

Polymerization amplified detection for nanoparticle-based biosensing

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SUPPORTING INFORMATION

Experimental Procedures

Materials: The 2-(*N*-morpholino)ethanesulfonic acid (MES), acac, chelex resin, metals, acrylic acid, D-glucose, HRP, GOx, catalase, and all other reagents were all purchased from Sigma Aldrich (Dorset, UK). The APMA was purchased from Polysciences (Eppelheim, Germany). The 20 and 5 nm AuNPs were purchased from BBI Life Sciences (Cardiff, UK) and the DNA (TAGGACTTACGC-SH) was purchased from Integrated DNA Technologies (IDT). The peptide was made on the solid phase and purified using prep-HPLC. All solutions were previously treated with Chelex resin for minimum of one hour to remove all heavy metals that might generate free radicals via the Fenton reaction with the hydrogen peroxide.

Dodecyl-trithiocarbonate propanoic acid RAFT agent: Dodecanethiol (3g, 15 mmol) was dissolved in acetone (60 ml) and a solution of potassium hydroxide (1.75g, 31 mmol) in water (10 ml) was added. Carbon disulfide (2.4 ml, 50 mmol) was added dropwise and the orange solution was stirred for 2.5 h at room temperature. To this, 2-bromo 2-methyl propionic acid (3.5g, 60 mmol) in acetone (5 ml) was added dropwise, which was then stirred overnight at room temperature. After removal of most of the acetone, water (50 ml) was added and the solution was acidified with HCl to pH 4. The aqueous solution was extracted with dichloromethane (2 x 75 ml). The combined organic fractions were then washed with acidified water (3 x 50 ml), brine (1 x 50 ml), and dried over MgSO₄. The product was purified over a silica column with a mixture of ethyl acetate / hexane (1:9) as the eluent to yield the acid RAFT agent as a yellow solid. ¹H-NMR (400 MHz, CDCl₃) δ (ppm): 3.28 (t, *J* = 7.6 Hz, 2H), 1.73 (s, 6H), 1.67 (quin, *J* = 7.4 Hz, 2H), 1.45 – 1.34 (m, 2H), 1.34 – 1.17 (m, 16H), 0.88 (t, *J* = 6.8 Hz, 3H).

General polymerization protocol for RAFT: RAFT agent, monomer and AIBN were dissolved in methanol in the desired ratios, and the oxygen was removed by bubbling argon through the solution for 10 min. Polymerisations were carried out at monomer concentrations of 0.35 M and 1 M for acrylic acid and APMA respectively. Reactions were performed at 65°C, and conversion was monitored by ¹H-NMR and used to estimate the molecular weight of the polymer. The polymers were purified by precipitation from diethyl ether. In the case of the acrylic acid polymers, the RAFT group was removed to expose a thiol in order to provide an anchor with which to functionalise the gold nanoparticles. Each polymer was dissolved in tetrahydrofuran (THF) and reacted with isopropyl amine with stirring overnight, and then precipitated from diethyl ether to purify.

Table S1. Reaction conditions and characterization data for polymers prepared by RAFT polymerization

Entry	Monomer	[RAFT]:[M]:[AIBN]	Time (h)	Conversion	DP	MW (Da)
1	AA	1 : 100 : 0.1	1.5	45%	45	3,605
2	AA	1 : 100 : 0.1	5.5	87%	87	6,905
4	APMA	1 : 30 : 0.2	16	90%	27	5,170
3	APMA	1 : 60 : 0.2	16	58%	35	6,560
4	APMA	1 : 120 : 0.2	16	53%	63	11,650
5	APMA	1 : 240 : 0.2	16	36%	87	15,790

Polymerization by HRP: APMA (5 M), acac (120 mM), hydrogen peroxide (80 mM) and HRP (16 mg/ml) in buffer was degassed by bubbling argon for at least 5 min then sealed and allowed to react for up to 24 h. At selected time points the polymer conversion was determined by ^1H -NMR in MeOD. The neat solution was then diluted in water and added directly to citrate capped AuNPs.

AuNP surface functionalization: Gold nanoparticles (AuNPs) were functionalised by addition of TAGGACTTACGC-SH DNA (100 μl , 15 μM in H_2O), C(GEP)₃ (100 μl , X μM in H_2O), or poly(acrylic acid) (100 μl , X μM in H_2O) to 1 ml of 20 nm AuNPs in H_2O (pH 9). The salt concentration was then gradually increased to 250 mM NaCl by addition of a concentrated brine solution (5 x 20 μl 1M NaCl over 2h, and then 3 x 10 μl 5M NaCl over 1h). The nanoparticles were left at 4°C overnight, washed with PBS using centrifugation (10,000 rpm, 10 min, x4) and re-suspended in PBS.

GOx model: The concentration of oxygen vs distance from the surface of the solution was modelled as a function of time and GOx concentration. The GOx was assumed to be homogeneously dissolved in solution, and the enzyme kinetics were assumed to be independent of glucose concentration. The rate of O₂ consumption by GOx was calculated from the Michaelis–Menten equation:

$$\frac{d[O_2]}{dt} = \frac{2k_{cat}[GOx][O_2]}{k_m + [O_2]}$$

Where $k_{cat} = 920 \text{ s}^{-1}$, $k_m = 3 \times 10^{-5} \text{ mol.cm}^{-3}$ and $[GOx] = 0.5, 1, \text{ or } 2 \times 10^{-10} \text{ mol.cm}^{-3}$

The solution was divided into 40 increments of $\Delta x = 0.025 \text{ cm}$ from the surface of the solution. The $[O_2]$ in the top fraction of the solution (x_0) was fixed at $2.4 \times 10^{-7} \text{ mol.cm}^{-3}$ (the concentration of dissolved O₂ in water under standard conditions) and standard diffusion kinetics at $T = 303\text{K}$ were assumed through the solution according to the following equations:

$$\frac{d[O_2]}{dt} = D_{O_2/H_2O} \frac{\partial^2 [O_2]}{\partial^2 x} \quad \text{where } D_{O_2/H_2O} = 7.4 \times 10^{-8} \frac{T(\varphi_{H_2O} \cdot M_{H_2O})^{0.5}}{\mu (V_{O_2})^{0.6}}$$

Where ψ (an ‘association’ parameter for water) = 2.26, M_{H_2O} (the molar volume of water) = 18 g.mol^{-1} , $V_{O_2} = 25.6 \text{ cm}^3.\text{g}^{-1}$, and μ (the viscosity at 30°C) = 0.819 cps.

Based on this, for any given x_i , the rate of O₂ consumption was determined by the sum of the GOx activity and diffusion out to the next x increment (x_{i+1}). The rate of O₂ in was determined only by diffusion in from the increment above (x_{i-1}), as is summarized in the equations below.

$$\left(\frac{d[O_2]}{dt}\right)_{out} = D_{O_2/H_2O} \frac{|[O_2]_{x=i} - [O_2]_{x=i+1}|}{(\Delta x)^2} + \frac{2k_{cat}[GOx][O_2]_{x=i}}{k_m + [O_2]_{x=i}}$$

$$\left(\frac{d[O_2]}{dt}\right)_{in} = D_{O_2/H_2O} \frac{|[O_2]_{x=i} - [O_2]_{x=i-1}|}{(\Delta x)^2}$$

These equations were used to calculate the concentration of O₂ at each x increment vs time according to the following equation:

$$[O_2]_{t=j+1} = [O_2]_{t=j} + \Delta t \left[\left(\frac{d[O_2]}{dt}\right)_{in} - \left(\frac{d[O_2]}{dt}\right)_{out} \right]$$

General assay format, data collection and analysis: Polymerization reactions were performed in light-protected 96-well plates containing 300 µl of APMA (0.25 M), glucose oxidase (200 nM), D-glucose (100 mM) and acetylacetone (0.4 mM) in a MES buffer (20 mM, pH 7.5) at 30°C for 30 min. To stop the reaction, the solution was diluted in oxygenated water (1:1 in most cases) and immediately added to a suspension of DNA-AuNPs until aggregation was observed. UV-vis spectra were recorded on a Perkin Elmer Lambda 25 UV-vis spectrometer. Transmission electron micrograph (TEM) images were captured using a JEOL 2000FX microscope on carbon coated copper grids without staining.

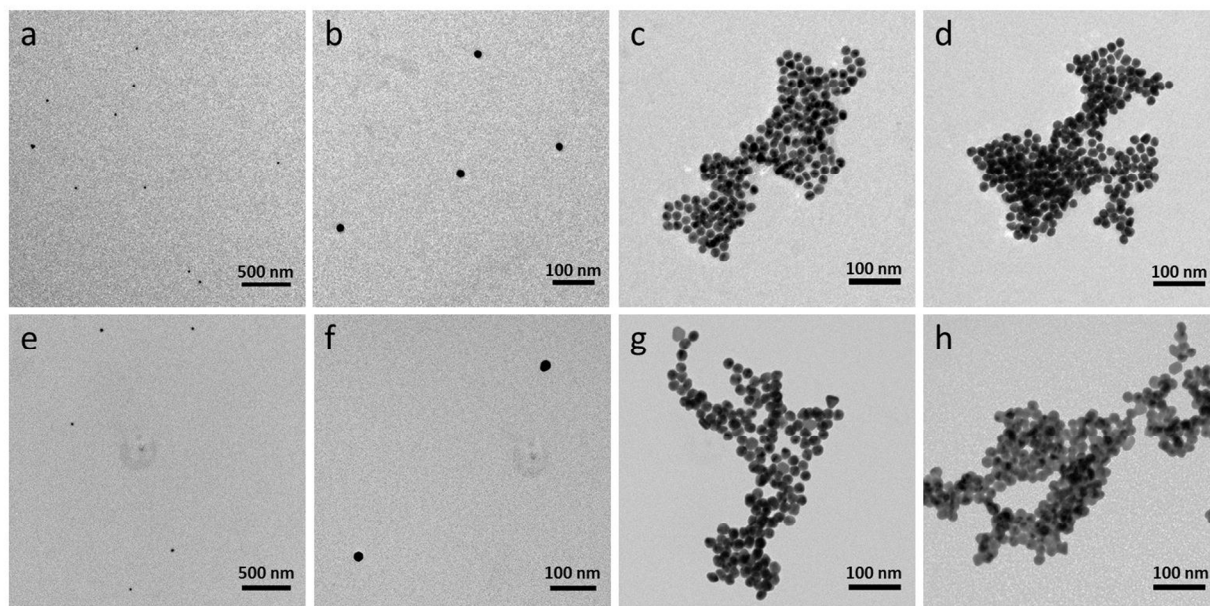


Figure S1. TEM images of Au-NPs aggregated with pAPMA₃₃. (a-b) DNA Au-NPs, (c-d) DNA Au-NPs with 0.05 mM pAPMA₃₃, (e-f) 20 nm citrate Au-NPs, (g-h) 20 nm citrate Au-NPs with 0.5 μM pAPMA₆₃

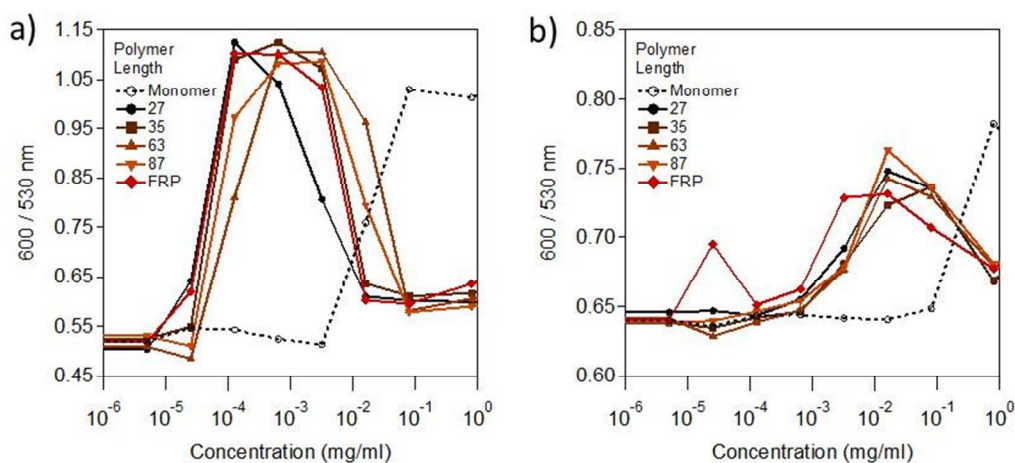


Figure S2. Aggregation of a) 20nm and b) 5 nm citrate capped AuNPs as monitored by a ratio of absorbance (600/530 nm) in the presence of varying concentrations of monomer and polymers of different lengths (DP).

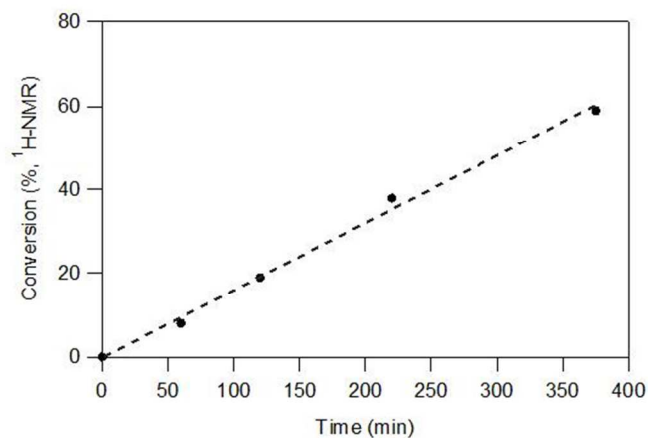


Figure S3. Conversion vs time as measured by $^1\text{H-NMR}$ for the polymerisation of APMA (5 M) with HRP (16 mg/ml) in water at 35°C. $[\text{acac}] = 120 \text{ mM}$, $[\text{H}_2\text{O}_2] = 80 \text{ mM}$

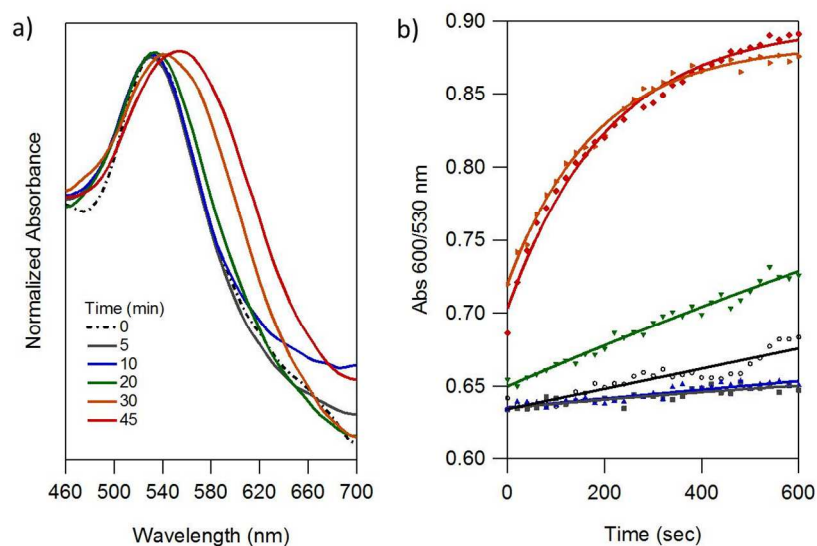


Figure S4. a) Absorbance spectra at 15 min and b) Ratio of absorbance at 600:530 nm after addition of the AuNPs to the assay reaction mixture. The assay was performed for 5 – 45 min. 200 nM of GOx, 1 mg/ml HRP, 400 μM Acac, 0.25 M APMA, and 0.1 M Glucose in MES (20 mM, pH 7.5) was used in all cases.

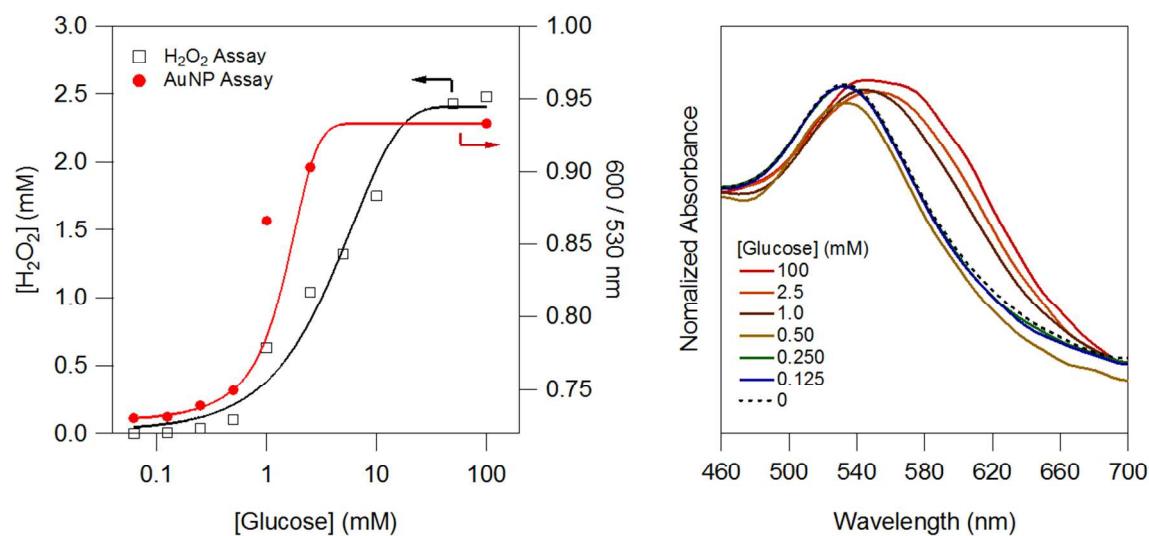


Figure S5. GOx and assay activity as a function of glucose concentration. Increasing the concentration of glucose results in increasing amounts of generated peroxide up to 10 mM. Above 10 mM, GOx activity is not limited by glucose concentration and the amount of peroxide generated plateaus. When these conditions were tested in the AuNP assay format (GOx = 200 nM, APMA = 0.25 M, acac = 0.4 mM, HRP = 1 μ g/ml), it was observed that at least 1 mM glucose is required to enable the GOx to sufficiently degas the solution and allow HRP initiated polymerization. The assays were performed in MES (20 mM, pH 7.5) for 30 minutes. Peroxide generation was measured using a standard Pierce quantitative peroxide assay (Thermo Scientific).

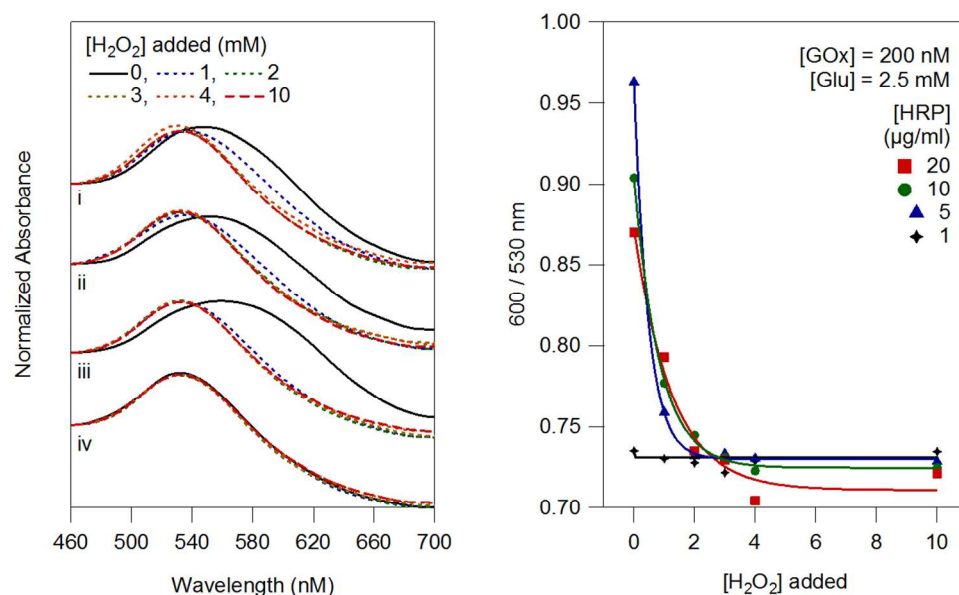


Figure S6. Assay activity as a function of peroxide concentration when the minimal amount of glucose (2.5 mM) was added. At 2.5 mM glucose, 1 mM peroxide is added to the system by GOx (Figure S4). Adding more peroxide (1, 2, 3, 4 or 10 mM) resulted in reduced HRP activity and thus inhibited polymer generation. When no peroxide was added and therefore the total concentration was 1 mM, strong AuNP aggregation occurred. However, raising the concentration to 2 mM total reduced the extent of aggregation. This was concentration dependent wherein 1 mM added peroxide resulted in more aggregation than 2 mM. The assays were performed in MES (20 mM, pH 7.5) for 30 minutes at $GOx = 200 \text{ nM}$, $APMA = 0.25 \text{ M}$, $acac = 0.4 \text{ mM}$. The concentration of HRP (i-iv) = 20, 10, 5, and 1 $\mu\text{g/ml}$.

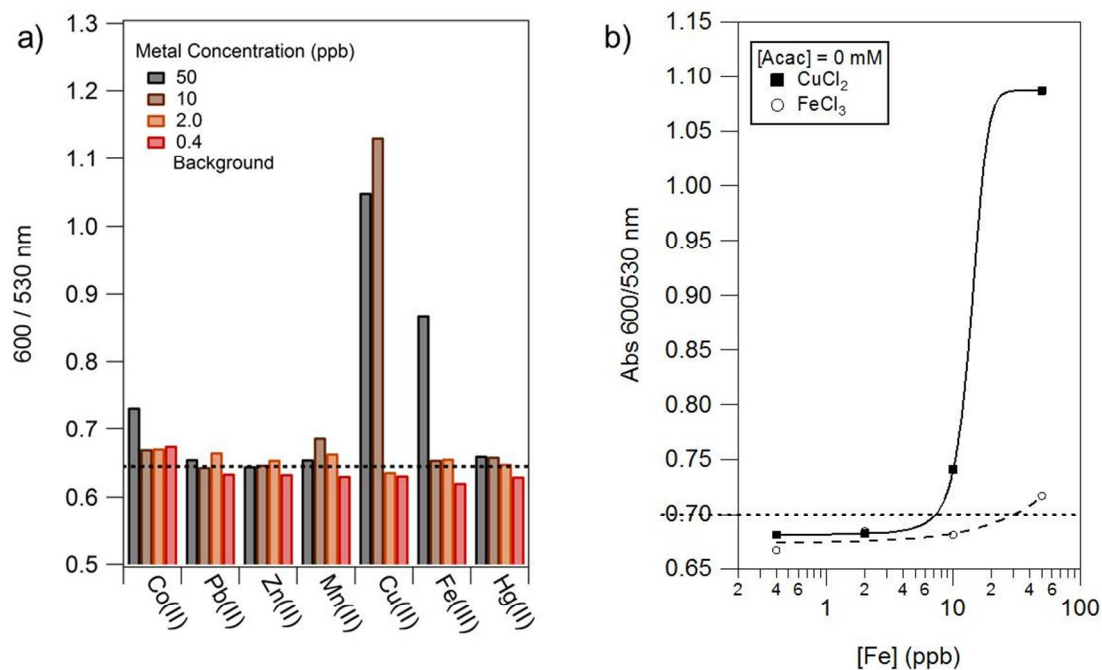


Figure S7. Ratio of absorbance at 600:530 nm after addition of AuNPs to reaction mixtures with APMA (0.25M), Glucose (0.1M) and transition metals at various concentrations. Reactions performed for 30 min at 30 °C. a) [GOx] = 100 nM, [Acac] = 2.4 mM; b) [GOx] = 200 nM, [Acac] = 0 mM

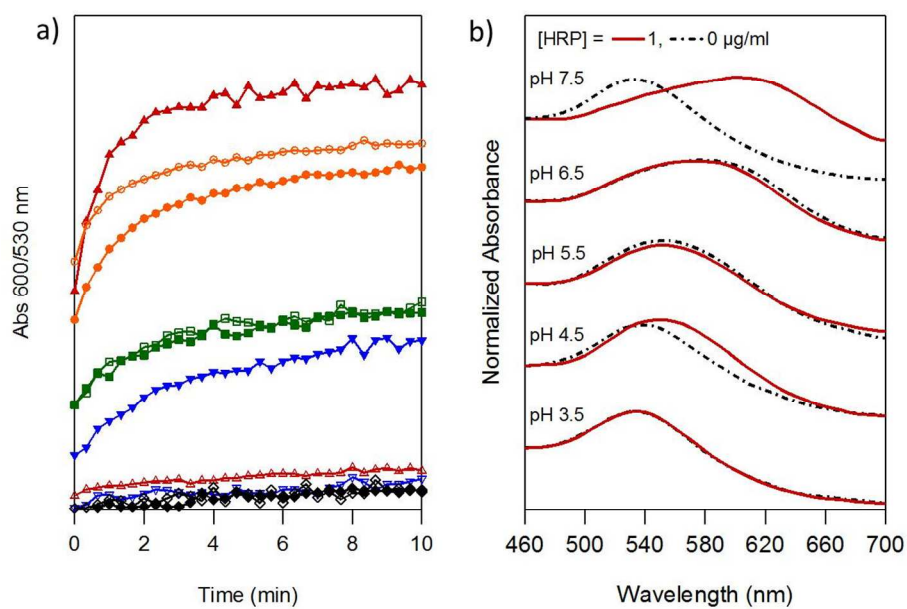


Figure S8. a) Ratio of absorbance at 600:530 nm and b) Absorbance spectra at 15 min after addition of the AuNPs to the assay reaction mixture performed at varying pH. In each case 200 nM of GOx, 400 μ M Acac, 0.25 M APMA, and 0.1 M Glucose in MES (20 mM, pH 7.5) was used. All assays were performed with and without 1 mg/ml HRP.