Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Facile colorimetric detection of human chorionic gonadotropin based on the peptide-induced aggregation of gold nanoparticles

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Experimental

Reagents and Apparatus

Gold chloride ($HAuCl_4$), human chorionic gonadotropin (hCG), ascorbic acid, glucose, uric acid, urea, and albumin were purchased from Sigma (St. Louis, MO, USA). Gold nanoparticles (AuNPs, 13 nm) were synthesized by the citrate reduction of $HAuCl_4$, and the maximum absorption peak of the AuNPs was 520 nm. The oligopeptide probe (PPLRINRHILTR) was synthesized from MB Mission Biotech (Taipei, Taiwan) at a purity of 95%. Ultrapure water was from a Milli-Q system (Millipore, Bedford, MA, USA) and was used in all experiments.

UV–vis absorption spectra were recorded at room temperature with a Varian Cary 50 spectrophotometer (Varian Medical Systems, Inc., Palo Alto, CA) in the wavelength range from 400–800 nm. The photographs were taken with a Sony Xperia S smartphone. Human serum samples were collected from healthy women. The study was approved by the Institutional Review Board of the Mackay Memorial Hospital.

Detection of hCG

Various concentrations of hCG (12 μL) were mixed with 500 nM peptide probe in a 10 mM phosphate solution (18.8 mM NaCl, pH 7.2) for 10 min, and the mixture was diluted to a volume of 170 μL with water. After a 10 min incubation, 30 μL of the AuNP solution (8.5 nM) was subsequently added to the hCG/peptide reaction solution. After 16 min of reaction, the absorption spectra of the resulting AuNP solutions were measured. Control experiments containing possible interferents, including ascorbic acid, glucose, uric acid, urea, and albumin, were performed under the same conditions. Real sample analysis was conducted using human serum from healthy women, and the samples were subjected to a 50-fold dilution before analysis. An examination was conducted similar to that described in the above assay process.

Results and discussion

Effect of peptide concentration, incubation time, and reaction time

Figure S1A shows the absorbance ratio A_{600}/A_{525} after adding 0, 250, and 750 mIU/mL hCG to different concentrations of peptide probes. The aggregation of the AuNPs was increasingly inhibited with increasing hCG concentrations at a constant concentration of peptide. Considering the sensitivity of the biosensor response and the signal-to-noise ratio, a 500 nM peptide concentration was selected as the appropriate amount. The effect of incubation time under the optimized peptide concentration was also investigated after 250 mIU/mL of hCG was added to the solution (Fig. S1B). The absorbance ratios of the AuNP solutions became constant after 10 min. Therefore, the mixture of hCG/peptide was incubated for 10 min. Figure S1C shows the change in the time-dependent absorbance ratio with various concentrations of hCG molecules. As expected, a high hCG concentration led to a noticeable decrease in the change in absorbance ratio because of the increased inhibition of peptide-triggered aggregation. The AuNP aggregation induced by the peptide was complete within 16 min.

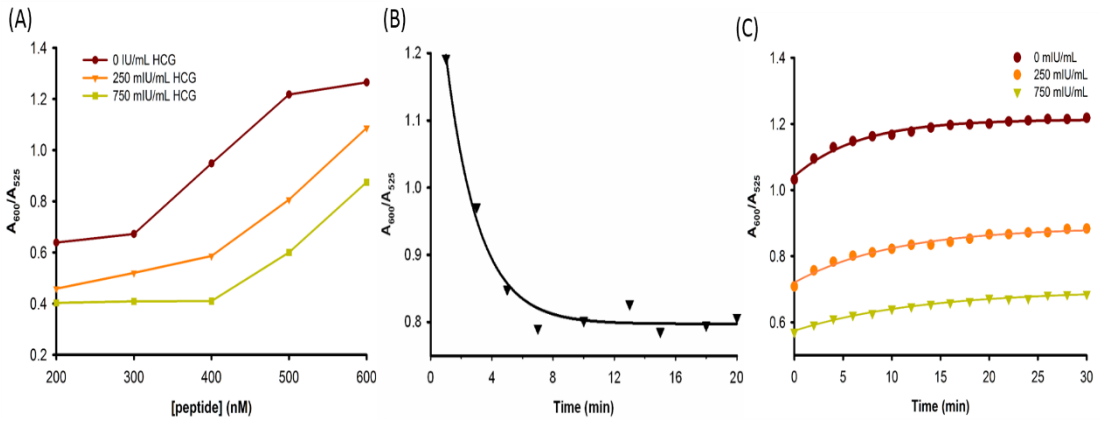

Fig. S1 Optimization of the colorimetric sensing efficiency using different (A) probe concentrations and (B) incubation times. (C) Kinetics of AuNP aggregation with different hCG concentrations.

Detection of hCG in complex sample matrices

As can be seen in Fig.S2, the ΔR increased with the increment of the dilution fold of serum samples. When the serum sample was diluted by more than 50-fold, the positive responses matched the result in the buffer solution much better. While the real reason is not quite clear to us, the interference from the matrix components such as albumin proteins may be one of the possibilities. This interference may be minimized by dilution of samples with the buffer solution, which would be convenient for the practice application in our study.

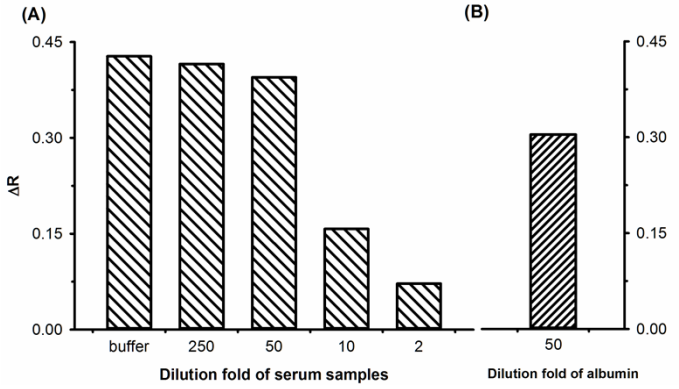

Fig. S2 The response of ΔR measured at different dilutions with and without the addition of 750 mIU/mL hCG (A) in different dilution fold of serum samples and (B) in the buffer solution containing 50-fold dilution of albumin (0.8 mg/mL).

Table S1 Analytical properties of different hCG immunosensors

Detection principle	Detection probe	Linear range (mIU/mL)	Detection limit (mIU/mL)	Reference
electrochemistry	antibody	0.1 - 20.0	0.024	1
electrochemistry	antibody	0.005 - 500	0.0026	2
immunochromatographic assay	antibody	100 - 6000	50	3
photoluminescence	antibody	not shown	500	4
photoluminescence	antibody	20 - 200	20	5
fluorescence	antibody	36 - 100	36	6
surface plasmon resonance	antibody	2500 - 1000	1000	7
liquid crystals	peptide	not shown	1000	8
colorimetric assay	peptide	50 - 1000	25	our work

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