Supplementary Information

Colorimetric detection of human chorionic gonadotropin using catalytic gold nanoparticles and a peptide aptamer

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Experimental section

Reagents and apparatus

Gold chloride (HAuCl$_4$), hCG, trisodium citrate, 4-nitrophenol (4-NP), sodium borohydride (NaBH$_4$), glucose, uric acid, urea, and albumin were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). Acetaminophen and acetylsalicylic acid were purchased from Taiwan Veterans Pharmaceutical Co., Ltd. (Taoyuan, Taiwan). AuNPs (13 nm) were synthesized based on published methods, and the maximum absorption peak of AuNP was 520 nm. In brief, 100 mL HAuCl$_4$ (41 mg, 1 mM) was brought to reflux, and 10 mL trisodium citrate (114 mg, 38.8 mM) was added while stirring. The AuNP solution was boiled for a further 30 min and left to cool to room temperature. Finally, the solution was filtered and stored at 4 °C until use. The customized synthesis peptide probe, Pro-Pro-Leu-Arg-Ile-Asn-Arg-His-Ile-Leu-Thr-Arg, was obtained from Mission Biotech Co., Ltd. (Taipei, Taiwan) at a purity of 95%.

A UV–vis spectrophotometer (Varian Cary® 50) from Varian Medical Systems, Inc. (Palo Alto, CA, USA) was used for all absorbance measurements, which were taken at room temperature with a wavelength range of 200–500 nm. Photographs were taken with a Sony Xperia S (Sony Mobile Communications, Tokyo, Japan). The clinical experiments were approved by the Institutional Review Board of the Mackay Memorial Hospital (Taipei, Taiwan).
**hCG detection**

For the catalysis-based colorimetric assay, various concentrations of hCG solution (12 μL), hCG-binding peptide (1 μL, 50 μM), and 50 μL of PBS buffer solution (1 mM sodium phosphate, 15 mM NaCl, pH 7.0) were sequentially added to AuNP solution (44 μL, 0.8 nM), and the mixtures were incubated for 5 min. Then, a reaction mixture containing 4-NP (22 μL, 0.01 M) and freshly prepared NaBH₄ (8 μL, 0.2 M) was added, and the mixtures were incubated for 90 min. Next, ultrapure water was introduced into the solution (263 μL) to stop the reaction. Subsequently, the mixtures were subjected to absorbance measurement.

For the aggregation-based colorimetric assay, various hCG concentrations (12 μL) were mixed with 1 μL of 100 μM peptide probe in PBS solution for 5 min, and the mixtures were diluted to 200 μL with water. After 5 min incubation, 200 μL of AuNP solution (1.7 nM) was added to the hCG/peptide reaction solutions. After 5 min reaction, the resulting AuNP solutions were subjected to absorption measurement.

Practical tests were conducted using human urine donated by the author and serum donated by a healthy woman. Pregnant samples were collected from the serum of pregnant women and were provided by the Department of Obstetrics and Gynecology, Mackay Memorial Hospital. Quantitative determinations of hCG in serum samples were made using a standard ELISA method. Prior to the measurement by the
colorimetric assay, these serum samples were diluted 100-fold with buffer due to our previous experiments (Fig. S4). Similar examinations were performed as for the catalysis-based colorimetric assays.

*Concordance correlation coefficient*

The concordance correlation coefficient (CCC) \(^1\) is used to simply evaluate how small the differences between two approaches by measuring the variation of their linear relationship from the 45° line through the origin. It includes a measurement of accuracy (Cb) and precision (r), and is calculated as follows: \(CCC = r \times Cb\), where \(r\) is the Pearson correlation coefficient and \(Cb\) is a bias correction factor. The value of CCC agreement is as perfect (>0.99), substantial (0.95-0.99), moderate (0.90-0.95), and poor (<0.90). The calculations of CCC were performed using MedCalc software (MedCalc Inc., Mariakerke, Belgium).
Fig. S1. Ultraviolet–visible spectrophotometry spectra and images (inset) of the mixtures with (a) 0.85 nM citrate-stabilized gold nanoparticles (AuNP); (b) 7.5 IU/mL human chorionic gonadotropin (hCG) + 0.85 nM AuNP; (c) 1.25 μM peptide + 7.5 IU/mL hCG + 0.85 nM AuNP; and (d) 1.25 μM peptide + 0.85 nM AuNP.
Fig. S2. Kinetics of the response change at various concentrations of hCG.
**Fig. S3.** Detection of human chorionic gonadotropin using an aggregation-based colorimetric method. (A) Absorption spectra of gold nanoparticle (AuNP) solution with increasing amounts of human chorionic gonadotropin (hCG; 0, 0.15, 0.3, 0.6, 1.875, 3.75, 7.5, and 15 IU/mL). (B) Calibration curve for the absorption ratio ($A_{600}/A_{522}$) as a function of hCG concentration using the aggregation-based colorimetric assay. Error bars represent the standard error of the mean, $n = 3$. The detection limit, based on a signal-to-noise ratio of 3, was calculated to be 300 mIU/mL.
Fig. S4 Spectral response at 400 nm ($A_{400}$) measured at different dilutions with the addition of 750 mIU/mL hCG. Error bars represent the standard error of the mean, $n = 3$. 
Fig. S5. The $\Delta A$ obtained from urine and serum samples spiked with human chorionic gonadotropin and comparison with the same concentrations examined in buffer.
Fig. S6. Correlation plot between sample concentrations as determined by referenced ELISA vs. our proposed assay.
**Table S1** Comparison of various methods for hCG assay

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Capture probe</th>
<th>Detection limit (^a) (mIU)</th>
<th>Linear range (mIU)</th>
<th>Practical application</th>
<th>Reference</th>
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<td>this work</td>
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\(^a\) 1 ng/mL=10 mIU/mL
References


