Supplementary Information for

Catalytic cooperativity between glucose oxidase and gold nanoparticles in the sequential oxidation of glucose to saccharic acid

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1. UV-Vis characterization of gold nanoparticles binding to glucose oxidase

The red-shift between the bound GOx-AuNP and bare AuNP is characterized with UV-vis over several orders of magnitude of GOx:AuNP ratios as shown in Figure S1.

$$Spectral\ shift = peak(AuNP{:}\,Enz)_{nm} - peak(AuNP)_{nm}$$

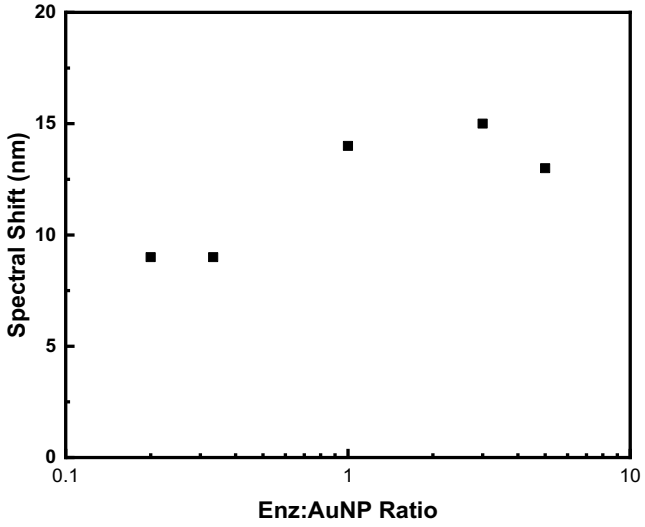


Fig. S1: Spectral red-shift of the surface plasmon resonance band between GOx-Au NP and bare AuNP materials. The greatest shift is exhibited for a ratio of about 3:1. As the ratio of GOx:AuNP decreases, the binding sites available to the enzyme are saturated and the spectra for unbound nanoparticles is observed, lowering the spectral shift.

2. Pseudo Steady State Hypothesis Modeling

For any continuous HRP assay to determine the generation rate of hydrogen peroxide, it is assumed that hydrogen peroxide is generated at a similar rate to its decomposition. In this case, the pseudo steady state approximation applies. In the experiments in this manuscript, we are only able to measure the activity of horseradish peroxidase (HRP) directly. HRP produces a H_2O_2 radical that will readily oxidize 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt (ABTS) to a radical ion. The oxidized form absorbs strongly at 415 nm in the visible light range with a molar extinction coefficient of the oxidized ABTS is 36 abs*cm*mM^{-1}.

2.1 Glucose Oxidase – Horseradish Peroxidase System

Horseradish peroxidase assays are utilized to determine the amount of hydrogen peroxide in any given system. However, by focusing on the concentration of active sites (i.e. enzyme concentration), we can elucidate the reaction network and any enhancements or loss in activity that occurs. The following mass balances are used in this work.

$$\frac{d[Glu]}{dt} = -k_{GOx}[GOx]\frac{[Glu]}{K_{m,GOx} + [Glu]} \quad (1)$$

$$\frac{d[H_2O_2]}{dt} = k_{GOx}[GOx]\frac{[Glu]}{K_{m,GOx} + [Glu]} - k_{HRP}[HRP]\frac{[H_2O_2]}{K_{m,HRP} + [H_2O_2]} \quad (2)$$

$$\frac{d[ABTS^{+\cdot}]}{dt} = -k_{HRP}[HRP]\frac{[H_2O_2]}{K_{m,HRP} + [H_2O_2]} \quad (3)$$

Glucose oxidase is assumed to follow Michaelis-Menten kinetics. First, we must show that the change in absorbance is due to the reaction in Equation 3. We can alter the concentration of enzyme or nanoparticle active sites to ensure that the desired step of the reaction is rate-determining and

all other parts are fast relative to the desired reaction. In order to satisfy the pseudo steady state approximation, we operate on this expression as a nondimensional form to normalize the kinetics with respect to the reaction time for glucose oxidase. In this case, the kinetics are normalized to $v_{max,HRP}$.

$$\frac{1}{k_{GOx}[GOx]}\frac{d[H_2O_2]}{dt^*} = \left(\frac{[Glu]}{K_{m,GOx} + [Glu]}\right) - R\left(\frac{[H_2O_2]}{K_{m,HRP} + [H_2O_2]}\right) \quad 4$$

Where

$$R = \frac{v_{max,GOx}}{v_{max,HRP}} = \frac{k_{GOx}[GOx]}{k_{HRP}[HRP]} \quad 5$$

In the case of R >> 1, any change in H_2O_2 is observed through the optical absorption of radical ABTS in Equation 6.

$$-\frac{[H_2O_2]}{K_{m,HRP} + [H_2O_2]} = -\left(\frac{[Glu]}{K_{m,GOx} + [Glu]}\right) \quad 6$$

Since the initial concentration of H_2O_2 is zero, the unsteady state term must therefore be equivalent to the generation of H_2O_2. The only generation term in this balance is due to the conversion of glucose. It is therefore necessary that the v_{max} of horseradish peroxidase be greater than v_{max} of glucose oxidase to characterize enzyme kinetics. This results in a pseudo steady state approximation that hydrogen peroxide concentration holds and the system with only glucose oxidase can be approximated as the following.

$$k_{GOx}[GOx]\frac{[Glu]}{K_{m,GOx}+[Glu]}=\frac{d[ABTS^{+\cdot}]}{dt} \quad (7)$$

While this is implied in glucose oxidase assay literature and is not necessary to further elaborate here, the concentration of active sites allows for determination of the rates of each specific component in the AuNPs coupled with GOx. This lays the framework for further analysis as AuNPs are introduced to the system.

2.2 Basis calculations for glucose oxidase activity when bound versus not bound to Au nanoparticles

To ensure glucose oxidase catalytic activity is not adversely affected by the bound nanoparticles, the activity must be monitored by similar analysis as above. Normalizing Equation 2 with the observable reaction time $v_{max,HRP}$ gives Equation 8.

$$\frac{d[H_2O_2^*]}{dt^*} = \frac{k_{GOx}[GOx]}{k_{HRP}[HRP]}\left(\frac{[Glu]}{K_{m,GOx} + [Glu]}\right) - \left(\frac{[H_2O_2]}{K_{m,HRP} + [H_2O_2]}\right) - \frac{k_{AuNP}[AuNP]}{k_{HRP}[HRP]}[H_2O_2]^n \quad (8)$$

In this case, if $k_{GOx}[GOx] \ll k_{HRP}[HRP]$ and $k_{AuNP}[AuNP] \ll k_{HRP}[HRP]$, then the solution converges to a similar unsteady state expression when using the glucose oxidase alone. This will be explained by the term R, the ratio of v_{max} of glucose oxidase to v_{max} of horseradish peroxidase, which is defined in Equation 9.

$$R = \frac{v_{max,GOx}}{v_{max,HRP}} = \frac{k_{GOx}[GOx]}{k_{HRP}[HRP]} \quad (9)$$

Similarly, when compared to glucose oxidase alone, the only generation of hydrogen peroxide comes from the glucose oxidase catalysed oxidation of glucose. Therefore, provided the stipulations on the concentrations of each enzyme (i.e. $k_{GOx}[GOx] \ll k_{HRP}[HRP]\ and\ k_{AuNP}[AuNP] \ll k_{HRP}[HRP]$) and assuming that the v_{max} for both the glucose oxidase and Au nanoparticles are below the v_{max} of the horseradish peroxidase, then the reaction rate observed is effectively the generation rate of H_2O_2 by GOx. So in effect, the degradation of H_2O_2 via Au nanoparticle catalysis is insignificant compared to the oxidation of ABTS by way of HRP.

$$k_{GOx}[GOx]\frac{[Glu]}{K_{m,GOx}+[Glu]} = k_{HRP}[HRP]\frac{[H_2O_2]}{K_{m,HRP}+[H_2O_2]} \quad (10)$$

If the observed H_2O_2 generation rates are similar between the glucose oxidase and the paired glucose oxidase-nanoparticle system, it can be inferred that the glucose oxidase is not hindered by the attachment of Au NPs. This result is shown in the main paper in Figure 2.

2.3 Basis calculations for gold nanoparticle activity

We can apply the pseudo steady state hypothesis so that the concentration of hydrogen peroxide is approximately constant. If the observed consumption of hydrogen peroxide from horseradish peroxidase production is linear, then the concentration of hydrogen peroxide is a constant; therefore, generation and consumption are balanced. For this reaction system we would like to ensure that all reactions are observable so the experiment is designed such that $R \approx 1$. So for this case, all terms are of order 1 and can be related directly. Reactions are performed both with and without gold nanoparticles under the same experimental conditions. The balance for reactions without Au nanoparticles can be described with Equation 11.

$$R\left(\frac{[Glu]}{K_{m,GOx}+[Glu]}\right)=\left(\frac{[H_2O_2]}{K_{m,HRP}+[H_2O_2]}\right) \quad 11$$

Conversely, the system with gold nanoparticles can be described through Equation 12.

$$R\left(\frac{[Glu]}{K_{m,GOx}+[Glu]}\right)-R_{AuNP}[H_2O_2]^n=\left(\frac{[H_2O_2]}{K_{m,HRP}+[H_2O_2]}\right) \quad 12$$

When the concentrations of each component are equal between the two systems, the systems can be compared directly. The two rates can be compared directly through subtraction in Equation 13.

$$\frac{k_{AuNP}[AuNP]}{k_{HRP}[HRP]}[H_2O_2]^n=\left(\frac{[H_2O_2]}{K_{m,HRP}+[H_2O_2]}\right)_{NoAuNP}-\left(\frac{[H_2O_2]}{K_{m,HRP}+[H_2O_2]}\right)_{AuNP} \quad 13$$

Both reactions are related to the observable rate and Equation 14 is the result.

$$k_{AuNP}[AuNP][H_2O_2]^n=\frac{1}{2\epsilon}\left(\frac{dABTS^{+\cdot}_{NoAuNP}}{dt}-\frac{dABTS^{+\cdot}_{AuNP}}{dt}\right) \quad 14$$

Two balances for the generation of hydrogen peroxide are provided, one for the GOx alone (Equation 2) and one for the hybrid AuNP-GOx system (Equation 8). The first balance is for the GOx only system. The second balance is for the hybrid AuNP-GOx system. Subtracting the two balances, and considering that the GOx term in this equation will be equivalent provided the pH is similar for reasons expressed above yields Equation 15.

$$([HRP]r_{HRP})_E - ([HRP]r_{HRP})_{E+AuNP} = \left(\frac{d[H_2O_2]}{dt}\right)_E - \left(\frac{d[H_2O_2]}{dt}\right)_{E+AuNP} - [AuNP]r_{AuNP} \quad 15$$

The term $[HRP]r_{HRP}$ is the rate observed as the production of the ABTS radical. We can determine the rate of hydrogen peroxide production in Equation 16.

$$\frac{1}{2}\left(\left(\frac{d[ABTS^{+\cdot}]}{dt}\right)_E - \left(\frac{d[ABTS^{+\cdot}]}{dt}\right)_{E+AuNP}\right) = \left(\frac{d[H_2O_2]}{dt}\right)_E - \left(\frac{d[H_2O_2]}{dt}\right)_{E+AuNP} - [AuNP]r_{AuNP} \quad 16$$

For long times, the production of H_2O_2 becomes pseudo steady-state and yields Equation 17.

$$r_{AuNP} = \frac{1}{2\epsilon}\frac{\left(\frac{d[abs_{ABTS^{+\cdot}}]}{dt}\right)_{E+AuNP} - \left(\frac{d[abs_{ABTS^{+\cdot}}]}{dt}\right)_E}{[AuNP]} \quad 17$$

This expression is utilized to determine the contribution of AuNPs to the overall kinetics of this reaction system through observations in kinetic experiments monitored by UV-Vis. The epsilon term is the molar extinction coefficient.

3. $ABTS^{\cdot+}$ radical formation rate data compared for enzyme and enzyme-Au nanoparticle reactions

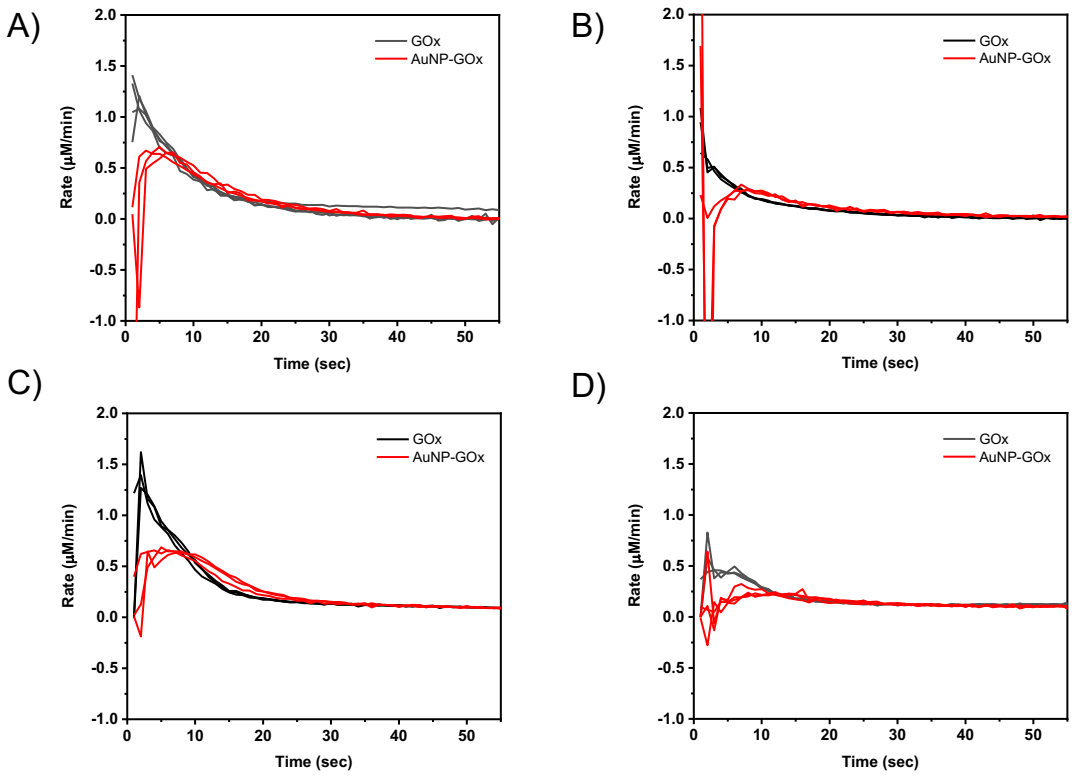


Figure S2: Rate of $ABTS^{\cdot+}$ production at 415 *nm* at a pH of 6.6 (A) 7.5 (B) 8.3 (C) and 9.2 (D). GOx alone (red) and GOx-AuNPs (black) show the observed spectra during hydrogen peroxide decomposition through the HRP pathway. 1.5 mL of 0.25 mg mL^{-1} of HRP is added with 0.5 mL of 0.05 mg mL^{-1} of GOx solution. Initial concentration of glucose is 16.6 mM. Under these conditions, the kinetics are first order. Each of these experiments correspond to a rate ratio of R=3.95 (A) R=4.94 (B) R=4.79 (C) and lastly R = 26.66 (D). Spikes in panels C and D are due to injections of glucose and localized eddies resulting in higher concentrations.

4. Hydrogen peroxide generation/consumption rates at different pH levels

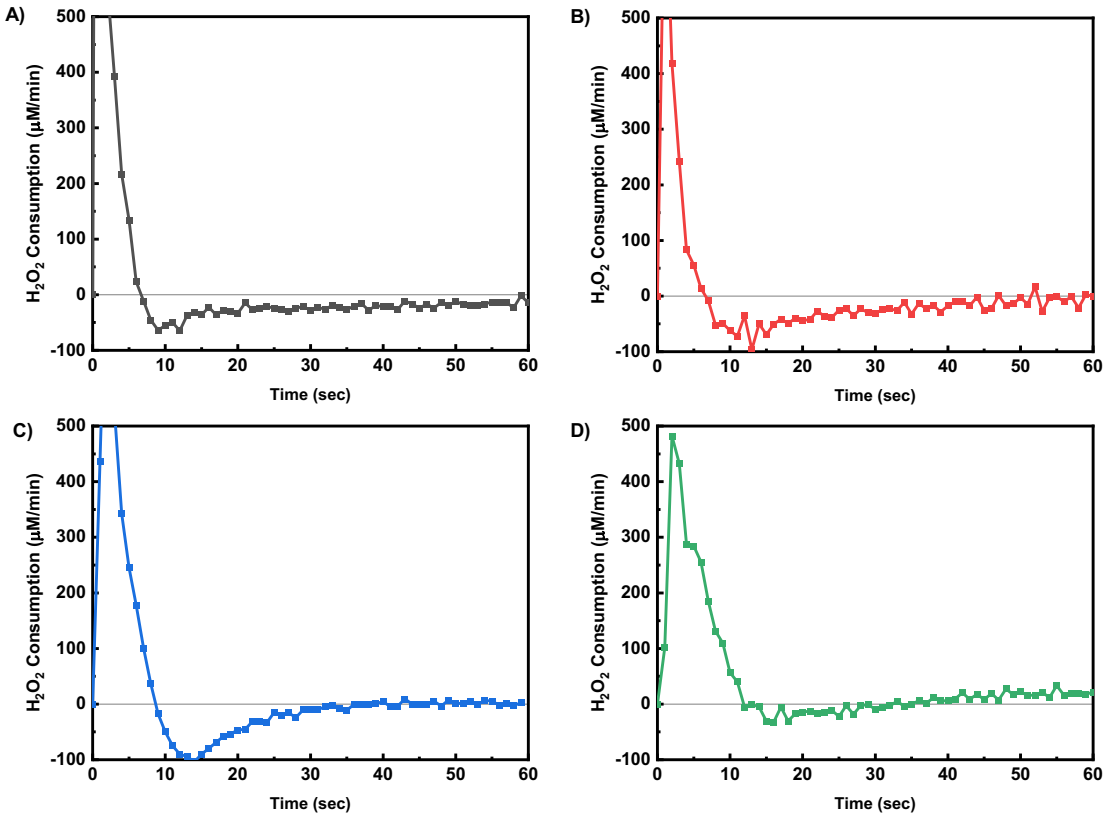


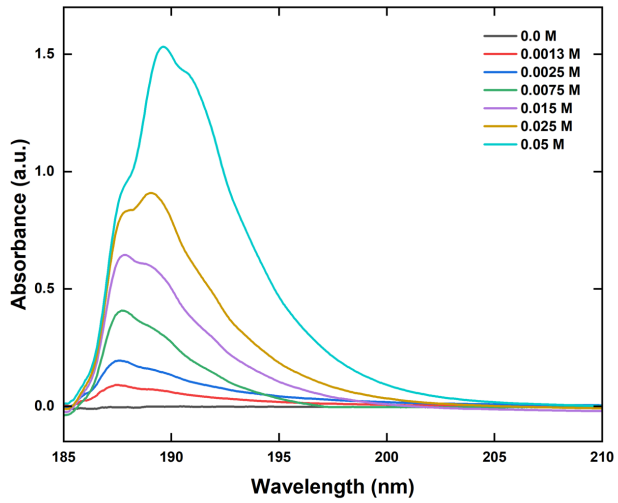
Figure S3. H_2O_2 consumption rates for Au-GOx materials at pH values of 6.3 (A), 7.1 (B) 8.3 (C), and 9.1(D). Initial concentration of glucose is 16.6 mM. Kinetics are observed in the first order regime for GOx. Note: positive values indicate a **consumption of H_2O_2** while negative values indicate a **generation of H_2O_2**.

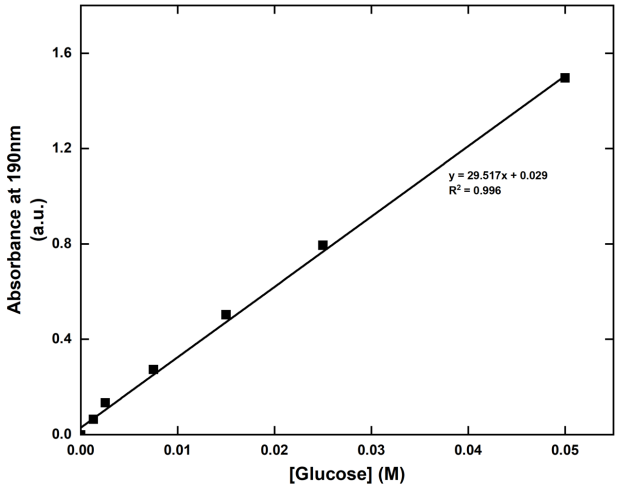
In Figure S3, the difference in the rates of H_2O_2 consumption between the AuNP-GOx system and the GOx systems are plotted as defined by Equation 17. This value is the H_2O_2 consumption rate by the AuNPs. Positive values indicate H_2O_2 consumption, and negative values indicate H_2O_2 generation. Initially for times less than 5 seconds, at all pH values tested, the H_2O_2 is consumed

when low initial concentrations of glucose are used. We hypothesize that the AuNPs are contributing to the significant initial consumption rates due to a proximity effect. Essentially, an initial production of H_2O_2 is decomposed on gold before the H_2O_2 can diffuse into the bulk solution and be reduced by the ABTS with HRP. At longer times the production of H_2O_2 exceeds the rate of nanoparticle consumption and is then balanced by HRP rate to oxidize ABTS. The initial consumption of H_2O_2 is prolonged at higher pH, suggesting that H_2O_2 is favored to decompose at higher pH. After a short time, H_2O_2 is generated rapidly and then approaches a steady-state rate. At times greater than 5s, the system is transient, and the H_2O_2 concentration increases until the generation of H_2O_2 due to GOx balances the loss of H_2O_2 via HRP or AuNP catalyzed pathways. The rate begins to approach a steady state with both the GOx and the AuNP-GOx systems after approximately 35 seconds in all samples.

5. Glucose oxidation with Au nanoparticles only

Calibration curves were developed for glucose using UV-Vis spectroscopic measurements to measure the rate of glucose oxidation with gold nanoparticles alone. Spectra were collected using UV-Vis at various glucose concentrations from 0.0500 M to 0.0013 M in Figure S4. At 190 nm, the relationship between the absorption and concentration is given by the equation, Abs = 29.517[glucose] + 0.029 (R^2 = 0.99) where glucose concentration is in units of M.





Glucose Conc. (M)	Absorbance (a.u.)	Std. Dev. (a.u.)
5.00E-02	1.50E+00	9.43E-04
2.50E-02	7.94E-01	6.02E-03
1.50E-02	5.03E-01	3.27E-03
7.50E-03	2.73E-01	1.70E-03
2.53E-03	1.33E-01	4.71E-04
1.30E-03	6.40E-02	4.24E-03
0.00E+00	-1.67E-03	4.71E-04

Figure S4. (top) UV spectra of glucose at concentrations from 0.0500 M to 0.00130 M with blank. (middle) Calibration plot relating concentration to UV absorbance. (bottom) Mean values and standard deviations for the calibration plot.

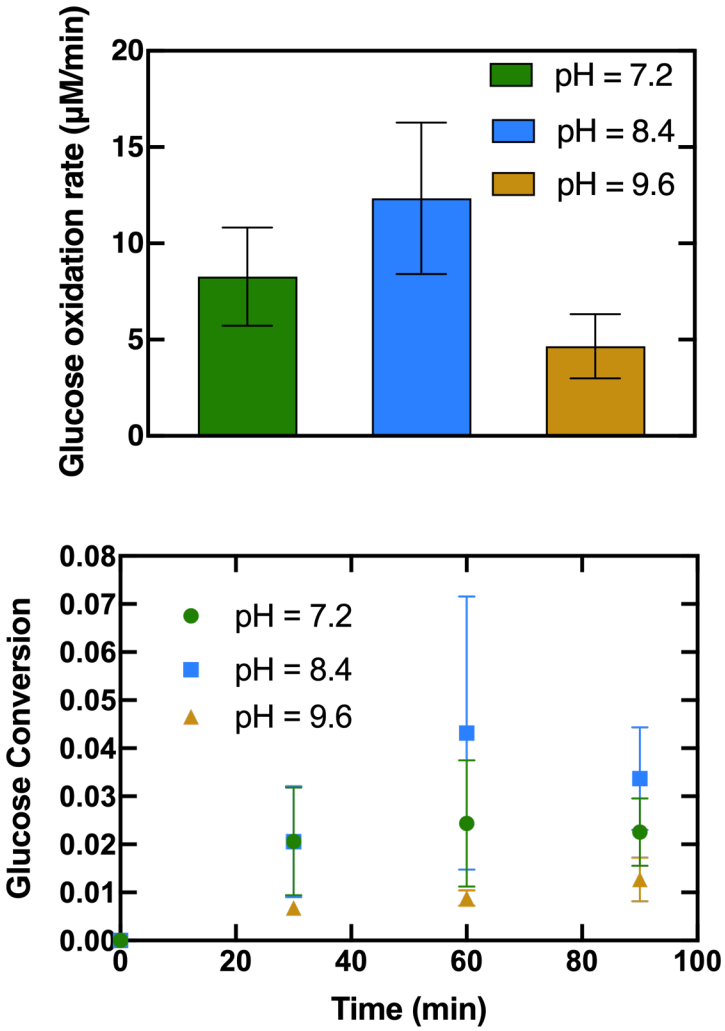


Figure S5. (top) Glucose oxidation rates at 90 minutes using only Au nanoparticles at pH 7.2 (green), 8.4 (blue), and 9.6 (brown). (bottom) Glucose conversion as a function of time from 0 to 90 minutes. The samples are measured every 30 minutes. The Au concentration is same in all systems. The absorbance of glucose was measured at 190 nm. Total volume for each reaction was 3 mL. To prepare the reaction mixture, 2 mL of 0.1 M phosphate buffer, 0.3 mL of 0.364 mM Au (0.141 uM AuNP for 4.8 nm average particles), and a volume of 1 M glucose (0.1 mL) were used. 18.2 MΩ H_2O was added to reach a total solution volume of 3 mL.

We observe that the rate of glucose oxidation with Au nanoparticles only is significant depending on the pH. Glucose conversions of nearly 2.4%, 4.3%, and 1.2% were observed at pH 7.2, 8.4, and 9.6, respectively. At pH = 8.4, the contribution of the Au nanoparticles for glucose oxidation is the most significant, representing up to approximately half of the conversion of the hybrid system in the case of the 3:1 sample as shown in Figure 5 in the main text. At pH = 9.6, the conversion of the Au nanoparticles alone reaches a maximum of nearly 10% of the hybrid system value in the case of the 5:1 ratio catalyst.

6. Additional HPLC Data

For **gluconic acid**, the correlation between HPLC peak area and concentration in mmol/mL is Peak Area = $1.76(10^9)$ * [gluconic acid].

For **saccharic acid**, the correlation between HPLC peak area and concentration mmol/mL is Peak Area = $7.92(10^8)$ * [saccharic acid].

Graphs of HPLC peak area for gluconic acid and saccharic acid versus time are shown in this section for GOx only systems (Figures S6A and S6C) and for AuNP + GOx (1:1 ratio) in Figures (S6B and S6D). This data was used to generate Figures 5A, B, C, and D in the main manuscript.

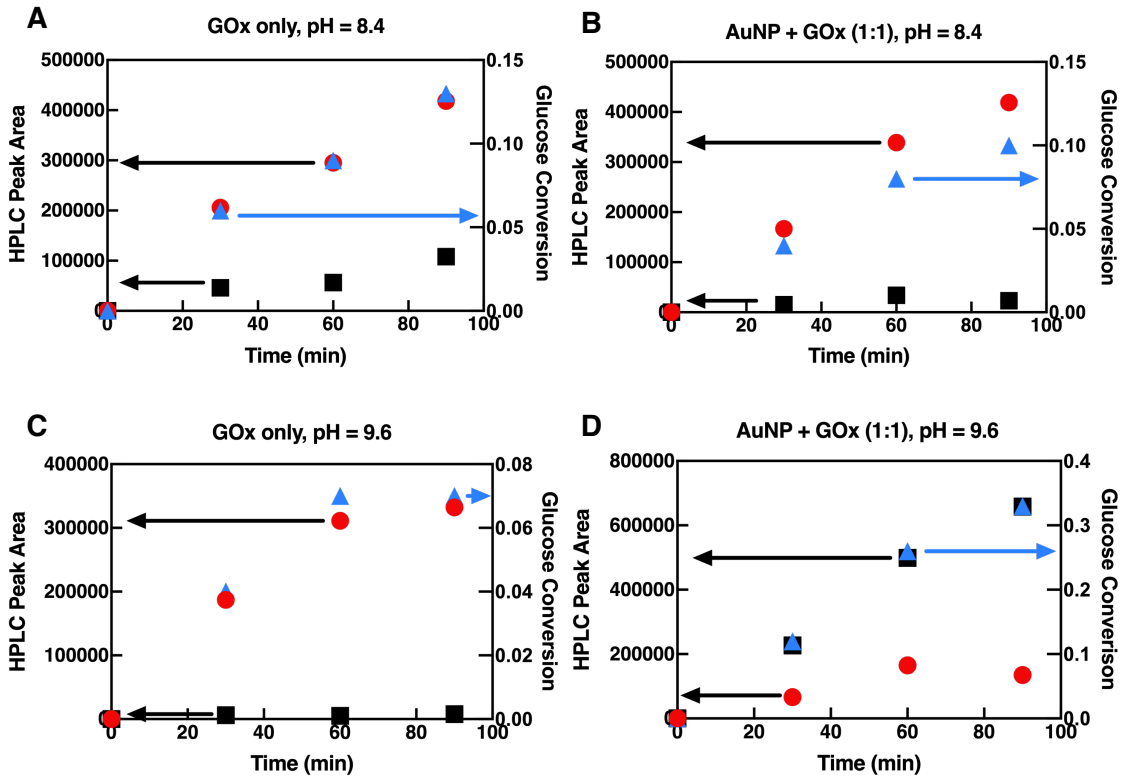


Figure S6. Kinetic curves showing change in HPLC peak area for gluconic acid (red circles) and glucaric acid (black squares) as a function of time. Glucose conversion as a function of time is shown with blue triangles on the secondary y-axis.