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A co-assembled probing system using the homoadenine self duplex signal

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Abstract

A co-assembly system consisting of fluorescent oligodeoxynucleotide (ODN) and biotin has been developed to recognize streptavidin and it shows a fluorescent discrimination between blue and red signals through recognizing streptavidin.

Self-assembled hydrogelators are promising supramolecules for use in the fields of gene/drug delivery systems, implanted materials, and diagnostic and other medical applications.[1] These important materials have the special potential to entrap peptides, proteins, enzymes, and nucleic acids without denaturation.[2] Hydrogels are also used to build up peptide/protein microarrays,[3] affording a semi-wet sensor as a matrix.[2–4] Here we report how a fiber patterned xerogel of a biotin hydrogelator[5] is co-assembled with a specific fluorescent oligodeoxyadenylate system.[6] This co-assembled system acts as a biosensor that can detect streptavidin[6,7] and discriminate it from other proteins through a fluorescent signal change (Fig. 1).

We used ODN **S1** (Fig. 2) as a probing material. ODN **S1** is a homoadenine self-duplex material that exhibits a reddish color in solution (sol) under aqueous buffer conditions.[8] However, it turned blue upon binding to the complementary sequence ODN **S2** (Fig. 3). We also used the modified synthetic biotin **G1** as hydrogelator.[9]

We first studied whether ODN **S1** can be used as a sol–gel transition signaling material. We added the hydrogelator **G1** to the ODN **S1** solution (0.3% MGC (minimum

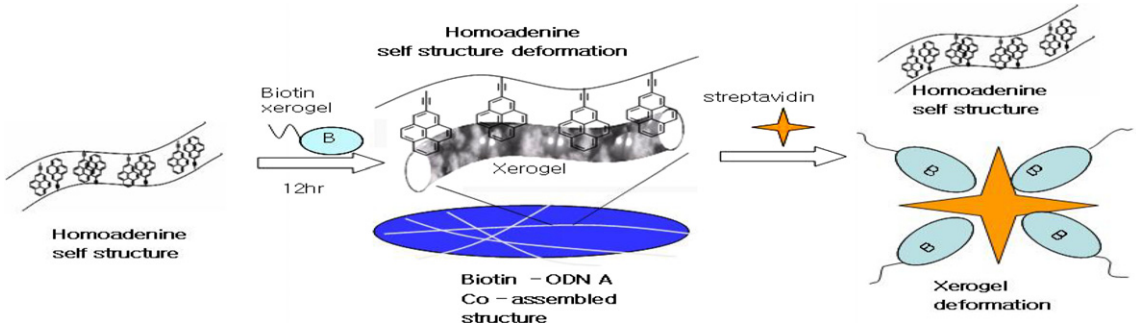

Fig. 1. Cartoon representation of the sol-'solid island'-sol transition involving the ODN **S1**, gelator **G1**, and **S1**–**G1** co-assembly to probe streptavidin.

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S1 5'- AAAA^{py}AAA^{py}AAA^{py}AAA^{py}AAAAATT
S2 3'- TTTTTTTTTTTTTTTTTTAA
S3 5'- AAAAAAAAAAAAAAAAAATT

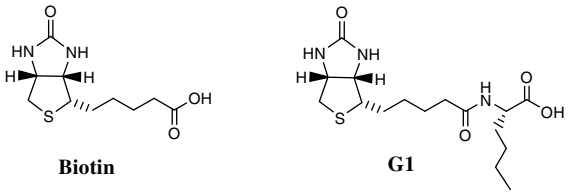

Fig. 2. Sequences of ODNs and the structure of the biotin hydrogelator (**G1**).

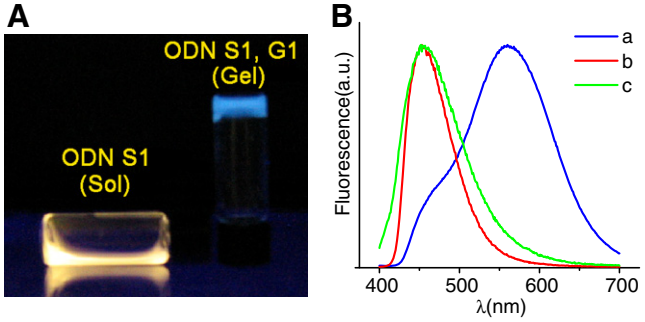

Fig. 3. (A) Photo image of sol to gel transition. (B) Normalized emission spectra of (a) ODN **S1** (sol), (b) duplex ODN **S1**·**S2** and (c) **G1**-gel containing ODN **S1**. All samples were prepared in buffer (100 mM Tris–HCl, 10 mM $MgCl_2$, 100 mM NaCl; pH 7.2) and imaged at 20 °C while irradiating at 386 nm.

gelation concentration)), and it formed the gel. We observed fluorescence changes of ODN **S1** during the sol–gel transitions of the hydrogelator **G1**. In the solution, ODN **S1** exhibited its original reddish orange color, whereas in the gel (0.3% MGC) it exhibited a blue color (Fig. 3). This shows that ODN **S1** can be used as a signal unit in the hydrogelator.

In the course of this experiment we observed an interesting phenomenon: ODN **S1** was adsorbed on the **G1** xerogel (solid) before gel formation with a concomitant color change from red to blue. Xerogel (solid) made of the biotin hydrogelator **G1** (1 mg, 2.8 mmol) was added to a solution of ODN **S1** (200 μL, 1.5 μM), and the resulting mixture was set aside. After 12 h, the reddish color of the ODN **S1** probe in the solution had changed to blue on the surface of the gelator particles (Fig. 4A).

From the emission spectra at different time intervals, we observed a λ_{max} change of ODN **S1** from 580 nm (red) to 465 nm (blue) (Fig. 4B). We believe that the homoadenine self-duplex structure that formed between the modified pyrene-containing ODN units in the solution state must be changed after adsorption on the **G1** xerogel.

The adsorption of ODN **S1** on the **G1** xerogel was confirmed using HPLC analysis. We synthesized an ODN **S3** (Fig. 2; same sequence as that of ODN **S1** but without

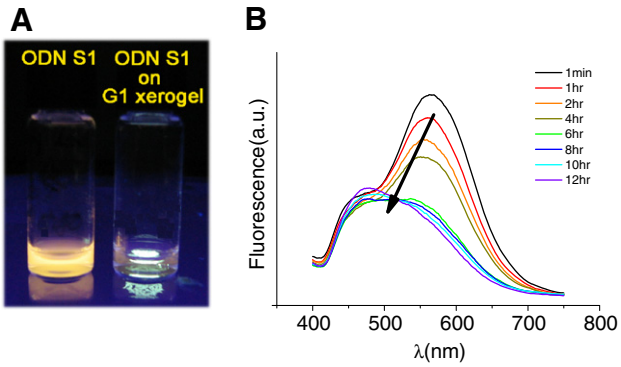

Fig. 4. (A) Photo image of a solution of ODN **S1** before and after the addition of **G1** xerogel (12 h). (B) Changes in the emission spectrum of ODN **S1** after adding **G1** xerogel at different intervals of time. All samples were prepared in buffer (100 mM Tris–HCl, 10 mM $MgCl_2$, 100 mM NaCl; pH 7.2) and the photo images were obtained at 20 °C using an SL-20 High Performance Visualizer (Bioscience Co., Ltd, Seoul). Emission spectra were obtained at 20 °C under irradiation at 386 nm.

pyrene) as control. First, a solution containing the ODN (**S1** or **S3**) was added to the **G1** xerogel. After 12 h incubation the solution from each experiment was subjected to HPLC analysis. In the chromatogram of the solution containing **S1**, a dramatic decrease in the intensity of ODN **S1** was observed, while in the chromatograms of the solutions containing ODN **S3**, the intensity of the ODN did not change. The HPLC study indicates that only ODN **S1** (containing pyrene attached to deoxyadenosines) binds to the **G1** biotin xerogel in the solid isolated state. Next we added unmodified biotin to the ODN **S1** solution to confirm that the color change of this system originated from the hydrophobic interaction between xerogel and ODN **S1**. Unlike modified biotin (xerogel), the color change and adsorption were not shown in the unmodified biotin. We believe that, in this case, the hydrophobic unit (pyrene) of ODN **S1** interacts with the hydrophobic pocket of the gel fibers.[10]

Based on the above result, we suggest that there is a certain hydrophobic interaction between the pyrenes of ODN **S1** and the **G1** xerogel; the only difference between ODN **S1** and ODN **S3** is the presence of the pyrenes (in **S1**), which are well-known hydrophobic planar aromatic bulky molecules.

Biotin binds to the tetrameric structure of streptavidin. This remarkable affinity has been utilized for bioanalysis, immunolabeling, diagnostics, and affinity targeting.[11] More recently, this technology has been applied in bioengineering and biomedicine, especially in drug targeting.[11c] Therefore, detection of this interaction and characterization of streptavidin are standard tasks.

We investigated the biological sensor activity of an isolated ODN **S1**–**G1** co-assembly system. We prepared four sample proteins—target protein streptavidin and BSA, mut-S and mut-L as control proteins—to clarify the sensitivity of ODN **S1**–**G1** co-assembly islands. The basic concept is that as our ODN **S1**–**G1** co-assembly islands

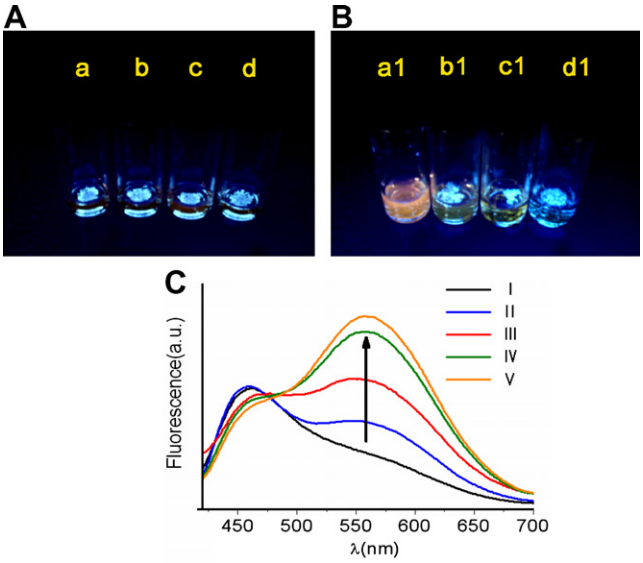

Fig. 5. Photo images of the ODN **S1**–**G1** co-assembly islands. (A) Before the addition of proteins, (B) After the addition of a1 = streptavidine, b1 = BSA, c1 = mut-S or d1 = mut-L. (C) Emission spectra at different amounts of streptavidin, I = 0.2 mg, II = 0.4 mg, III = 0.6 mg, IV = 0.8 mg, V = 1 mg. (Spectral conditions were the same as in Fig. 3.)

contain biotin, which can bind with streptavidin, the addition of streptavidin to this system must induce some physical change in the island system. In fact we did observe disruption of the xerogel structure and, also, an intense color change from blue to red after the addition of streptavidin to **S1**–**G1** co-assembly islands. Other proteins such as BSA, mut-S and mut-L did not show the color change (Fig. 5A and B). We also observed a consistent increase in emission intensity on increasing the amount of the streptavidin (Fig. 5C).[9]

At the molecular level, after the addition of streptavidin to the ODN **S1**–**G1** co-assembly islands, the ureido group of biotin must bind with streptavidin. As a consequence, the co-assembled xerogel collapses. This disruption results in the release of ODN **S1** to the solution, where it regains its original homoadenine self structure.

In conclusion, we have developed a co-assembly system to discriminate streptavidin from other general proteins using fluorescent ODN **S1** and biotin-based hydrogelator **G1**. At first, we observed a fluorescence signal change from red to blue on addition of ODN **S1** to the **G1** xerogel. The adsorption could be due to hydrophobic interactions between the pyrene units of ODN **S1** and the **G1** xerogel fibers. Such ODN **S1-G1** 'co-assembly system' is disrupted on addition of only streptavidin, as a result, the homoadenine self-duplex of ODN **S1** is reproduced with fluorescence signal change. Thus, our ODN **S1**–**G1** xerogel 'co-assembly system' is promising as a new optical streptavidin sensor.

Acknowledgments

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