

Influence of Antibody Immobilization Strategy on Carbon Electrode

Immunoarrays

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Supporting Information

Table of Contents

	Page No.
Synthesis of glutathione-gold nanoparticles (GSH-AuNP)	2
Synthesis of Graphene oxide	2
BCA assay for anti-HRP	3
Diffusion controlled antigen (HRP) interaction with anti-HRP	4
Theoretical calculation of no. of HRP available to bind to anti-HRP	5
HRP calibration using SIGMAFAST OPD assay	6
Study of electrode to electrode variation	7
Study of electrodes' stability	8

Synthesis of Glutathione-Protected AuNPs (AuNPs) and LBL Growth

Glutathione-protected gold nanoparticles (AuNP) with diameter 5 nm were formed by the reduction of gold salt with the use of sodium borohydride in the presence of glutathione¹. To a mixture of 3.0 mL methanol and 0.5 mL acetic acid, 19.7 mg $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ and 7.7 mg glutathione were dissolved by stirring for 5 minutes producing a clear yellow solution. A sodium borohydride solution with 30 mg NaBH_4 dissolved in 1.5 g of nanopure water was added dropwise into the HAuCl_4 solution with rapid stirring. After the total volume of the NaBH_4 solution was added, the HAuCl_4 solution changed from yellow to brown. The solution was rapidly stirred for 2 hours. The glutathione-protected gold nanoparticles (GSH-AuNP) formed were soluble in water. The solution with the particles were filtered through a 50 KDa MW cutoff, centrifuged at 3500 rpm and washed with nanopure water four times. The particle solution then was dissolved in 20 mM HEPES buffer (pH 8) before the particles were characterized by transmission electron microscopy and plasmon resonance spectra and the diameter was determined to be 5.05 ± 1.4 nm.

PDDA was spotted onto the eight electrode surfaces incubated for twenty minutes, rinsed with distilled water and dried with nitrogen. Once dried AuNP were spotted onto the electrodes, incubated for twenty minutes, again rinsed with DI water and dried with nitrogen

Synthesis of Graphene oxide (Go):

GO was prepared using modified Hummer's method². In an ice bath with 69 mL of concentrated sulfuric acid (Sigma-Aldrich), 3.00 g graphite powder (Fisher Scientific) and 1.5 g sodium nitrate, NaNO_2 , (Sigma) were mixed. To avoid overheating above 20 °C, 9 g of potassium permanganate, KMnO_4 , was added in small portions. The solution was then heated to 35 °C with constant stirring and kept at that temperature for 7 hours. Another 9 g of KMnO_4 was added and the solution was stirred for another 12 hours at 35 °C. The solution was cooled to room temperature and 400 mL of

ice and 3 mL of 30% H₂O₂ were mixed in. The solution was then centrifuged at 4000 rpm. The supernatant was decanted and washed multiple times with water and ethanol until the supernatant was neutral on litmus paper. The black GO was then dried under vacuum at 40 °C for 6 hours.

BCA Assay for anti-HRP

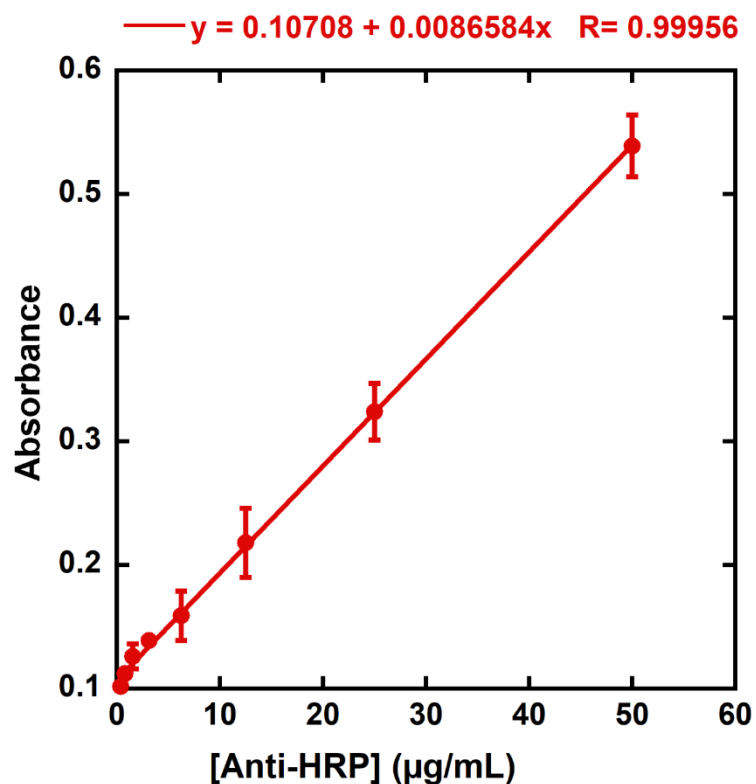


Figure S1: BCA assay for standard anti-HRP. Absorbance was measured at 562 nm after incubation of BCA reagent with series of standard anti-HRP for 2 hours at 37°C. (Error bars represent standard deviation, n=3)

Diffusion controlled interaction between antigen and immobilized antibodies on electrode surface: Concentration of HRP that would interact with antibodies immobilized on each electrode, from bulk solution inside microfluidic chamber, was calculated using Einstein's diffusion equation (Eq. 1) assuming that the interaction is only diffusion controlled³. Assuming the diffusion coefficient of HRP to be $5 \times 10^{-7} \text{ cm}^2/\text{s}$, the calculated distance that can be travelled by HRP in 20 minutes would be 0.012 mm⁴⁴. The partition of solution that would have HRP available to bind to anti-HRP immobilized on each electrode would be represented as a hemisphere with radius of 0.012 mm and its volume was estimated to be $7.24 \times 10^{-6} \mu\text{L}$ (Fig. S1, Supporting Information).

$$d = \sqrt{2 D t} \dots\dots\dots \text{Eq. 1}$$

Where d: distance travelled in cm, D is the diffusion coefficient and t is time in seconds.

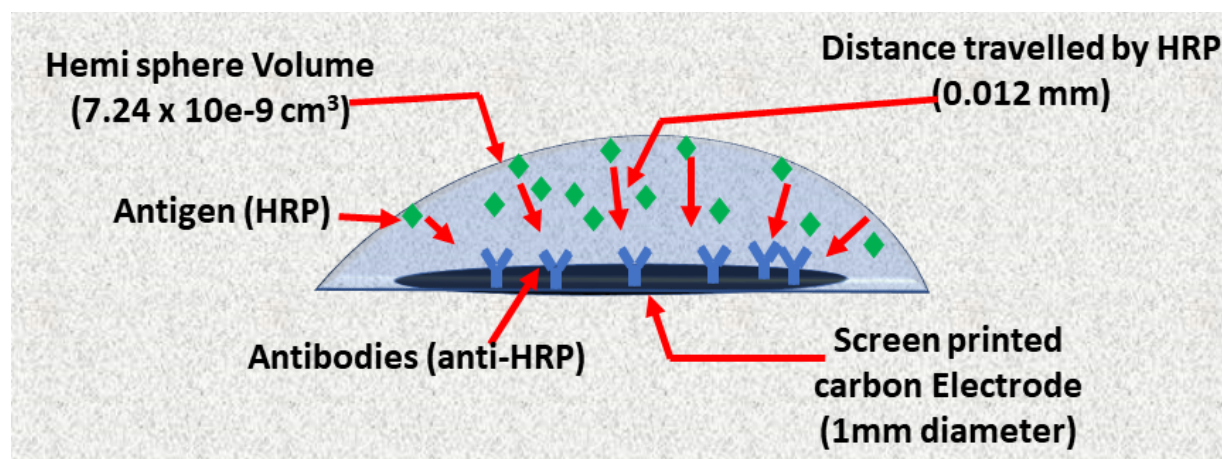


Fig. S2: Schematic illustration of the diffusion-controlled interaction between antigen and antibodies immobilized on electrode surface; maximum distance that can be travelled by antigen with diffusion coefficient of $5 \times 10^{-7} \text{ cm}^2/\text{s}$ in 20 min was estimated to be 0.012 mm. This result in a hemisphere with a volume of $7.24 \times 10^{-6} \mu\text{L}$.

Table S1: Theoretical calculation of no. of HRP available to bind to anti-HRP immobilized on electrodes at each concentration of HRP standards under diffusion control

[HRP] (pg mL ⁻¹)	[HRP] available to bind anti-HRP/electrode (pg mL ⁻¹)	No. of HRP available to bind anti-HRP/electrode
0.008	5.79×10^{-15}	8.20×10^4
0.04	2.90×10^{-14}	4.10×10^5
0.2	1.45×10^{-13}	2.05×10^6
1	7.24×10^{-13}	1.03×10^7
5	3.62×10^{-12}	5.13×10^7
25	1.81×10^{-11}	2.56×10^8
125	9.05×10^{-11}	1.28×10^9
250	1.81×10^{-10}	2.56×10^9
500	3.62×10^{-10}	5.13×10^9
1000	7.24×10^{-10}	1.03×10^{10}

HRP calibration using SIGMAFAST OPD assay

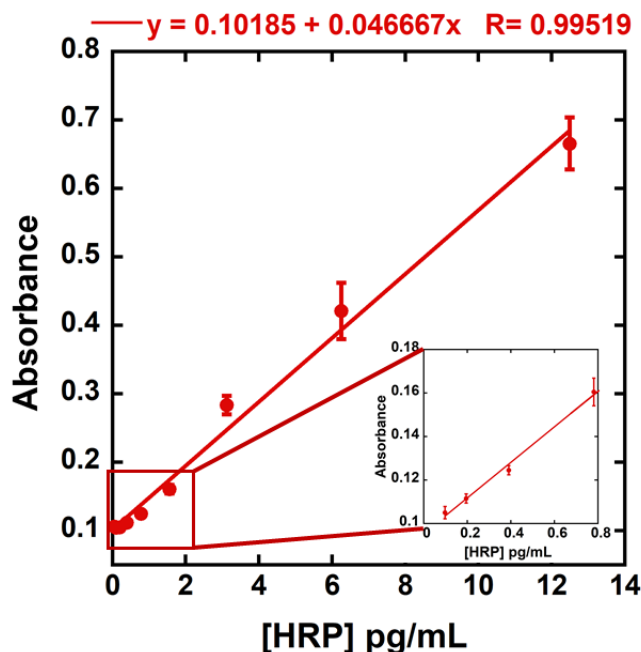


Fig. S3: Calibration curve of series of standard HRP using SIGMAFAST OPD assay. 100 μ L O-phenylenediamine (4 mg/mL) in 0.05 M phosphate-citrate buffer (pH 5.0) was incubated with 100 μ L of HRP standard for 1 hour, reaction was stopped using 25 μ L 3M HCL and absorbance was measured at 492 nm. Error bars represent standard deviation, n=3.

Electrode to electrode variation: 5 different electrodes prepared with the same antibody immobilization strategy was challenged against 5 pg mL^{-1} of HRP in the same day to estimate intra-day signal variations for each immobilization strategy (Fig. S4 supporting information).

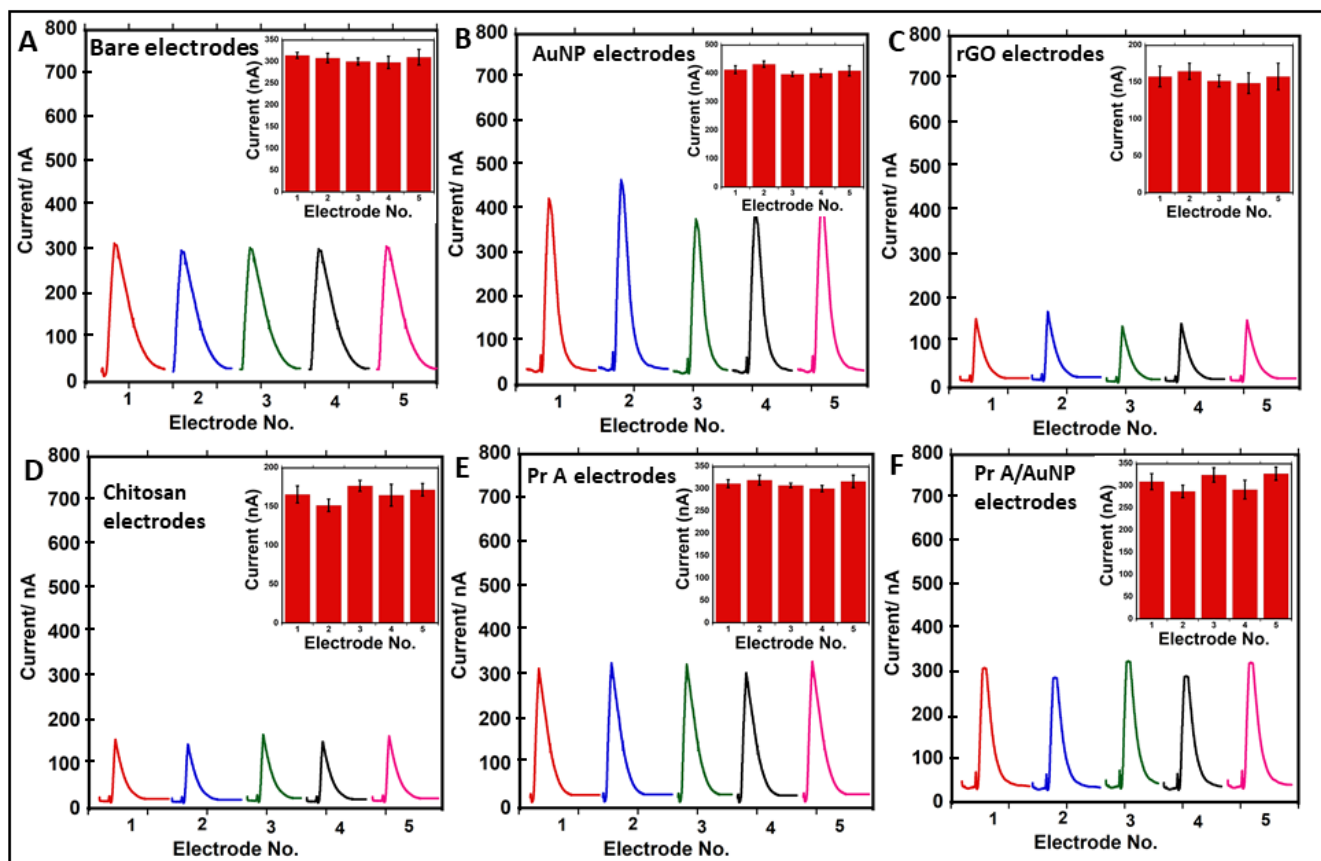


Fig. S4: Inter-day electrode to electrode variation showing amperometric peaks obtained from 5 different electrodes challenged against 5 pg mL^{-1} HRP; anti-HRP antibodies (A) passively adsorbed on bare electrodes; (B) covalently bound to AuNP decorated electrodes; (C) covalently bound to rGO coated electrodes; (D) covalently bound to chitosan modified electrodes; (E) oriented on protein A passively adsorbed on bare electrodes; (F) Anti-HRP oriented on protein A covalently immobilized on AuNP decorated electrodes. Insets are columns representing peak currents from different electrodes

Stability. Arrays were challenged with the same concentration of HRP over 5 days. Interestingly, Chitosan coated electrodes showed the best stability with no obvious loss in signal

over 5 days ($\leq 7\%$). Electrodes with passively adsorbed antibodies were the fastest to deteriorate with 15-20% loss of activity each day in the first 3 days. AuNP and rGO electrodes were relatively stable for the first three days of storage ($\sim 5\%$ decrease/day) followed by larger decrease in signal in 5th day of storage ($\sim 10\%$). Antibodies adsorbed on protein A electrodes had good stability over

References

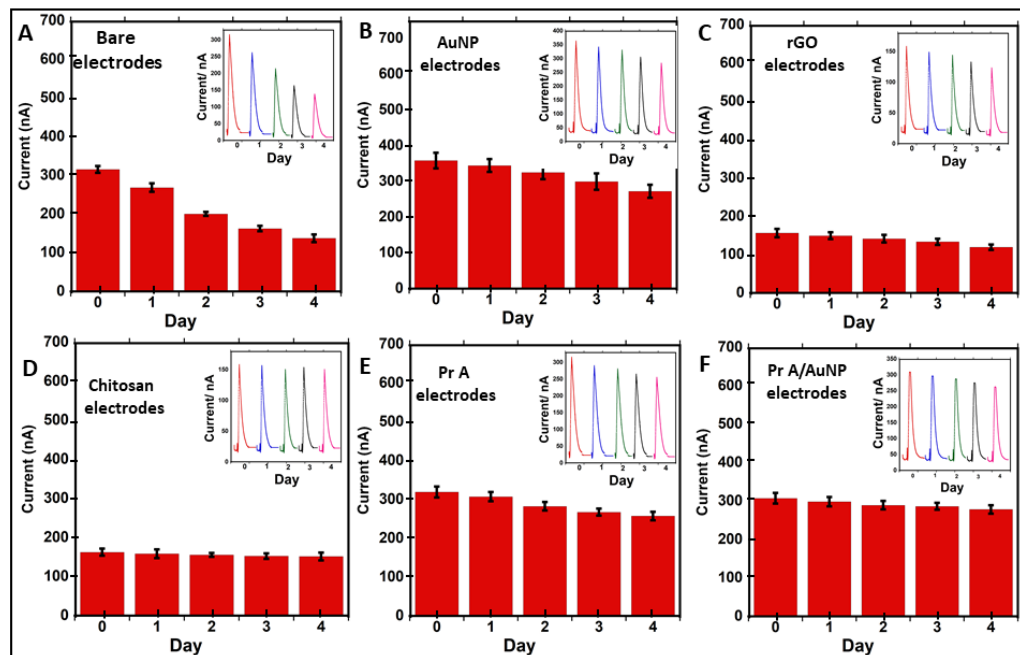


Fig. S5. Stability of electrodes as a function of change of the electrochemical amperometric signal challenged against 5 pg mL^{-1} HRP over a period of 5 days; Anti-HRP antibodies were (A) Passively adsorbed on bare electrodes; (B) Covalently immobilized on AuNP decorated electrodes; (C) Covalently immobilized on rGO coated electrodes; (D) Covalently immobilized on chitosan modified electrodes; (E) Oriented onto protein A passively adsorbed on bare electrodes; (F) Oriented onto protein A covalently immobilized on AuNP decorated electrodes. Insets are amperometric peaks. (Error bars represent standard deviation, $n=8$).

- (1) V. Mani, B. V. Chikkaveeraiah, V. Patel, J. S. Gutkind and J. F. Rusling, *ACS Nano* 2009, **3**, 585–594.
- (2) M. I. Kim, M. S. Kim, M.-A. Woo, Y. Ye, K. S. Kang, J. Lee and H. G. Park, *Nanoscale*, 2014, **3**, 1529–1536.
- (3) M. S. Wilson and W. Nie, *Anal. Chem.* 2006, **78**, 2507–2513.