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Fluorescence Patterns from Supramolecular Polymer Assembly and Disassembly for Sensing Metallo- and Nonmetalloproteins

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Abstract: Critical aggregation concentration (CAC) of surfactants is lowered when polyelectrolytes act as counterions. At a concentration in between the CACs of the surfactant and the polymer−surfactant complex, protein-induced disassemblies can be achieved. This is because, when proteins competitively bind to the polyelectrolytes, the surfactants are not capable of sustaining a micelle-type assembly at this concentration. Since these amphiphilic aggregates are capable of noncovalently sequestering hydrophobic guest molecules, the protein binding induced disassembly process also results in a guest release from these assemblies. We show here that the change in fluorescence with different proteins is dependent not only on the nature of the polymer−surfactant complex, but also on the fluorescent transducer. Two processes can be responsible for the observed fluorescence change: fluorophore guest release from the hydrophobic interior of the assembly and excited state quenching due to complementary components in the analyte. The latter mechanism is especially possible with metalloproteins. We show here that an excited state quenching is possible at nanomolar concentrations of the proteins, while the disassembly based fluorescence reduction is the dominant pathway at micromolar concentrations.

Introduction

Developing materials for sensing biological analytes has received significant attention in recent years due to implications in important areas such as medical diagnostics, proteomics, and bioterrorism.[1] In a simple description, a sensor design needs two components: (i) an analyte binding unit or receptor; (ii) a transducer that translates the binding event into a readable signal.[2] An obvious requirement for a design to be effective is that the sensing device should be able to report on the targeted analyte with high sensitivity and selectivity. Nature has tackled this requirement for its signaling pathways by evolving highly specific lock-and-key type receptors. Mimicking this capability in an artificial sensor design is an enormous challenge.[3] Fortunately, however, nature also provides the inspiration for a less design-intensive approach to sensing. This approach involves the generation of an array of less selective receptors and then utilizes the differential response from each of these to different analytes to develop patterns. Prominent examples of this in nature are the senses of smell and taste.[4] Recently it has been shown by several reports that such naturally occurring events can be mimicked by taking advantage of differential binding receptors to generate analyte-specific patterns. A wide variety of compounds have been sensed using this strategy, including amino acids, proteins, volatiles, aromatic amines, and carbohydrates.[5] While this strategy circumvents the intricacies of precise molecular design for a particular ligand, the synthetic demands of this approach are still rather high. This is due to the number of receptors needed for obtaining reliable patterns for analyte identification.[5,6] The applicability of the pattern recognition would be greatly enhanced, if the number of required

(1) For examples, see: (a) Wright, A. T.; Anslyn, E. V. *Chem. Soc. Rev.* **2006**, *35*, 14–28. (b) Ikebukuro, K.; Kiyohara, C.; Sode, K. *Biosens. Bioelectron.* **2005**, *20*, 2168–2172. (c) D'Auria, S.; Lakowicz, J. R. *Curr. Opin. Biotechnol.* **2001**, *12*, 99–104. (d) Zhu, H.; Snyder, M. *Curr. Opin. Chem. Biol.* **2001**, *5*, 40–45. (e) Zhou, H.; Baldini, L.; Hong, J.; Wilson, A. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **2006**, *128*, 2421–2425.

(2) (a) De Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566. (b) Ambade, A.; Sandanaraj, S. B.; Klaikherd, A.; Thayumanavan, S. *Polym. Int.* **2007**, *56*, 474–481. (c) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537–2574.

(3) (a) Ojida, A.; Mito-oka, Y.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 2454–2463. (b) Korbakov, N.; Timmerman, P.; Lidich, N.; Urbach, B.; Sa'ar, A.; Yitzchaik, S. *Langmuir* **2008**, *24*, 2580–2587.

(4) (a) *Handbook of Olfaction and Gustation*, 2nd ed.; Doty, R. L., Ed.; Marcel Dekker: New York, 2003. (b) Axel, R. *Angew. Chem., Int. Ed.* **2005**, *44*, 6111–6127. (c) Toko, K. *Biosens. Bioelectron.* **1998**, *13*, 701–709. (d) Adler, E.; Hoon, M. A.; Mueller, K. L.; Chandrashekar, J.; Ryba, N. J. P.; Zuker, C. S. *Cell* **2000**, *100*, 693–702.

(5) (a) Dirinck, I.; Van Leuven, I.; Dirinck, P. *Dev. Food Sci.* **2006**, *43*, 521–524. (b) Goodey, A.; Lavigne, J. J.; Savoy, S. M.; Rodriguez, M. D.; Curey, T.; Tsao, A.; Simmons, G.; Wright, J.; Yoo, S. J.; Sohn, Y.; Anslyn, E. V.; Shear, J. B.; Neikirk, D. P.; McDevitt, J. T. *J. Am. Chem. Soc.* **2001**, *123*, 2559. (c) Litvinchuk, S.; Tanaka, H.; Miyatake, T.; Pasini, D.; Tanaka, T.; Bollot, G.; Mareda, J.; Matile, S. *Nat. Mater.* **2007**, *6*, 576–580. (d) You, C.-C.; Miranda, O. R.; Gider, B.; Ghosh, P. S.; Kim, I.-B.; Erdogan, B.; Krovi, S. A.; Bunz, U. H. F.; Rotello, V. M. *Nat. Nanotechnol.* **2007**, *2*, 318–323. (e) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2001**, *40*, 3118–3130. (f) Racow, N. A.; Suslick, K. S. *Nature* **2000**, *406*, 710–713. (g) Greene, N. T.; Shimizu, K. D. *J. Am. Chem. Soc.* **2005**, *127*, 5695–5700. (h) Folmer-Adersen, J. F.; Kitamura, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2006**, *128*, 5652–5653. (i) Buryak, A.; Severin, K. A. *J. Am. Chem. Soc.* **2005**, *127*, 3700–3701. (j) Lee, J. W.; Lee, J.-S.; Chang, Y.-T. *Angew. Chem., Int. Ed.* **2006**, *45*, 6485–6487.

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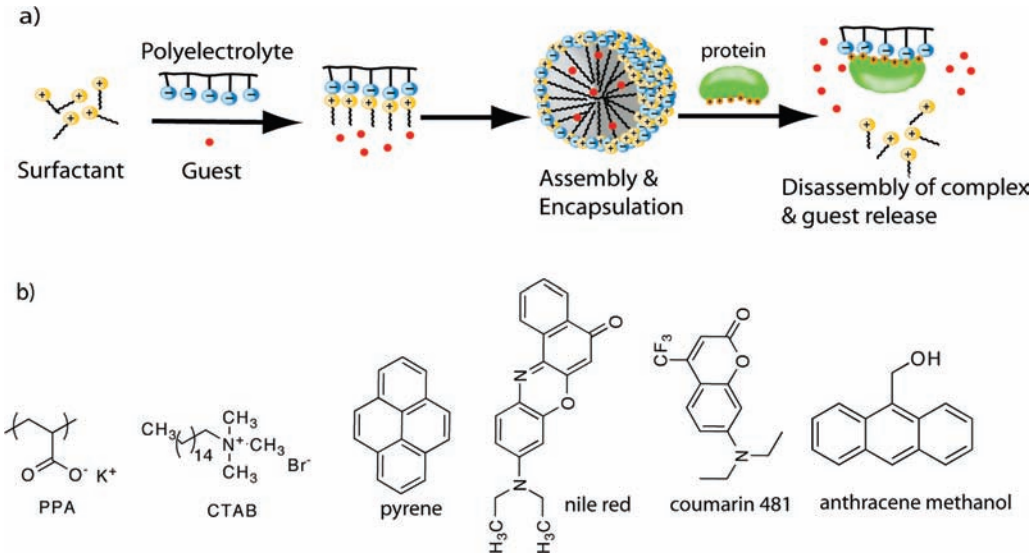


Figure 1. (a) Polymer−surfactant complex assembly and protein-induced disassembly. (b) Structures of the polymer, surfactant, and dye molecules used in the experiment.

synthetic receptors were reduced or if synthetic strategies were significantly simplified.[7]

We have recently introduced a new approach to generating patterns, where differential responses can be generated from a single receptor,[8] but by varying the transducer. The key feature of this design is to choose noncovalent transducers with significant variations in their frontier molecular orbital levels. The orthogonality in this approach, i.e., varying the transducer rather than varying the receptor, provides a unique opportunity to generate multiple data points necessary for the fidelity of pattern-based sensing with relatively lesser synthetic efforts. This is made possible, especially because the fluorescent transducers are incorporated into our amphiphilic receptor assemblies through noncovalent binding. In this approach, it is necessary that the frontier orbital energy levels of the transducers complement those of the analytes to experience fluorescence quenching. Thus, among proteins, this approach is restricted to metalloproteins as these have the appropriate cofactors to quench the fluorescence of several common organic fluorophores.

More recently, we have reported on our preliminary findings on a fluorescence-based pattern generation approach that is also applicable to nonmetalloproteins.[10] In this case, we exploited the observation that the complex between a polyelectrolyte and a surfactant exhibit lower critical aggregation concentration compared to the surfactant by itself.[11] We envisaged that when one has the polyelectrolyte−surfactant complex at an intermediate concentration between the two critical aggregation concentrations (CACs), removing the polyelectrolyte from the complex through a competitive binding event to the analyte would cause disassembly. This disassembly can then be quantified for fluorescence reporting, when a noncovalently sequestered fluorescent guest molecule is released upon disassembly (see Figure 1 for a schematic illustration). By varying the structure and charge of the surfactant and the polyelectrolyte structures, we were able to generate analyte-specific patterns for proteins that do not have electronic complementarity to the fluorescent guest molecules. Another important feature of this work is that both the receptor and the fluorescent transducer are noncovalently assembled from simple, commercially available molecular components. This paper elaborates on the details of the findings in our communication along with several new insights in this sensing approach.[10] First, it is interesting to ask whether noncovalently assembled receptors can be utilized to generate analyte-dependent patterns by varying the fluorescent guest molecules. Note that the underlying mechanism of this approach

(6) (a) Albert, K. J.; Lewis, N. S.; Schauer, C. L.; Sotzing, G. A.; Stitzel, S. E.; Vaid, T. P.; Walt, D. R. *Chem. Rev.* **2000**, *100*, 2595–2626. (b) Grandini, P.; Mancin, F.; Tecilla, P.; Scrimin, P.; Tonellato, U. *Angew. Chem., Int. Ed.* **1999**, *38*, 3061–3064. (c) Hong, M.-Y.; Lee, D.; Kim, H.-S. *Anal. Chem.* **2005**, *77*, 7326–7334. (d) Kolusheva, S.; Zadmard, R.; Schrader, T.; Jelinek, R. *J. Am. Chem. Soc.* **2006**, *128*, 13592–13598. (e) Palacios, M. A.; Nishiyabu, R.; Marquez, M.; Anzenbacher, P., Jr. *J. Am. Chem. Soc.* **2007**, *129*, 7538–7544. (f) Ponnu, A.; Edwards, N. Y.; Anslyn, E. V. *New J. Chem.* **2008**, *32*, 848–855. (g) Baldini, L.; Wilson, A. J.; Hong, I.; Hamilton, A. D. *J. Am. Chem. Soc.* **2004**, *126*, 5656–5657. (h) Kumar, N.; Parajuli, O.; Hahm, J. *J. Phys. Chem. B* **2007**, *111*, 4581–4587. (i) Christman, K. L.; Maynard, H. D. *Langmuir* **2005**, *21*, 8389–8393.

(7) (a) Zadmard, R.; Schrader, T. *J. Am. Chem. Soc.* **2005**, *127*, 904–915. (b) Zhou, H.; Baldini, L.; Hong, J.; Wilson, A. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **2006**, *128*, 2421–2425. (c) Wright, A. T.; Anslyn, E. V.; McDevitt, J. T. *J. Am. Chem. Soc.* **2005**, *127*, 17405–17411.

(8) We use the term "receptor" in the supramolecular chemistry sense, i.e., the molecular assembly that is capable of binding an analyte. This is not to be confused with the use of "receptor" in biology.

(9) Sandanaraj, S. B.; Demont, R.; Thayumanavan, S. *J. Am. Chem. Soc.* **2007**, *129*, 3506–3507.

(10) Savariar, E. N.; Ghosh, S.; González, D. C.; Thayumanavan, S. *J. Am. Chem. Soc.* **2008**, *130*, 5416–5417.

(11) (a) Goddard, E. D.; Ananthapadmanabhan, K. P. *Interactions of Surfactants with Polymers and Proteins*; CRC Press: Boca Raton, FL, 1993. (b) Macknight, W. J.; Ponomarenko, E. A.; Tirrell, D. A. *Acc. Chem. Res.* **1998**, *31*, 781–788. (c) Zhou, S.; Chu, B. *Adv. Mater.* **2000**, *12*, 545–556. (d) Bronich, T. K.; Kabanov, A. V. *Macromolecules* **1997**, *30*, 3519–3525. (e) Bronich, T. K.; Cherry, T.; Vinogradov, S. V.; Eisenberg, A.; Kabanov, V. A.; Kabanov, A. V. *Langmuir* **1998**, *14*, 6101–6106. (f) Kabanov, A. V.; Bronich, T. K.; Kabanov, V. A.; Yu, K.; Eisenberg, A. *J. Am. Chem. Soc.* **1998**, *120*, 9941–9942. (g) Wang, C.; Tam, K. C. *Langmuir* **2002**, *18*, 6484–6490. (h) Mizusaki, M.; Morishima, Y.; Yoshida, K.; Dubin, P. *Langmuir* **1997**, *13*, 6941–6946. (i) Hashidzume, A.; Yoshida, K.; Morishima, Y.; Dubin, P. *J. Phys. Chem. B* **2002**, *106*, 2007–2014. (j) Hayakawa, K.; Tanaka, R.; Kurawaki, J.; Kusumoto, Y.; Satake, I. *Langmuir* **1999**, *15*, 4213–4216. (k) Zhou, S.; Burger, C.; Chu, B. *J. Phys. Chem. B* **2004**, *108*, 10819–10824. (l) Hayakawa, K.; Satake, I.; Kwak, J. C. T. *Colloid Polym. Sci.* **1994**, *272*, 876–883. (m) Kong, L.; Cao, M.; Hai, M. *J. Chem. Eng. Data* **2007**, *52*, 721–726.

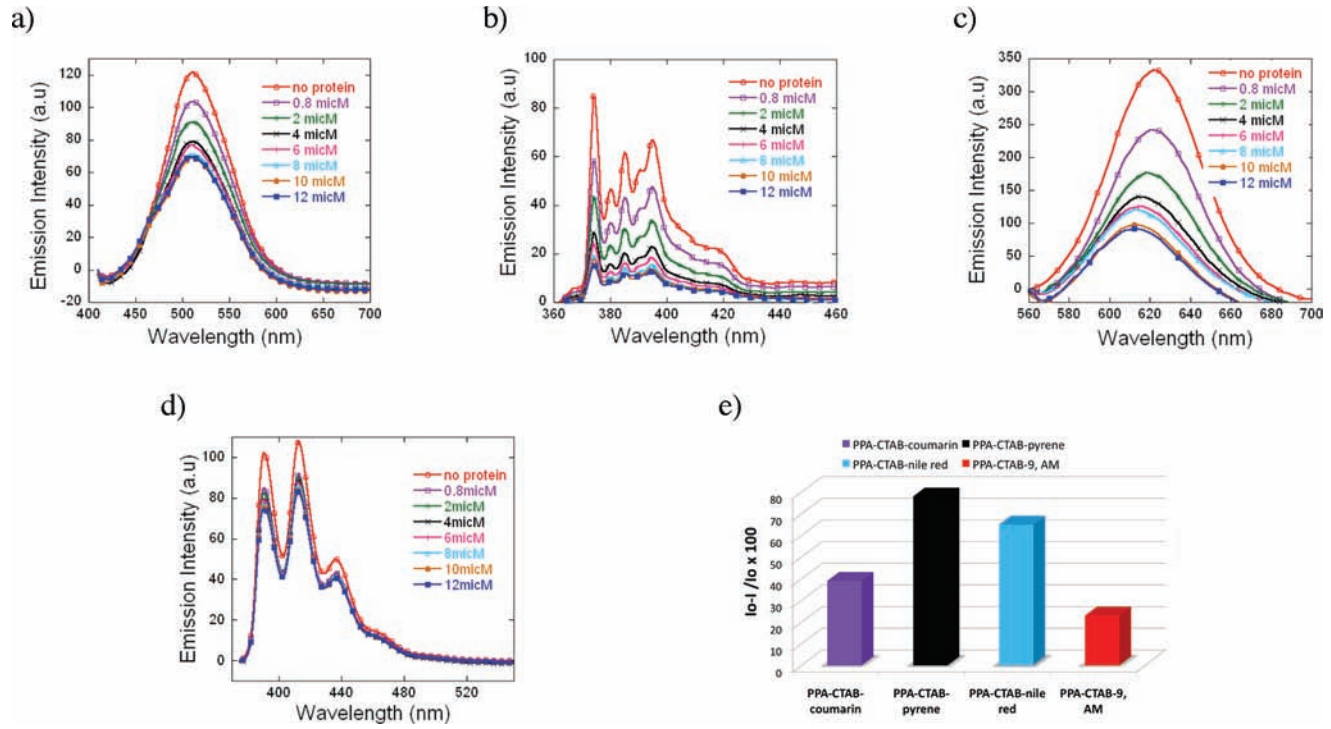


Figure 2. Emission intensity changes of dye molecules in PPA−CTAB complex in response to different concentrations of the protein β-Glu: (a) coumarin (10^{-6} M; $\lambda_{ex} = 400$ nm); (b) pyrene (10^{-6} M; $\lambda_{ex} = 339$ nm); (c) nile red (10^{-6} M; $\lambda_{ex} = 550$ nm); (d) 9-anthracene methanol (10^{-5} M; $\lambda_{ex} = 365$ nm) as the transducers. (e) Transducer-based pattern at 8 μM concentration of β-Glu.

is very different from our previous work. In our previous approach, we relied on the variations in the frontier orbital energy level complementarity.[9] Here, since our change in fluorescence is based on binding-induced disassembly, electronic complementarity is unlikely to play any significant role. Second, we investigate the disassembly approach to sense metalloproteins. With these analytes, two different mechanisms are possible for sensing: (i) fluorescence quenching based on photoinduced energy or electron transfer; (ii) disassembly based guest release. It would be interesting to ask which of these two processes will dominate the metalloprotein sensing. Third, based on our findings, we ask whether we could utilize this approach to selectively sense metalloproteins with higher sensitivity compared to nonmetalloproteins.

Results and Discussion

Patterns from Fluorophore Variations in Nonmetalloprotein Sensing. Can we generate analyte-specific variations in fluorescence response to generate patterns by changing the nature of the fluorophore in the binding-induced disassembly approach? We use poly(potassium acrylate) (PPA), potassium salt of the commercially available poly(acrylic acid), that complexes to the surfactant cetyltrimethylammonium bromide (CTAB). The negatively charged carboxylate functionalities of the polyelectrolyte and the positively charged amine functionalities of the surfactant complex to form amphiphilic aggregates with hydrophobic interiors. The CAC of this complex is 4×10^{-5} M, while that of CTAB is 1.3×10^{-3} M.[10] Thus, the binding-induced disassembly studies here are carried out at a CTAB concentration of 1.0×10^{-4} M, with 1 equivalent of carboxylate units from PPA. Note that this concentration is above the CAC of the complex and below that of the surfactant. A binding interaction between the polyelectrolyte and the protein will dissociate the surfactant from the polymer−surfactant complex. Since the concentration of the surfactant is now below its CAC, a disassembly will ensue and cause the release of the fluorescent guest molecule. This guest release is monitored to report on the binding between the polyelectrolyte and the protein. Pyrene, nile red, coumarin 481, and 9-anthracene methanol were chosen as the fluorescent dye molecules for this study. All these molecules are hydrophobic and have been shown to be good guest molecules to be sequestered within the hydrophobic interiors of amphiphilic assemblies.[12]

We have previously shown that β-glucosidase (β-Glu) binds to a pyrene-encapsulated PPA−CTAB complex and releases the fluorophore.[10] In order to investigate if fluorophore-dependent differences could be generated in response to protein binding, we encapsulated coumarin 481 and pyrene within the hydrophobic interiors of the PPA−CTAB complex and monitored the decrease in fluorescence intensity with varying concentrations of protein, β-Glu. At 8 μM concentration of β-Glu in 1.0×10^{-4} M solution of the PPA−CTAB−coumarin complex, a significant decrease in the fluorescence of coumarin 481 was indeed observed. The extent of decrease in fluorescence at this concentration of the protein was 39%, as shown in Figure 2a. This is very different from the 78% decrease observed for pyrene in Figure 2b for the same polyelectrolyte−surfactant combination at identical concentration of the protein. These results support for our hypothesis that it is possible to generate patterns by simply varying the fluorescent transducer in the

(12) (a) Bromberg, L. E.; Barr, D. P. *Macromolecules* **1999**, *32*, 3649–3657. (b) Wilhelm, M.; Zhao, C.-L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J.-L.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033–1040. (c) Astafieva, I.; Zhong, X. F.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7339–73352. (d) Satpati, A. K.; Kumbhakar, M.; Nath, S.; Pal, H. *J. Phys. Chem. B* **2007**, *111*, 7550–7560. (e) Webber, G. B.; Wanless, E. J.; Armes, S. P.; Tang, Y.; Li, Y.; Biggs, S. *Adv. Mater.* **2004**, *16*, 1794–1798. (f) Jiang, J.; Tong, X.; Zhao, Y. *J. Am. Chem. Soc.* **2005**, *127*, 8290–8291.

Table 1. Percent Dye Release at 8 μM Concentration of Nonmetalloproteins

		$I_0 - I/I_0 \times 100$ (%)			
protein	p*I*	pyrene	nile red	coumarin 481	9-anthracene methanol
BSA	4.8	16	4	−100	3
Avd	10.0	35	81	72	21
Lys	11.0	29	31	47	29
ChT	8.8	11	16	5	18
β-glu	5.5	78	65	39	23
Pep	1.0	13	16	−17	11
Thr	7.6	26	6	20	13
Rnase A	9.6	38	24	16	17

disassembly based sensing. To further verify this, we carried out similar experiments with nile red and 9-anthracene methanol. We observed that the extents of fluorescence decrease were 65% and 23%, respectively, as shown in Figure 2c and 2d. The differential response from the four different dyes for β-Glu is shown in Figure 2e.

Next, we were interested in investigating whether these differences observed for β-Glu with four different dye molecules translate to generation of patterns for different analytes. For this, it is necessary that these dye molecules respond differently to various proteins. We investigated the response of these dye molecules with PPA−CTAB combination for seven other nonmetalloproteins, viz., bovine serum albumin (BSA), avidin (Avd), lysozyme (Lys), chymotrypsin (ChT), pepsin (Pep), thrombin (Thr), and ribonuclease A (Rnase A). The extents of the release of different dye molecules from the hydrophobic interiors of the PPA−CTAB complex in response to 8 μM concentrations of different proteins are shown in Table 1 and Figure 3. With only four dye molecules as the variation, we were gratified to find that analyte-specific patterns do emerge. These results provide the evidence that the fluorophore variations can indeed be utilized to generate patterns for disassembly based protein sensing.

It is clear that proteins with different p*I* values and sizes will interact differently with charged species such as the polyelectrolytes. Note however that the correlation between the p*I* and extent of dye release is not necessarily straightforward in all cases, because (i) proteins are polyampholytes and thus can interact both with anionic and cationic polyelectrolytes with different affinities;[13] (ii) proteins are capable of not only competitively interacting with the polyelectrolyte, but also with the surfactant assembly of opposite charge. The question however remains: why would one expect differential response from the dye molecules, when the mechanism of fluorescence change is based on the release of the dye molecule from a hydrophobic environment? In other words, if the disassembly of the micelle is based on the interaction between the protein and the polymer and/or the surfactant, why should the variation in dye molecules provide differences in their responses? To generate patterns for sensing, it is necessary that the interactions between different analytes and a receptor have overlapping features, but there should be factors embedded that provide subtle differences in their interactions. In our case, the difference has to come from the transducers. The extent of sequestration of the hydrophobic guest molecules within the hydrophobic pockets of an amphiphilic assembly depends on the distribution coefficient of those molecules between the bulk solvent (water) and the hydrophobic pocket. This distribution coefficient is different for different dye molecules, and therefore the extent of the dye release with respect to the extent of the disassembly of the amphiphiles will be different.[14] We envisaged that this feature, combined with the fact that certain fluorophores have inherent differences in their fluorescence quantum yields depending on changes in the polarity and rigidity of their microenvironments, will provide analyte-dependent variations in fluorescence.[15]

We were gratified to find that there are additional features that provide further opportunities for pattern generations. These came from our investigations on the reasons for the increase in the fluorescence of coumarin 481 with the dye molecules in response to BSA and Pep. We hypothesized that it is possible that some of the proteins themselves are capable of sequestering coumarin 481 in an even better hydrophobic pocket and thus the fluorescence of these dye molecules increases. In order to test this, we analyzed the fluorescence of coumarin 481 in the presence of these proteins without the polyelectrolyte or the surfactant. The fluorescence of coumarin 481 indeed increased with the concentration of the protein, as shown in Figure 4. These results suggest that a number of factors contribute to the overall pattern generation from variations in transducers, even when there is no electronic complementarity between the frontier orbital energy levels of the analyte and the fluorophore. The complex interplay of these factors contributes to pattern generations for nonmetalloproteins by simply varying the noncovalently bound fluorescent transducers in the polymer−surfactant complex assembly. Note that we have already shown that analyte-specific patterns can be generated by varying the structure of the polymer and the surfactant.[10] Our demonstration here with the transducer variations affords an useful orthogonal dimension to generate the large number of data points needed for obtaining reliable sensing patterns.

Interaction with Metalloproteins. In the case of metalloproteins, emission from the encapsulated fluorophore can be modulated through two different mechanisms, viz., the disassembly based pathways shown above or the photoinduced charge or energy transfer quenching (see Figure 5 for a schematic illustration). We used four metalloproteins, viz., cytochrome *c* (Cc), ferritin (Ferr), hemoglobin (Hb), and myoglobin (Myo), as candidates for these experiments. These proteins were tested against the PPA−CTAB complex using the same four dye molecules above. First, when a micromolar concentration of Cc was added to a 1×10^{-4} M solution of the pyrene-encapsulated PPA−CTAB complex, we noticed a rather drastic change in fluorescence. This observation led us to investigate the minimum concentration at which there will be a significant change in the emission from the encapsulated fluorophore. We found that significant changes in fluorescence were observed at even nanomolar concentration of the protein. Upon investigating all four metalloproteins with different dye molecules, we consistently found that a significant decrease in emission was observed at nanomolar concentrations for all metalloproteins with all fluorophores. Moreover, the extent of the emission quenching was dependent not only on the nature of the dye molecule, but

(13) Roy, R.; Sandanaraj, B. S.; Klaikherd, A.; Thayumanavan, S. *Langmuir* **2006**, *22*, 7695.

(14) Soo, P. L.; Luo, L.; Maysinger, D.; Eisenberg, A. *Langmuir* **2002**, *18*, 9996–10004.

(15) (a) Dutta, A. K.; Kamada, K.; Ohta, K. *J. Photochem. Photobiol., A: Chem.* **1996**, *93*, 57–64. (b) Hou, Y. W.; Bardo, A. M.; Martinez, C.; Higgins, D. A. *J. Phys. Chem. B* **2000**, *104*, 212–219. (c) Hendriks, J.; Gensch, T.; Hviid, L.; Van der Horst, M. A.; Hellingwerf, K. J.; Van Thor, J. J. *Biophys. J.* **2002**, *82*, 1632–1643.

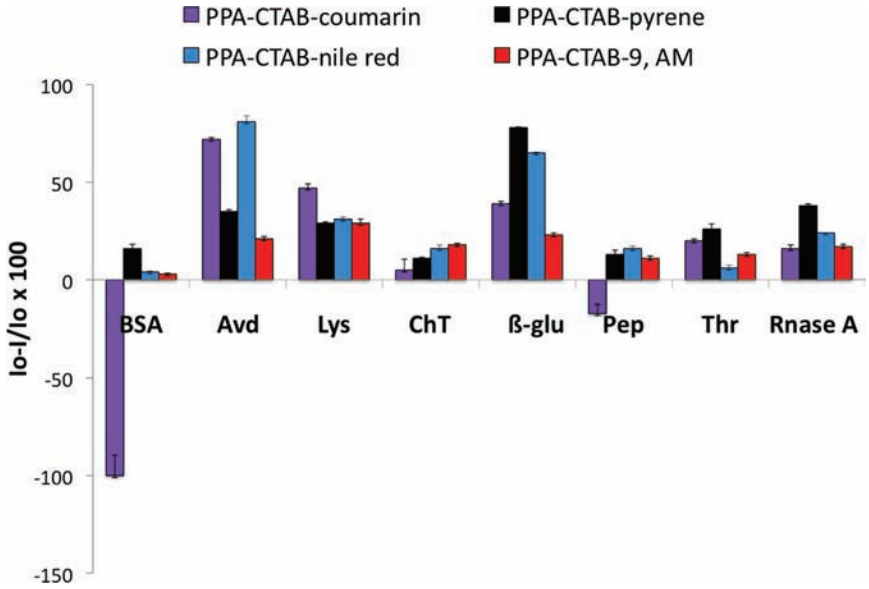


Figure 3. Analyte-dependent patterns from emission changes at 8 μM concentration of the protein.

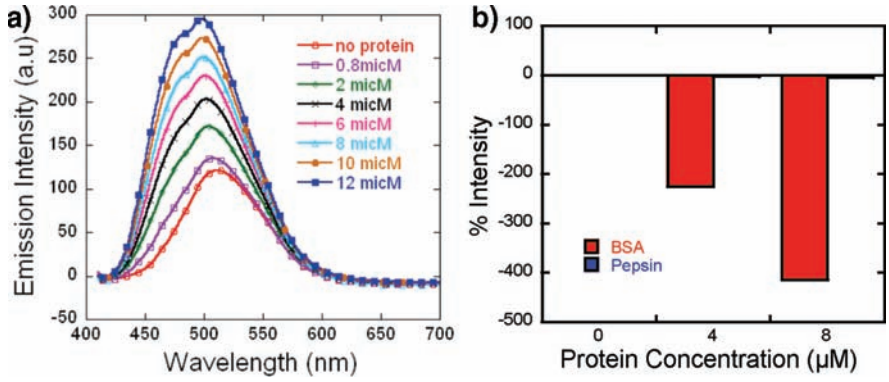


Figure 4. (a) Increase in emission intensity with increase in BSA concentration in PPA−CTAB−coumarin 481 (10^{-6} M at λ_{ex} = 400 nm). (b) Control experiment showing that the increase in emission is due to the direct binding of dye to the proteins BSA and pepsin.

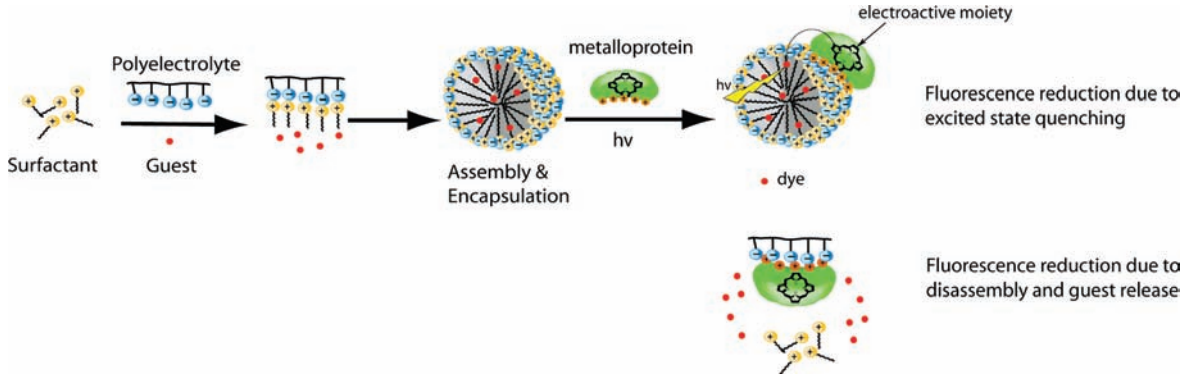


Figure 5. Schematic of the fluorescence response due to metalloproteins either due to the disassembly or due to the energy/electron transfer based quenching.

also on the protein, thus creating an analyte-dependent pattern (Figure 6b and Table 2).

It is interesting to note that we have previously reported on sensing metalloproteins using a different polymer-based amphiphilic assembly, but with the same dye molecules.[9] The sensitivity in that case seemed much lower, as the fluorescence response was observed usually with micromolar concentrations of proteins. We were first interested in quantifying the relative sensitivities of these two polymeric systems. Stern−Volmer quenching is a useful method for quantifying the relative sensitivities.[17] In the Stern−Volmer experiment, the fluorescence

(16) Sandanaraj, B.; Demont, R.; Aathimanikandan, S.; Savariar, E. N.; Thayumanavan, S. *J. Am. Chem. Soc.* **2006**, *128*, 10686–10687.
(17) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Kluwer Academic/Plenum: New York; 1999.
(18) Wang, J.; Wang, D.; Miller, E. K.; Moses, D.; Bazan, G. C.; Heeger, A. J. *Macromolecules* **2000**, *33*, 5153–5158.

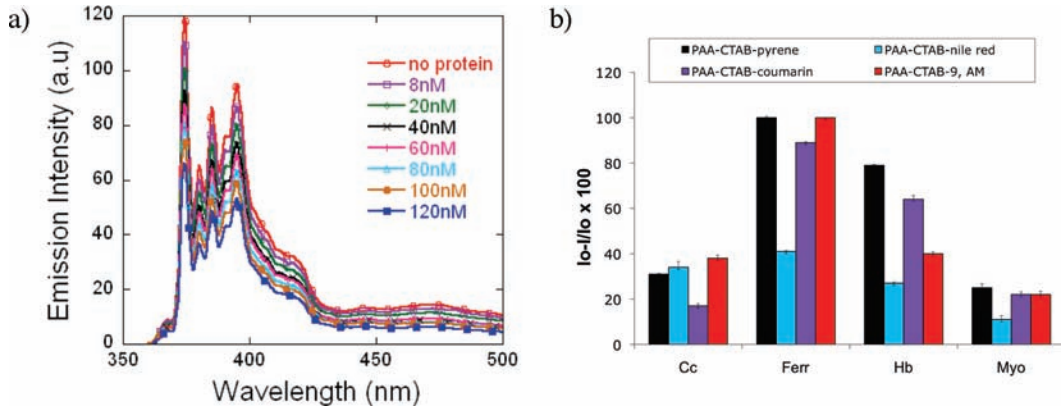


Figure 6. (a) Emission spectral change in pyrene in response to nanomolar concentration of Cc. (b) Analyte-specific sensing patterns for metalloproteins with different dye molecules at 80 nM concentration of proteins (coumarin (10^{-6} M; $\lambda_{ex} = 400$ nm); pyrene (10^{-6} M; $\lambda_{ex} = 339$ nm); nile red (10^{-6} M; $\lambda_{ex} = 550$ nm); 9-anthracene methanol (10^{-5} M; $\lambda_{ex} = 365$ nm)). See also Table 2.

Table 2. Percent Dye Release at 80 nM Concentration of Metalloproteins

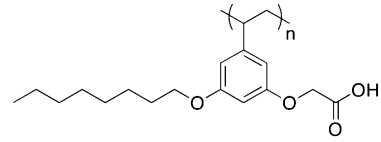
		$I_0 - I/I_0 \times 100$ (%)			
protein	p*I*	pyrene	nile red	coumarin 481	9-anthracene methanol
Cc	10.2	31	34	17	38
Ferr	6.0	100	41	89	100
Hb	6.8	79	27	64	40
Myo	7.2	25	11	22	22

quenching is related to the concentration of the quencher, i.e. protein, using eq 1:

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

where I_0 is the emission intensity of the fluorophore in the absence of the quencher and I is the emission intensity of the fluorophore in the presence of the quencher at concentration [Q]. The slope obtained from plotting I_0/I vs [Q] is the Stern−Volmer quenching constant K_{SV}. The K_{SV} value represents the ability of the protein to quench the excited state of the fluorophore. Assuming that the emission quenching is not due to the disassembly and release of the fluorophore from the assembly, the excited state can be quenched through either energy or electron transfer. Also, we have established in the case of amphiphilic homopolymers that the observed decrease in fluorescence is due to static quenching, i.e., binding-induced quenching and not collision-based dynamic quenching.[9,16] Since both energy and electron transfer processes are distance dependent, binding events significantly enhance quenching. Thus, the K_{SV} value represents both the binding affinity of the protein to the polymer assembly and the inherent ability of the protein to quench the excited state of the fluorophore. Note that we have shown that the interplay between these factors along with the extent of energy vs electron transfer processes in different protein−fluorophore combinations can afford analyte-dependent pattern generations.[9]

The decrease in the fluorescence intensity of pyrene with increasing concentration of hemoglobin is shown in Figure 7a. When the emission intensity change at 374 nm was plotted against the concentration of Hb, a linear plot emerges at lower concentrations (Figure 7b). The slope of this plot affords the Stern−Volmer quenching constant K_{SV}. At higher protein concentrations, the relationship approaches nonlinearity and the linear fit of the data is less convincing (Figure 7c). This behavior is a hallmark of static quenching. The rather different exponential behavior in the quenching has been rationalized based on the possibility of the analyte being within the so-called sphere of action of the fluorophore.[18] K_{SV} can also be extracted through an exponential fitting of the data in Figure 7c. The Stern−Volmer constants from this fit were found to be similar to the linear fit at lower concentrations. For consistency, we have measured the K_{SV} value at lower concentrations of proteins for all the polymer−surfactant−fluorophore combinations. As expected, protein-dependent patterns could be generated upon analyzing the data with all four dye molecules, as shown in Figure 7d. Note that the pattern from Figure 7d represents the same phenomena; however, it can be distinguished from that shown in Figure 6b. Figure 6b represents the extent of emission quenching at a specified concentration of the protein (80 nM), while Figure 7d represents the overall ability of a protein to quench the emission of a particular fluorophore. The Stern−Volmer constants for the proteins are shown in Table 3. The K_{SV} values are consistently about 2 orders of magnitude higher than that observed with the assemblies based on the amphiphilic homopolymer PS8M.[9] For example, the K_{SV} value for Hb is 5.5×10^7 in the case of pyrene in the PPA−CTAB complex, compared to the 3.6×10^6 observed for PS8M. Similarly, a K_{SV} of 6.6×10^6 was observed in the present case for Cc, compared 1.1×10^6 with PS8M.



PS8M

As mentioned above, both binding affinity and the inherent ability of the protein to quench the fluorophore in an assembly contribute to quenching. Binding affinity is unlikely to be the consistent reason for the difference. This is because we have shown previously that the electrostatic binding affinity of poly(acrylic acid) to a positively charged protein (such as chymotrypsin) is actually lower than that of PS8M.[19] However, the sensitivity of the PPA−CTAB complex is higher in most cases. It is likely however that the proximity between the protein and the dye molecules is higher in the PPA−CTAB complex relative to the PS8M-based assembly. This is reasonable, considering the fact that we have shown that the PS8M-based assemblies are most likely based on a complex micelle type

(19) Sandanaraj, B. S. Ph.D. Thesis, University of Massachusetts, Amherst, 2007.

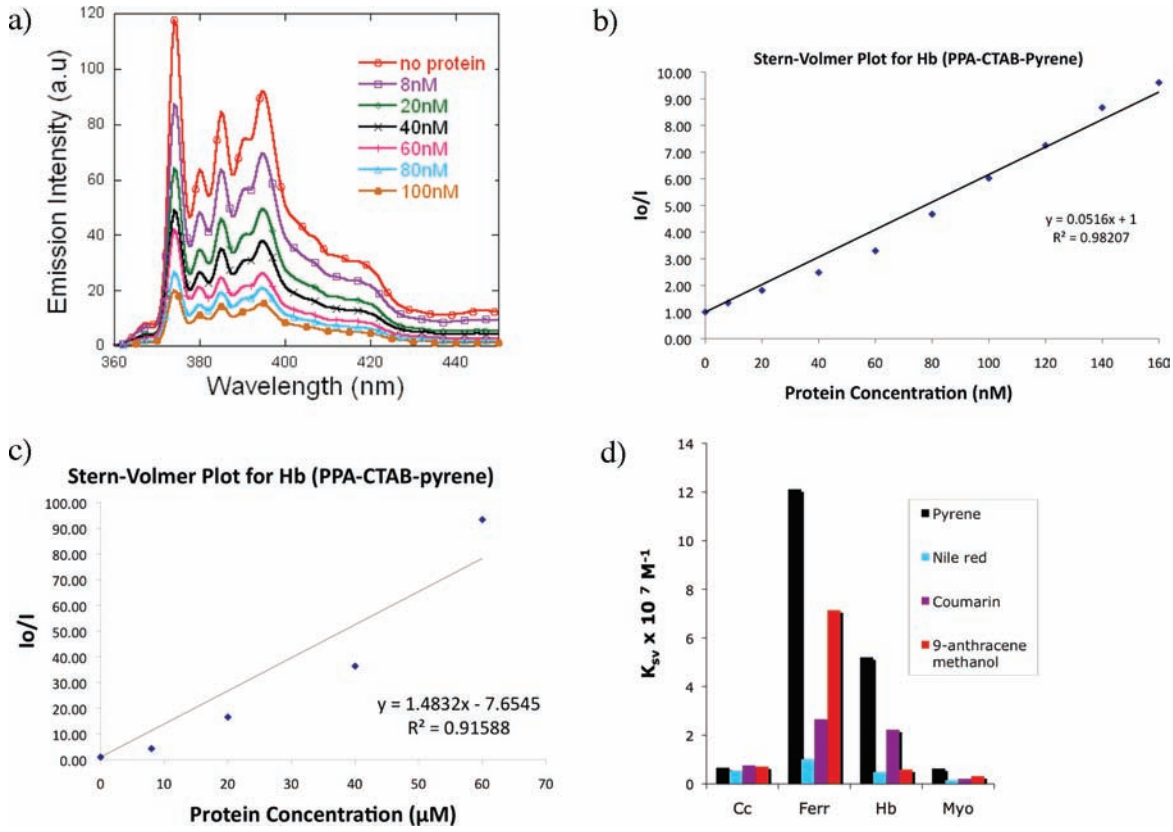


Figure 7. (a) Fluorescence intensity of pyrene noncovalently bound in the PPA−CTAB complex at different concentrations of Hb. (b) Stern−Volmer plot of the data at low protein concentrations. (c) Stern−Volmer plot of the data at higher protein concentrations. (d) Protein-dependent patterns based on K_{SV} values (coumarin (10^{-6} M; λ_{ex} = 400 nm); pyrene (10^{-6} M; λ_{ex} = 339 nm); nile red (10^{-6} M; λ_{ex} = 550 nm); 9-anthracene methanol (10^{-5} M; λ_{ex} = 365 nm)).

Table 3. K_{SV} Values of Metalloproteins with Different Dye Molecules

	Stern−Volmer values (M^{-1})			
protein	pyrene	nile red	coumarin 481	9-anthracene methanol
Cc	6.6×10^6	7.8×10^6	8.9×10^6	7.7×10^6
Ferr	3.38×10^8	1.0×10^7	8.7×10^7	1.0×10^9
Hb	5.5×10^7	4.8×10^6	2.3×10^7	7.4×10^6
Myo	6.5×10^6	1.5×10^6	2.8×10^6	4.0×10^6

assembly.[20] Note, however, that this explanation is provisional in the absence of higher resolution structural information on both these assemblies.

Mechanism of Fluorescence Change. The decrease in the emission intensity of the fluorophore upon increase in the concentration of nonmetalloproteins is attributed to the disassembly of the micelles and release of the noncovalently sequestered fluorophore. On the other hand, fluorescence changes in response to metalloproteins can occur either due to the disassembly mechanism or due to the photoinduced energy or electron transfer mechanism. It is reasonable to suggest that the latter mechanism is the operating pathway for the decrease in fluorescence, because the key difference between the metallo- and nonmetalloproteins involves the metal-containing cofactors (such as porphyrins) that are buried in the interiors of the proteins. The binding between the polyelectrolyte and proteins is attributed to the electrostatic interaction between the charged functionalities in the polymer and the surface charges of the

(20) Savariar, E. N.; Aathimanikandan, S. V.; Thayumanavan, S. *J. Am. Chem. Soc.* **2006**, *128*, 16224–16230.

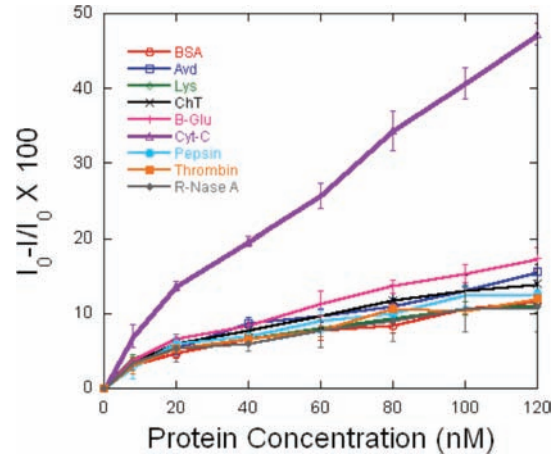


Figure 8. Comparison of percent dye release at nanomolar concentrations of nonmetalloproteins and Cc (a metalloprotein).

proteins. Since the surface charge of functionalities would be independent of the cofactors buried in the interiors, it is reasonable to assume that the binding-induced disassembly would be independent of whether the metal-based cofactors are present in proteins. In order to test this hypothesis, we first tested the responsiveness of the PPA−CTAB assembly to nanomolar concentrations of nonmetalloproteins by monitoring the decrease in the emission of the fluorophore nile red. A comparison of nonmetalloproteins with Cc is shown in Figure 8. The percent decrease in fluorescence was about 10% for concentrations up to 120 nM concentration of the nonmetalloproteins, while the metalloprotein Cc caused a decrease of about 47% of the

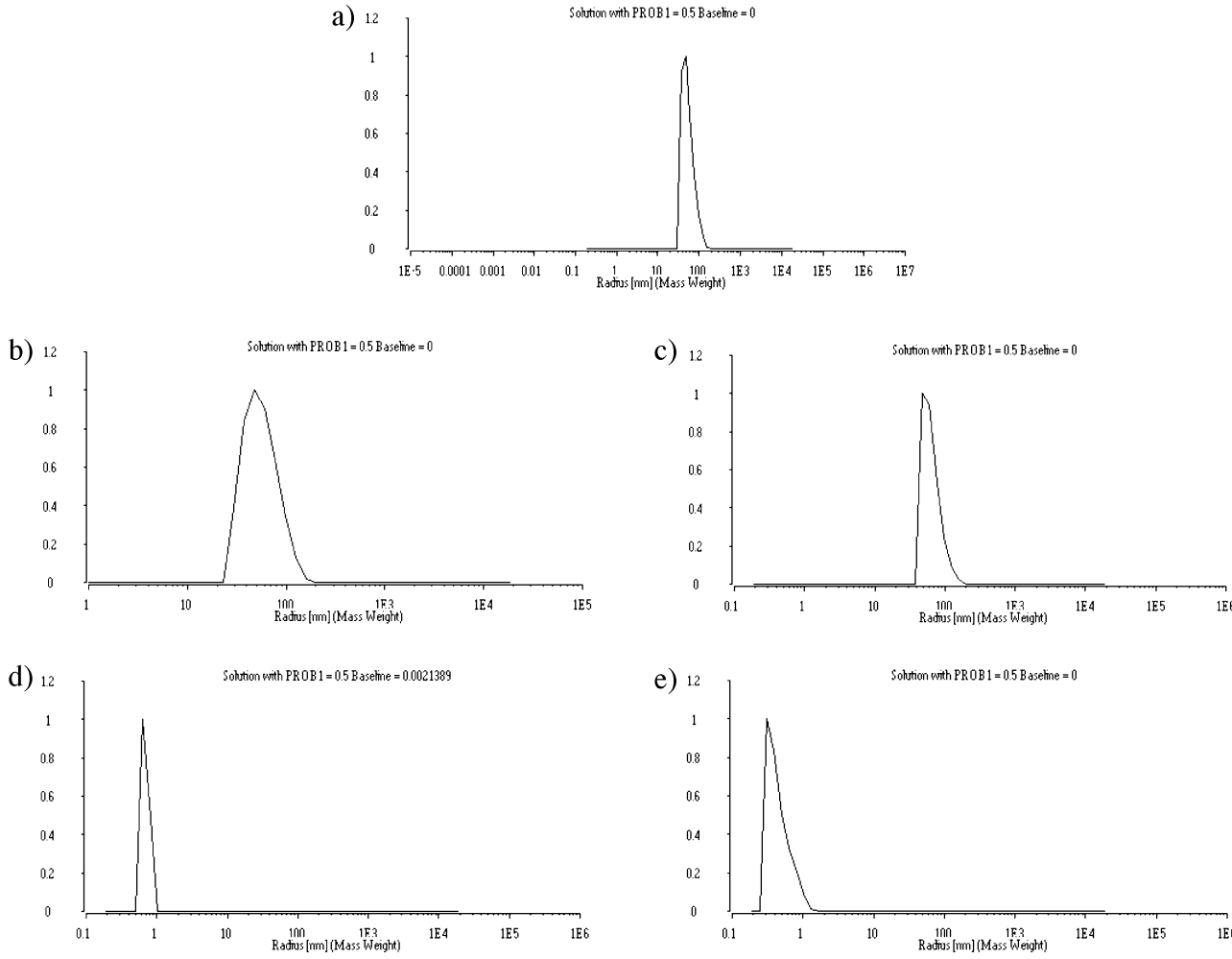


Figure 9. DLS of PPA−CTAB complex: (a) before adding protein; (b) after adding 120 nM Lys; (c) after adding 80 nM Cc; (d) after adding 12 μM Lys; (e) after adding 8 μM Cc.

fluorophore. These results indeed support our hypothesis that the fluorescence decrease in metalloproteins is likely due to a different mechanism, compared to the nonmetalloproteins.

While the above results provide the supporting evidence, these do not unambiguously provide the reason for the mechanistic divergence with these two classes of proteins. These mechanistic suggestions would be much better supported if we were to show that there is no dye release at nanomolar concentrations of the proteins and that the disassembly and release is observed only at micromolar concentrations of proteins. Since there is a direct correlation between the concentration of the dye molecule in solution and absorbance through Beer's law, we tried to determine the change in the absorbance at the λ_{max} of the dye molecule. If our hypothesis were correct, then the concentration of the dye molecule in solution would be identical for both classes of proteins at nanomolar concentration, since there would be no disassembly. However, the fluorescence quenching would be observed for metalloproteins, indicating an excited-state quenching mechanism. However, since the absorbance of our solution is already very low before the addition of any protein, we were unable to unambiguously come to a conclusion on the change in the concentration of the dye molecule through this method.

Another method that can be used to test this possibility involves dynamic light scattering (DLS). DLS estimates the size of our amphiphilic assembly in solution. If our mechanistic hypothesis were correct, then the amphiphilic assembly should retain its size at nanomolar concentrations of metallo- or nonmetalloproteins. Similarly, we should also notice complete disassembly at micromolar concentration of either of these types of proteins. Results of the DLS experiments are shown in Figure 9. The size of the assembly prior to the addition of proteins was about 100 nm (Figure 9a). Upon addition of nanomolar concentrations of the nonmetalloprotein Lys, the size of the assembly is essentially unchanged (Figure 9b). Similarly, addition of the metalloprotein Cc also afforded no size change in the assembly (Figure 9c). On the other hand, addition of micromolar concentration of either of these proteins results in complete disassembly (Figure 9d,e). Although we observe a peak at low sizes, note that the correlation function for this measurement is very low, and it is impractical to obtain any reasonable estimate of these very small sizes. Thus, this measurement only suggests that there is a disassembly, but the plots in Figure 9d,e are not good indicators of actual sizes of the assemblies present in solution. In any case, these results do clearly show that disassembly is not the reason for the significant fluorescence change at nanomolar concentrations of metalloproteins. Therefore, these results suggest that excited state quenching is most likely the operating mechanism with metalloprotein sensing and that this difference provides the op-

portunity to selectively sense these proteins at nanomolar concentrations.

Summary

In summary, we have shown the following: (i) Since the CAC of a polyelectrolyte−surfactant complex is significantly lower than the surfactant itself, polyelectrolyte−protein binding interaction can be used to disassemble the amphiphilic assembly from this complex. This is achieved when the concentration of the polymer and the surfactant is between the two CACs. (ii) By varying the noncovalently sequestered fluorescent transducers within these amphiphilic assemblies, protein-specific sensing patterns can be generated. The patterns generated in this case are due to the differential binding affinity of proteins with the polyelectrolyte and thus different extents of disassembly, inherent differences in the behavior of the fluorophores in the aqueous phase vs the amphiphilic interior, and the possibility that the fluorophores can bind to some of the proteins. (iii) When analyzed for metalloproteins, the sensitivity of sensing is enhanced by 1−2 orders of magnitude, which allows for generating analyte-specific patterns even at nanomolar concentration of proteins. (iv) Both metallo- and nonmetalloproteins behave similarly with respect to the disassembly of the polyelectrolyte−surfactant complex. However, the difference arises from the fact that there is a binding interaction between the complex and the proteins at nanomolar concentrations, where there is no disassembly. Since metalloproteins have cofactors that can quench the excited state energy of the fluorophores, the proximity due to the binding interaction results in decreased emission from the fluorophore. The versatility of our approach arises from the fact that these assemblies are achieved noncovalently from their easily accessible components, viz., the polymer, surfactant, and the fluorophore. Protein binding, but without disassembly, at nanomolar concentrations allows for the unique opportunity to distinguish metalloproteins from other proteins through sensing patterns. Although we have used proteins as the model analytes, we believe that these new pathways based on fully noncovalent assemblies to obtain sensing patterns will have a broad impact in the sensing arena beyond protein sensing.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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