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Generating Patterns for Sensing Using a Single Receptor Scaffold

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Taking advantage of the differential binding affinity of a set of nonspecific receptors to generate analyte-specific patterns for identification is an attractive concept in sensor applications.[1] This is mainly because it obviates the rather challenging task of achieving a highly specific lock-and-key receptor and thus significantly simplifies the sensor design. This approach will be even more attractive if the components of the sensor are assembled noncovalently[2] in such a fashion that the differential response could be achieved from a single receptor for multiple analytes. This paper describes a new approach in which a single receptor that binds nonspecifically to proteins is capable of generating analyte-dependent patterns; the key is the difference in reporter elements noncovalently bound to the receptor.

Conjugated polyelectrolytes (polymeric electrolytes) have been used extensively for sensors because of the inherent fluorescence properties and the ability to bind biomacromolecules using polyvalent interactions. Since metalloproteins have cofactors that could accept energy or charge from the excited state of these conjugated polymers, these scaffolds have been used for sensing metalloproteins.[3] However, it has been shown that the response is not selective to metalloproteins; the possible binding-induced conformational changes from any protein could result in modified fluorescence properties of conjugated polyelectrolytes.[4] We have recently demonstrated that water-soluble nonconjugated polyelectrolytes containing fluorescent pendant units are capable of selectively sensing metalloproteins,[5] although the binding itself is nonselective. To enhance the repertoire of such systems, it is necessary that these polymers not only respond to metalloproteins selectively but also be able to differentiate one from the other. We utilize a new pattern generation strategy based on a single receptor to achieve this differentiation and identification.

In this approach, we take advantage of the fact that our recently reported amphiphilic homopolymers bind to proteins[6] and that these polymers form micelle-type assemblies in water.[7] We have previously shown that these micellar assemblies are capable of sequestering hydrophobic guest molecules such as pyrene.[8] We conceived the possibility that if the excited state of such a noncovalently bound dye molecule is able to transfer the energy or electron to a bound protein, then an opportunity exists to create patterns by simply varying the hydrophobic guest molecule sequestered within the micellar assembly. The main advantage of this approach is the possibility of easy interchange of the different hydrophobic dyes to create an array of fluorescent nanosensors without having to go through the rather time-consuming design and syntheses of multiple receptors (Figure 1a).

In order to test our hypothesis, we first tested whether an energy or a charge-transfer-based fluorescence quenching from a noncovalently bound fluorophore is possible. A hydrophobic fluorophore (pyrene, **1**) was encapsulated within the hydrophobic core of the micellar assemblies formed in polymer **P1** (Figure 1b) in aqueous media. The ability of this "polymer−dye" complex to sense metalloproteins was studied by monitoring the decrease in fluorescence intensity of **1** with increasing concentration of cytochrome *c* (Figure 2a). The concentration-dependent quenching was used to generate a Stern−Volmer plot (Figure 2b) and to extract the Stern−Volmer quenching constant (K_{SV}). For cytochrome *c*, this value was 1.1×10^5. This value compares quite favorably with the covalently attached dye-molecule-based strategy.[5] To further test whether these fluorescent assemblies would respond differently to other proteins, we investigated the response of the pyrene-encapsulated polymer assembly in water for three other metalloproteins, *viz.*, ferritin, hemoglobin, and myoglobin. The K_{SV} values obtained were 1.0×10^6, 4.8×10^5, and 5.2×10^5, respectively. This suggests that generating patterns for recognition using this strategy is viable.

The key feature of the current approach is that a variety of patterns could be obtained just by varying the noncovalently bound dye molecule. This is easily accessible because of the rather broad range of hydrophobic dyes that the micellar interior is capable of sequestering. We haven chosen eight different dye molecules for this purpose. The results are summarized in Figure 3. Of the systems investigated, the highest K_{SV} value of 2.0×10^6 was observed for

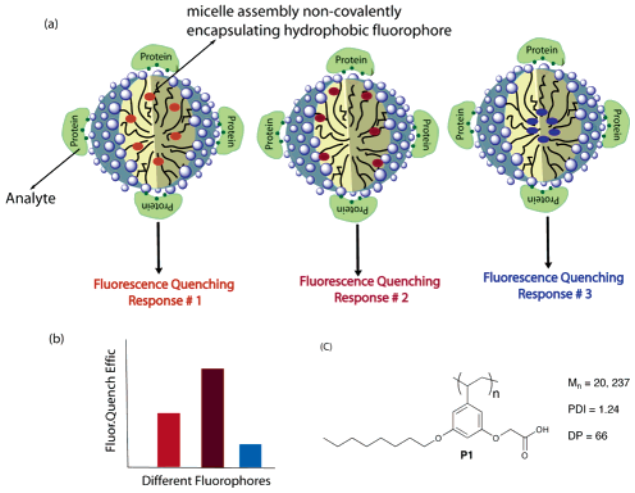

Figure 1. (a) Schematic of the differential transducer approach using amphiphilic homopolymer micelles. (b) Expected fluorescence quenching response for a hypothetical analyte. (c) Structure of polymer **P1**.

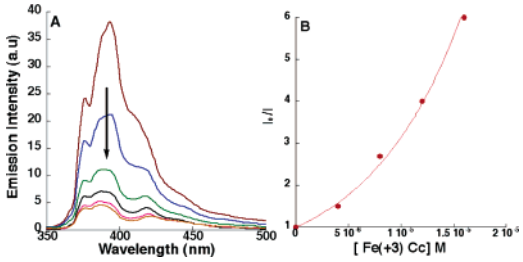

Figure 2. (A) Fluorescence quenching of pyrene-containing **1** with concentrations of metalloprotein (Cc). (B) Stern−Volmer plot for the data in panel A. Excitation wavelength = 338 nm, emission wavelength = 376 nm.

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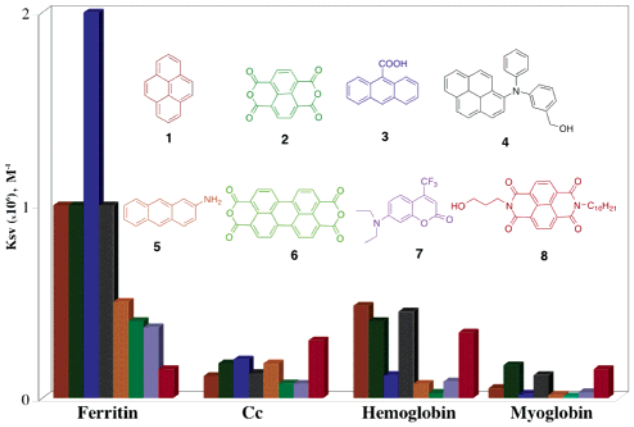

Figure 3. Patterns of Stern−Volmer quenching constants (K_{SV}) of four different metalloproteins with respect to eight different hydrophobic fluorescent dye molecules noncovalently bound to the micellar interiors of the amphiphilic homopolymer **1** in aqueous solutions.

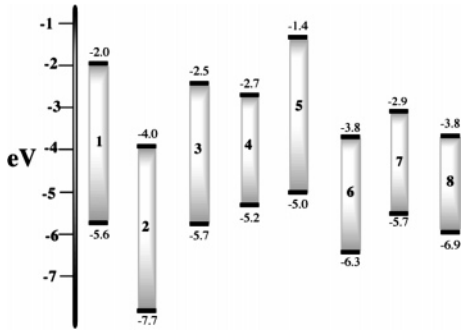

Figure 4. Band diagram of fluorophores **1**−**8** with respect to vacuum level.

ferritin when **3** is used as the fluorescent transducer. Similarly, the polymer assembly containing **6** exhibits the lowest K_{SV} value of 9.0×10^3 for myoglobin. The most important feature is that the fluorescence quenching of each polymer−dye complex is different for the metalloproteins investigated, which resulted in a unique fingerprint for each protein. Thus, we show that the strategy indeed works. The error in the K_{SV} values is less than 5%. Therefore, the fingerprint is reproducible for a particular analyte.

While we have demonstrated the principle that one could indeed generate patterns for the identification of an analyte using a single receptor, but by varying the transducers, important questions that would arise include the following: Why would one expect differential response from these fluorophores? How does one choose the appropriate dye molecule to provide these variations? The differential response is most likely due to the complex mix of variables that dictate the observed fluorescence quenching. For example, we had shown that both energy transfer and electron transfer processes could contribute to the overall quenching process in our covalently bound fluorophore system.[5] The factors that determine the percentage contribution between these pathways are different. Those include (i) excited state lifetime of a fluorophore; (ii) frontier orbital energy levels of the fluorophore and the protein cofactor responsible for quenching (redox potentials and ΔE_{0-0}); (iii) spectral overlap between fluorophore and the protein cofactor; and (iv) distance between the fluorophore and the protein cofactor. These factors are very different for the fluorophores, and these are in fact the criteria used to choose the dye molecules in this study. For example, the redox potentials of the fluorophores are such that **1** is likely to accept an electron from the porphyrins upon excitation, whereas **4** is likely to donate an electron. In fact, we have estimated the frontier orbital energy levels of the dye molecules used in this study using a combination of cyclic voltammetry, UV−visible absorption, and emission spectra. The estimated relative energy levels for the eight fluorophores studied here are shown in Figure 4. These are the inherent variations in the transducers. In addition, the polymer−protein binding is also likely to cause variations in the proteins, which provides an additional avenue for differences in the quenching response. For example, we have shown that amphiphilic homopolymers, such as **P1**, bind to chymotrypsin and cytochrome *c*. While there is no evidence for any conformational change in chymotrypsin, there seems to be a significant structural change in cytochrome *c*.[6] This causes change in the redox potential of the metalloporphyrin cofactor in cytochrome *c*.[6b] These protein-dependent binding-induced changes provide the necessary, additional variations for a pattern generation.

In summary, (i) we have shown that a single receptor containing a variety of fluorescent transducers could be used for generating patterns in sensing. (ii) This principle has been demonstrated using an amphiphilic homopolymer scaffold that is capable of encapsulating a broad range of hydrophobic fluorophores within its micellar interior noncovalently. (iii) The noncovalent incorporation of these molecules provides access to a wide variety of differentially responding systems with high facility. (iv) The variations in the frontier orbital energy levels of the dye molecules, distance dependence in energy/electron transfer, and conformational changes in protein all likely contribute to the differential response and therefore the patterns. The demonstration that one receptor can provide variable responses by varying a noncovalent guest molecule significantly enhances the capabilities of multianalyte recognition approach in general. This is because the combination of the multiple transducers approach demonstrated here, combined with the multiple receptors approach reported previously,[1] provides two orthogonal dimensions for variations in obtaining the volume of data necessary for reliable analyte-specific patterns relatively easily.

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Supporting Information Available: Fluorescence spectra, the Stern−Volmer plots, and other experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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