Supporting Information

Nanoparticle Film Assemblies as Platforms for Electrochemical Biosensing – Factors Affecting Amperometric Signal Enhancement of Hydrogen Peroxide

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Contents:

► TEM image and UV-Vis spectra for citrate-stabilized gold nanoparticles (CS-NPs)

► Cyclic voltammetry of potassium ferricyanide electrochemical probe at various stages of the PLL-linked CS-NP film assembly.

► Amperometric I-t curves for peroxide injections at PLL-linked CS-NP film assemblies at MHDA and MHA SAMs.

► Detailed synthetic procedures and characterization for alternative gold nanoparticles (NPs), including TEM, UV-Vis, and some NMR characterization, as well as examples of film growth tracking (electrochemical and spectroscopic) for the following NPs:
  · Thioctic-acid stabilized nanoparticles (TAS-NPs)
  · Alkanethiolate or monolayer-protected clusters (MPCs)
  · 3-mercaptopropionic acid stabilized nanoparticles (MPA-NPs)
  · Cholate-stabilized nanoparticles (CHO-NPs)
  · Poly(N-vinyl-2-pyrrolidone) nanoparticles (PVP-NPs)
  · 4-mercaptobenzoic acid nanoparticles (MBA-NPs)

► Amperometric I-t curves for peroxide injections at PLL-linked CS-NP and TAS-NP film assemblies with a MHA SAM modified electrode base

► Permeability results for PLL-linked CS-NP film assemblies with comparison to polyurethane outer layer.

► Amperometric I-t curves showing effect of polyurethane outer layer on signal-to-noise when employing NP film assemblies as components of biosensing schemes (glucose sensing example)

► Preliminary chronoamperometry results.

► Preliminary results with PFA-coated gold “needle” or wire electrode and photograph.

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Figure SM-1. Example TEM imaging and UV-Vis spectrum of CS-NPs.
Figure SM-2. (A) Cyclic voltammetry and (B) peak splitting ($\Delta E_p$) tracking of 5 mM potassium ferricyanide (Fe(CN)$_6^{3-4}$) at a gold electrode with various modifications during PLL-linked CS-NP film assembly including, bare gold, MUA SAM, the first PLL layer, the first exposure to CS-NPs, and the second exposure to CS-NPs (after a second exposure to PLL) – see experimental details for film growth procedure. Voltammetry was recorded at 100 mV/sec in 0.5 M KCl.
Figure SM-3. Representative amperometric $I$-$t$ curves during 10 µL injections (black arrows) of 0.25 M H$_2$O$_2$ to a stirred 4.4 mM phosphate buffer (pH=7) solution containing gold electrodes modified with (a) PLL-linked CS-NP film assembly at a SAM or (b) a SAM where the underlying SAM is (A) MHDA and (B) MHA. Each injection (black arrows) of H$_2$O$_2$ resulted in a 0.1 mM H$_2$O$_2$ increase of the bulk solution 4.4 mM potassium phosphate buffer (pH = 7).
Synthetic Details and Characterization of Alternative Gold Nanoparticles Embedded in PLL-linked PEM film assemblies:

**Hexanethiolate Monolayer Protected Clusters (MPCs).** MPCs were synthesized, characterized, and subsequently modified via place-exchange reactions with 11-mercaptooundecanoic acid as has been previously reported. Characterization of the MPCs used for this study (NMR, UV-Vis, and TEM analysis) were consistent with these prior reports and are not included here. Three different average gold core diameters (1.6, 2.0, 2.85 nm) of MPCs were synthesized by varying experimental conditions (temperature, thiol-to-gold ratio, and speed of reductant addition) as has been previously reported.

**Cholate-Stabilized NPs (CHO-NPs).** Cholate-stabilized gold nanoparticles were synthesized according to a procedure published by Huang et al. Briefly, to 10.0 mL of a 150 mM sodium cholate solution (aq), stored for at least 3 hours at ~20°C, 10.0 mL of a 1.0 mM HAuCl₄ solution (aq) was added and intensely vortexed for 5 minutes. The mixture was then stored in the dark and uninterrupted at ~20°C for 5 days during which time it changed color from very pale yellow/colorless to pale reddish purple. CHO-NPs were characterized with UV-Vis spectroscopy and TEM imaging (Supporting Information). The average core diameter for CHO-NPs is reported as 13.4 ± 2.0 nm.

**Poly(N-vinyl-2-pyrrolidone) nanoparticles (PVP-NPs)** Polyvinylpyrrolidone or PVP-stabilized gold NPs were created with an adaptation to procedures by Xia and Silvert et al. designed for PVP-stabilized Ag NPs. For PVP-NPs with gold cores, 100.0 mL of 0.36 mM HAuCl₄ (aq) was heated to reflux in a 500 mL round bottom flask (heating mantle and water condenser) before 20.0 mL of a 3.63 mM PVP (Mₖ = 55,000 g/mol) in ethylene glycol was quickly added. A color transition, from pale yellow to purple, was observed and the solution was allowed to reflux for an additional 10 minutes before being removed from heat and cooled to room temperature. The PVP-NPs were centrifuged for 30 minutes at 16,000 rpm and 10°C, causing a pellet to form. The supernatant was discarded and the pellet was re-suspended in an equal amount of ultra-pure H₂O (18.2 MΩ). PVP-NPs were characterized via UV-Vis spectroscopy and TEM imaging (Supporting Information). The range of average core diameter for gold PVP-NPs is 6-10 nm.

**Thioctic Acid Stabilized NPs (TAS-NPs).** TAS-NPs were created with CS-NPs as a precursor material in a place-exchange reaction with thioctic acid (lipoic acid). This procedure has been described in detail in a previous report by our lab as well as by other laboratories. In brief, immediately following the preparation of CS-NPs, the pH of CS-NP aqueous solution was adjusted to 11.00 using aliquots of 0.5 M NaOH. Thiocetic acid was added to the solution in a 1:1 molar ratio with the HAuCl₄ used to create the CS-NP precursors. In a typical procedure, for example, if 0.0339 g of HAuCl₄ were used to make a 100 mL batch of CS-NPs, 0.0206 g of thioctic acid would be added. The mixture was stirred in the dark overnight before centrifugation for 20-30 minutes at 15,900 rpm and 10°C. The supernatant was decanted with the remaining TAS-NPs re-suspended in ultra-pure H₂O (18.2 MΩ) and stored in the dark until further use. Characterization, including UV-Vis and TEM analysis, was consistent with our prior results in published reports (average core diameter of ~10.5 nm) and are not included as part of the current study.
Synthetic Details and Characterization of Alternative Gold Nanoparticles Embedded in PLL-linked PEM film assemblies (continued):

3-Mercaptopropionic Acid-Stabilized NPs (MPA-NPs)  As in the synthesis of TAS-NPs, MPA NPs were made from CS-NP precursors according to a procedure published by Ren and co-workers. After the combination of 1 mM HAuCl4 with 38.8 mM sodium citrate, described in the Experimental Details section for synthesis of CS-NPs, the CS-NP solution (100 mL) was subsequently treated further with the dropwise addition of 30 mL of 5 mM MPA solution to ensure thiolate ligand concentration would displace the adsorbed citrate molecules on the NPs (0.23 MPA / Au atom ratio). Excess or unreacted MPA molecules as well as displaced citrate molecules were removed using membrane dialysis (Spectrum Labs, cellulose ester membranes, MWCO: 8-10K) with the dialysis tubes soaking for 24 hours in slowly stirred, ultra-pure H2O (18.2 MΩ) that was renewed four times during the purification. Purified MPA NPs were stored in the dark until further use and characterized with NMR, UV-Vis spectroscopy and TEM imaging (Supporting Information). The average core diameter for MPA-NPs is ~15-17 nm.43

4-Mercaptobenzoic acid NPs (MBA-NPs). Following an adopted procedure by Kornberg et al., 3.94 mL UP H2O, 5.64 mL methanol, 1.286 mL of aqueous 28 mM HAuCl4 solution, 1.134 mL of 95 mM MBA (methanol) were combined in a 50 mL Falcon centrifuge tube (12 mL total volume of mixture solution). To this mixture 0.1446 g NaOH were added and the mixture was lightly agitated (vortex) for 1 hour at room temperature before 0.48 mL of fresh 150 mM NaBH4 solution was added, causing an immediate color change to black. The reaction was allowed to proceed for 2 additional hours, after which time the MBA NPs had precipitated. The supernatant was discarded and the product was allowed to dry in air overnight followed by ten minutes of drying under a stream of N2 gas. The pellet precipitate was re-dissolved in UP water and the MBA-NPs were characterized with UV-Vis spectroscopy and TEM imaging (Supporting Information). The average core diameter for MBA-NPs is reported as approximately 1.3 nm.48

† Note: Due to their small size TEM imaging of MBA was accomplished by dropcasting samples on Formvar microscopy grids modified with 0.1% wt/vol PLL (rinsed with drops of purified water).48
Figure SM-4. Typical UV-Vis spectra of poly-L-lysine (PLL) linked MPA-NP films during assembly procedure. * PLL-linked CS-NP films must be kept wet at all times as they are very susceptible to aggregation if exposed to air for any time.\textsuperscript{28-29} Note: Electrochemical tracking of PLL-linked CS-NP films is shown as part of Figure 1.
Figure SM-5. Example TEM imaging and UV-Vis spectrum of CHO-NPs (Au).
Figure SM-6. Cyclic voltammetry tracking film growth of PLL-linked CHO-NP assemblies showing typical increasing double-layer capacitance with increasing exposures and incorporation of CHO-NPs into film assembly. Voltammetry was recorded at 100 mV/sec in 4.4 mM potassium phosphate buffer (pH = 7). Note: Growth for CHO-NP films was clearly more sluggish relative to the other NP films tested.
Figure SM-7. Examples TEM imaging and UV-Vis spectrum of PVP-NPs (Au).
Figure SM-8. Cyclic voltammetry tracking film growth of PLL-linked PVP-NP assemblies showing typical increasing double-layer capacitance with increasing exposures and incorporation of PVP-NPs into film assembly. Voltammetry was recorded at 100 mV/sec in 4.4 mM potassium phosphate buffer (pH = 7). Note: Growth for PVP-NP films was obvious in initial exposures but plateaued in subsequent exposures.
**Figure SM-9.** Cyclic voltammetry tracking film growth of PLL-linked TAS-NP assemblies showing typical increasing double-layer capacitance with increasing exposures and incorporation of TAS-NPs into film assembly. Voltammetry was recorded at 100 mV/sec in 4.4 mM potassium phosphate buffer (pH = 7).
Figure SM-10. Example TEM imaging and UV-Vis spectrum of MPA-NPs.
Figure SM-11. (A) Cyclic voltammetry tracking film growth of PLL-linked MPA-NP assemblies showing typical increasing double-layer capacitance with increasing exposures and incorporation of MPA-NPs into film assembly. Voltammetry was recorded at 100 mV/sec in 4.4 mM potassium phosphate buffer (pH = 7); (B) Typical UV-Vis spectra of PLL-linked MPA-NP films during assembly procedure.
Figure SM-12. Example TEM imaging and UV-Vis spectrum of MBA-NPs (Au).

**Note:** Based on literature reports on MBA-NPs, the estimated *average* diameter of these polydisperse NPs is ~1.3 nm, at or near our TEM instrument’s limitation.
Figure SM-13. Cyclic voltammetry tracking film growth of PLL-linked MBA-NP assemblies showing typical increasing double-layer capacitance with increasing exposures and incorporation of MBA-NPs into film assembly. Voltammetry was recorded at 100 mV/sec in 4.4 mM potassium phosphate buffer (pH = 7).
Figure SM-14. Representative amperometric I-t curves during 10 µL injections (black arrows) of 0.25 M H₂O₂ to a stirred 4.4 mM phosphate buffer (pH=7) solution containing gold electrodes modified with (a) PLL-linked CS-NP film assembly at a MHA SAM; (b) PLL-linked TAS-NP film assembly at a MHA SAM; and (c) at a MHA SAM; Each injection (black arrows) of H₂O₂ resulted in a 0.1 mM H₂O₂ increase of the bulk solution 4.4 mM potassium phosphate buffer (pH = 7).
Figure SM-15. (A) Example amperometric $I$-$t$ curves of single 10 $\mu$L H$_2$O$_2$ injections (0.25 M) at a bare gold electrode, a MUA-SAM modified gold electrode, a standard PLL-linked CS-NP film, (CS-NP/PLL)$_3$/MUA/Au), and a standard PLL-linked CS-NP with a polyurethane coating. (B) Corresponding permeability index for different film layers. Each injection (black arrows) of H$_2$O$_2$ resulted in 0.1 mM H$_2$O$_2$ bulk solution of 4.4 mM potassium phosphate buffer (pH = 7).
Figure SM-16. Example amperometric $I$-$t$ curves of single 25 μL injections (black arrows) of 1 M glucose at gold electrodes modified with MPTMS sol-gel doped with MPC gold nanoparticles and glucose oxidase (GOx) enzyme with (a) and without (b) an outer polyeurathane (PU) coating. The outer PU layer adds both selectivity and improved signal-to-noise ratios (i.e., dampening background noise) to the nanoparticle film assemblies as part of a biosensing scheme. Each injection (black arrows) of glucose resulted in a 1 mM increase in glucose concentration of the bulk solution of 4.4 mM potassium phosphate buffer (pH = 7).
Figure SM-17. Example Cottrell plots for the forward step of double-step chronoamperometry (CA) experiments on 0.1 mM H₂O₂ solutions at bare gold, MUA SAM modified gold, and a PLL-linked CS-NP film assemblies. Solution conditions are 4.4 mM potassium phosphate buffer (pH = 7). CA parameters include an initial potential of 0.4 V and pulse widths of 250 ms for a forward step of 0.75 V and a reverse step of 0.1 V (vs. Ag/AgCl reference).
Figure SM-18. (A) Photograph of PFA-coated gold wire, or “needle” electrode, used to assess amperometric amplification capability of CS-NP/poly-L-lysine film assemblies. The bare gold WE end of the wire was cleaned with cyclic voltammetry (0.1 M H₂SO₄) prior to described modifications; (B) Representative amperometric I-t curves during a single H₂O₂ injection (black arrow; 100 s) to an unstirred, 4.4 mM phosphate buffer solution containing a gold “wire” electrodes that is (a) bare or modified with (b) a MUA SAM or (c) PLL-linked CS-NP film assembly at a MUA SAM (Scheme I); Note: The current is offset displayed to facilitate direct comparison of the amperometric responses to H₂O₂ at each system. The original data is shown in the Supporting Information. The 10 μL injection of 0.25 M H₂O₂ results in a concentration of 0.1 mM H₂O₂ in the unstirred bulk solution.
Figure SM-19. Additional representative amperometric $i$-$t$ curves during a single 10 μL H$_2$O$_2$ (0.25 M) injection (black arrow, 100 sec) to an *unstirred* solution (4.4 mM KPB, 25 mL) containing a bare and MUA SAM or PLL-linked CS-NP film assembly modifications of the wire electrode. **Inset:** Expansion of $i$-$T$ curves for the bare ware and MUA SAM modified wire.
Figure SM-20. Additional representative amperometric I-t curves during a single 10 μL H₂O₂ (0.25 M) injection (black arrow, 100 sec) to an unstirred solution (4.4 mM KPb, 25 mL) containing a bare and MUA SAM or PLL-linked CS-NP film assembly modifications of the wire electrode. Inset: Expansion of i-T curves for the bare ware and MUA SAM modified wire. Note: In addition to enhancing the amperometric H₂O₂ signal, the CS-NP modified wire electrode also exhibits a substantial increase in capacitive charging current (see Fig. SM-18) as well as behavior suggestive of a transition from steady-state microelectrode activity at a bare wire to more diffusional-based, macroelectrode electrochemical activity at the CS-NP modified wire.