Spontaneous Self-Assembly and Disassembly of Colloidal Gold Nanoparticles Induced by Tetrakis(hydroxymethyl) Phosphonium Chloride

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Supporting Information

Experimental Methods

Materials and Reagents: Citrate-stabilized 15 nm gold nanoparticles (AuNPs) and citrate-stabilized 20 nm silver nanoparticles (AgNPs) stored in 2 mM citrate buffer were purchased from Ted Pella, Inc. Tetrakis(hydroxymethyl) phosphonium chloride (THPC) and tri(hydroxymethyl) phosphine (THP) were purchased from Sigma-Aldrich. Tri(hydroxypropyl) phosphine (THPP) was purchased from EMD Millipore. Copper TEM grids were purchased from Ted Pella, Inc. All other materials, reagents, and buffers were purchased from either Sigma-Aldrich or Fisher Scientific and used without further modification. Deionized water used in this investigation was purified with a resistivity greater than or equal to 18.2 MΩ•cm.

Instrumentation: UV-vis spectra were collected on an Agilent 8453 spectrophotometer with a photodiode array detector or a Biotek Synergy H4 plate reader. Dynamic laser light scattering measurements were performed on a Malvern Zetasizer. Transmission electron microscopy (TEM) images were captured on a Tecnai Osiris instrument at an accelerating voltage of 20 kV. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was performed on a Perkin Elmer Optima 7000 DV instrument. X-ray photoelectron spectroscopy (XPS) was performed on a PHI 5000 Versaprobe instrument. ^{31}P nuclear magnetic resonance (NMR) spectra were acquired on a Bruker 600 MHz spectrometer with a CPQCI probe. Color photographs of suspended particles were captured with a Nikon Coolpix 18.1 Megapixel digital camera.

ICP-OES Analysis of THPC Ligand Adsorbed to AuNPs: To an aqueous suspension of 15 nm citrate-stabilized AuNPs (2.3 nM, 4 mLs), 100 μM THPC was added and incubated for 2 days. The particles were then each split into 1 mL aliquots into 1.5 mL Eppendorf microcentrifuge tubes and washed by centrifuging for 45 minutes at 7400 rcf. The supernatant was removed and the THPC AuNPs were resuspended in 1 mL 0.1 M N-cyclohexyl-2-aminoethanesulfonic acid (CHES) buffer containing 0.025% v/v Tween 20 (pH 9.0). It should be noted that this buffer system contains an undetectable quantity of phosphorus. This centrifuge/wash procedure was repeated three additional times before finally suspending each aliquot in 1 mL deionized water. The particles were spun down a final time for 45 minutes at 7400 rcf and the supernatant was removed. The pellets from the 4 aliquots were then combined into a 15 mL conical tube, and 800 μLs of fresh aqua regia (3:1 v/v trace-metal grade HCl:HNO_3) solution was added to dissolve the gold nanoparticles. This solution was finally diluted to a total volume of 4 mLs with deionized water. The phosphorus emission line at 213.618 nm was used to quantify the phosphorus concentration in each gold nanoparticle sample. Samples were calibrated with fresh phosphoric acid ICP standard solution.

XPS Analysis of Phosphorus: A 100 μM THPC solution was first incubated with an aqueous suspension of 15 nm citrate-stabilized AuNPs (2.3 nM). At 5 minute, 30 minute, 60 minute, and 48 hour time intervals, respectively, the particles were centrifuged at 21,100 *g* for 1 minute, the supernatant was removed, and the THPC AuNPs were resuspended in 250 μLs deionized water. Next, a 100 μL aliquot of the THPC particle suspension in water was immediately drop-cast on a B-doped silicon wafer and allowed

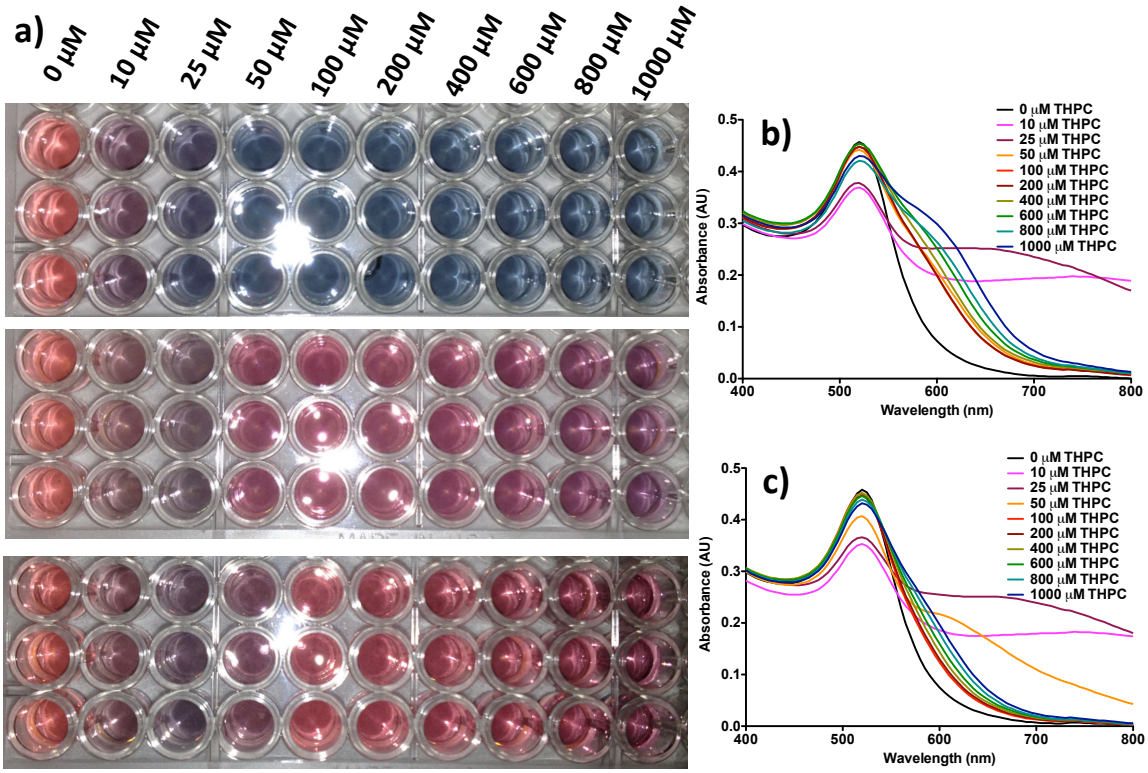
to dry. Unmodified citrate-stabilized AuNPs were also drop-cast on a B-doped silicon wafer as a control. Survey scans and high resolution scans were performed with a monochromated Al Kα source (hν = 1486.6 eV) with a 100 μm beam diameter operating at 25 W. The analyzer was oriented 45° with respect to the normal. For survey scans, the samples were analyzed at a path energy of 187.85 eV, while high resolution scans were collected at 23.5 eV. Peak analysis was performed with CasaXPS software and fitted with Gaussian/Lorentzian lines with 30% Gauss character. The Shirley method was utilized to model the background of the spectra. Phosphorus P 2p and Au 4f peak binding energy positions were calibrated relative to the C 1s unsaturated hydrocarbon binding energy at 284.8 eV, while all P 2p and Au 4f peaks were normalized to the counts of the most intense peak.

^{31}P NMR on-particle: In order to perform ^{31}P NMR on 15 nm THPC-functionalized AuNPs, particles were quenched by centrifugation and resuspension in acidic buffer at 1 minute, 30 minute, 4 hour, and 48 hour intervals, respectively, after adding 100 μM THPC to citrate-stabilized AuNPs. First, 100 μM THPC was added to 10 1 mL aliquots of 2.3 nM citrate-stabilized AuNPs and allowed to incubate for the aforementioned time intervals. Afterwards, the particles were centrifuged for 5 minutes at 16,100 rcf (aggregated particles) or 45 minutes at 7400 rcf (monodisperse particles). The supernatant was then removed and each aliquot of particles was suspended in 100 μLs 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) with 0.025% v/v Tween 20 (pH 4.4). The 10 aliquots were next combined into one aliquot and centrifuged again. After removing the supernatant, the particles were resuspended in 180 μLs 0.1 M MES with 0.025% Tween 20 (pH 4.4), effectively concentrating the AuNPs by a factor of 50 relative to their stock concentration. This solution was added to a 3 mm NMR tube and run with a cold ^{31}P NMR channel. A minimum of 30,000 scans was performed to acquire each spectrum of THPC functionalized to the AuNPs. For analysis of free THPC in solution, a concentrated THPC sample was stored in deionized water for 24 hours before acquiring spectra.

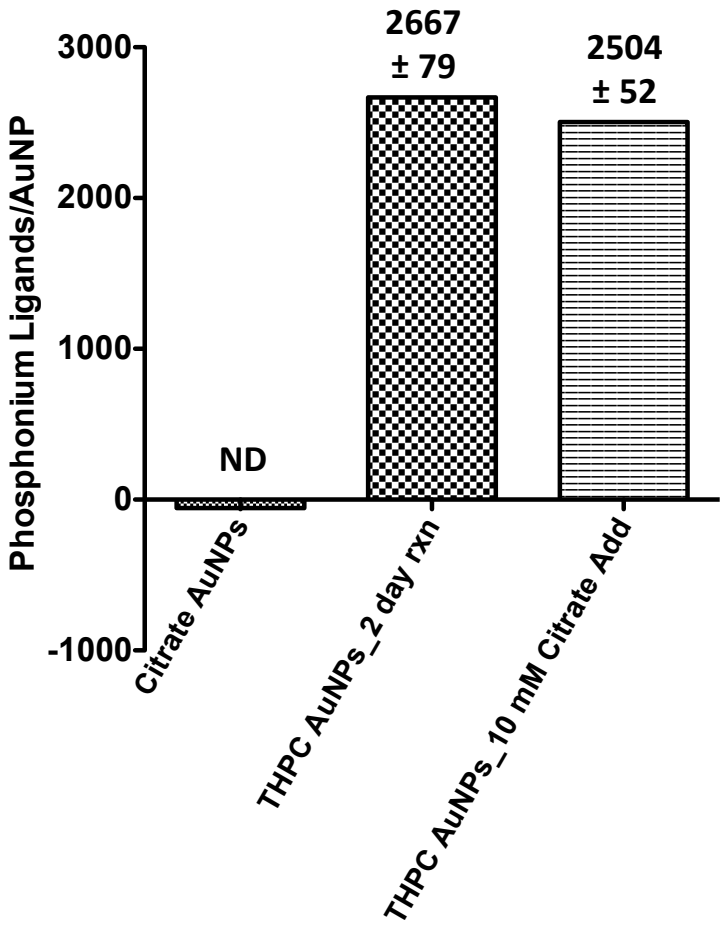
^{31}P NMR off-particle: Ten 1 mL aliquots of citrate-stabilized AuNPs (2.3 nM) were incubated with 100 μM THPC for 30 minutes. The particles were centrifuged for 5 minutes at 16,100 rcf and the supernatant was removed. Each aliquot was resuspended in 100 μLs of 0.1 M MES with 0.025% v/v Tween 20 (pH 4.4). The 10 aliquots were combined and centrifuged again. The supernatant was removed and the particles were resuspended in 180 μLs of 1 M potassium cyanide for 30 minutes with shaking. The solution was centrifuged briefly to pellet debris and the supernatant was used for ^{31}P NMR analysis.

TEM Sample Preparation: For TEM sample preparation without HCl quenching (Figure 1), the THPC AuNPs were centrifuged once at 21,100 *g* at the appropriate time intervals after THPC addition. The supernatant was removed and the particles were resuspended in 250 μLs deionized water, effectively concentrating them by a factor of four. Finally, a 5 μL samples was deposited onto a copper grid and the solvent was wicked away using filter paper. For TEM sample preparation with HCl quenching (Figure 2), 6.6 mM HCl was added to the THPC AuNPs at the appropriate time intervals

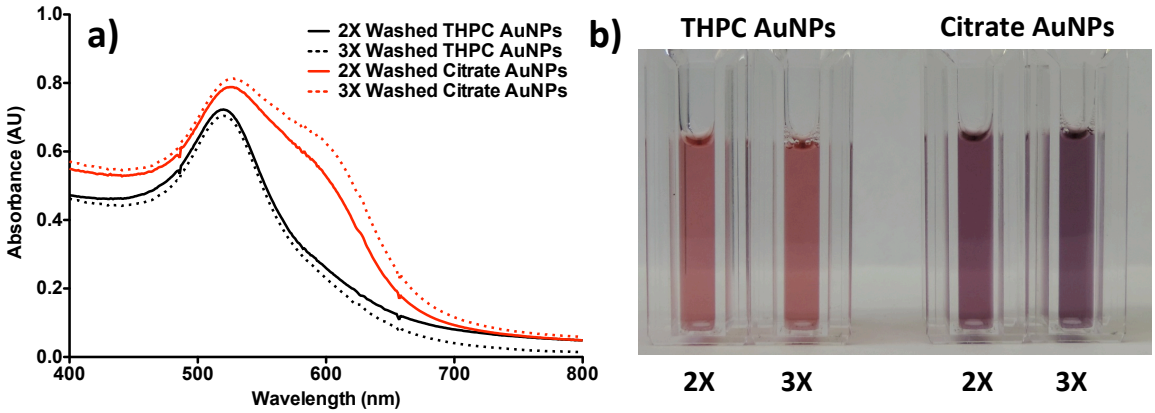
after THPC addition. Approximately 24 hours after HCl addition, samples were prepared by depositing a 10 μL sample onto a copper grid and wicking away the solvent with filter paper.



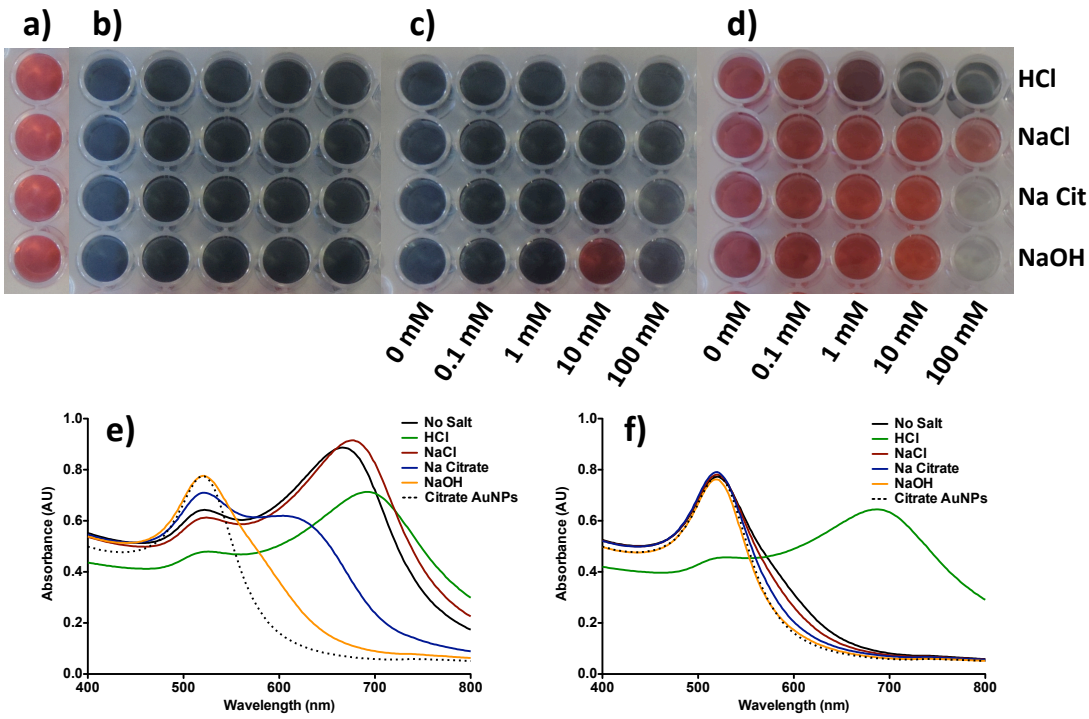
Supplemental Figure 1. Concentration dependence of THPC AuNP assembly and disassembly. a) Color images of THPC AuNPs in a 96-well plate upon incubating 2.3 nM citrate-stabilized AuNPs (15 nm) with THPC between 0 and 1000 μM concentrations. Concentration dependent color changes are evident 5 minutes after THPC addition (top), 24 hours after THPC addition (middle), and 48 hours after THPC addition (bottom). Each nanoparticle reaction was performed in triplicate. Acquired UV-vis spectra b) 24 hours and c) 48 hours after THPC addition. A 100 μM THPC concentration was chosen because of the most rapid rates of gold nanoparticle assembly and disassembly.



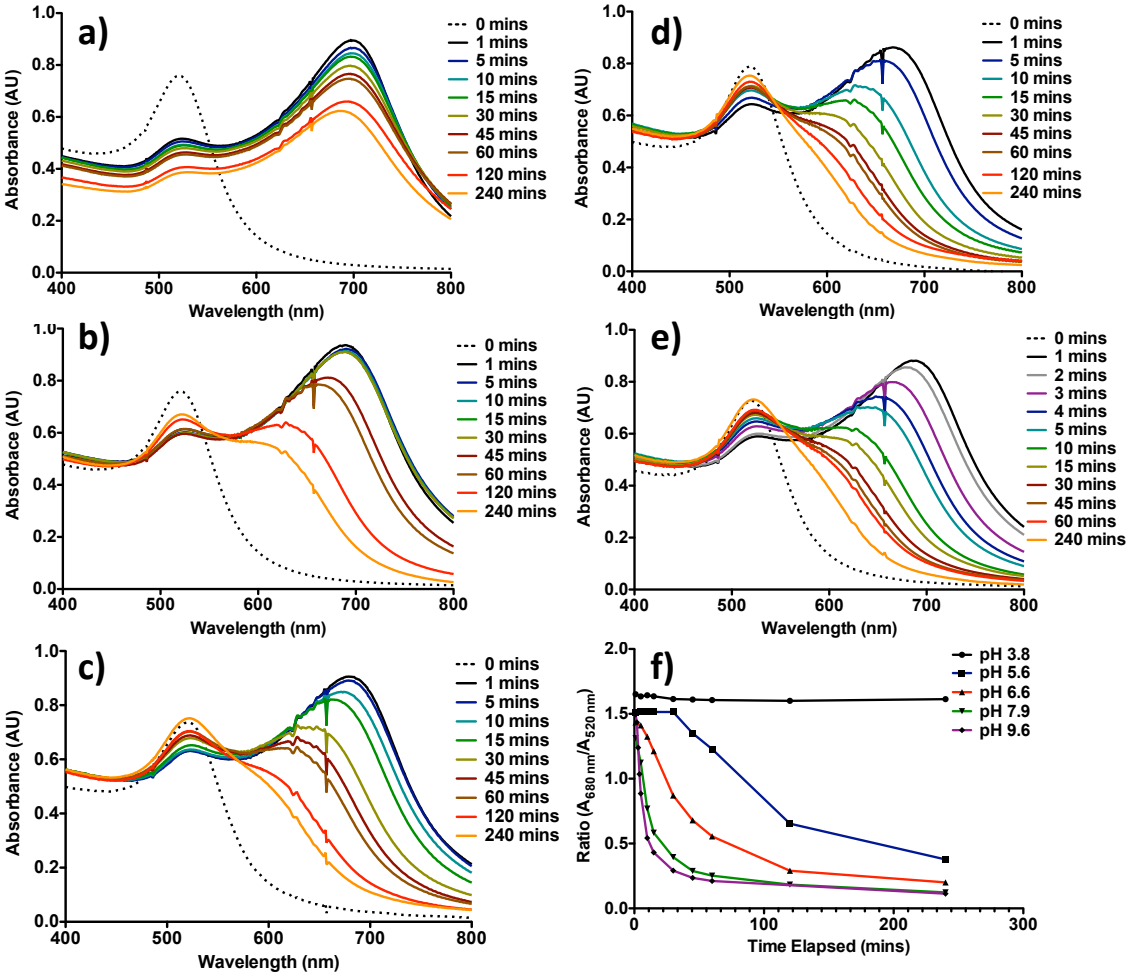
Supplemental Figure 2. ICP-OES analysis to quantify the number of phosphonium ligands per gold nanoparticle. The 15 nm citrate-stabilized nanoparticles (left bar) possess an undetectable quantity of phosphorus, while the THPC AuNPs (middle bar) possess nearly 2700 ligands after a 2-day incubation. In order to determine if free citrate can displace the THPC ligand, 100 μM THPC was added to 15 nm citrate-stabilized AuNPs (2.3 nM) and allowed to incubate for five minutes, so that the particles transmitted a blue color. Next, 10 mM sodium citrate solution was added and the particles were subsequently washed five times by centrifugation. The similar ligand densities in the middle and right bars suggest that free citrate does not displace the strong-binding phosphorus ligands adsorbed to the gold surface. All of these measurements were performed in triplicate.



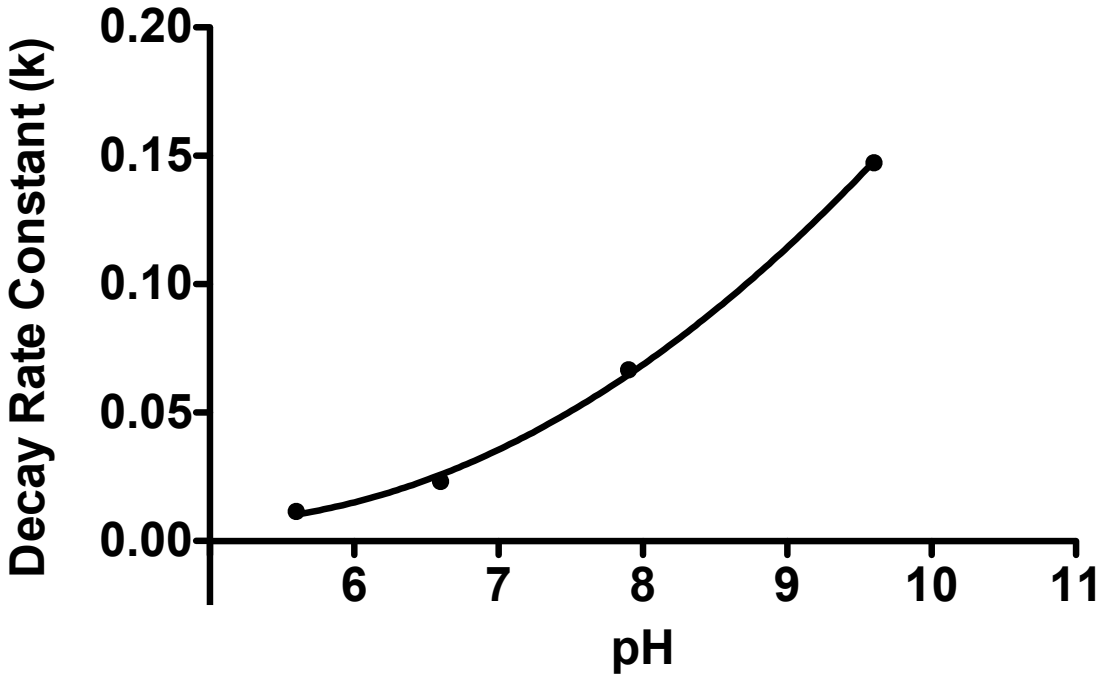
Supplemental Figure 3. Qualitative determination of THPC adsorption to the AuNP surface. a) UV-vis spectra and b) color images of washed THPC AuNPs and washed citrate AuNPs, respectively, after resuspending in CHES buffer. Upon incubating 100 μM THPC for 2 days with 15 nm citrate-stabilized AuNPs (2.3 nM), the particles were centrifuged for 45 minutes at 7400 rcf and resuspended in 1 mL 0.1 M CHES buffer containing 0.025% v/v Tween 20 (pH 9.0). This process was repeated additional times in order to acquire UV-vis spectra and capture images of washed THPC AuNPs and citrate AuNPs, respectively. The citrate AuNPs begin to aggregate upon washing, which is evident from the formation of an additional peak in the UV-vis spectrum and a red-to-purple color change in solution. As citrate anions are weakly adsorbed to the AuNP surface, the physical forces imposed by centrifugation and resuspension desorb the species from the particle surface, reducing stability. On the contrary, the THPC ligand is more strongly adsorbed to the particle surface, so that the particles can endure wash cycles.



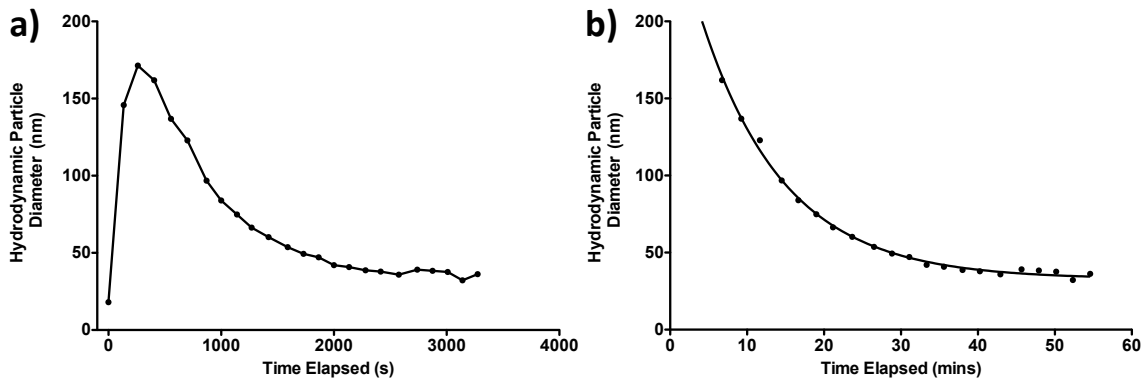
Supplemental Figure 4. Self-assembly and disassembly of THPC AuNPs upon the addition of HCl, NaCl, sodium citrate (Na Cit), and NaOH. a) Images of 15 nm citrate-stabilized AuNPs (2.3 nM) captured in 96 well plates. b) Images of gold nanoparticles 5 minutes after a 100 μM THPC addition (all 20 wells contain 100 μM THPC). It should also be mentioned that although many nanoparticle reactions are inconsistent, these reactions are extremely reproducible, as indicated by the nearly identical shades of blue colors transmitted in all 20 wells. c) Images captured after serial titrations with HCl, NaCl, Na Cit, and NaOH, respectively. The images were captured 5 minutes after salt addition. d) Images captured 18 hours after salt addition. e) UV-vis spectra of THPC AuNPs 30 minutes after adding 10 mM salt. f) UV-vis spectra of THPC AuNPs 24 hours after adding salt. The addition of HCl to THPC AuNPs quenches disassembly, while NaOH expedites THPC AuNP disassembly. This experiment suggests that NaCl does not play a significant role in THPC AuNP disassembly, while sodium citrate disassembles the particles at a slower rate than NaOH, but still faster than NaCl.



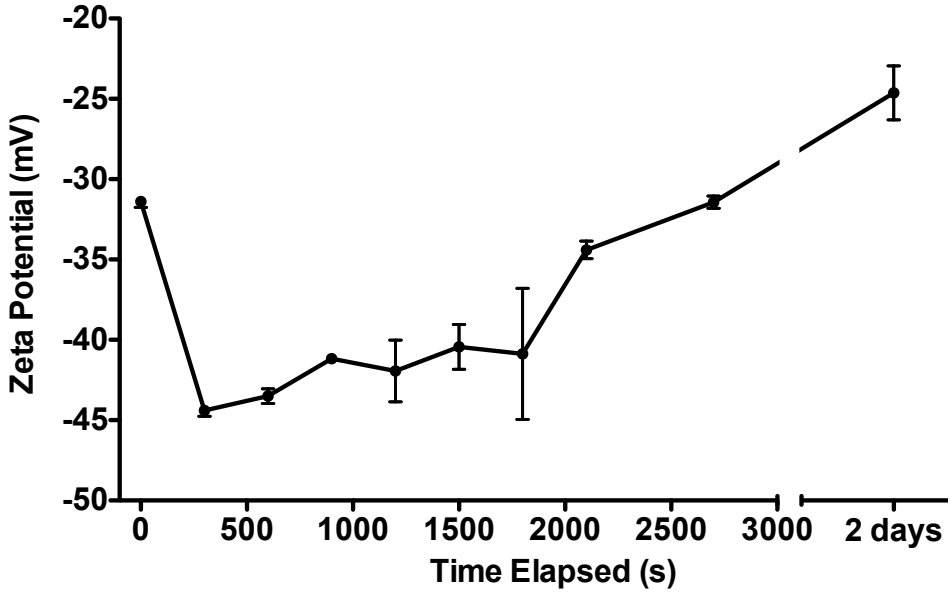
Supplemental Figure 5. UV-vis spectra of THPC AuNPs suspended in 2 mM tribasic sodium citrate at a) pH 3.8, b) pH 5.6, c) pH 6.6, d) pH 7.9, and e) pH 9.6. f) Plot of the absorbance ratio ($A_{680\ nm}/A_{520\ nm}$) over a 4-hour time interval. Citrate-stabilized AuNPs were centrifuged one time at 7400 rcf for 45 minutes, and the supernatant was removed. The particles were next resuspended in 2 mM sodium citrate (tribasic) at different pH values to generate particle concentrations of 2.3 nM (1 mL aliquot). When 100 μM THPC is added to the particles suspended in more basic conditions, the rate of disassembly increases. However, at acidic pH values below the pKa of the phosphonium ligand, disassembly does not occur, and the particles ultimately aggregate.



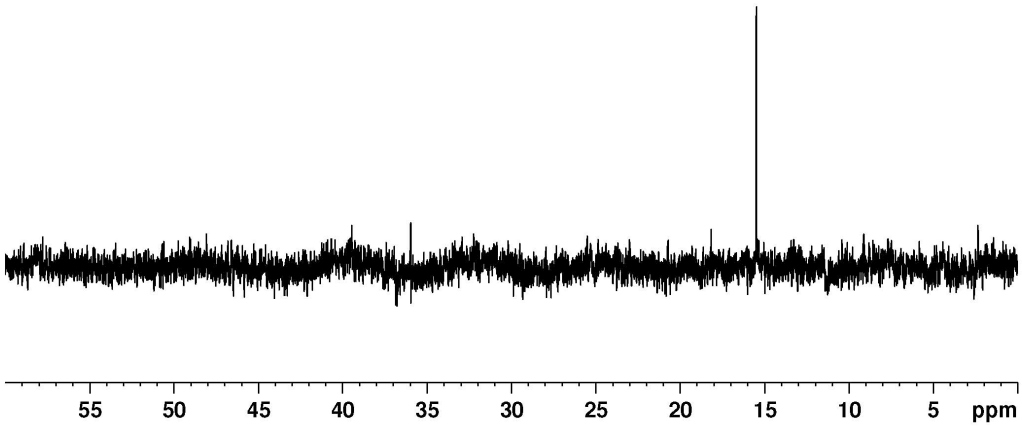
Supplemental Figure 6. Plot of the decay rate constant as a function of the pH of solution. The rate of decay or dissociation was calculated using the plasmon shifts ($A_{680\ nm}/A_{520\ nm}$) provided from the data in Figure S5. The rate of decay increases exponentially as the pH of the solution increases. When the particles are suspended in a solution possessing a pH value greater than 10, no color change is evident after THPC addition, and the particles remain red.



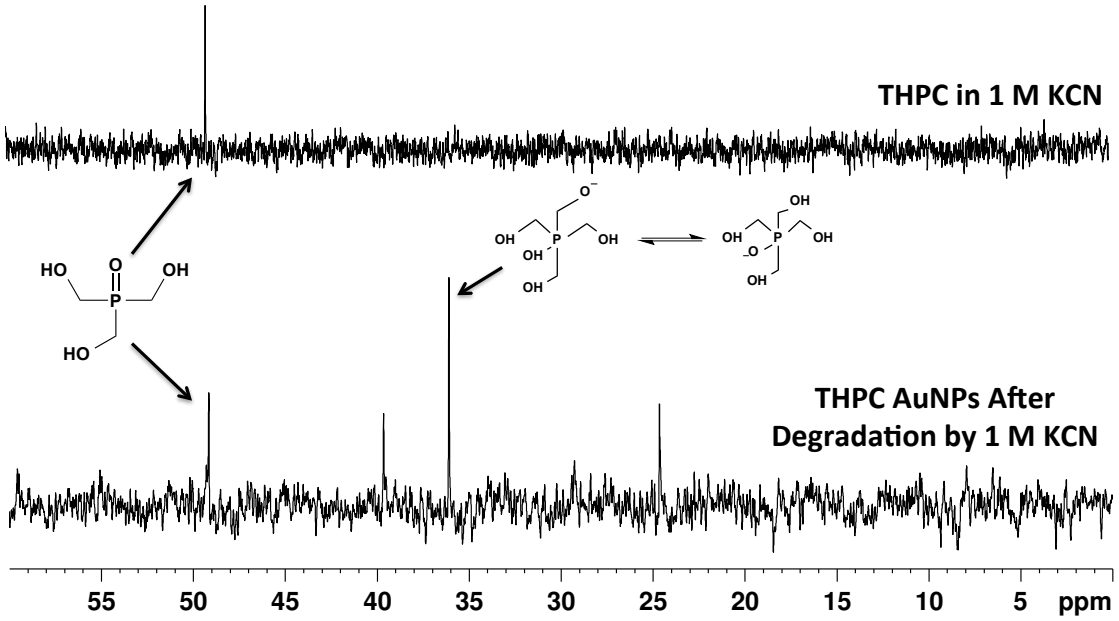
Supplemental Figure 7. Dynamic light scattering (DLS) of 15 nm citrate AuNPs upon addition of THPC. In a glass cuvette with a square opening, 100 μM THPC was added to 1 mL of a 2.3 nM suspension of AuNPs. The hydrodynamic diameter of the particles was recorded at regular time intervals as the reaction progressed. a) Plot of hydrodynamic particle diameter upon exposure to THPC, highlighting that the particles immediately form assemblies. The particle chains begin dissociating minutes after addition of THPC, which correlates with the time required for the UV-vis spectra to begin to blueshift. b) The THPC AuNPs fit a decay curve, which is similar to the generated decay curves monitoring the plasmon bands in the UV-vis spectra.



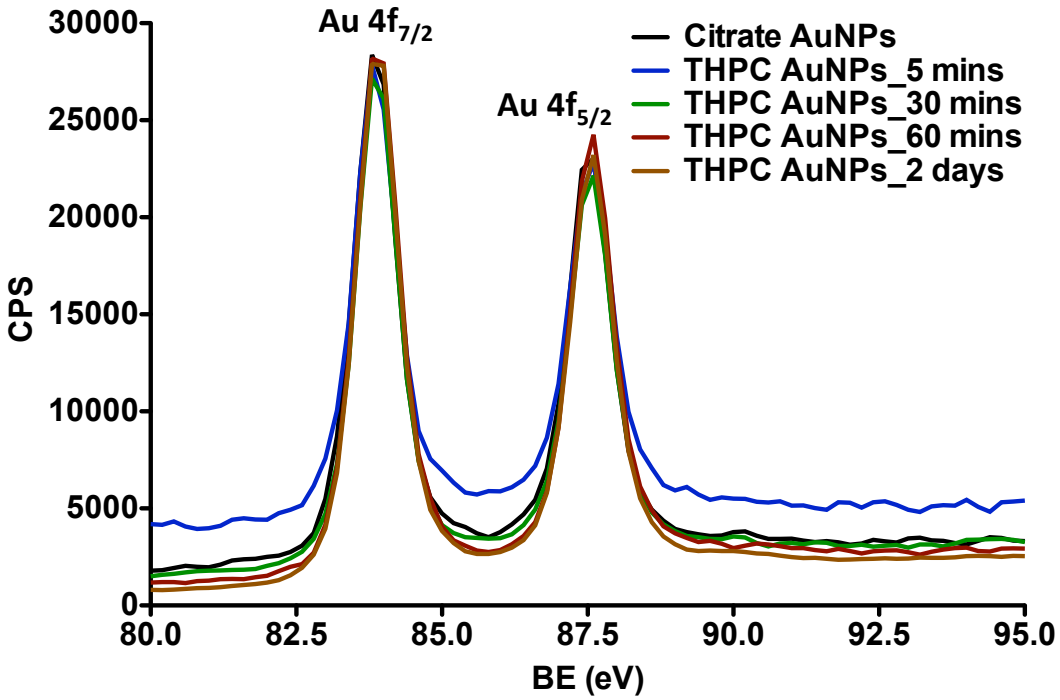
Supplemental Figure 8. Zeta potential of 15 nm AuNPs upon incubation with THPC. In 10 separate 1.5 mL Eppendorf tubes, 100 μM THPC was added to a 2.3 nM suspension (1.0 mL) of 15 nm citrate AuNPs simultaneously. Each measurement was performed with a fresh particle suspension at different time points. The zeta potential at t = 0 is representative of 15 nm citrate AuNPs. The zeta potential increases as a function of incubation time, moving towards more positive values as the assemblies begin to dissociate. When the particles achieve monodispersity after two days, the final zeta potential value is slightly more positive than citrate AuNPs, which correlates with a negatively charged species adsorbed to the surface of the particle. Since citrate is more negative than THPOH, the final zeta potential after two days correlates with an adsorbed THPOH species.



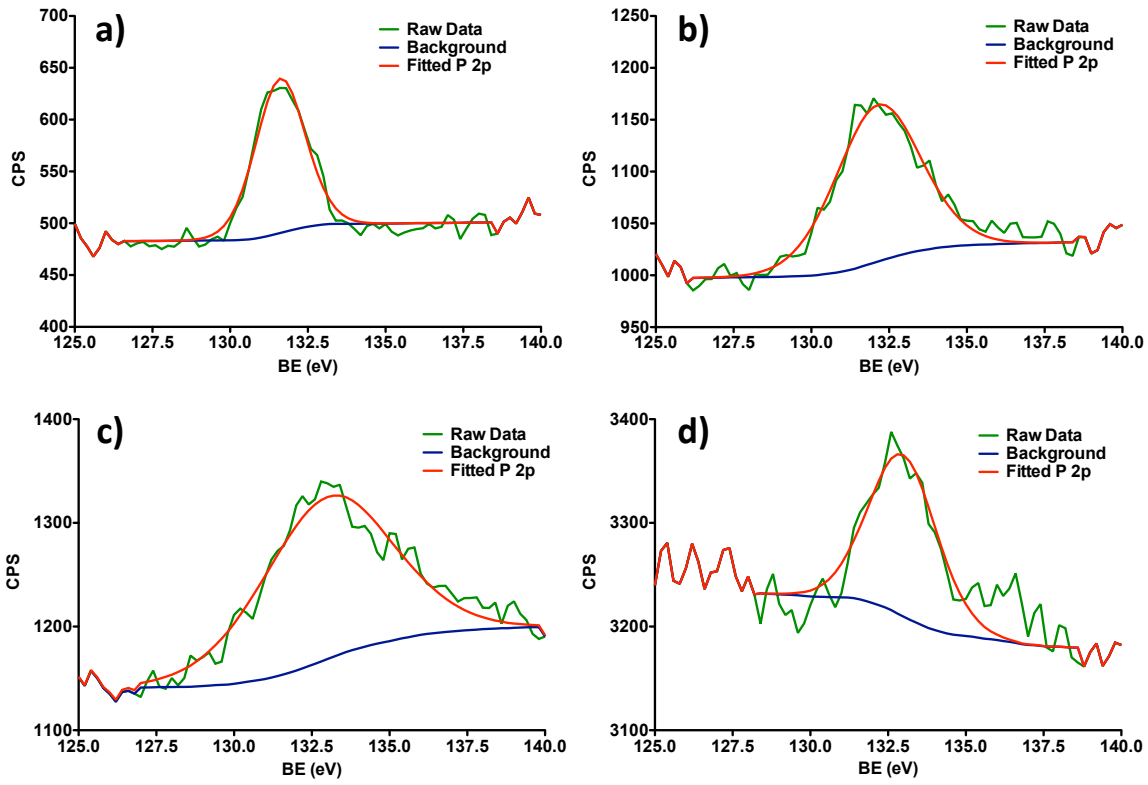
Supplemental Figure 9. ^{31}P NMR spectrum of THPC AuNPs (100 nM) stored in basic buffer (pH 9.0). Citrate AuNPs (2.3 nM, 10 mLs) were first reacted with 100 μM THPC and allowed to incubate undisturbed for 48 hours. Afterwards, the particles were centrifuged at 7400 rcf for 45 minutes (10 1 mL aliquots) and the supernatant was removed. Next, each aliquot was resuspended in 100 μL 0.1 M CHES with 0.025% Tween 20 (pH 9.0). Afterwards, each aliquot was combined into one aliquot, and the centrifugation process was repeated. After removing the supernatant, particles were resuspended in 180 μLs CHES buffer, concentrating the particles by a factor of approximately 50. After storing at room temperature for 24 hours, this solution was added to a 3 mM NMR tube for ^{31}P spectral acquisition. No peak for THPO (δ, +49 ppm) is observed, suggesting that THPC cannot fully oxidize to THPO on-particle.



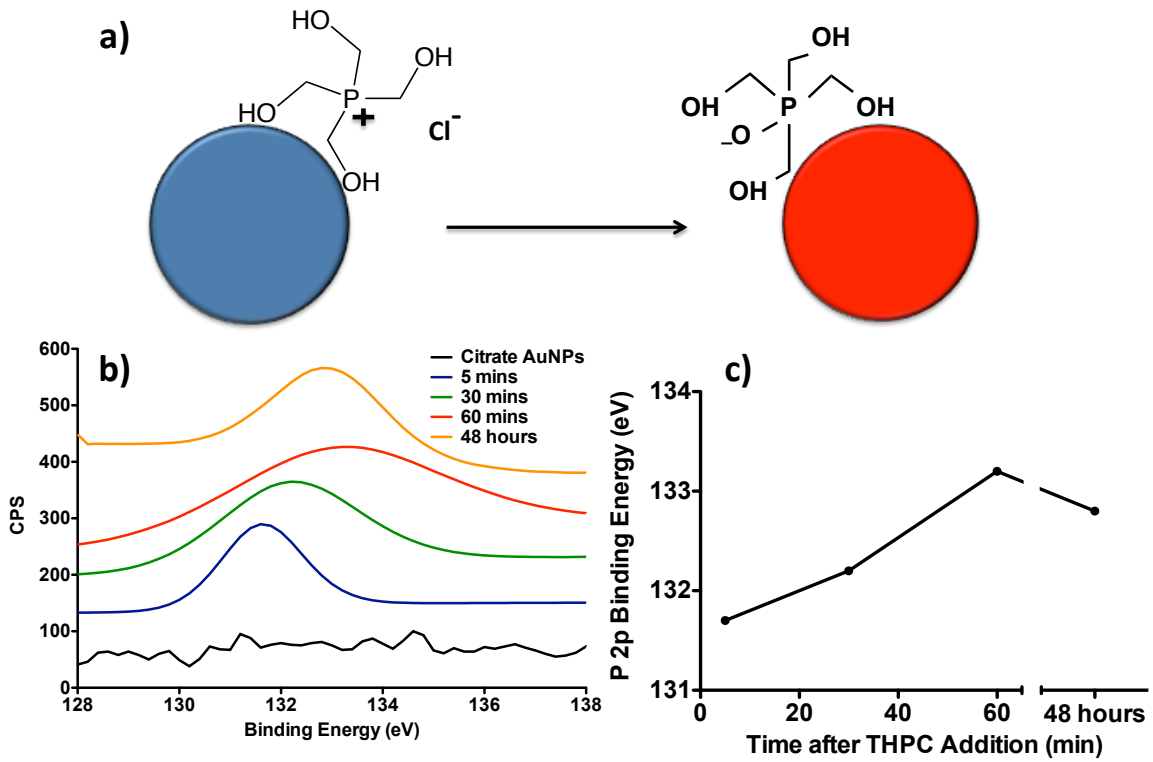
Supplemental Figure 10. ^{31}P NMR spectra of concentrated THPC and THPC AuNPs after chemical degradation by 1 M potassium cyanide (KCN). The THPC ligand is converted entirely to THPO (δ, +49 ppm) upon exposure to KCN. Following chemical digestion of the THPC AuNPs, four peaks are observed: THPO (δ, +49 ppm), THPOH (δ, +36 ppm), and two unidentified peaks (δ, +39.6 ppm and +24.6 ppm) resulting from the chemical degradation process. These results are consistent with the proposed mechanism of the particle preventing complete oxidation of THPC to THPO, however chemical degradation introduces changes to the ^{31}P spectrum that are not observed in on-particle NMR.



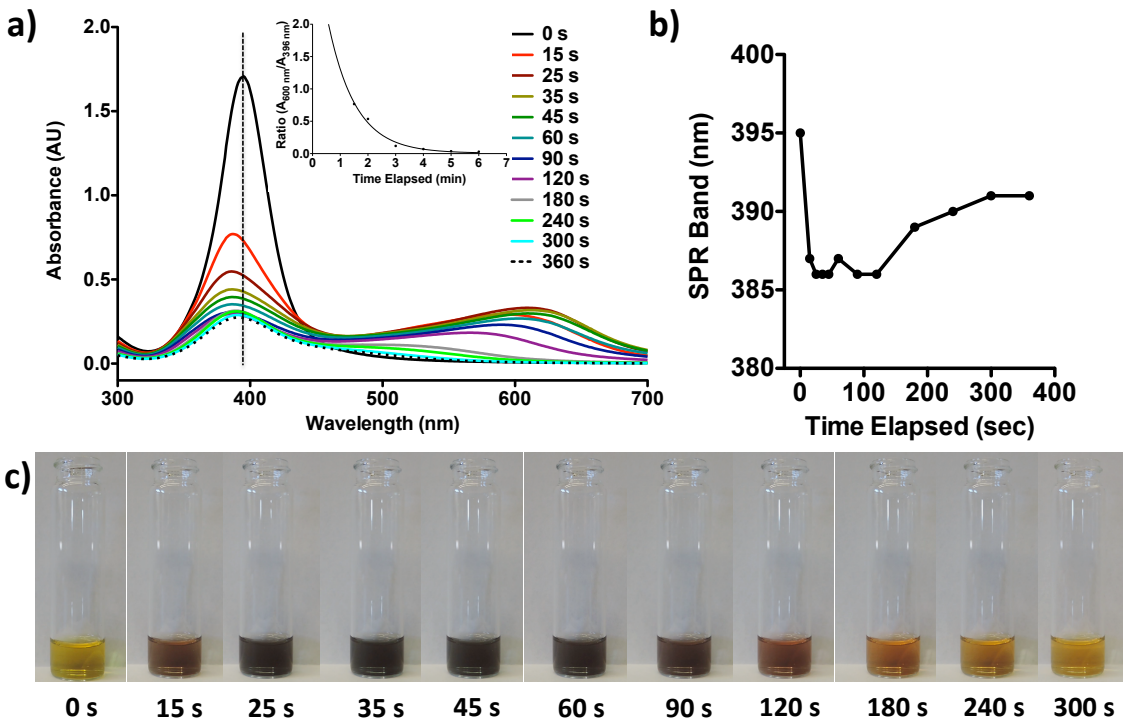
Supplemental Figure 11. Raw XPS spectral data of the Au 4f peaks of THPC AuNPs after different reaction time intervals with THPC. The Au $4f_{7/2}$ peak for all samples possess binding energies of 83.8 eV, while the Au $4f_{5/2}$ peak for each sample possesses binding energies of 87.6 eV. These peaks are consistent with Au^0, and demonstrate that the instrument is calibrated. Additionally, the THPC does not induce chemical changes to the gold nanoparticle surface.



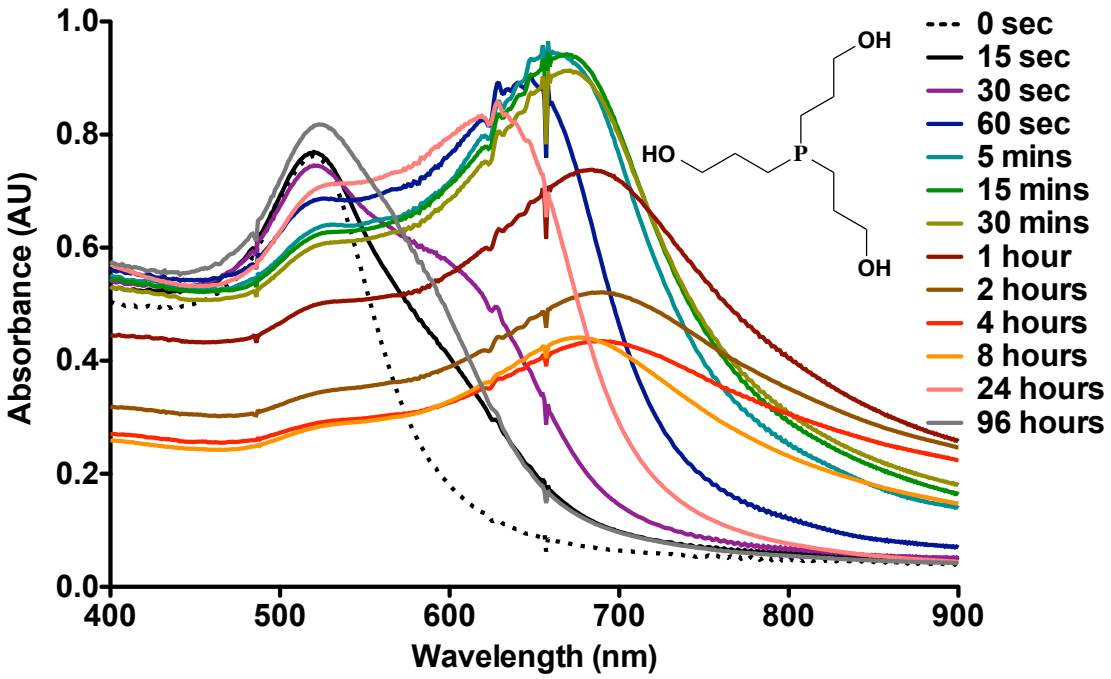
Supplemental Figure 12. Raw data, background modeling, and fitted P 2p XPS spectra of THPC AuNPs after a) 5 minutes, b) 30 minutes, c) 60 minutes, and d) 48 hours of THPC incubation.



Supplemental Figure 13. Evidence to support the THPC oxidation to pentavalent THPC hydroxide. a) Hypothesized schematic of AuNP disassembly. As THPC-functionalized particles assemble, they form linear chains and a blue color is observed. As the oxidation reaction occurs at the particle's surface, the particles disassemble into more monodisperse entities because there is too much negative surface charge to maintain the assembly. b) Fitted P 2p XPS spectra as a function of time elapsed after THPC addition. Since no significant phosphorus signal is observed for citrate AuNPs, no fit is necessary. c) The P 2p binding energy increases relative to THPC incubation time, suggesting the presence of a pentavalent phosphorus species adsorbed to the particles.



Supplemental Figure 14. Rapid assembly and disassembly of THPC AgNPs. a) UV-vis of 1 mM THPC added to 20 nm citrate-stabilized AgNPs (400 pM). Note the dotted black line centered on the localized surface plasmon resonance band (SPR) of citrate-stabilized AgNPs, highlighting the blue shift after initial THPC addition. Inset is the rate of decay versus time by monitoring the ratio of plasmon bands ($A_{600\ nm}/A_{396\ nm}$). The rate constant (k = 1.015) for AgNPs is significantly greater than for AuNPs, suggesting in this application, Ag has greater catalytic activity. b) Plot of the primary silver localized SPR band over time and c) THPC AgNP color changes over a five-minute timespan. The initial blue shift of the localized SPR band at 395 nm can be attributed by the vast presence of charge carriers encompassing the nanoparticles, as the oxidation reaction progresses. After approximately two minutes, the primary localized SPR band of the THPC AgNPs begins to redshift. The UV-vis spectra and images of the THPC AgNPs after 6 minutes are not shown, as they begin to lose stability and crash out of solution.



Supplemental Figure 15. Slow reversible assembly and disassembly of THPP AuNPs at pH 6.3. To a 2.3 nM solution of 15 nm citrate-stabilized AuNPs (pH 6.3), a 100 μM solution of THPP was added and UV-vis spectral kinetics were monitored over 96 hours.