Supporting Information

For

Enzyme-catalysed deposition of ultrathin silver shells on gold nanorods: A universal and highly efficient signal amplification strategy for translating immunoassay into litmus-type test

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Experimental details

Preparation of AuNRs: Seeding solution of the AuNRs was prepared as follows: 0.25 mL of 0.20 M cetyl trimethylammonium bromide (CTAB) solution was mixed with 0.25 mL of 0.5 mM $HAuCl_4$. To the stirred solution, 0.03 mL of ice-cold 0.01 M $NaBH_4$ was added, which resulted in the formation of a brownish-yellow solution. Vigorous stirring of this seeding solution was continued for 2 min after which the solution was kept at 25 ºC. The growth solution was prepared by mixing together in 50 ml flask 10 mL of 0.2 M CTAB, 0.56 mL of 4 mM $AgNO_3$, 0.65 mL of 23 mM $HAuCl_4$ and 9.5 mL of Milli-Q water. Approximately 0.25 mL of 80 mM ascorbic acid was slowly added to the mixture. The addition of ascorbic acid was conducted dropwise until the mixture became colourless after which one quarter more of the total number of droplets to that point was added. The final step was the addition of 0.18 mL of the seed solution to the growth solution at 25 ºC. The colour of the solution gradually changed within 10-20 min. The temperature of the growth medium was kept at 25 ºC during the full procedure. 5 mL of the as-prepared solution was centrifuged and redispersed in 5 ml water before use.

Synthetize of biotinylated silica nanoparticles: The silica nanoparticles were prepared as Stober method. Briefly, 4.5 mL of tetraethyl orthosilicate (TEOS) was mixed with 45.5 mL ethanol. 3.33 mL H_2O and 7.55 mL $NH_3.H_2O$ (25%) were mixed with 39 mL ethanol under stirring (1100 rpm). Then the TEOS ethanol solution was added to the above solution and the stirring speed was slowed down to 400 rpm. 3 h later, 0.25 mL of 3-mercaptopropyl trimethoxysilane was added. The silica nanoparticles solution was centrifuged and washed with water after 1 h. Then 5 mg/mL of the thiol modified silica nanoparticles in ethanol was reacted with biotin-malemide (1 mg/mL). After 3 h, the nanoparticles were centrifuged and washed with ethanol. Finally, the nanoparticles were mixed with PEG_{24}-malenide solution for 6 h. After centrifugation, wash and redispersed in PBS steps, the biotin modified silica nanoparticles were prepared.

Assay procedure: 96-well polystyrene plates were modified with mouse monoclonal anti-PSA (100 ml 6μg/mL, Abcam) in PBS at 4 °C overnight. After washing the plates three times with wash buffer, the plates were blocked with blocking buffer (1 mg/ml BSA in PBS) for 1 h at room temperature. Subsequently, the plates were washed three times with wash buffer, and PSA (100 ml, Abcam) was added to the desired final concentration by diluting block buffer. After 2 h, the plates were washed three times with wash buffer, and biotinylated rabbit polyclonal anti-PSA (100 ml, 5μg/mL, Abcam) in blocking buffer was added for 2 h at room temperature. The plates were then washed three times, and 20 μg/mL streptavidin in blocking buffer for 1 h at room temperature. After three times of thorough washing, 20 μg/mL the biotinylated silica nanoparticles were added. Subsequently, 20 μg/mL streptavidin linked alkaline phosphatase was added. Finally, the plates were washed three times with wash buffer and two times with HEPES buffer (100 μL, 10 mM, 5 mM $Mg(Ac)_2$, pH 7.9). The alkaline phosphatase substrate p-aminophenyl phosphate in HEPES buffer (5 mM, 100 μL) was added in each well and incubated for a period of 60 min to produce the reducing agent p-aminophenol. Finally, 20 μL of the AuNRs was added, followed by the addition of fresh prepared $AgNO_3$ with a final concentration of 0.25 mM. After a period of 30 min incubation, the spectrum of each well was recorded.

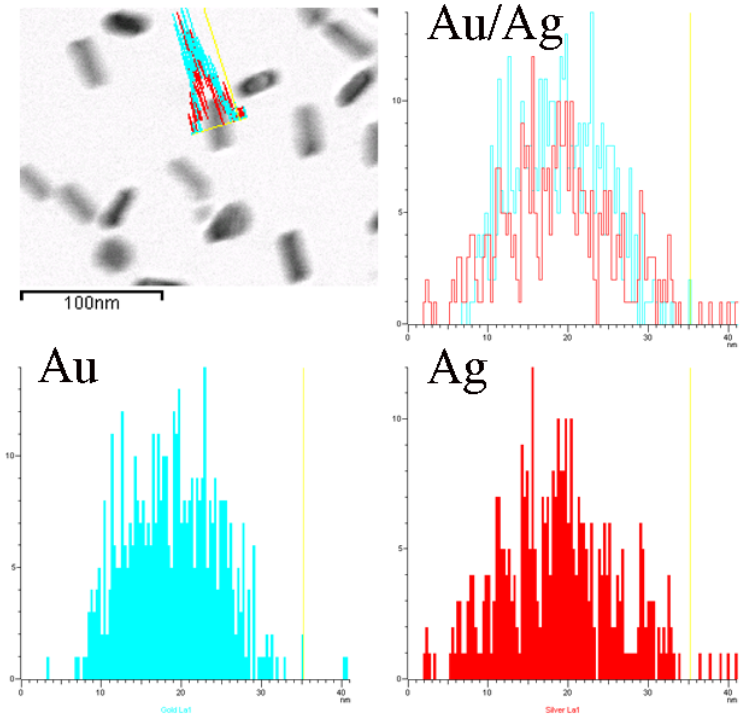

Figure S1 Liner element mapping of the AuNRs after the enzyme-catalysed deposition of the ultrathin silver shells.

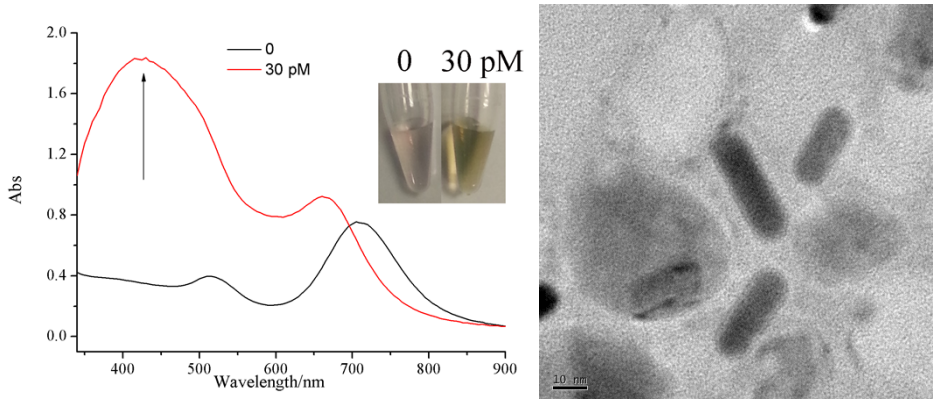

Figure S2 (A) UV-vis spectrum of the AuNRs in the presence of 30 pM alkaline phosphatase and (B) the corresponding TEM image.

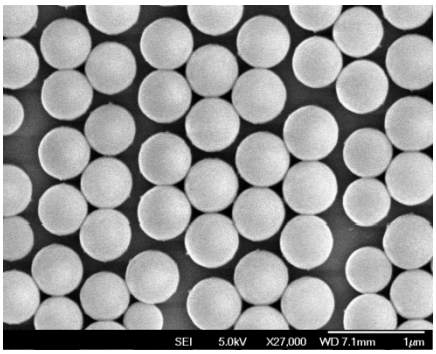

Figure S3. SEM image of the biotinylated silica nanoparticles.

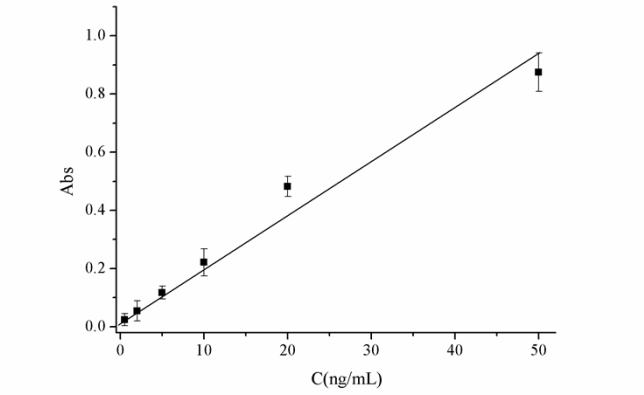

Figure S4. Performance of HRP-based ELISA for PSA.

Table S1 Summary of sensitivities of different protein assays

Method	Analyte	Detection limit	Reference
LSPR chip	PSA	17 ng/mL	1
Enzyme catalyzed gold particle growth	PSA	93 aM	2
Plasmonic ELISA	PSA/p24	10^{-18} g/ml	3
Electrochemical immunoassay	human IgG	5 pM	4
Chemiluminescent immunoassay	Human IgG	7.1 pM	5
Rolling circle amplification	Human IgG	0.9 fM	6
Surface-enhanced Raman spectroscopy	THR/MYG/CRP	100 pM	7
Dissolution-Enhanced Luminescent Bioassay	CEA	0.5 fM	8
DNA-based hybridization chain	IgG	0.1 fg/mL	9
Electronic ELISA	sFlt1	1.25 ng/mL	10

[1] S. S. Aćimović, M. A. Ortega , V. Sanz, J. Berthelot , J. L. Garcia-Cordero, J. Renger , S. J. Maerkl, M.P. Kreuzer , R. Quidant . *Nano Lett.* 2014, **14**, 2636-41.

[2] D. Liu, J. Yang, H. F. Wang, Z. Wang,X. Huang, Z. Wang, G. Niu, A. R. Hight Walker, X. Chen. *Anal Chem.* 2014, **86**, 5800-6.

[3] R. de la Rica, M.M. Stevens. *Nat Nanotechnol*. 2012, **7**,821-4.

[4] Z. Zhong, M. Li, D. Xiang, N. Dai, Y. Qing, D. Wang, D. Tang. *Biosens Bioelectron.* 2009, **24**, 2246-9.

[5] Y. M. Liu, L. Mei, L. J. Liu, L. F. Peng, Y. H. Chen, S. W. Ren. *Anal Chem*. 2011, **83**,1137-43.

[6] Q. Xue, Z. Wang, L. Wang, W. Jiang. *Bioconjug Chem.* 2012, **23**, 734-9.

[7]N. Guarrotxena, G. C. Bazan. *Adv Mater.* 2014, **26**, 1941-6.

[8] S. Zhou, W. Zheng, Z. Chen, D. Tu, Y. Liu, E. Ma, R. Li, H. Zhu, M. Huang, X. Chen. *Angew Chem Int Ed Engl.* 2014, **53**, 12498-502.

[9] B. Zhang, B. Liu, D. Tang, R. Niessner, G. Chen, D. Knopp. *Anal Chem*. 2012, **84**, 5392-9.

[10] M. L. Hammock, O. Knopfmacher, T. N. Ng, J. B. Tok, Z. Bao. *Adv Mater.* 2014, **26**, 6138-44.