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A displacement assay for the sensing of protein interactions using sugar–tetraphenylethene conjugates

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ABSTRACT

A displacement sensor array based on sugar-substituted tetraphenylethenes with the aggregation-induced emission feature for proteins to perform screening of protein–protein interactions is demonstrated.

In biological systems, protein–protein interactions underlie many aspects of cellular processes, such as cellular recognition, adhesion, signal transduction, DNA transcription, and protein antigen/antibody recognition, and these interactions are often involved in diseases as well.[1] The detection of proteins and the subsequent understanding of their functions and regulation mechanism are important issues.[2] A variety of techniques including protein microarrays have been developed. In principle, a conventional protein microarray technique (e.g., Enzyme-linked immunosorbent assay, ELISA) makes it possible to simultaneously map proteins in a high-throughput fashion, which requires protein receptors of high affinity and specificity. However, the structural diversity and complexity of the target proteins produce difficulties in preparing the receptors. Label-free techniques, for example, surface plasmon resonance (SPR) imaging, are also available.[3] Alternatively, a sensor array based on the analyte-specific patterns arising from the differential binding affinity of a set of nonspecific receptors is attractive.[4] This sensing approach simplifies the sensor design. In fact, sensor-array approaches based on this principle for protein detection have been addressed.[5] The development of sensitive, convenient, and precise protein-sensing methods still remains challenging.

Recently, we[6] and others[7] have demonstrated that aggregation-induced emission (AIE)-active materials, first reported by Tang and co-workers,[8,9] have a potential utility in biosensor fields. Here, we describe a displacement sensor array based on the AIE feature for proteins to perform screening of protein–protein interactions.

The design for sensing proteins is schematically illustrated in Figure 1. Tetraphenylethenes (TPEs), which show no emission in solution but an intense emission in a state of aggregation, that is, are AIE active,[7a–c] are used as basic sensor elements. The basis for the sensing is as follows: (1) sugar-substituted TPE binds carbohydrate-binding proteins (lectins) to aggregate via multivalent interactions, resulting in displaying of an intense emission, which has been employed for the fluorescence turn-on detection of lectins;[6] (2) upon addition of another protein interactive with the lectin, the aggregate is disrupted, at least in part, due to agglutination between the lectin and the protein to release the sugar–TPE conjugate resulting in quenching of the emission, which we use in the present study for the fluorescence turn-off detection of proteins. Because the agglutination is associated with the binding affinity of the protein with the lectin, the fluorescence response could be used to differentiate the proteins.

An α-mannopyranoside TPE derivative **1a** was synthesized by the reaction of 4-hydroxy-substituted TPE and acetyl-protected D-mannopyranoside followed by deprotection.[10] Another TPE with α-mannopyranoside linked by a diethylene glycol unit **1b** and α-lactopyranoside congener **1c** were also synthesized in the same way. Importantly, these conjugates **1a**–**c** are water soluble, which is usually required for biosensing in aqueous media.

As we have already reported, **1a**, which is practically nonluminescent in a buffer solution, displays an intense emission upon the addition of concanavalin A (Con A) due to aggregation-induced by

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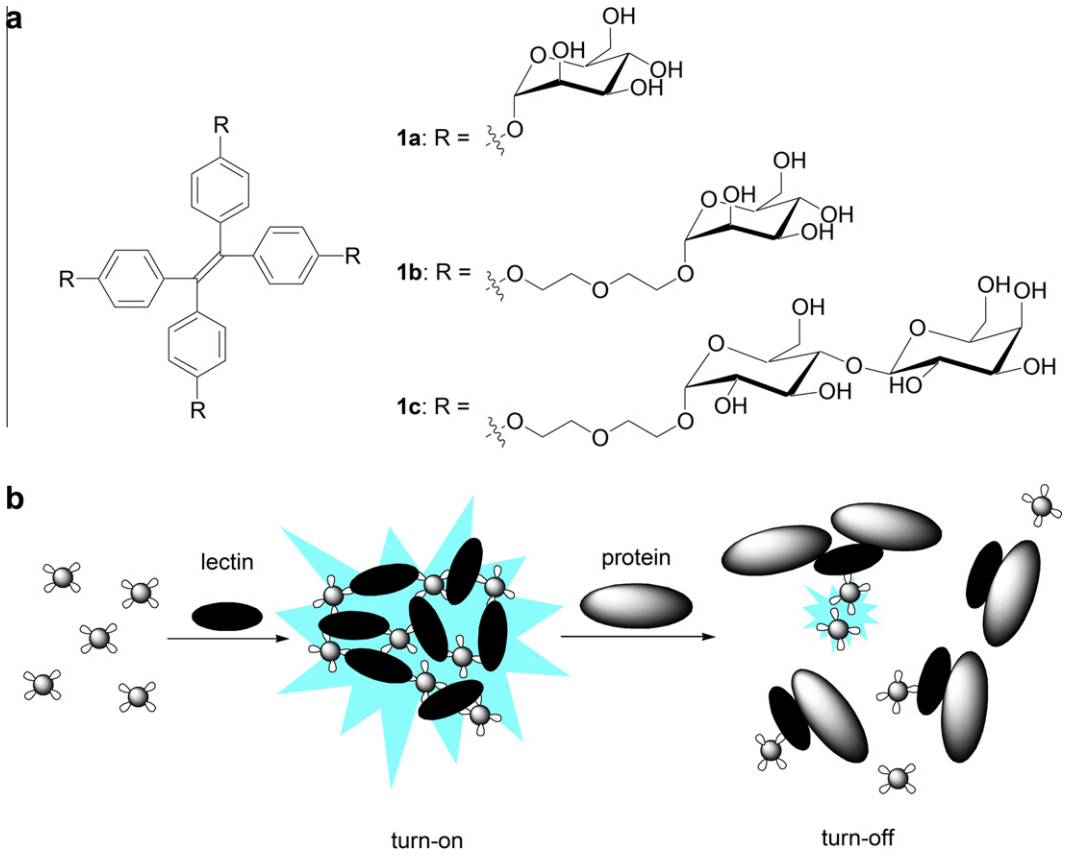

Figure 1. (a) Chemical structures of sugar-modified tetraphenylethenes **1a–c**. (b) Schematic representation of the displacement assay for detection of protein–protein interactions based on aggregation-induced emission (AIE)-active materials. A complex of sugar-modified tetraphenylethene and lectin (fluorescence turn-on) is employed as the sensor element. Displacement of sugar-modified tetraphenylethene by protein analyte generates the fluorescence response pattern (fluorescence turn-off) to differentiate protein interactions.

complexation between **1a** and Con A.[6b] The resulting **1a**/Con A complex can be used as a sensor element for the displacement assay for protein sensing. Thus, upon mixing **1a**/Con A complex with thyroglobulin (Tg) in a buffer solution, the fluorescence intensity decreased progressively with an increase in Tg concentration, as shown in Figure 2 (and also graphical abstract). The fluorescence quenching extent was approximately 70% relative to the initial fluorescence intensity at 460 nm of **1a**/Con A. The observation appears to indicate that the agglomeration of **1a** and Con A has been disrupted to release **1a** because of the stronger binding of Tg with Con A, as illustrated in Figure 1b.

In the sensor array studies, agglomeration complexes of **1a**/Con A, **1b**/Con A, and **1c**/RCA120 (RCA120: ricinus communis agglutinin, a galactose-binding lectin)[11] were employed as the sensor elements and the following eight proteins with a variety of sizes were considered as analytes: bandeiraea simplicifolia (BS-I), bovine serum albumin (BSA), cytochrome C (Cyto), histone (His), peanut agglutinin (PNA), soybean agglutinin (SBA), Tg, and wheat germ agglutinin (WGA). In the experiments, samples were prepared with protein concentrations set at a normalized UV absorbance (A_{280} = 0.06). After the addition of proteins to agglomeration complexes and stirring for 4 h, the samples were subjected to the fluorescence measurements.

The results clearly show that this displacement sensor assay indeed works. Thus, addition of the proteins results in quenching of the fluorescence of the solution, but the extent of quenching is dependent on the particular combination of the agglomeration complexes and the proteins (Fig. 2). For instance, **1a**/Con A, **1b**/Con A, and **1c**/RCA120 behaved similarly toward BS-I to result in approximately 40% quenching upon mixing. Likewise, the sample obtained by mixing **1a**/Con A, **1b**/Con A, or **1c**/RCA120 with Cyto or SBA also displayed the quenching to nearly the same extent (35–50%). BSA displayed approximately 40% quenching for **1a**/Con A and **1b**/Con A, indicating that **1c**/RCA120 was less effective for the recognition of BSA. PNA and Tg showed more drastic difference, depending on the agglomeration complex used. Thus, they showed, upon addition to **1a**/Con A and **1b**/Con A, an efficient quenching up to 60–90% while the addition to **1c**/RCA120 resulted in only 25–35% quenching. On the other hand, His showed only marginal changes (5–14% quenching) for all the complexes. **1b**/Con A was more effective for the recognition of WGA but **1a**/Con A and **1c**/RCA120 were not effective. The fluorescence response patterns observed are envisioned to be associated with the binding affinity of the proteins being dependent on the surface characteristics such as size, shape, and hydrophobicity. For example, Con A has high affinity for mono- and bi-antennary-structured glycoproteins with high-mannose contents, such as Tg, but it exhibits less affinity for the branched structures of tri- and tetra-antennary glycoproteins.[12] The obtained order of the binding affinity of Con A to the glycoproteins is in good agreement with the known binding strengths between Con A and glycoproteins (Tg < BS-I < WGA).[2c,3,13]

In summary, we have developed a displacement sensor array for the screening of protein interactions based on AIE-active material. This method does not require any special protein modifications, labeled lectins, or special analytical instruments. Further experiments are required to demonstrate the practical use of this assay. For instance, the sensitivity of this assay was not considered. The normalized concentrations of the proteins were only examined at this stage, aiming at the analysis of an unknown sample analyte.

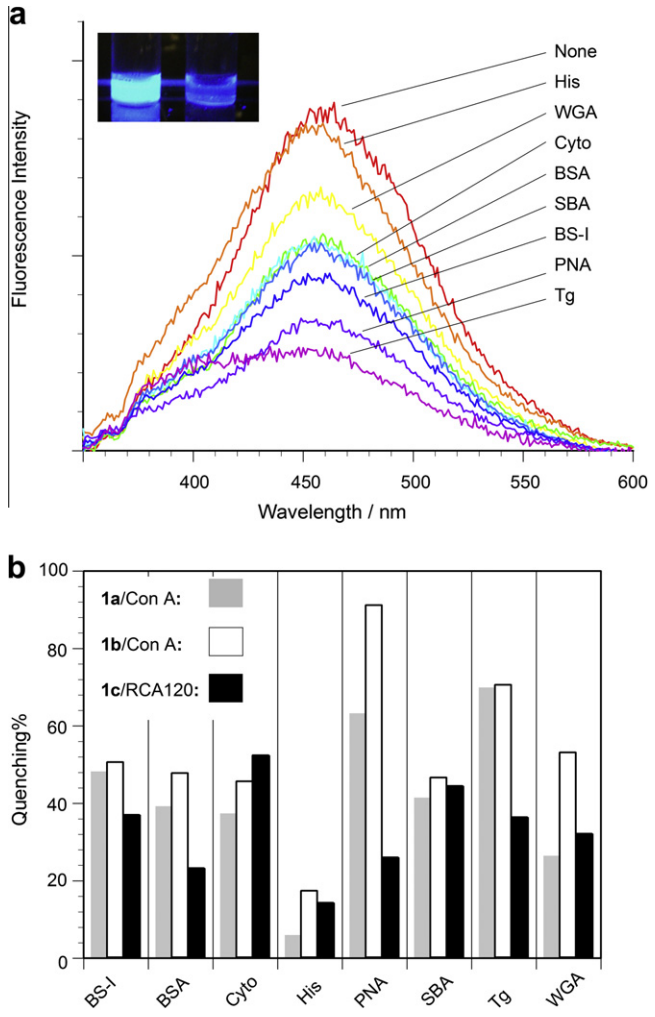

Figure 2. (a) Change in fluorescence spectra of mannose-modified TPE **1a**/Con A upon addition of protein analytes (BS-I, BSA, Cyto, His, PNA, SBA, Tg, and WGA) at a standard UV absorbance ($A_{280} = 0.06$) in a buffer solution ($\lambda_{ex} = 320$ nm). (b) Fluorescence response patterns (fluorescence quenching extent) of sugar-modified TPE/lectin sensor array against the protein analytes. The fluorescence 'Quenching%', $(I_0 - I)/I_0 \times 100$, was based on the fluorescence intensity (I) relative to the initial fluorescence intensity of the sensor element (I_0), where the fluorescence intensities were monitored at 460 nm for **1a**, **b** and 400 nm for **1c**. All measurements were performed in a buffer solution (for **1a**, **b**, 10 mM Tris–HCl, 1 mg/mL $CaCl_2$, 1 mg/mL $MnCl_2$, pH 7.6; for **1c**, 10 mM phosphate buffer, pH 7.4).

In addition, the assay must be tested in a more complex environment, that is, biologically relevant fluids and mixtures of proteins, for their ability to detect species that are present at very low concentrations. However, the observed fluorescence response patterns of each analyte would be statistically analyzed to differentiate and identify glycoproteins. Further study is currently in progress.

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Supplementary data

Supplementary data (experimental details and fluorescence spectral changes of **1a–c** with Con A or RCA120 and fluorescence patterns to proteins) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.09.112.

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10. See Supplementary data for details.
11. The complex **1c**/RCA120 showed an emission at about 400 nm (Fig. S1), which is blue-shifted compared with the complexes of **1a–b** and Con A (460 nm). TPEs in the aggregate state usually emit in the blue spectral region (460–480 nm), in which the position of the emission is dependent on the conformation resulting from restriction of the intramolecular rotation of the phenyl group. Because lactose-modified TPE **1c** is partially bound to RCA120 through only one or two of its four lactose arms and/or its binding mode (spot) is different from that of mannose-Con A, partial intramolecular rotations are possible, leading to emission in the region of about 400 nm.[7a–c]
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