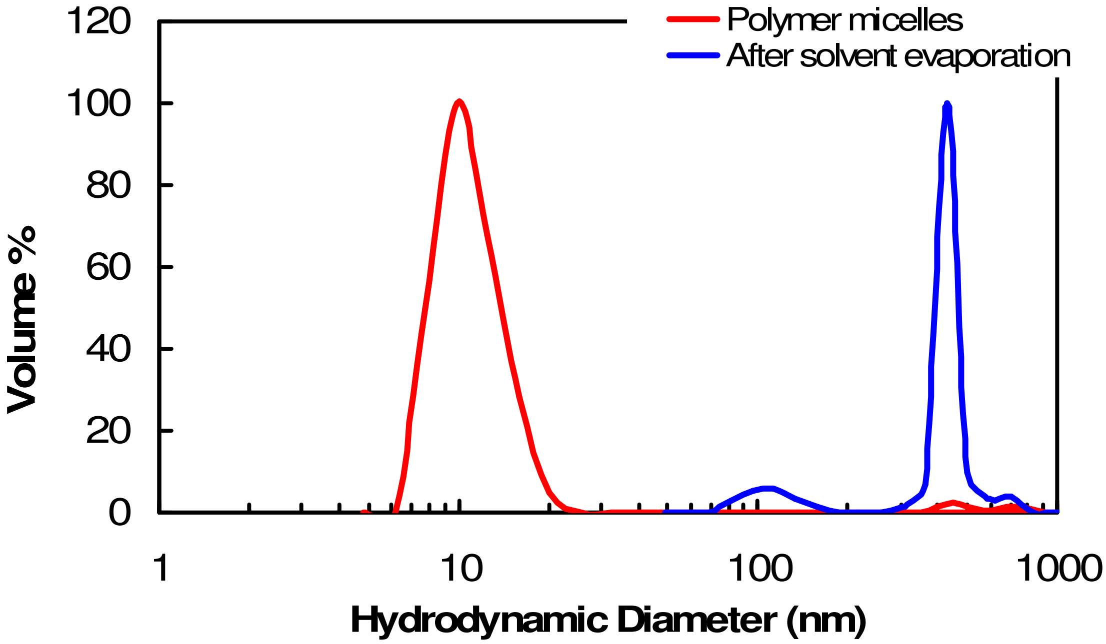

Figure S8: DLS measurement of PLA-*b*-PEG-*b*-PLA micelles prior to solvent evaporation and after solvent evaporation. A 50 mg/mL polymer solution was prepared. To measure the micelle size, the solution was diluted to 1 mg/mL for analysis by DLS. To determine the effect of solvent evaporation on the polymer, the solution was evaporated to dryness and then redispersed in DI water to a concentration of 5 mg/mL.

Table S1: Particle sizes, as determined by DLS, of citrate/lysine-capped nanoclusters formed when varying the starting concentration of the colloidal gold solution. The starting PLA-*b*-PEG-*b*-PLA concentration was 50 mg/mL.

Sample	Particle Size Range (nm)
C_0 = 0.19 mg/mL Au	74-118 (12%), 380-608 (80%)
C_0 = 0.38 mg/mL Au	82-122 (62%), 502-613 (38%)
C_0 = 0.75 mg/mL Au	44-57 (68%), 250-377 (32%)
C_0 = 1.5 mg/mL Au	56-100 (89%), 316-562 (11%)
C_0 = 3.0 mg/mL Au	54-101 (82%), 236-359 (18%)