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Aptamer-Embedded DNA Origami Cage for Detecting (Glycated) Hemoglobin with a Surface Plasmon Resonance Sensor

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ABSTRACT

DNA origami-based cages functionalized with aptamer motifs, were used to detect hemoglobin and glycated hemoglobin. The binding between the cages and hemoglobin was monitored using a surface plasmon resonance (SPR) sensor. One DNA strand in the nano-cage was replaced with an aptamer that demonstrated a high affinity to hemoglobin (Hb) or glycated hemoglobin (gHb). Three types of the DNA nano-cages designed to fit the size and shape of hemoglobin were evaluated: one without an aptamer, one with the Hb-affinity aptamer (HA) and one with the gHb-affinity aptamer (GHA). Both DNA nano-cages embedded with HA and GHA showed significantly more stable binding with Hb and gHb by 5 and 9 times, respectively, than the aptamers directly immobilized on the SPR surface. HA-embedded DNA and GHA-embedded DNA improved the sensor selectivities by 9 times and 37 times between Hb and gHb.

Keywords**:** DNA origami; glycated hemoglobin; aptamer; surface plasmon resonance sensor; dissociation constant

1. Introduction

DNA aptamer-based surface plasmon resonance (SPR) sensing has drawn interest due to its advantages in high sensitivity and selectivity with label-free monitoring for proteins [1-3]. This technique

can be used to detect refractive index changes based on the amount of binding proteins on the sensing surface. The high binding affinity of aptamer sequences with analytes, and aptamers' secondary loop structure are essential to target molecule recognition and stability of binding [4,5]. However, the selected sequences in the loop region often show unsatisfactory binding affinities with target molecules, and, as a result, further sequence refinement and optimization are required [6,7]. To enhance the binding stability of aptamer with hemoglobin (Hb) and glycated hemoglobin (gHb), which are biomarkers for determination of the long-term average blood glucose level and used as a standard measurement for assessing glycemic control and diabetes diagnosis, DNA nano-cages synthesized via DNA origami technique were used in the current study [8-10]. DNA aptamers were hemoglobin aptamer (HA) and glycated hemoglobin aptamer (GHA) through Systematic Evolution of Ligands by EXponential Enrichment (SELEX) [11]. The 3-D DNA cage containing two selected aptamer-equipped confined cavities were designed for fitting, capturing and enhancing the binding and selectivity of HA and GHA toward Hb and gHb, respectively.

Fig. 1 shows the computer-generated 3-D model of the DNA structure and a transmission electron microscopy (TEM) image of the DNA nano-cages of rectangular box shape with a cavity in the middle (Fig. 1b) similar to a previous nanocapsule design [12]. The two thiol-modified halves of the cage as depicted in Fig. 1a are connected *via* a flexible unhybridized DNA scaffold strand, where the green DNA strands with a thiol group at the end attach to the sensor surface (Fig. 1d). The staple strands at the bottom of the cavities (cavity size: 14×11×6 nm^3) were extended with the aptamer sequence, HA or GHA (Fig. 1c, orange-color strands). The detailed sequences of the cage design are provided in Supporting Information.

2. Materials and methods

The preparation of SPR sensor chips and the experimental protocols for detecting (glycated) hemoglobin are explained in Supporting Information.

3. Results and discussion

Comparing the results of the HA and HA-embedded DNA nano-cages, the adsorption signals of HA (Fig. 2c) for Hb appear to increase more rapidly (430 RU/420s for 0.1 mg/mL) than those of the HA-embedded DNA nano-cage (325 RU/420s for 0.1 mg/mL) in Fig. 2e. This prompt increase is also observed in the

adsorption of gHb on GHA compared to the GHA-embedded DNA nano-cage as shown in Figs. 2d and 2f. It is thought that the surface concentrations of the aptamer-embedded DNA nano-cages were lower than those of aptamers, and as a result, the number of protein molecules bound to the aptamer-embedded DNA nano-cages during the adsorption phase was lower than that bound to aptamers. In the dissociation phase beginning at 420 s, however, the protein molecules appear to be desorbed more rapidly from the aptamers than from the aptamer-embedded DNA nano-cages. To compare binding stabilities of sensing elements, the dissociation constant, K_D, was used. K_D is defined as the ratio between the association and dissociation rate constant of target protein with aptamer, k_d/k_a, on sensor surface calculated by the Langmuir model [13] Eq. (1):

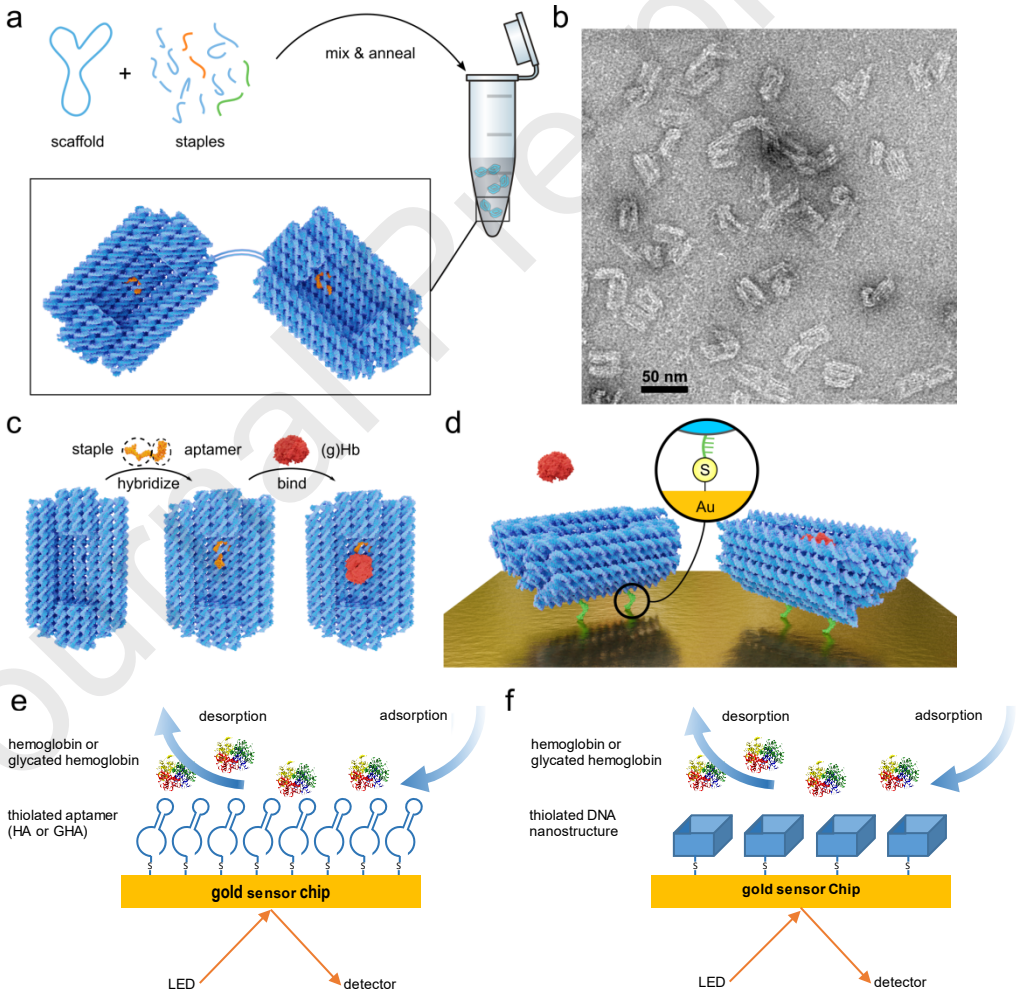

Fig. 1. (a) 3D computational model of the designed DNA origami structure with two cavities. (b) TEM image of the DNA nano-cages after they were folded. (c) Embedment of an aptamer within the cavities

by hybridization and a following adsorption of a gHb to the aptamer. (d) Immobilization of the aptamer-embedded DNA origami on a gold chip and a gHb binding. Inset shows the thiolated ssDNA strand extruded from DNA origami and covalently bound to the gold surface. SPR sensor experiments for (e) thiolated HA or GHA and (f) thiolated aptamer-embedded DNA nano-cage.

$$\textbf{Response} = \frac{\boldsymbol{Pr\,Fac_1(k_a)[F](1-e^{-(k_a[F]+k_d)t})}}{\boldsymbol{(k_a[F]+k_d)}} + \frac{\boldsymbol{Pr\,Fac_2[F](1-e^{-k_{a_{NS}}[F]t})}}{\boldsymbol{[F]}} \quad (1)$$

PrFac$_1$ and *PrFac*$_2$ are proportionality factors that combine the conversion ratio of the bound element concentrations to the SPR response and the total number of binding sites for specific sites and non-specific sites, respectively. A non-linear least square global fitting technique was applied to fit the data with Eq. (1) for generating solid lines in Figs. 2c-f to determine rate constants using MATLAB. In Fig. 2g, the adsorption signal of Hb on HA was slightly faster than the HA-embedded DNA nano-cage. However, the latter maintained the binding better than HA in the desorption period. Besides, the HA-embedded DNA nano-cage showed the largest decrease in the desorption period against gHb, which is attributed to a higher affinity of the HA-embedded DNA cage with Hb than with gHb. As for the GHA-embedded DNA nano-cage (Fig. 2h), it shows the highest binding stability with gHb in the desorption period compared to the GHA, HA, and HA-embedded DNA nano-cage. This is due to the synergistic effects of the embedded-GHA with the sequences exclusive to gHb and the shape of nano-cage matching with gHb (Supporting Information).

As in Table 1, when K_D is large, desorption of bound proteins is significant during the desorption period, and that means the binding is not stable. The aptamer-embedded DNA nano-cages show better binding stabilities than the aptamers. For 0.1 mg/mL proteins, the K_D of HA-embedded DNA nano-cage with Hb was 88 nM where that of HA was 447 nM demonstrating a factor of 5 enhanced binding stability of the HA-embedded nano-cage with Hb. Likewise, the GHA-embedded DNA nano-cage shows a lower K_D with gHb (2.4 nM) than that of GHA (22.4 nM) indicating the affinity of GHA was improved by a factor of 9.4 when GHA was embedded in the DNA nano-cage. The K_D value of HA-embedded nano-cage with gHb was 1900 nM as compared to 88 nM for Hb, which means the HA-embedded DNA nano-cage binds with Hb 22 times more strongly than with gHb indicating its high selectivity with Hb against gHb. In Table 1, 4th column, the ratio of K_D's between Hb and gHb, [K_D for Hb]/[K_D for gHb], verifies the selectivity of aptamer toward

proteins. A low ratio of K_D's of HA and HA-embedded nano-cage means a high selectivity for Hb. On the other hand, for GHA and the GHA-embedded nano-cage, a high K_D ratio represents a high selectivity toward gHb over Hb. The selectivity of HA-embedded nano-cage (0.046) toward Hb is 9 times higher than that of HA (0.42) for 0.1 mg/mL. Likewise, the GHA-embedded nano-cage shows 37 times higher selectivity toward gHb compared to GHA in 0.1 mg/mL. The same trend was found for all the tested protein concentrations as shown in Fig. 3. These experimental results underline the advantage of DNA nano-cage to improve the aptamer's selectivity toward the targeted proteins.

4. Conclusions

The aptamer-embedded DNA nano-cages show significant improvement in both selectivity and binding stability with target proteins compared to directly immobilized aptamers (HA and GHA). The current work demonstrates that the embedment of aptamers within DNA nano-cages is the promising technique to enhance the efficiency of DNA aptamer-based surface plasmon resonance (SPR) sensing for selective detect of glycated blood proteins.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

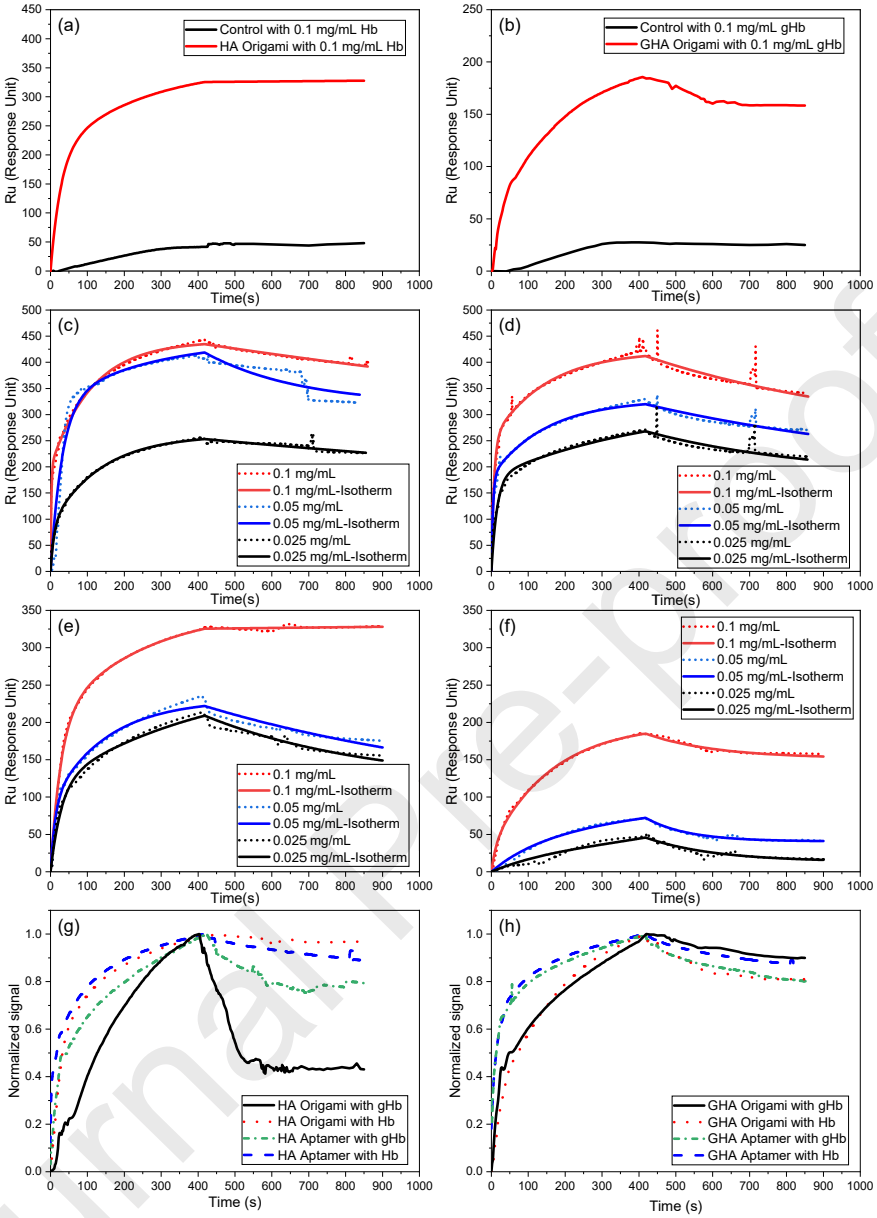


Fig. 2. SPR responses from (a) normal cage and HA-embedded nano-cage for Hb, (b) normal cage and GHA-embedded nano-cage for gHb, (c) HA aptamer for Hb, (d) GHA aptamer for gHb, (e) HA-embedded DNA nano-cage for Hb, (f) GHA-embedded DNA nano-cage for gHb. Normalized SPR signals of (g) the HA and HA-embedded DNA nano-cage associated with Hb and gHb (0.1 mg/mL) and (h) the GHA and GHA-embedded DNA nano-cage associated with Hb and gHb (0.1 mg/mL).

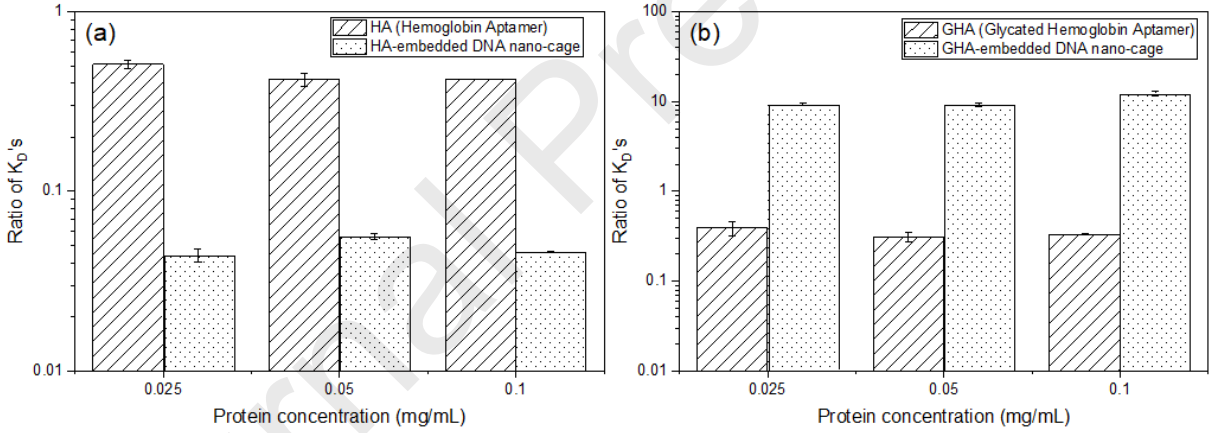
Table 1

Dissociation constants, K_D, of the HA, HA-embedded DNA nano-cage, GHA, and GHA-embedded DNA

nano-cage with Hb and gHb at 0.1 mg/mL.

SPR Sensor Element	Protein	Dissociation Constant, K_D (nM)	Ratio of K_D's
HA	Hb	447 ± 23	0.42
	gHb	1060 ± 60	
HA-Embedded DNA Nano-Cage	Hb	88 ± 28	0.046
	gHb	1900 ± 130	
GHA	Hb	7.5 ± 2.3	0.33
	gHb	22.4 ± 3.3	
GHA-Embedded DNA Nano-Cage	Hb	29.1 ± 1.1	12.2
	gHb	2.4 ± 0.7	

Fig. 3. Ratios of K_D's of (a) HA aptamer and HA-embedded DNA nano-cage for Hb and (b) GHA aptamer



and GHA-embedded DNA nano-cage for gHb with 0.025, 0.05 and 0.1 mg/mL protein concentrations.

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Highlights

- DNA nano-cage is synthesized for glycated hemoglobin detection
- Aptamer for glycated hemoglobin is embedded in DNA nano-cage
- Aptamer-embedded DNA nano-cages on SPR sensor chips bind with glycated hemoglobin
- Aptamer/DNA nanocage showed stable and selective binding with glycated hemoglobin

Credit Author Statement

Surachet Duanghathaipornsuk: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - Original draft preparation, Writing - Reviewing and Editing

Boxuan Shen: Methodology, Software, Validation, Data curation, Visualization, Resources, Writing - Review & Editing

Brent D. Cameron; Methodology, Resources, Writing - Review & Editing

Heini Ijäs: Software, Resources, Writing - Review & Editing

Veikko Linko: Software, Validation, Resources, Supervision, Writing - Review & Editing

Mauri A. Kostiainen: Conceptualization, Software, Validation, Resources, Visualization, Supervision, Project administration, Funding acquisition, Writing - Review & Editing

Dong-Shik Kim: Conceptualization, Validation, Formal analysis, Data curation, Visualization, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition