Supplementary Material

Label-free colorimetric aptasensor for IgE using DNA pseudoknot probe

Chia-Chen Chang, Chen-Yu Chen, Xihong Zhao, Tzu-Heng Wu, Shih-Chung Wei, and Chii-Wann Lin

Experimental

Quartz Crystal Microbalance (QCM) Measurements

To perform quartz crystal microbalance measurements, 200 nM of DNA capture probe was first immobilized on gold-coated quartz crystals overnight, followed by thorough rinsing with distilled water. Then, the gold surface was incubated in 10 mM mercaptohexanol for 10 min to eliminate nonspecific adsorption. After establishing a stable baseline with PBS used as the running buffer, the mixture solution of 200 nM psuedoknot DNA probes and 5 nM IgE was introduced into the flow channel of the QCM chamber at a flow rate of 10 μL/min.

Results and Discussion

QCM Measurement

To realize our design, we used QCM to monitor the binding reactions of the IgE/aptamer probe complex (Fig. S1A). As seen from curve B in Fig. S1B, the DNA_C-modified gold substrate showed a slight frequency shift in the absence of IgE ($\Delta F_B = -0.9$ Hz). After incubation with the IgE and aptamer probe, the frequency shift was significantly increased, indicating the binding of the IgE/aptamer complex onto the DNA_C-modified gold surface (curve A, $\Delta F_A = -11.1$ Hz). Moreover, we also observed that the frequency shift was only slightly changed when the DNA_R-modified QCM probe was incubated with the IgE/aptamer probe complex (curve C, $\Delta F_C = -1.2$ Hz). The results revealed that the IgE/aptamer probe complex could be absorbed onto the QCM probe through hybridization between the DNA_C capture probe and 3′ terminus of the aptamer probe. In addition, this difference provides support for the detection mechanism for IgE by using a pseudoknot DNA switch probe.

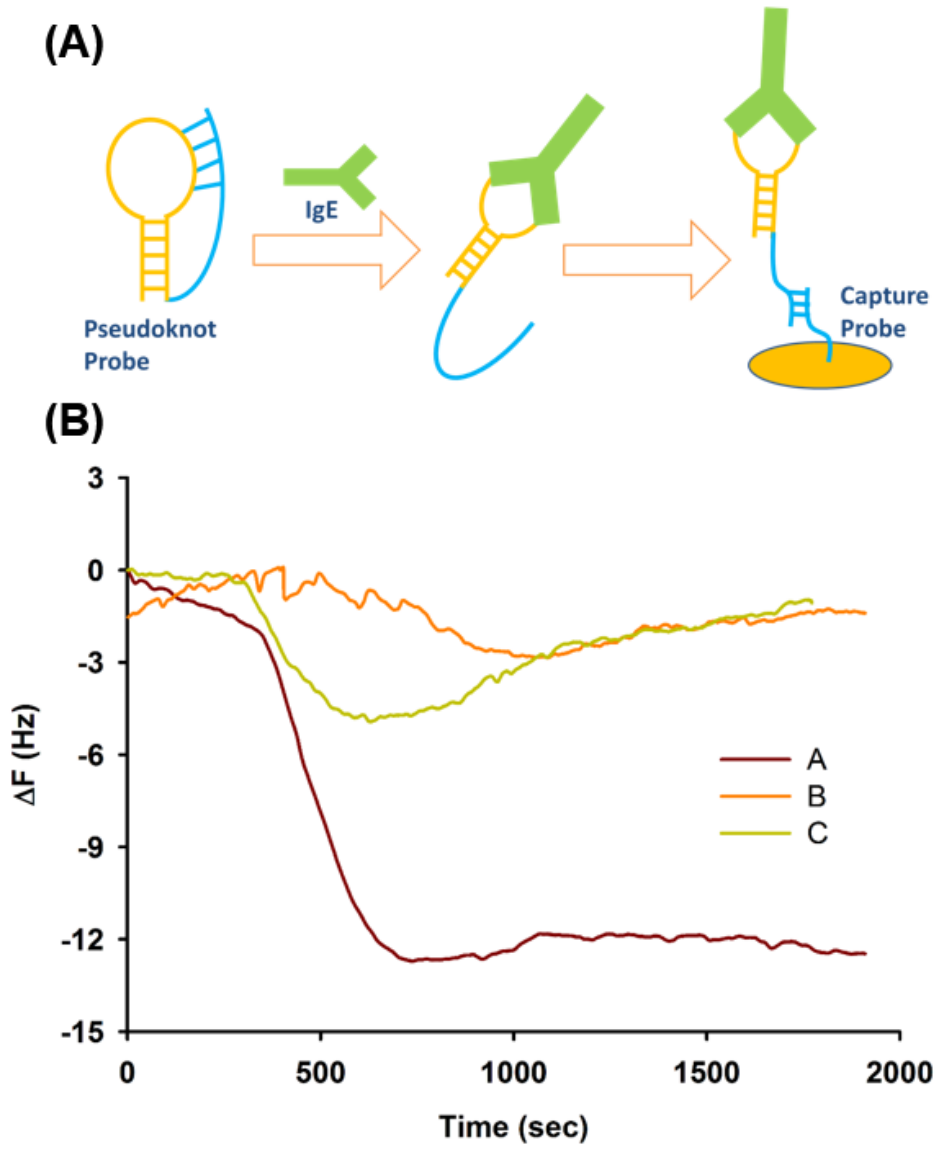

Fig. S1. (A) Schematic for the recognition of IgE by using the structure switching IgE aptamer probe. (B) Real-time frequency responses of the (a) IgE/pseudoknot probe, (b) pseudoknot probe to a DNA_C-immobilized QCM chip, and (c) IgE/pseudoknot probe to a DNA_R-immobilized QCM chip.

Assay Optimization

We further investigated the effect of the concentration of the pseudoknot DNA probe and salt ion and the incubation time between the pseudoknot aptamer/IgE and DNA_C in order to minimize the reagent consumption and reduce the assay time. The effect of the concentration of the pseudoknot aptamer was studied in the range 50–400 nM. The absorption ratio increased at higher concentration; however, the noise also increased when the concentration exceeded 100 nM (Fig. S2A). A similar response had also been obtained by the addition of sodium ion above 35 mM (Fig. S2B). Considering the high signal-to-noise ratio of the spectral ratio, we chose 100 nM pseudoknot probe and 35 mM sodium ion in the developed method. The effect of the IgE/aptamer probe and DNA_C interaction time was investigated between 5 and 75 min. From Fig. S2C, it could be observed that the blank ratio (without IgE) hardly changed as time passed. Conversely, the absorption ratio of the IgE/aptamer probe/capture probe complex gradually increased from 5 to 15 min, and almost remained constant after 15 min of interaction time. Thus, the optimal interaction time was considered as 15 min. The time-dependent spectral ratio of AuNP was monitored in the presence of 5 nM IgE (Fig. S2D). The absorption ratio increased with the binding time and reached a maximum at about 20−25 min; therefore, the response time of this colorimetric assay after addition of IgE is 20 min.

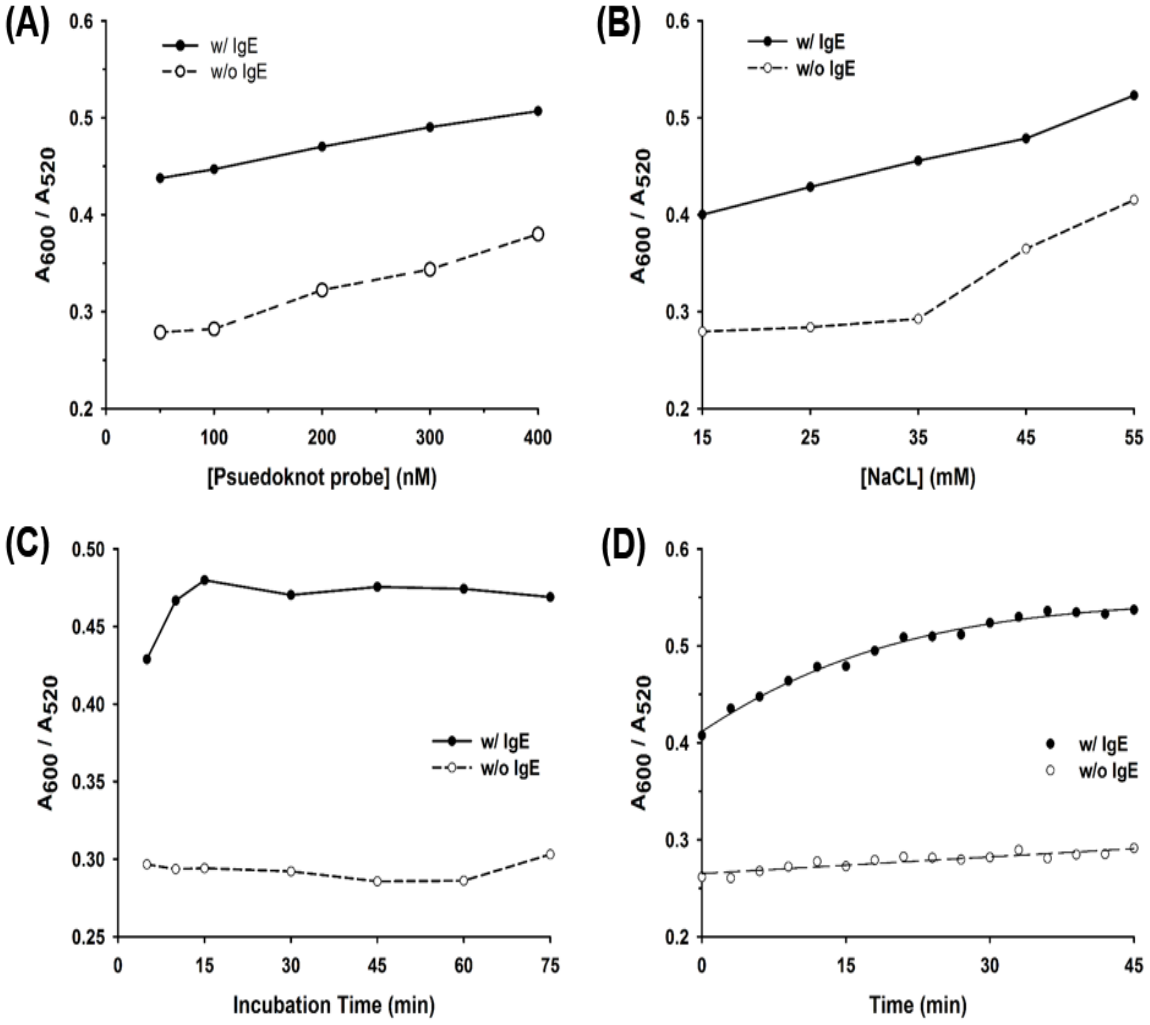

Fig. S2 Optimization of the sensing efficiency for 5 nM IgE using different (A) pseudoknot probe concentration, (B) NaCl concentration, and (C) incubation time. (D) Kinetics of AuNP aggregation with 5 nM IgE.

Detection of IgE in complex sample matrices

Recent research reported that the expression levels of IgE in vaginal fluid might depend on Candida infections [1,2]. To further demonstrate the feasibility of this assay, experiments were carried out by standard addition approaches in diluted vaginal fluid. In Fig. S3A, when the vaginal fluid was diluted by less than 50-fold, the positive responses were at least 80% lower than those from the buffer system, and a large variation was observed. At this stage, while the real cause is not fully understood, the interference from the heterophilic molecules or matrix components may be one of the possibilities [3, 4]. Nevertheless, with over 75-fold dilution, the testing matched the results in homogeneous solution much better, and a good correlation between the detection of spiked concentrations of IgE in the buffer system and in the diluted vaginal fluid was also observed (Fig. S3B). These results indicated that the interference could be greatly reduced by high dilution, which results in a better recovery.

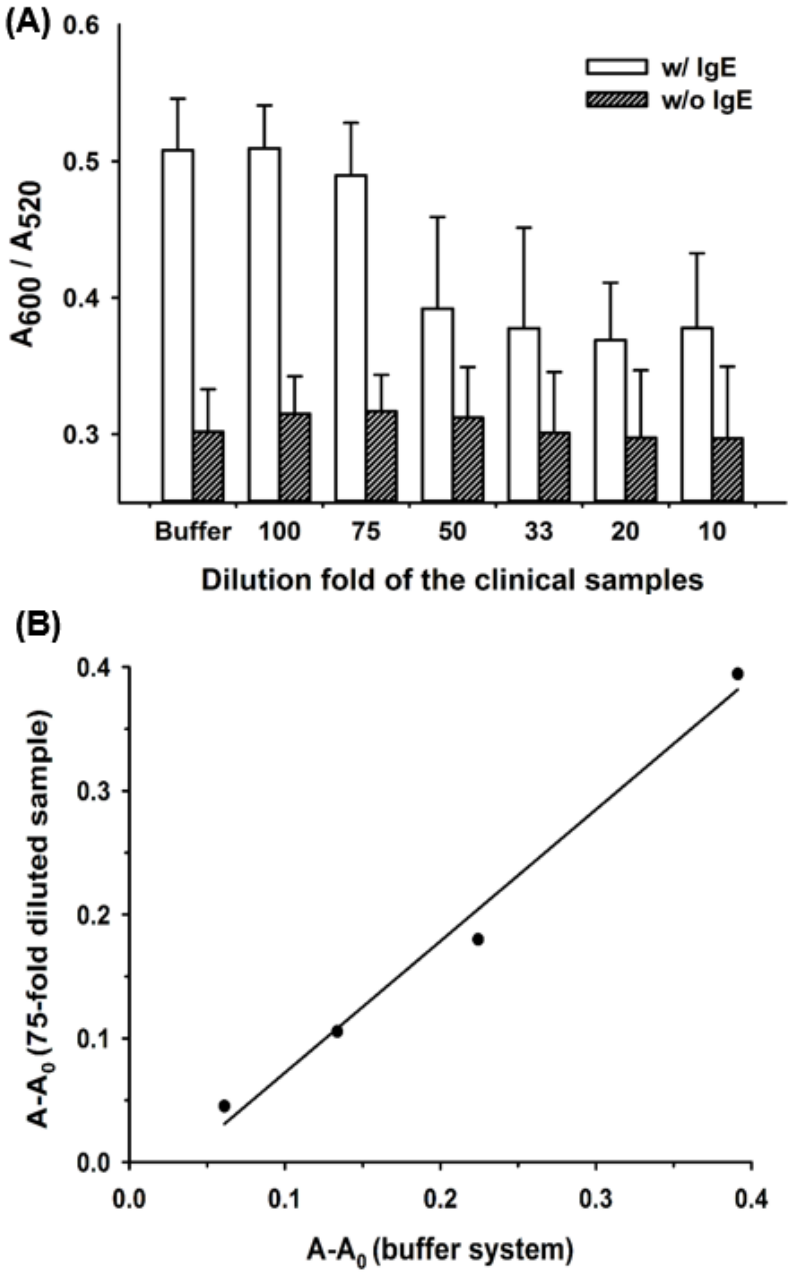

Fig. S3 (A) Spectral ratio of the colorimetric aptasensor measured at different dilutions with and without the addition of 5 nM IgE. Error bars represent the standard deviations of three replicates. (B) Correlation between the spectral response obtained in the clinical samples and the standard samples of spiked IgE at four concentrations (1, 2.5, 5, and 25 nM) (R^2 = 0.986).

References

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