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Solid-state colorimetric polydiacetylene liposome biosensor sensitized by gold nanoparticles

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Polydiacetylene (PDA), a conjugated polymer, has attracted attention for realization of a label-free real-time colorimetric biosensor because it exhibits large and rapid colorimetric responses upon the binding of biomolecules. This is due to the conformational distortion of its conjugated backbone. However, solid-state PDA biosensors for point-of-care diagnosis remain unexplored. We describe a highly sensitive solid-state biosensor based on PDA liposomes. We employed gold nanoparticles (AuNPs) on PDA liposomes as the molecular-binding-signal sensitizer, which provides additional conformational distortion in the backbone structure of PDA by exerting steric repulsion to the attached biomolecules. To prove the concept, AuNPs and a thrombin-binding-aptamer were individually functionalized on PDA liposomes, which were attached to a substrate for the detection of thrombin. We found that the sensitivity was enhanced 2.5 times in the presence of AuNPs compared with the case without AuNPs. Because the steric repulsion of the AuNPs is target-independent, we believe that our solid-state biosensor provides a path toward advanced solid-state biosensors.

1. Introduction

Polydiacetylene (PDA) has potential as a sensing unit for diverse sensors because of its large and rapid colorimetric transition upon various stimuli, such as changes in temperature[1,2] and pH,[1,3] chemical modifications,[4] and mechanical stress induced by biomolecular binding.[5–7] The colorimetric transition of PDA arises from the conformational distortion of both its structural backbone and side chains. For decades, biomolecular sensing by PDA was primarily demonstrated in an aqueous solution because PDA naturally forms liposomal micelles for efficient color transitions. PDA liposomes were typically implemented in relatively high concentrations to facilitate mechanical stresses by either repulsion or aggregation of the PDA liposomes under biomolecular binding.[8–10] Despite remarkable progress, liquid-state PDA biosensors have latent problems in storage, transportation, handling, and reliability. Therefore, the development of solid-phase biosensors has become important for applications in line with the enormous growth of healthcare markets. However, solid-state platforms for PDA biosensors have hardly been developed because their sensitivity is typically inferior to that of liquid-state PDA biosensors.

To realize highly sensitive PDA biosensors, metallic nanoparticles have been employed through diverse approaches. In a recent study, gold nanoparticles (AuNPs) were used as a biosensing label that induced mechanical stress on PDA. However, this biosensor required a complex sandwich detection scheme, which is undesirable in a point-of-care biosensor.[7] Another approach is using AuNPs as a sensitizer for the photo-polymerization of diacetylene to amplify signal generated from the PDA.[11,12] However, this approach is also unsuitable for point-of-care detection because it requires an external UV light source to initiate polymerization *in situ*. Therefore, despite these notable efforts, a practical PDA biosensor that provides a label-free real-time solid-state biosensing platform, remains unexplored.

We developed a highly sensitive solid-state biosensor based on PDA liposomes by employing AuNPs as the molecular-binding-signal sensitizer. The conformational distortion in the backbone structure of PDA was amplified by the steric repulsion of AuNPs to the attached biomolecules. We demonstrated the detection of thrombin using the AuNPs and a thrombin-binding-aptamer, which were individually functionalized on PDA liposomes attached to a substrate. The sensitivity, due to

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the steric repulsion effect of AuNPs, was enhanced 2.5 times that without AuNPs. We also found that the size and the grafting number of AuNPs are important for obtaining maximum sensitivity. Our strategy for sensitizing the colorimetric responses of PDA liposomes is target-independent, therefore, we believe that our solid-state biosensor paves the way for highly sensitive label-free real-time solid-state PDA biosensors.

2. Experimental section

2.1. Materials

The following chemicals were used without further purification: (3-aminopropyl)triethoxysilane (APTES), *n*-(3-dimethylaminopropyl)-*n*′-ethylcarbodiimide hydrochloride (EDC), *n*-hydroxysuccinimide (NHS), triethylamine (TEA), sodium boron hydride ($NaBH_4$), cysteamine hydrochloride, $HAuCl_4$, chloroform (anhydrous, >99%), and thrombin purchased from Sigma-Aldrich (St Louis, MO, USA); 10,12-tricosadiynoic acid (TCDA) purchased from GFS chemicals (Powell, OH, USA), and amine-modified thrombin-binding-aptamer (TBA) DNA oligonucleotide purchased from Bioneer Co. Ltd. The 0.8 μm cellulose acetate filter used in this study was purchased from Advantec Toyo. Ltd.

2.2. Synthesis of amine-functionalized AuNPs

First, 22.8 μL of $HAuCl_4$ (1.42 mM) was mixed with 400 μL of cysteamine hydrochloride in 40 mL of deionized water. The mixture was vigorously stirred at room temperature for 20 min, then 10 μL of freshly prepared $NaBH_4$ (10 mM) solution was rapidly added into the mixture under vigorous stirring for 60 min. The color of the solution changed from yellowish to red-wine-colored as the AuNPs synthesized. The solution was stored in a refrigerator (4 °C) before use. The optical absorption spectra were examined by a UV–Vis spectrometer and morphological characterization was conducted using field emission scanning electron microscopy (FE-SEM) and dynamic light scattering (DLS).

2.3. Preparation of TCDA liposomes

TCDA liposomes were prepared by a bulk method. TCDA monomers were dissolved in anhydrous chloroform and the solvent was evaporated by a rotary evaporating system under N_2 steam, generating a thin film on the surface of a flask. Next, deionized water (18.2 MΩ cm) was added into the flask to make 1 mM of TCDA lizposome solution. The solution was sonicated at 70 °C for 15 min until it was dissolved and appeared clear. Then, the solution was filtered through a 0.8 μm cellulose acetate syringe filter to remove aggregated and oversize liposomes. This was followed by cooling at 4 °C overnight before use.

2.4. Functionalization of AuNPs on TCDA liposomes attached on a glass substrate

A glass substrate was functionalized using APTES, as described in a previous work,[13] for immobilization of the amino group on the glass surface. It is necessary to immobilize TCDA liposomes before the grafting of AuNPs and TBA because we exploit EDC/NHS coupling method for the further sequential modifications. Equal volumes of EDC/NHS solution (500 μL, 10 mM) were mixed and added to the prepared TCDA liposome solution (50 μl) for 30 min at 4 °C. Then, the glass substrate was dipped into the mixture solution at room temperature for 3 h to allow the reaction between the carboxyl group of the TCDA liposomes and the amine group of the glass surface. This forms amide bonding for immobilization of TCDA liposomes on the glass surface. After the reaction, the substrate was washed with deionized water to remove un-bonded TCDA liposomes and then dried with pure N_2 gas. Next, equal volumes of EDC/NHS solution (500 μL, 1 mM) and amine-functionalized AuNP solution (250 μL) were mixed. Then, the glass substrate with the TCDA liposomes was dipped in the mixture solution for 30 min to attach AuNPs to the TCDA liposomes. After gentle washing with deionized water to remove unbonded AuNPs, the glass substrate was dried with pure N_2 gas. We controlled the grafting amount of AuNPs using triethylamine solution (1 mM), for regulating the pH from 8.5 to 9.5, for an efficient EDS/NHS reaction.

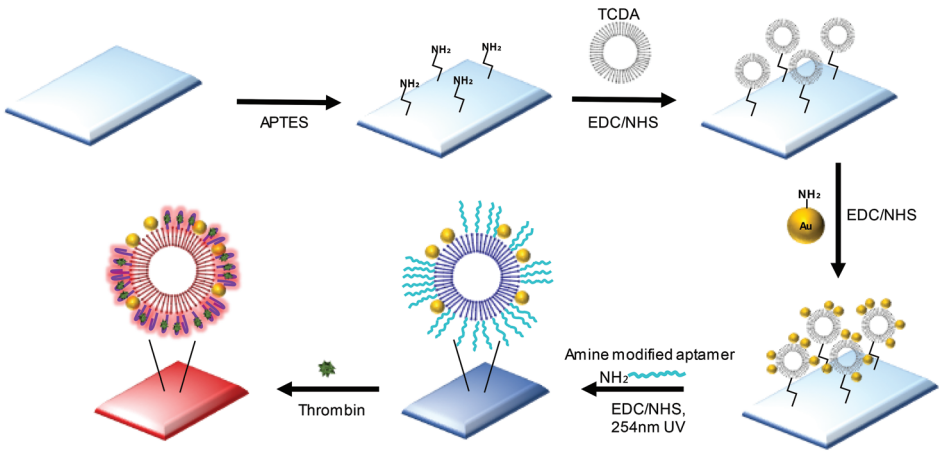
2.5. Grafting TBA on AuNP-PDA liposomes

AuNP-TCDA liposomes on the glass substrate were modified with TBA through a coupling reaction with EDC/NHS. To form an amide bond, 500 μL of EDC (1 mM), 500 μL of NHS (1 mM), and 5 μL of TBA (0.05 mM) were added to the AuNP-TCDA liposomes and incubated for 3 h. After incubation, the substrate was washed with deionized water three times and dried with pure N_2 gas. Finally, to polymerize TCDA liposomes into PDA liposomes, we irradiated the glass substrate using UV light at 254 nm for 15 min. As a result, the color of the glass substrate changed to blue, as described in Scheme 1. The whole process was monitored with a UV–Vis spectrometer (JASCO V770) and a field emission scanning electron microscope (FE-SEM; JSM7500F, JEOL).

3. Results and discussion

We investigated the sensitization effect of AuNPs on the colorimetric response of the PDA liposome biosensors, immobilized on a solid substrate, by employing TBA-thrombin binding as stimuli for triggering the color conversion from blue to red. The overall fabrication procedure is illustrated in Scheme 1.

First, we prepared sensors with AuNPs of two different diameters sizes, 27.8 and 48.1 nm (Fig. 1). The AuNPs with diameters of 27.8 nm were chosen for the primary experiments since it has the similar size to most proteins. TCDA liposomes



Scheme 1 Fabrication steps for the solid-state AuNP-PDA liposome biosensor.

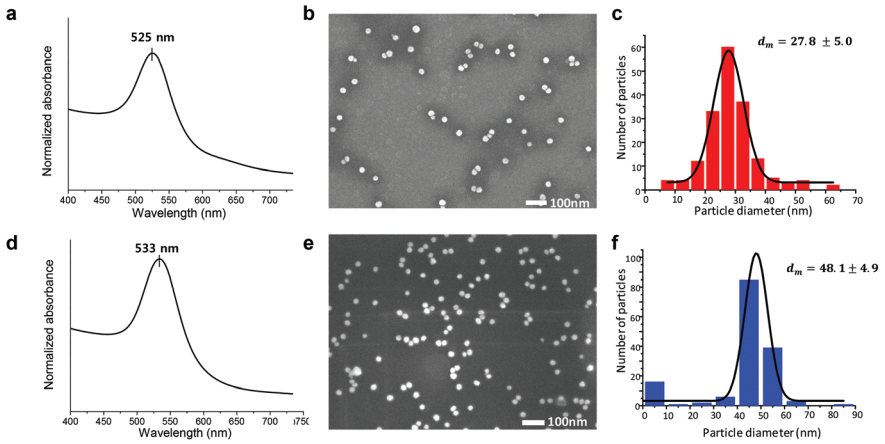


Fig. 1 (a–c) UV–Vis spectrum (a), SEM image (b), and size distribution (c) of 27.8 nm AuNPs. (d–f) UV–Vis spectrum (d), SEM image (e), and size distribution (f) of 48.1 nm AuNPs.

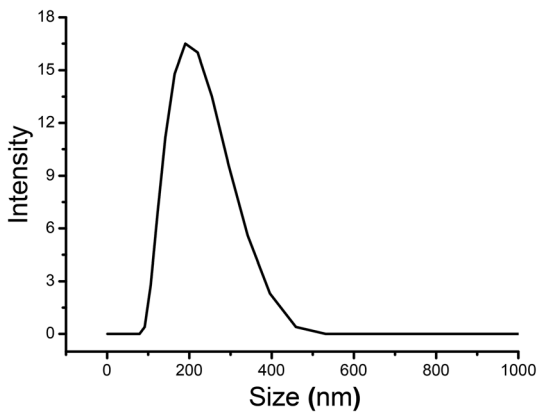


Fig. 2 Dynamic light scattering (DLS) of TCDA liposomes.

were immobilized on the amine-functionalized glass substrate. The size of the prepared TCDA liposomes was measured by DLS to be 200 nm in diameter (Fig. 2). Then, the previously prepared amine-functionalized AuNPs were grafted on the surface of the TCDA liposomes. A distinct absorption peak at 525 nm appeared because of the localized surface plasmon resonance (LSPR) of the 27.8 nm-diameter AuNPs, which decorated the surface of the TCDA liposomes (Fig. 3a, black curve). The TBA functionalization was confirmed by the shift of the AuNP LSPR peak from 525 nm to 528 nm (Fig. 4).

After the TBA functionalization on TCDA liposomes, TCDA was polymerized under 254 nm UV irradiation to form PDA liposomes through the 1,4-addition reaction. This reaction alternates the conjugated polymer backbone chain to create a blue color with absorption bands at 580 and 640 nm (Fig. 3a, blue dotted curve). Typical spectrum of PDA liposomes is depicted in Fig. 5. For the case of the AuNP-grafted PDA liposomes, an additional absorption peak at 525 nm was observed because of the LSPR of the AuNPs[14] (Fig. 3a, red curve). To examine the effect of the amount of grafted AuNPs on the PDA liposomes, different amounts of AuNPs were grafted on the PDA liposomes using 6.6 μM, 20 μM, and 26.5 μM, referred to as low, medium, and high concentration, respectively, of the

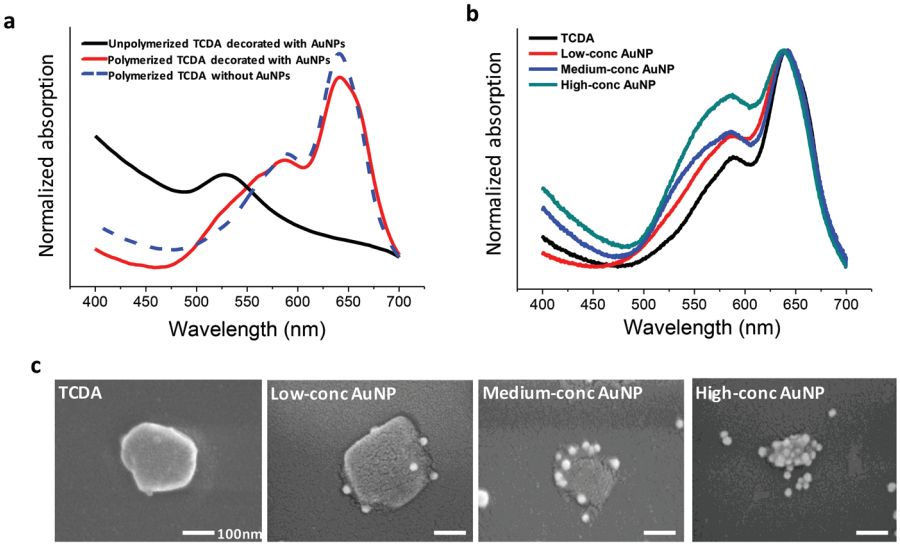


Fig. 3 (a) UV–Vis absorption spectra of TBA-PDA liposomes (blue dotted curve), TBA-AuNP-TCDA liposomes (black curve) and TBA-AuNP-PDA liposomes (red curve). (b and c) UV–Vis absorption spectra (b) and SEM images (c) of TBA-AuNP-PDA liposomes with various amounts of AuNPs synthesized by AuNP solutions of 6.6 μM (low conc. AuNP), 20 μM (med. conc. AuNP), and 26.5 μM (high conc. AuNP).

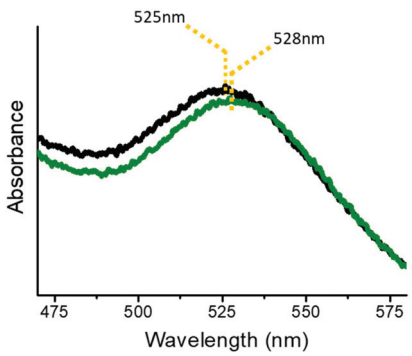


Fig. 4 UV–Vis spectrum of the red-shift of the LSPR peak from 525 to 528 nm after introducing TBA on the AuNP-TCDA liposomes.

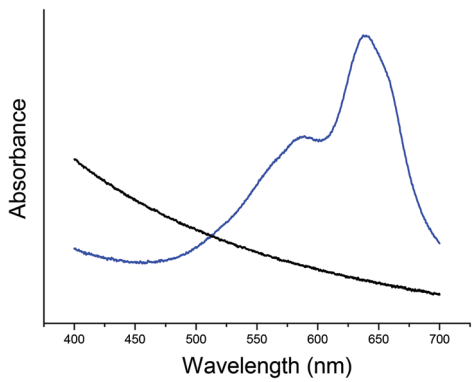


Fig. 5 UV–Vis spectrum of the monomeric assembled TCDA liposomes (black line) and the polymerized diacetylene after UV irradiation (blue line).

AuNP solution described in section 2.4 (the AuNPs concentration was defined by the amount of the input $HAuCl_4$, and the solution was diluted to attain designated amount of AuNPs). As shown in Fig. 3b, the intensity of the absorption band of PDA at 580 nm increases as the amount of AuNPs increases. This indicates that the grafted AuNPs induce conformational distortion on the PDA backbone structure. As confirmed by SEM images of the TBA-AuNP-PDA liposomes in Fig. 3c, the liposome structure appears shrunk and distorted, presumably because of the mechanical stress from the attached AuNPs on the PDA liposome surface.[9]

The prepared TBA-AuNP-PDA changed color, from blue to red, in the presence of thrombin because of the specific binding of thrombin to TBA, which destroys the surface structure of the PDA liposomal membrane. This leads to the insertion of the hydrophobic domain portion of thrombin into the PDA liposome for perturbation of the conjugated backbone.[15–17] The colorimetric response (CR, %) of TBA-AuNP-PDA liposomes after the addition of the thrombin was calculated by determining the change in the intensity ratio of the absorbance peaks at 543 nm and 640 nm in the absence (PB_0) and the presence (PB_1) of thrombin, respectively, according to the following equation:[18,19]

$$\mathrm{CR}\ (\%) = \left(\frac{\mathrm{PB}_0 - \mathrm{PB}_1}{\mathrm{PB}_0}\right) \times 100.$$

Without TBA and AuNPs, PDA liposomes showed a slight change in the absorption spectrum, even with a high thrombin concentration (Fig. 6a). This change is due to non-specific binding of thrombin to the negatively charged alkyl tail group of the PDA liposomes. By functionalizing TBA, the CR was improved because of the specific binding

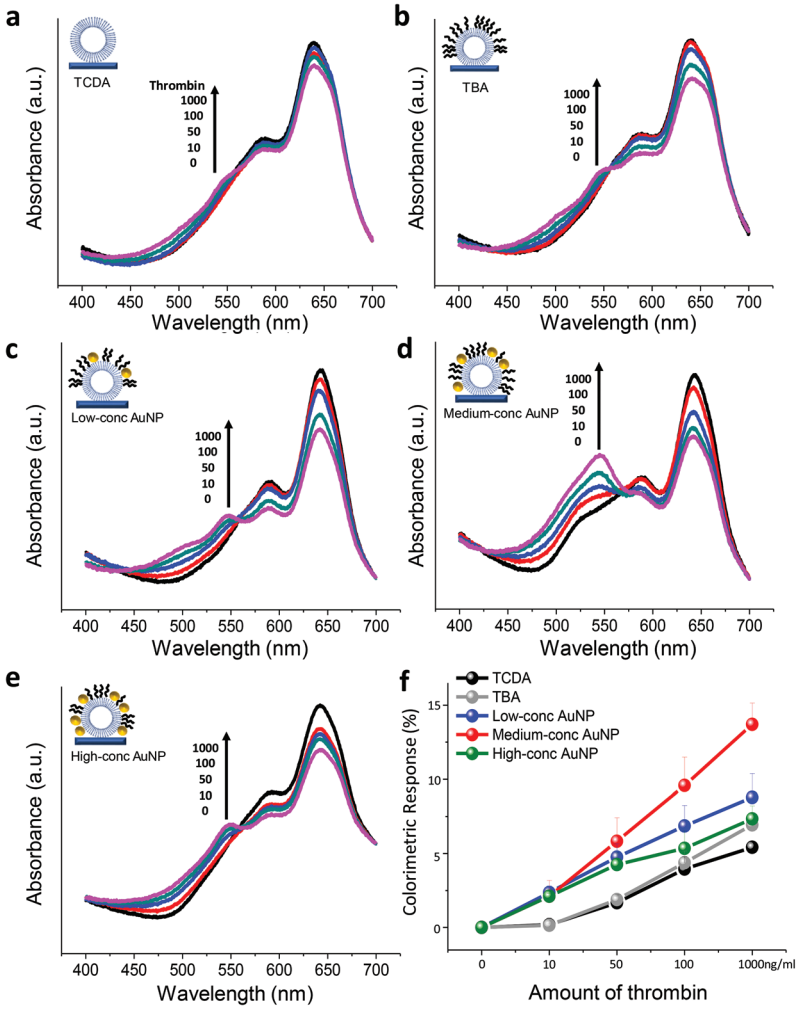


Fig. 6 UV–Vis spectra of different polymerized TCDA liposome toward various concentrations of thrombin (10, 50, 100, and 1000 ng ml^{-1}) for (a) PDA liposomes, (b) TBA-PDA liposomes, and TBA-AuNP-PDA liposomes with low conc. AuNP (c), med. conc. AuNP (d) and high conc. AuNP (e). (f) is the CR comparison of the TBA-AuNP-PDA liposomes toward thrombin detection. From bottom to top: PDA liposome (black), TBA-PDA liposome (gray), TBA-AuNP-PDA liposomes of high conc. AuNP (green), low conc. AuNP (blue), and med. conc. AuNP (red).

of thrombin and TBA (Fig. 6b). The CR of PDA liposomes was sensitized by AuNPs. When small amounts of AuNPs are grafted on the PDA liposome surface, the CR on thrombin target addition was slightly enhanced (Fig. 6c). When medium amounts of AuNPs are grafted on the PDA liposome surface, the CR was significantly enhanced as depicted in Fig. 6d. However, large amounts of AuNPs induced a decrease of CR compared with that of the medium amount of AuNPs (Fig. 6e). This is because, when excessive amounts of AuNPs are grafted on the PDA liposome surface, the AuNPs are rigidly packed together and chemically bonded to the side chain of the PDA liposome, making the side chain more rigid and decreasing the degree of conformational distortion of the backbone.[20,21]

As summarized in Fig. 6f, the medium concentration of AuNP has a 2.5 times higher CR than that of PDA liposomes without AuNPs. Note that the disappearance of the AuNPs absorption peak is attributed to that the relatively small amount of AuNPs were attached on PDA liposomes so the absorption of AuNPs were not enough to be detected. From these results, we concluded that the sensitivity of the solid-state PDA liposomal biosensors could be effectively sensitized by AuNPs. The AuNPs lead to more morphological stress on the backbone structure, which perturbs and destroys the structural planarity and, consequently, more significant colorimetric responses are observed.[9,17] Because the CR of TBA-AuNP-PDA liposomes was the most effective for the medium concentration of AuNPs, the AuNP grafting degree will be fixed to this concentration for future experiments.

To confirm the size effect of AuNPs on the sensitization, we compared the CR of the PDA liposomes grafted with 27.8 and 48.1 nm AuNPs (Fig. 7a and b). As a result, the case of 48.1 nm AuNPs showed the inferior CR and this is possibly attributed

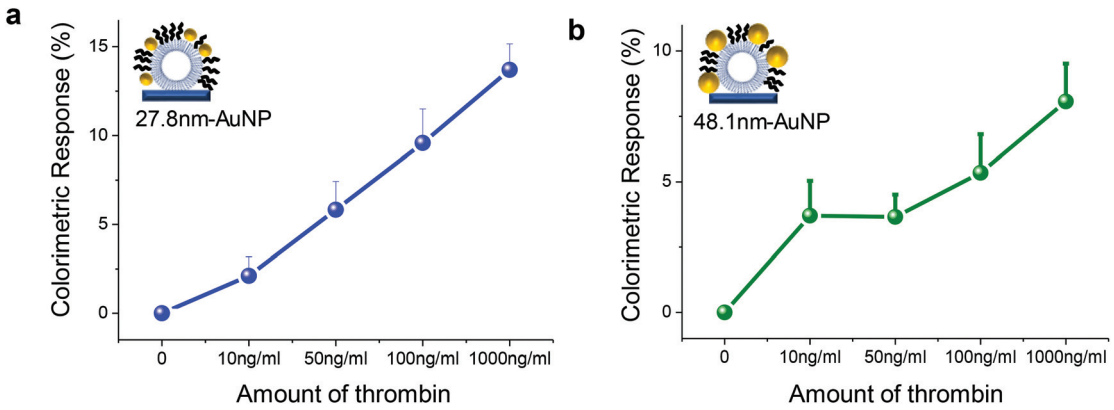


Fig. 7 The size effect of AuNPs on the PDA liposome biosensors by comparing the colorimetric response upon the grafting 27.8 (a) and 48.1 nm (b) AuNPs.

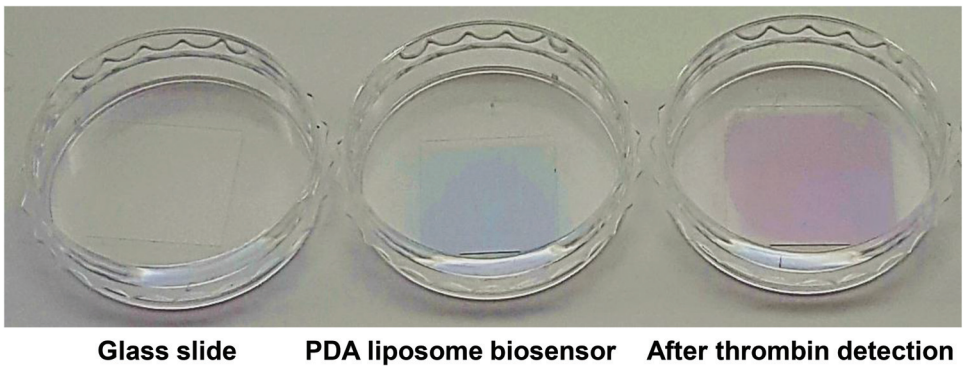


Fig. 8 The colorimetric responses of the developed PDA liposome biosensor.

to that the 48.1 nm AuNPs sterically hinders the binding of thrombin, therefore, the sensing ability was retarded than the case of 27.8 nm AuNPs.

Fig. 8 shows the solid-state colorimetric biosensing of thrombin using the optimal conditions as described in Fig. 6 and 7. The clear visualization of thrombin detection was observed by the color change from blue to red.

4. Conclusion

We investigated the sensitizing effect of AuNPs for solid-state PDA liposomal biosensors by demonstrating the detection of thrombin. We found that the sensitivity of PDA liposomes could be effectively amplified by the morphological stress from the steric effect of AuNP toward biomolecules. The AuNP-grafted PDA liposome surface, with medium concentration of AuNPs (20 μM) having a diameter of 27.8 nm, shows high sensitivity for thrombin recognition. The steric repulsion of the gold nanoparticles is target-independent. Therefore, we believe that our solid-state biosensor offers insight for the fabrication of chromogenic materials in portable biological and chemical solid sensing systems.

Author contribution

J. Kim, E. Hwang, and W. Lee conducted the experiments. B.-S. Moon, S. Shaban, and D.-G. Pyun analyzed the data. D. H. Lee, and D.-H. Kim conceived the concepts and supervised the entire process. All the authors wrote the manuscript.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

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