Ultrasensitive Electrochemical Immunosensors for Antibodies to Peanut Allergen Ara h2 Using Gold Nanoparticle-Peptide Films

Hongyun Liu, Ruchika Malhotra, Mark W. Peczuh, James F. Rusling

Supporting Information

1. TEM (Transmission electron microscopy).

The glutathione (GSH) protected gold nanoparticle (GSH-AuNP) dispersion was analyzed for core dimensions and size distribution by TEM. Figure S1A shows typical TEM image of GSH-AuNP. All particles are spherical and well separated. The size histogram obtained from analysis of TEM images shown in Fig. S1B reveals that the average diameter of GSH-AuNP was 5 ± 1 nm, a relatively narrow size distribution. TEM investigations of AuNPs were carried out on a Tecnai Spirit Twin transmission electron microscope (FEI) operating at 80 kV. The TEM specimens were prepared by depositing a drop of dilute HEPES buffer of AuNPs onto an ultrathin (abouț 10 nm) amorphous carbon substrate supported on a copper-mesh grid.

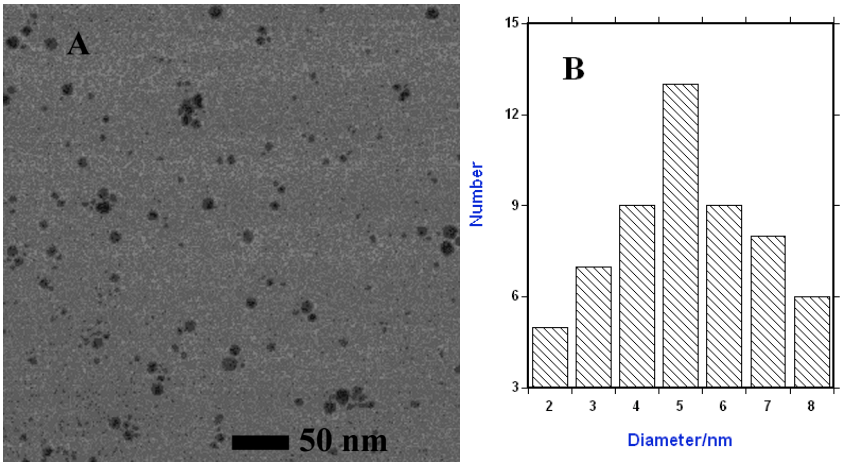

Figure S1. (A) TEM image of GSH-AuNPs. (B) Size distribution histogram of GSH-AuNPs.

2. QCM (quartz crystal microbalance)

Before adsorbing, QCM resonators that were treated with 3-mercaptopropane sulfuric acid (MPA) to mimic the partly negative PG surface before adsorbing the layers.[1] Adsorbed mass per unit area (Γ, μg/cm^2) of each layer for dried films was obtained from the measured QCM frequency change (ΔF) and the Sauerbrey equation.[2] Based on the Sauerbrey equation, the relationship between frequency shift (ΔF, Hz) and micro-mass change (ΔM, g) for each adsorption step can be expressed as:

$$\Delta F = \frac{-2f_0^2 \Delta M}{A\sqrt{\mu\rho}} \qquad (1)$$

where ΔM is mass change, ΔF is the frequency shift of the quartz crystal resonator, f_0 is the

fundamental resonant frequency of the quartz crystal, μ is the shear modulus of quartz (2.947 × 1011 g cm-1 s^{-2}), ρ is the density of the crystal (2.648 g cm^{-3}), and A is the geometric area of the QCM electrode. By taking into account the properties of quartz resonator used in this work, f_0 = 9 MHz, A = 0.16 cm^2, therefore:

$$\Delta M/A = \Delta F/(-1.83 \times 10^8) \quad (2)$$

$$\Delta F\ (Hz) = -11.4 \times 10^8\ \Delta M \quad (3)$$

This equation is reliable for a dry adsorption layer when the measurement is performed in air, and the viscoelastic effect of the adsorbed layer on frequency that is significant in solution can be ignored in air.[3] If assuming that the layer was densely packed on the surface without any imperfection, the QCM data can also be used to estimate the nominal thickness (d, cm) of each adsorption layer according to the equation of:

$$d = \frac{\Delta M}{2\rho' A} \quad (4)$$

where ρ′ (g cm^{-1}) is the density of the layer material, and A is the area of the QCM electrodes. As for A = 0.16 cm^2, d (cm) = $-2.7 \times 10^{-9}\ \Delta F/\rho'$.

For polymer poly (diallydimethylammonium chloride) (PDDA) the density is about 1.2 ± 0.1 g cm^{-3},[4] while for protein, the density is assumed to be 1.3 ± 0.1 g cm^{-3}.[5] For GSH-AuNP, the density is estimated by the weightings according to the volume ration of GSH and gold (~ 4 g cm^{-3}). Table S1 provides estimates of surface concentration (Γ), nominal thickness (d) of each layer adsorbed on PDDA/MPA surface.

Table S1. Average characteristics of different adsorption layer obtained from QCM data

Layers	- ΔF/Hz	Δm/ng	Γ/μg cm^{-2}	d/nm
PDDA	33 ± 8	29 ± 7	0.2	0.7
AuNP	614 ± 87	539 ± 78	3.4	4.1
Ara h 2-2 (peptide)	2175 ± 380	1908 ± 333	12	45
Ig Y (10 ng mL^{-1})	578 ± 167	507 ± 146	3.2	12
Ab_2-HRP[a]	350 ± 93	307 ± 82	1.9	6.4

[a]: Ab_2-HRP = horseradish peroxidase (HRP) conjugated goat anti-chicken IgY antibody.

3. SPR (surface Plasma resonance)

SPR instrument is equipped with a cuvette, and a gold sensor disk (diameter 17 mm) is mounted to the optical lens through index-matched oil. An auto-sampler is used to inject or remove test solutions with a 250 μL volume loop. Measurements were performed with mixing, where the solution was continuously aspirated and dispensed at a flow rate of 1 μL s^{-1}. An SR7000 gold sensor slide (Reichert Inc.) with the carboxyl function group and thiol self-assemble membranes was then flushed by 400 mM EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride) and 100 mM NHSS (N-hydroxysulfosuccinimide) to activated the carboxyl group for half an hour at room temperature. The activated Au-film slide was then rinsed with the reaction buffer solution (10 mM pH 7.0 PBS buffer). The solutions of peptide Ara h 2-2, IgY, BSA (bovine serum albumin) and Ab_2-HRP (horseradish peroxidase (HRP) conjugated goat anti-chicken IgY antibody) with

the same concentration as mentioned in Immunosensor Procedure Section were successively injected by the auto-sampler.

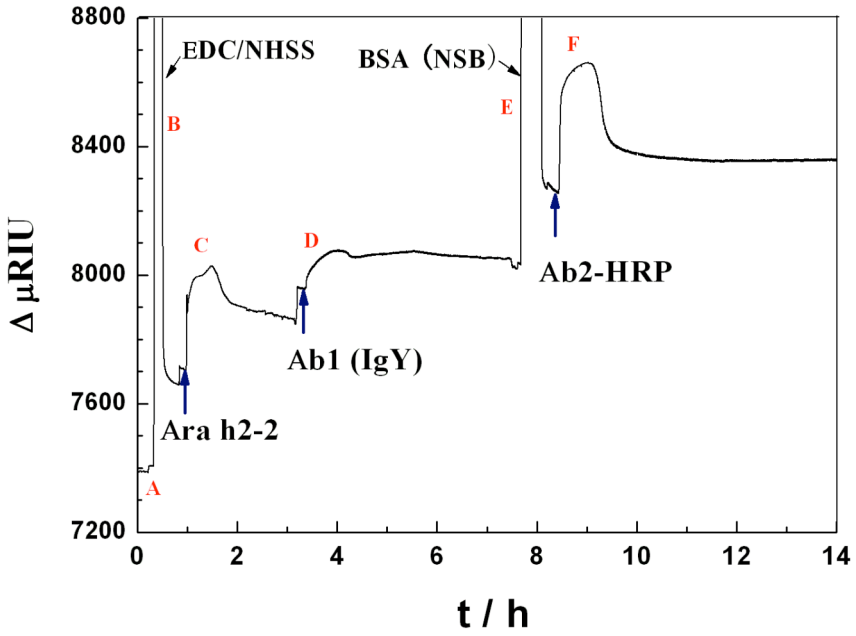

Figure S2. SPR response curve for attaching of Ara h 2-2 onto carboxylic group-functionalized gold surface and binding the antibodies onto peptide interface. (A) Baseline absorbance in pH 7.2 PBS + 0.05% (v/v) Tween 20 (PBST) as a function of time. (B) 250 μL of EDC (0.4 M) and NHS (0.1 M) was injected at 10 μL min^{-1}. (C) 350 μL 0.5 mg mL^{-1} Ara h 2-2 was injected at 5 μL min^{-1}. (D) 250 μL 150 ng mL^{-1} IgY in pH 7.2 PBST was incubated at 5 μL min^{-1}. (E) Nonspecific binding process was processed with 250 μL pH 7.2 PBST buffer containing 2% BSA running at flow rate of 10 μL/min. (F) Incubation of 250 μL Ab_2-HRP in pH 7.2 PBST containing 0.2% BSA at 5 μL min^{-1}. Washing was processing between each two steps with pH 7.2 PBS at 50 μL min^{-1}.

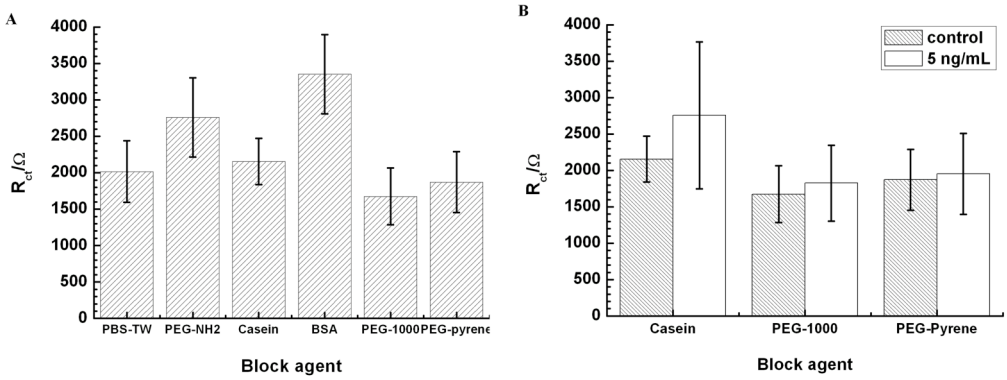

Figure S3. (A) Resistance of charge transfer (R_{ct}) for PG/PDDA/AuNP/Ara h 2-2/serum using different block agent. (B) R_{ct} of PG/PDDA/AuNP/Ara h 2-2/IgY with control and 5 ng mL^{-1} IgY.

EIS were performed in 1 mM $Fe(CN)_6^{3-/4-}$ at 0.3 V from 0.1 to 100 kHz. EIS were performed in 1 mM $Fe(CN)_6^{3-/4-}$ at 0.17 V from 0.1 to 100 kHz with 5 mV amplitude.

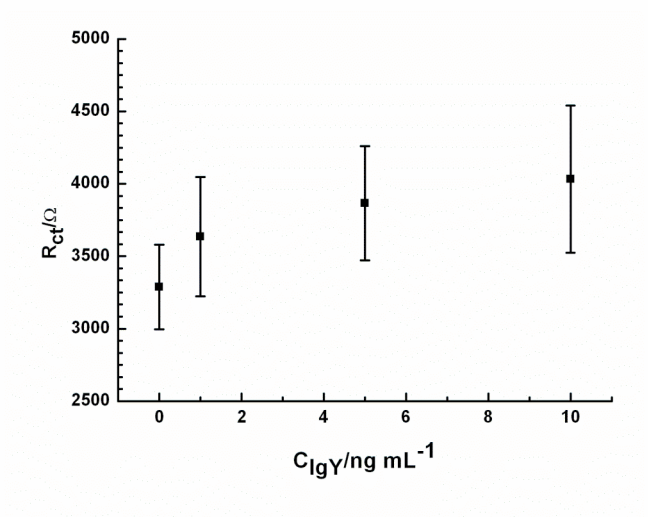

Figure S4. The resistance of charge transfer (R_{ct}) for different concentration of IgY in serum for System III (with Ab_2-HRP **without amplification**) immunosensor. Electrochemical impedance spectroscopy (EIS) was performed in 1 mM $Fe(CN)_6^{3-/4-}$ at 0.17 V from 0.1 to 100 kHz with 5 mV amplitude.

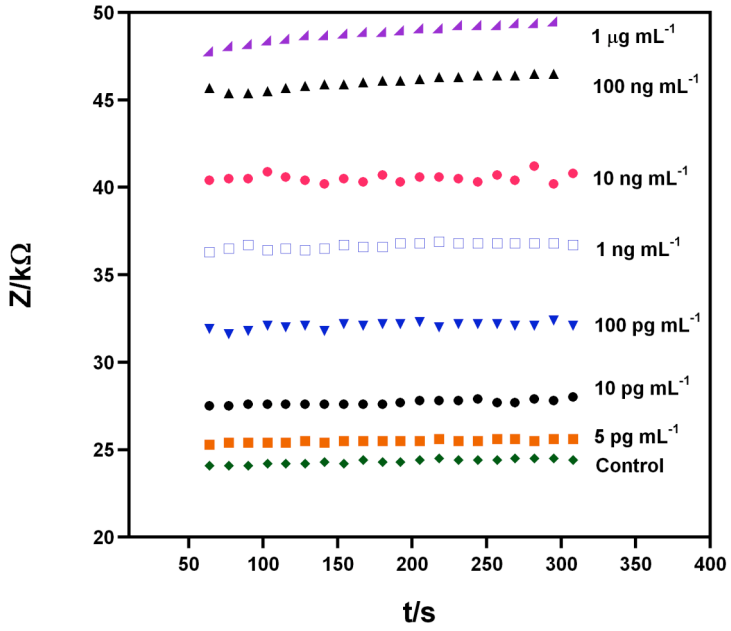

Figure S5. Impedance dependence of time of functionalized PG immunosensor with Ab_2-HRP after the biocatalyzed precipitation for 20 min in the presence of TMB-H_2O_2 substrate solution (System IV) to detect different concentration of IgY in pH 7.0 PBS buffer at 1 Hz and 0 V vs SCE. TMB = 3,3′,5,5′-tetramethylbenzidine.

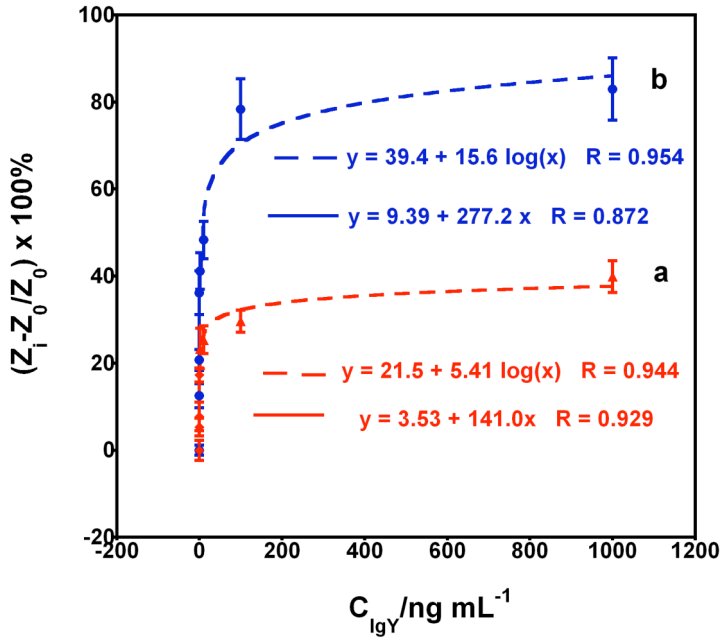

Figure S6. Calibration curve of IgY in serum by (a) Faradaic EIS and (b) non-Faradaic EIS using Ab_2-HRP after biocatalyzed precipitation for 20 min in the presence of TMB-H_2O_2 (System IV) in pH 3.3 acetate buffer. Faradaic EIS was done in 1 mM $Fe(CN)_6^{3-/4-}$ at 0.17 V from 0.1 to 100 kHz , 5 mV amplitude. non-Faradaic impedance using System IV in pH 7.0 PBS buffer at 1 Hz and 0 V. The solid lines are the linear fitting from 0.005 – 0.1 ng mL^{-1}.

References:

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(2) Sauerbrey, G. *Z. Phys.* **1959**, *155*, 206-222.
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(4) Brandrup, J.; Immergut, E.H (Eds.) *Polymer Handbook*, Wiley: New York, 1975.
(5) Creighton, T. E. *Protein Structure: A Practical Approach*; IRL Press, Oxford, U.K., 1990.