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# Noncovalent Immobilization of Proteins on a Solid Surface by Cucurbit[7]uril-Ferrocenemethylammonium Pair, a Potential Replacement of Biotin−Avidin Pair

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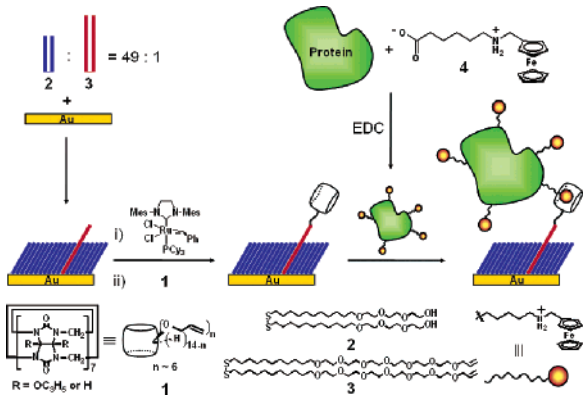
The biotin−avidin (or streptavidin) system is the strongest noncovalent interaction found in nature with a binding constant in the order of $10^{13}-10^{15}$ $M^{-1}$.[1,2] The strong and specific interaction makes it one of the affinity pairs most widely used in many applications such as molecular, immunological, and cellular assays.[3,4] In particular, it has been extensively used for immobilization of biomolecules on solid surfaces.[5] However, it suffers some shortcomings including denaturation by organic solvents or elevated temperatures and high cost. Developing a synthetic ligand−receptor pair that can replace the biotin−avidin system is thus important not only for deeper understanding of noncovalent interactions but also for practical applications. We and others recently reported that cucurbit[7]uril (CB[7]), a member of the host family cucurbit[*n*]-uril (CB[*n*], $n = 5-10$)[6,7] with a hydrophobic cavity and two identical carbonyl-fringed portals, binds ferrocenemethylammonium (FA) or adamantylammonium ion with an exceptionally high binding constant ($\sim 10^{12}$ $M^{-1}$) and good specificity in aqueous solution.[8] We have also achieved the direct functionalization of CB[*n*] by oxidation with potassium persulfate,[9] which allowed us to synthesize a wide variety of tailor-made CB[*n*] derivatives and to study their applications.[10,11] These new developments in the CB-[*n*] chemistry led us to explore applications of the exceptionally stable CB[7]−FA pair. Here we report a novel noncovalent method to immobilize a protein on a solid surface using the CB[7]−FA pair, which may serve as a replacement of the avidin−biotin system for this and other applications.[12]

As illustrated in Scheme 1, our approach to the noncovalent immobilization of a protein on gold involves (1) anchoring CB[7] units on an alkanethiolate self-assembled monolayer (SAM) on gold, (2) attachment of ferrocenemethylammonium units to a protein to be immobilized, and (3) immobilization of the "ferrocenylated" protein to the CB[7]-attached SAM on gold.

For surface immobilization of CB[7], allyloxyCB[7] (**1**)[13] was synthesized as shown in Scheme S1 in the Supporting Information. Immobilization of **1** was achieved by olefin cross-metathesis reaction between **1** and a vinyl-terminated, mixed self-assembled monolayer (SAM) on gold, which had been prepared by immersing a gold substrate in an ethanol solution of disulfide compounds **2** and **3** in a ratio of 49:1 for 1 day.[14,15] The successful immobilization of **1** on the mixed SAM was evidenced by the appearance of the strong CO and CN stretching bands of **1** at 1748 and 1468 $cm^{-1}$, respectively, in the reflectance IR spectrum (Figure S1b).

Our approach to the protein immobilization on a solid surface requires ferrocenylated proteins. We chose glucose oxidase (GOx) as a model protein for the surface immobilization because it is one of the most well-known redox proteins, extensively studied for glucose sensor applications, and the synthesis of ferrocene attached GOx has been well established.[16] The ferrocenylated GOx (FA-GOx) was prepared by EDC coupling reaction of GOx with **4**.[17,18] The average number of ferrocene units attached to GOx was determined to be ~19 and the activity of FA-GOx was ~60% of that of unmodified GOx. Immobilization of FA-GOx on the CB-[7]-anchored gold substrate was achieved by immersion of the substrate in a buffer solution containing FA-GOx for 1 day followed by washing. As shown in Figure S1c, new peaks corresponding to the amide bonds of FA-GOx were observed at 1670 and 1540 $cm^{-1}$ in the IR spectrum. The amount of unmodified GOx adsorbed on the CB[7]-anchored gold substrate under the same conditions was less than 20% compared to that of FA-GOx as judged by the IR intensity of the amide bonds of GOx (Figure S1d).

***Scheme 1.*** Immobilization of a Protein on Gold Using the FA-CB[7] Interaction.




The immobilization of FA-GOx on the CB[7]-anchored gold substrate was also monitored by surface plasmon resonance (SPR) technique. The sensorgrams obtained with FA-GOx and unmodified GOx are compared in Figure 1, which showed ~5.5 times higher protein loading on the surface for the case of FA-GOx (1820 RU for FA-GOx vs 330 RU for GOx; 1000 RU = 1 ng/$mm^2$), which is consistent with the above IR result. The surface density of FA-GOx calculated from the SPR data is $1.1 \times 10^{-12}$ mol/$cm^2$, which corresponds to ~60% of a densely packed GOx monolayer on the surface.[19,20]

The utility of the FA-GOx immobilized on gold as a glucose sensor has been investigated by cyclic voltammetry and glucose-concentration-dependent current measurements. FA-GOx was immobilized on a gold electrode in the same way as described above. Cyclic voltammograms obtained with the FA-GOx immobilized gold electrode as a working electrode in the presence of various concentrations of glucose are shown in Figure 2. While no appreciable current was observed in the absence of glucose, the catalytic current increased with increasing glucose concentration and reached a plateau above 30 mM of glucose (inset of Figure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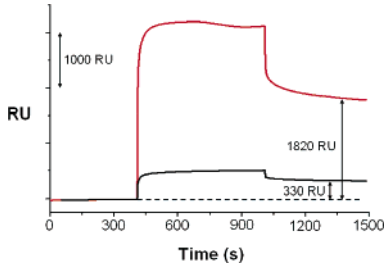


***Figure 1.*** SPR sensorgrams showing the adsorption of FA-GOx (red line) and unmodified GOx (black line) on a CB[7]-anchored gold substrate (1000 RU = 1 ng/mm$^2$).

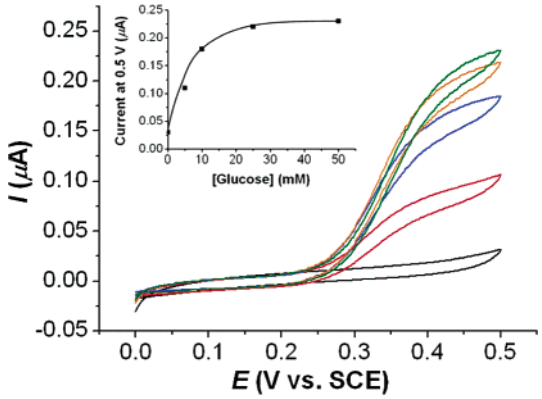


***Figure 2.*** Cyclic voltammograms obtained with a FA-GOx immobilized gold electrode and various glucose concentrations in phosphate buffer (0.10 M, pH 7.3). Electrode surface area = 0.020 cm$^2$; scan rate, 2 mV s$^{-1}$; $T$ = 294 K. Glucose concentration (mM): 0 (black line), 5.0 (red line), 10 (blue line), 25 (orange line), and 50 (green line). The inset shows a plot of current at 0.5 V as a function of glucose concentration.

2),[21] which suggested that the FA-GOx immobilized gold electrode can be used as a glucose sensor.

In conclusion, we report a novel noncovalent method to immobilize a protein on a solid surface using the CB[7]−FA pair. As a proof of concept, the immobilization of ferrocenylated GOx on a CB[7]-anchored gold substrate, and its use as a glucose sensor have been demonstrated. In principle, this approach can be applied to the immobilization of any biomolecules including nucleic acids on any surfaces including glass, silicon, silica, and polymers. The synthetic host−guest pair with exceptional affinity, chemical robustness, simple preparation, and easy handling may thus replace the biotin−avidin system not only in the immobilization of biomolecules on solid surfaces, but also in other applications such as affinity chromatography and immunoassay. We are currently exploring the possibility.

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**Supporting Information Available:** Complete ref 10c, synthesis of **1**−**4**, preparation and characterization of **1**-anchored SAM, synthesis and characterization of ferrocenylated GOx, SPR, and glucose sensing experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Based on the SPR data and size of the protein, we estimated that one or two CB[7]−FA pairs are involved in immobilization of each FA-GOx on the surface.
(21) On the basis of the catalytic current, the turnover number of FA-GOx attached on gold was estimated to be ∼55 s$^{-1}$, which is ∼20% of that in solution. The origin of the reduction in the activity is not clear at the moment, but it may be due to the inefficiency of the electron transfer between FA-GOx and the electrode surface.

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