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Supplementary material

DNA base-stacking assay utilizing catalytic hairpin assembly-induced gold nanoparticle aggregation for colorimetric protein sensing

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1. Material and Methods

1.1 Materials and Apparatus

All chemicals were purchased from Merck (Darmstadt, Germany), TCI Japan (Tokyo, Japan), or Sigma-Aldrich Inc. (St. Louis, MO, USA), and were of analytical grade without further purification. Fibronectin and vitronectin were obtained from Abcam (Cambridge, MA, USA). Interleukin-1 and human chorionic gonadotropin were acquired from Sigma-Aldrich Inc. Ultrapure deionized water was generated using a Millipore water purification system (18.2 MΩ cm–1; Milli-Q). DNA oligonucleotides were synthesized by Purigo Biotech (Taipei, Taiwan) and were purified on OPC cartridges. The DNA substrates are listed in Table S1. The underlined letters in sequence HS1 are the fibronectin aptamer sequence[1], which is partially blocked by the hairpin stem region. All colorimetric measurements were performed on a Cary-50 spectrometer (Varian Medical Systems, Palo Alto, CA, USA) at room temperature.

Table S1. DNA sequences

	Sequence
HS1	CATGCGCCTTCCCCCTGTGGTTGGTGTCAGTCGGCCTGTG AAGGCGCATG
HS2	CATGCGCCTTCACAGGCCGACTGACACCAACCACAGGGG GAAGGCCTGTGAAGGT
RP1 (7-nt)	TTCACAG
RP2 (8-nt)	CTTCACAG
RP3 (9-nt)	CCTTCACAG
RP4 (10-nt)	ACCTTCACAG

1.2. Synthesis of Gold Nanoparticles

Bare gold nanoparticles were prepared using the citrate-mediated reduction method. Briefly, 10 mL of trisodium citrate (114 mg, 38.8 mM) solution was rapidly added to 100 mL boiling $HAuCl_4$ (41 mg, 1.0 mM) solution. The resulting solution was boiled for an additional 10 min and then cooled to room temperature with continuous stirring. The AuNP solution was then filtered through a 0.22 μm membrane filter and stored at 4 °C prior to further use.

1.3. Enzyme-Linked Immunosorbent Assay

The cervical-vaginal fluid sample was obtained with the approval of the institutional review board committee of Mackay Memorial Hospital, Taiwan. The sample was determined by Department of Obstetrics and Gynecology, Mackay Memorial Hospital, using enzyme immunoassay kits (Abcam, Cambridge, UK) with the microplate reader. The procedures for fibronectin were followed by the commercial protocols.

1.4. Colorimetric Detection of Fibronectin

Twenty microliters of fibronectin solutions at different concentrations were mixed with 6 μL of HS1 (10 μM) and 6 μL of HS2 (10 μM) in a running buffer solution (90 mM Tris, 90 mM Boric acid, 2 mM Na_2EDTA, and 14 mM NaCl) and the HS1/HS2/protein mixture was brought up to a total volume of 100 μL with buffer. Meanwhile, 1 μL of RP (20 μM) was added to 80 μL of AuNP solution and the RP-capping AuNP solution was brought up to a total volume of 100 μL with ultrapure water. Following 60 min incubation at room temperature, these two solutions were mixed together for 20 min. Subsequently, 1 μL of NaCl (500 mM) was added into the

reaction mixture and incubated for 5 min. Finally, the mixed solution was observed using the naked eye or was quantified by UV–vis absorption spectroscopy.

1.5 Quartz Crystal Microbalance (QCM) Measurements

The QCM system and 9 MHz AT-cut quartz chips available from ANT technology (Taipei, Taiwan) were operated at room temperature. Prior to the immobilization of the reporting probe, the quartz chips were cleaned sequentially with 1 M NaOH for 30 min and 1 M HCl for 5 min, then rinsed thoroughly with water and dried under nitrogen. The cleaned chips were immersed in a solution of 500 nM thiolated reporting probe (SH-CCTTCACAG) overnight at room temperature. Following surface modification, the chips were incubated with 1 mM mercaptohexanol for 10 min to reduce non-specific binding effects. Following attachment of the reporting probes to the chip surface, the mixture solution containing the target protein, HS1, and HS2 was applied to the chip surface to determine the validity of this method.

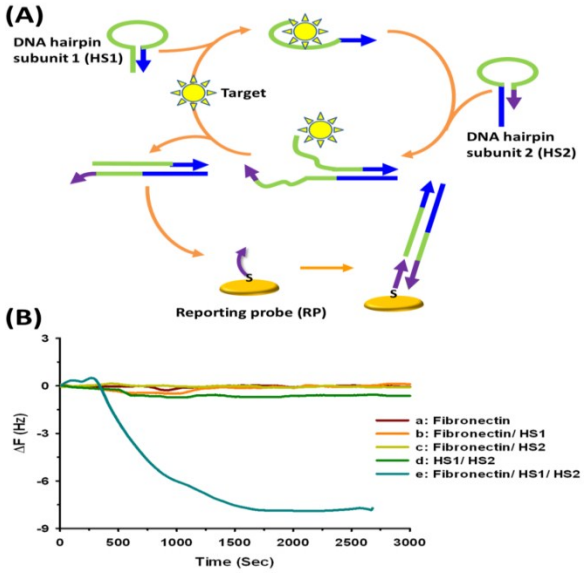

Fig. S1 (A) Schematic of protein detection using a combination of the CHA strategy and base stacking hybridization. The presence of target proteins triggers the activation of the CHA reaction cascade, resulting in the generation of abundant CHA products. Next, the CHA products are captured by reporting probe because of the base stacking effect and are recorded by QCM. (B) The frequency of change following the addition of different mixtures: (a) fibronectin, (b) fibronectin/HS1, (c) fibronectin/HS2, (d) HS1/HS2, and (e) fibronectin/HS1/HS2.

The concentration of fibronectin used was 100 ng/mL and the concentrations of HS1 and HS2 were 300 nM.

As exemplified by Fig. S1, the change of frequency is associated with the adsorbed mass. The addition of the mixture solution (e) can induce a frequency decrease of approximately 7.5 Hz, indicating that the CHA products were captured on the surface of the AuNPs. In contrast, the addition of different mixture solutions induced only a small or negligible frequency change, which also indirectly suggests that only target fibronectin can cause hairpin DNA assembly between HS1 and HS2 and propagate the subsequent base stacking interaction between the CHA products and the reporting probes. The QCM results confirm the feasibility of target protein catalyzed hairpin assembly strategies for base stacking hybridization.

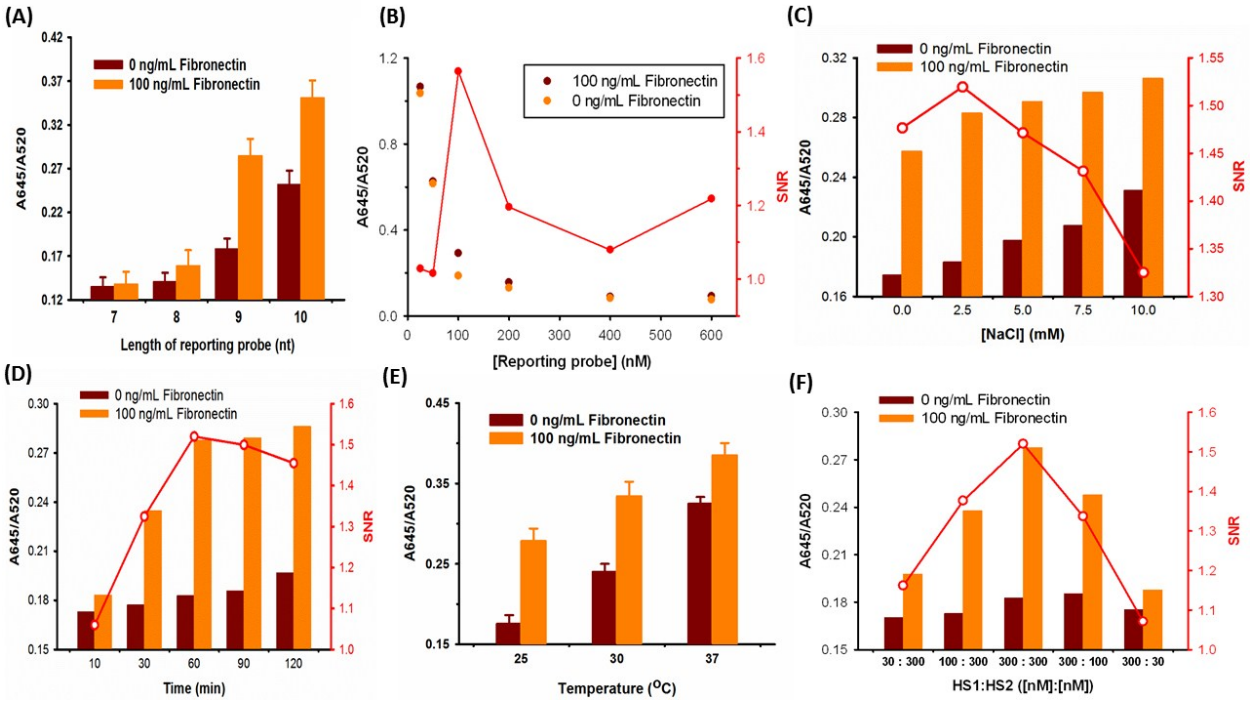

Fig. S2 Effect of (A) reporting probe length, (B) reporting probe concentration, (C) NaCl concentration, (D) CHA reaction time, (E) reaction temperature, and (F) the concentration ratio of HS1 to HS2 on the colorimetric assay. SNR; signal-to-noise ratio.

Reference

1. A. Ogawa, N. Tomita, N. Kikuchi, S. Sando, and Y. Aoyama, *Bioorg. Med. Chem. Lett.* 2004,14, 4001.