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Array-Based Sensing of Proteins Using Conjugated Polymers

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Convenient, precise, and rapid protein sensing methods are of great importance in medical diagnostics and proteomics.[1] Widely used specific interaction-based sensing protocols (e.g., ELISA) require protein receptors of high affinity and specificity requiring the generation of pertinent protein receptors/ligands for multiprotein detection.[1] In this regard, sensor array approaches are attractive, using differential binding interactions that are selective rather than specific.[2] This "electronic nose/tongue" strategy provides highly versatile sensors.[3,4] Recently, this principle has been used for protein detection through either fluorescence quenching[5] or indicator displacement.[6] While these sensors have been effective, they feature high limits of detection, and only relatively small sets (4−5 proteins) were studied.

Effective protein sensing requires efficient protein receptors and competent signal transducers. Water-soluble conjugated polymers with pendant-charged residues provide an excellent scaffold for sensor design.[7,8] These materials can bind protein surfaces through multivalent interactions. Moreover, their optical properties are sensitive to minor conformational or environmental changes,[7,9] enabling efficient signal transduction of the binding events. In this work, we use six functionalized poly(*p*-phenyleneethynylene)s (PPEs)[10] to build a protein sensor array (Figure 1). These highly fluorescent polymers possess various charge characteristics and molecular scales. Such structural features provide tremendous binding diversity upon interaction with protein analytes, generating distinct fluorescence response patterns for protein discrimination.

We have chosen 17 proteins as the sensing targets (Table 1). These proteins possess diverse structural characteristics including metal/nonmetal-containing, molecular weight (MW), isoelectric point (pI), and UV absorbencies. Notably, many protein targets have comparable MW and pI values, thereby providing excellent objects for examining the differentiation ability of the PPE-based sensor array.

In the sensing studies, the fluorescence of the polymer solution (100 nM, on the basis of number-averaged molecular weight) in phosphate buffered saline (PBS) was recorded before and after addition of protein analytes. The six polymers display substantial overlap in their absorption and emission spectra (Figure S1), allowing the same excitation wavelength (430 nm) and emission wavelength (465 nm) to be used for all polymers to expedite their analysis on the microplate reader. To facilitate the quantitative detection of proteins, we generated patterns at protein concentrations at a standard UV absorbance ($A_{280} = 0.005$), the lowest concentration for all proteins to induce substantial emission changes of the polymers. With this as the detection limit of the system, protein identification was readily achieved in combination of UV measurements (vide post). Besides metalloproteins, such as CytC, Fer, Hem,

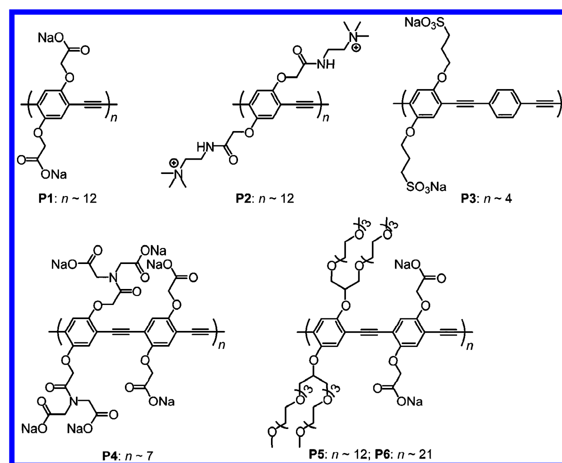

Figure 1. Chemical structures of PPE polymers (**P1**−**P6**).

Table 1. Basic Properties of the Proteins Used as Sensing Targets

protein	metal	MW (kDa)	pI	ϵ_{280} (M^{-1} cm^{-1})
α-amylase (α-Am)	Y	50	5.0	130000
bovine serum albumin (BSA)	N	66.3	4.8	46860
α-chymotrypsin (ChT)	N	25	8.7	51000
cytochrome *c* (CytC)	Y	12.3	10.7	23200
ferritin (Fer)	Y	750	4.5	950000
β-galactosidase (β-Gal)	N	540	4.6	1128600
hemoglobin (Hem)	Y	64.5	6.8	125000
histone (His)	N	21.5	10.8	3840
human serum albumin (HSA)	N	69.4	5.2	37800
lipase (Lip)	N	58	5.6	54350
lysozyme (Lys)	N	14.4	11.0	38000
myoglobin (Myo)	Y	17.0	7.2	13940
papain (Pap)	N	23.0	9.6	57500
acid phosphatase (PhosA)	N	110	5.2	257980
alkaline phosphatase (PhosB)	N	140	5.7	62780
ribonuclease A (RibA)	N	13.7	9.4	10000
subtilisin A (SubA)	N	30.3	9.4	26030

and Myo, nonmetalloproteins also generally quench the polymer emission (Figure 2), indicating that the electronic states of the polymers are modulated by protein binding. In comparison with polymers in the absence of proteins, the fluorescence quenching extent ranges from 5 to 50%. These fluorescence responses are not correlated with the pI and MW of the proteins. Significantly, the fluorescence response patterns are characteristic and highly reproducible for particular proteins, indicating the possibility of protein discrimination.

The fluorescence response patterns were subjected to linear discriminant analysis (LDA);[11] LDA converts the patterns of the training matrix (6 polymers × 17 proteins × 6 replicates) to canonical scores. The first three canonical factors contain 65.0, 20.8,

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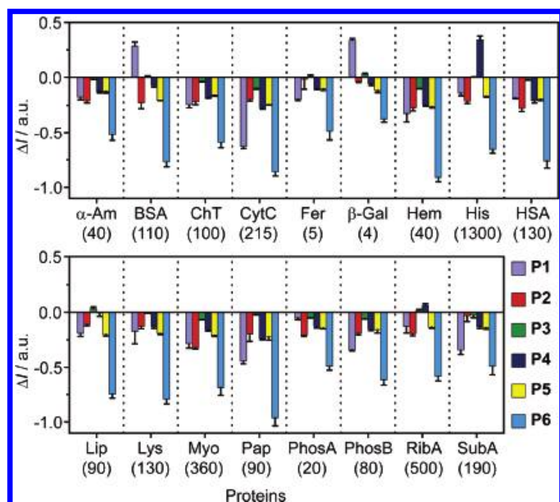

Figure 2. Fluorescence response (ΔI) patterns of the PPE polymer array (**P1**−**P6**) against protein analytes (A_{280} = 0.005) as an average of six parallel measurements. The values in the parenthesis indicate the protein concentrations in nM.

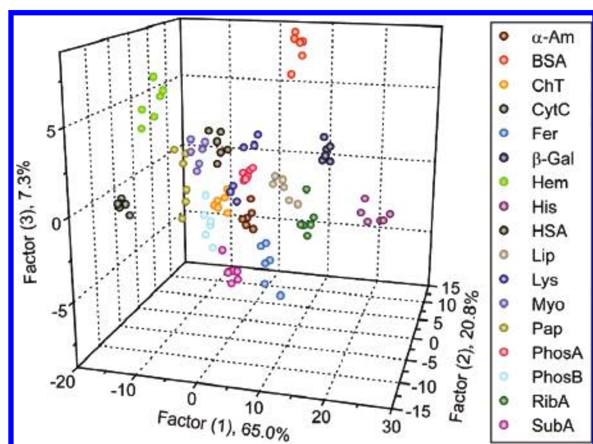

Figure 3. Canonical score plot for the first three factors of simplified fluorescence response patterns obtained with PPE polymer array against 17 protein analytes (A_{280} = 0.005).

and 7.3% of the variation, respectively, occupying 93.1% of total variation (Figure 3, 2-D correlations are shown in Figure S2). The canonical patterns are clustered into 17 different groups, and the jackknifed matrix with cross-validation reveals a classification accuracy of 100%. For a single polymer, however, the classification accuracies range only from 26 to 56% (Table S2), indicating that an array of different sensors is essential for protein discrimination. Significantly, if the polymer with the least differentiation ability (**P2**, 26%) is removed from the array, a classification accuracy of only 97% is obtained, confirming the effect of each individual polymer.

We next focused on detection and identification of protein samples with both unknown concentration and identity. The unknowns from the training set were submitted to an analysis protocol including determination of UV absorbance at 280 nm, dilution of solution to A_{280} = 0.005, generation of fluorescence response patterns against the sensor array, and LDA. During LDA, the new cases were classified to the groups generated through the training matrix according to their shortest Mahal distances to respective groups. Once the protein ID was established, the initial protein concentration was obtained through using ϵ_{280} values (Table 1). Out of 68 protein samples that were randomly selected from the 17 protein species, only 2 samples were misclassified, affording an identification accuracy of 97%. Moreover, the protein concentrations were generally determined within ±5% deviation (Table S3).

In summary, we have demonstrated that a PPE-based sensor array can effectively detect and identify proteins. Benefiting from their high fluorescence sensitivity as well as inherent amplification effects, this array of six conjugated polyelectrolytes displays an unprecedented discrimination ability of 17 protein analytes. Further experiments are, however, required to demonstrate the robustness of the system, as cross-reactive arrays are still prone to errors. In particular, the array must be tested with complex mixtures of proteins for their ability to detect species present at very low concentrations in the presence of large amounts of potentially interfering species.

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Supporting Information Available: Experimental procedures, two figures, and three tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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