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Color Fingerprinting of Proteins by Calixarenes Embedded in Lipid/Polydiacetylene Vesicles

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Abstract: "Naked eye" color detection of proteins was achieved by embedding calixarene receptors within vesicles comprising phospholipids and the chromatic polymer polydiacetylene. Dramatic visible absorbance changes were induced through electrostatic interactions between the protein surface and the vesicle-incorporated hosts. The colorimetric responses could be induced by micromolar protein concentrations, and furthermore, specific protein fingerprints could be obtained by incorporating different receptors within the vesicles. Fluorescence and circular dichroism experiments confirmed the relationship between the colorimetric phenomena and protein docking on the surface of the chromatic vesicles. The colorimetric assay constitutes a generic platform for high-sensitivity detection of soluble proteins and for evaluation of protein surface charge distribution.

Introduction

Protein detection is important in diverse biological, medical, and diagnostic applications.[1,2] Protein sensing by artificial molecules is a challenging endeavor, especially if the recognition event is desired to be coupled to a simple quantifiable readout. Different chemical approaches have been developed for protein recognition and sensing.[3−5] Recently we have developed protein recognition schemes based on amphiphilic calixarenes with ionic groups at their upper rims specific for charged amino acid residues.[6] These synthetic protein receptors were incorporated into lipid monolayers and facilitated protein discrimination through multivalent interactions between the calixarenes and the soluble macromolecules. That work exploited the concept of multivalent electrostatic attraction between lipid-embedded receptors and proteins drawn from the water subphase, with protein detection achieved through conventional film-balance experiments.[6]

Generation of *color changes* is particularly attractive as a tool for protein detection, however demonstrated only in very few instances.[7] Here we describe a generic approach for high-sensitivity and p*I*-specific *colorimetric* detection of water-soluble proteins through phospholipid/polydiacetylene (PDA) vesicles anchoring ionic calixarene modules. PDA exhibits unique chromatic properties that have been widely exploited for biosensing applications. Specifically, PDA appears intense blue due to absorption of the ene−yne cross-linked framework in the visible region. Furthermore, phase transitions of the conjugated backbone of the polymer can be induced, resulting in modification of the delocalized conjugated electronic network—thereby resulting in dramatic blue−red transformations. The structural/colorimetric transformations of PDA can be generated by varied external factors, such as heat,[8] ionic strength,[9] or mechanical pressure,[10] that interact with or disrupt the pendant side chains of the polymer. In particular, *surface perturbations* in PDA matrixes, induced through molecular recognition events at the PDA/water interface, are a potent factor affecting the blue−red transformations of the polymer.[11−13] In recent years, innovative chemically derivatized PDA systems have exploited surface binding reactions, making PDA a vehicle for chromatic sensing of molecular recognition.[14−16]

Several studies published in recent years have demonstrated that PDA undergoes blue−red transitions also in vesicles and films that further comprise *lipid assemblies* interspersed within the polymer matrix in response to interactions with varied analytes.[14,17,18] In such mixed systems it was shown that surface

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perturbation and reorganization occurring within the *lipid* moieties gave rise to the structural transitions in the conjugated (ene−yne) PDA backbone.[19] In particular, these indirectly induced structural transitions are most likely transmitted through the molecular interfaces between the lipid and PDA domains in the mixed assemblies.[20] Application of the mixed lipid/PDA sensors for studying varied biological processes has been reported, including the study of antimicrobial peptides,[21] membrane penetration enhancers,[22] ion binding,[18] drug permeation through lipid barriers,[23] and antibody/epitope binding.[11]

We have recently demonstrated that chromatic detection of *biomolecular recognition* events is feasible in mixed phospholipid/PDA assemblies through embedding amphiphilic receptor molecules in the phospholipid scaffolding.[18,24] Specifically, we have demonstrated that synthetic hosts incorporated within lipid/PDA vesicles confer dramatic chromatic selectivity of the vesicles toward different ligands.[24] Here we show that a facile "naked eye" detection of proteins can be achieved through incorporation of acidic and basic amphiphilic calixarenes in phospholipid/PDA vesicles. The mixed assemblies facilitate "colorimetric fingerprinting" of proteins, pointing to potential utilization of the system for diagnostic applications.

Experimental Section

Materials. Dimyristoylphosphatidylcholine (DMPC) was purchased from Sigma. The diacetylene monomer 10,12-tricosadiynoic acid was purchased from GFS Chemicals (Powell, OH). Lipids were dissolved in chloroform and filtered through 0.8 μm filters. Preparation of vesicles containing phospholipids and PDA (2:3 mole ratio) has been described previously.[18,25] Briefly, the lipid constituents are dried together in vacuo, followed by addition of deionized water and probe sonication at 70 °C. The vesicle solution is then cooled, kept at 4 °C overnight, and polymerized using irradiation at 254 nm. The resulting solution exhibits an intense blue appearance.

Visible Spectroscopy. Samples were prepared by adding calixarene receptors and proteins to 60 μL vesicle solutions at 0.5 mM total lipid and 25 mM Tris. The pH in the solutions was maintained at 8.0 in all experiments. Proteins were dissolved in Tris buffer (host **1** and host **3**) and water (host **2**). Following addition, the solutions were diluted to 1 mL, and visible spectra were acquired. All measurements were carried out at 27 °C on a Jasco V-550 spectrophotometer, using a 1 cm optical path length cell. A quantitative value for the extent of the blue-to-red color transitions of the vesicle solutions is given by the colorimetric response (CR, %), which is defined as follows:[21]

$$\mathrm{CR} = [(\mathrm{PB}_0 - \mathrm{PB}_1)/\mathrm{PB}_0] \times 100$$

where $\mathrm{PB} = A_{blue}/(A_{blue} + A_{red})$, A is the absorbance either at the "blue" component in the UV−vis spectrum (640 nm) or at the "red" component (500 nm) (note that "blue" and "red" refer to the visual appearance of the material, not its actual absorbance). PB_0 is the blue/red ratio of the control sample (before induction of a color change), and PB_1 is the value obtained for the vesicle solution after colorimetric transition occurs.

The net colorimetric effect was calculated as

$$\Delta(\mathrm{CR}) = \mathrm{CR}_1 - \mathrm{CR}_0$$

where CR_1 (%) is the CR after addition of the proteins to the DMPC/PDA vesicles containing the calixarene receptors, while CR_0 (%) is the colorimetric response recorded after addition of the same protein concentration to DMPC/PDA vesicles *not containing* the receptors.

Fluorescence Quenching Measurements. The fluorescent probe NBD-PE [*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt] was purchased from Molecular Probes, Inc. (Eugene, OR). NBD-PE was dissolved in chloroform, added to phospholipids at 1 mol %, and dried together in vacuo before sonication. Addition of the NBD-labeled lipids did not affect either the initial blue color of the vesicles or the blue-to-red transitions. The quenching reaction was initiated by adding sodium dithionite ($Na_2S_4O_2$; Aldrich) from a 0.6 M stock solution, prepared in 50 mM Tris base buffer, pH 11.0, to a final concentration of 10 mM. The fluorescence emission was recorded for 5.5 min at 27 °C using 467 nm excitation and 535 nm emission on an Edinburgh Co. (Edinburgh, Scotland, U.K.) FL920 spectrofluorimeter. The fluorescence decay was calculated as a percentage of the initial fluorescence measured before the addition of dithionite.

Tryptophan Fluorescence Measurements. Changes in tryptophan intrinsic emission were measured for 0.1 μg protein solutions added to the receptor/DMPC/PDA vesicles. Fluorescence emission spectra were acquired at 27 °C on an Edinburgh Co. FL920 spectrofluorimeter, using excitation at 280 nm and emission at 345 nm. Excitation and emission slits were both set to 8 nm. Total sample volumes were 1 mL, and the solutions were placed in a quartz cell having a 1 cm optical path length. Light scattering from the vesicles was confirmed to account for less than 5% of the emission intensity.

Circular Dichroism (CD). CD spectra were acquired on an Aviv 62A-DS circular dichroism spectrometer (Aviv Inc., Lakewood, NJ). Four scans were recorded between 190 and 250 nm with 1 nm acquisition steps. A 0.2 mm optical path length was used. All vesicle solutions had a total lipid concentration of 0.5 mM in 25 mM Tris−HCl at pH 8. The pepsin concentration was 0.1 μg/mL.

Results and Discussion

Figure 1A depicts the structures of the synthetic host compounds employed in this study, which are known to form complexes with charged amino acid side chains in solution. Calixarene **1** ($pK_a = 9.5$) binds aspartate and glutamate, **2** ($pK_a = 1.8$) was specifically designed for arginine and lysine, and **3** is much less active due its intermediate pK_a of 4.5.[26] The protein sensing mechanism is schematically described in Figure 1B, showing a receptor/phospholipid/PDA assembly displaying phospholipid-flanked calixarenes which are open to the aqueous solution and bind to a protein surface through multivalent electrostatic interactions.

Colorimetric Analysis. Figures 2−4 depict examples of the visual color effects and spectral transitions following protein/receptor interactions in the phospholipid/PDA environment.

Figure 2 presents data recorded for the acidic protein pepsin ($pI = 1$) and the effect of preaddition of the three calixarene receptors to the DMPC/PDA vesicles. Figure 2A shows that addition of pepsin to the vesicles did not give rise to a noticeable color transformation (the solution remained blue) due to the negative charge on the PDA surface. However, mixing of pepsin with DMPC/PDA vesicles to which calixarene **1** was preadded gave rise to a distinct blue-to-purple color change, clearly

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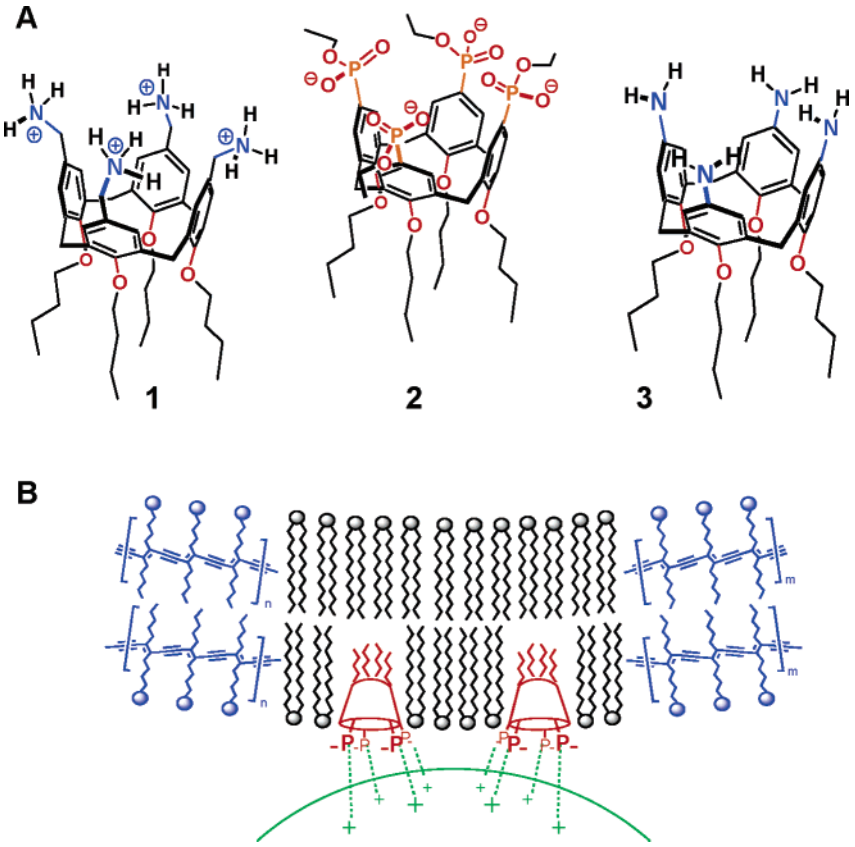

Figure 1. (A) Structures of the three receptors. (B) Schematic structure of the receptor/phospholipid/PDA assemblies (depicting **2** as an example). Key: blue, PDA; black, phospholipids; red, receptor units; green, protein.

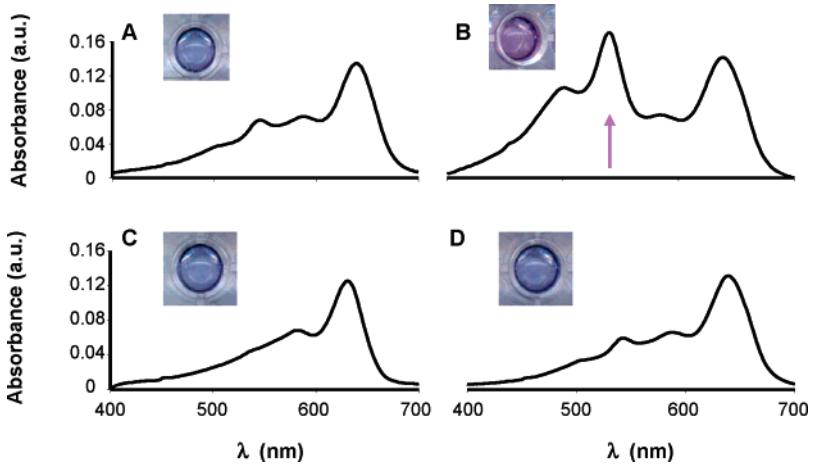

Figure 2. Pictures of DMPC/PDA vesicle solutions and corresponding visible spectra following addition of pepsin (pI = 1) to (A) DMPC/PDA vesicles (no receptor added), (B) DMPC/PDA vesicles with **1** preadded, (C) DMPC/PDA vesicles with **2** preadded, and (D) DMPC/PDA vesicles with **3** preadded. The arrow indicates the increase of the visible peak corresponding to the blue−purple change. The protein concentration was 0.1 μg/mL, and the receptor concentration was 1.1 μM.

reflected in the visible spectrum of the solution mixture (Figure 2B). In contrast to the pronounced color effect of **1**, preaddition of either receptor **2** (Figure 2C) or receptor **3** (Figure 2D) to the DMPC/PDA vesicles did not affect the color of the solutions (i.e., the same blue color was observed as when pepsin was added to receptor-free DMPC/PDA vesicles, Figure 2A).

Different colorimetric phenomena were apparent in the case of the basic protein histone (pI = 10.4), as depicted in Figure 3. Histone induced a significant blue−red transformation when added to receptor-free DMPC/PDA vesicles (Figure 3A)—ascribed to the strong electrostatic attraction between the positive charges on the protein and the negative vesicle surface. Similar intense red colors were induced by histone even when receptors **1** and **3** were added to the DMPC/PDA vesicles prior to protein mixing (parts B and D of Figure 3, respectively). However, when the tetraphosphonate calixarene **2** was incorporated into the lipid/PDA vesicles, histone induced a much less pronounced color transition—giving rise to a purple-blue solution as depicted in Figure 3C.

We carried out a similar comparative colorimetric analysis for the case of the relatively neutral protein albumin (pI = 6.0, Figure 4). Albumin did not induce a color transition when added

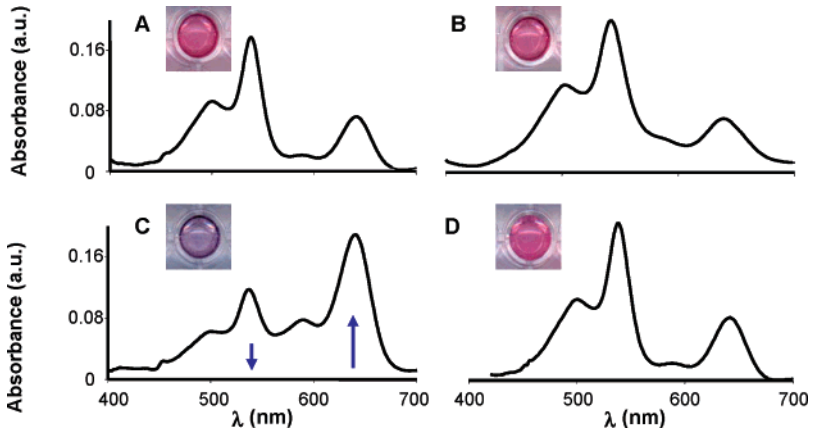

Figure 3. Pictures of DMPC/PDA vesicle solutions and corresponding visible spectra following addition of histone (pI = 10.4) to (A) DMPC/PDA vesicles (no receptor added), (B) DMPC/PDA vesicles with **1** preadded, (C) DMPC/PDA vesicles with **2**, and (D) DMPC/PDA vesicles with **3** preadded. Arrows indicate the changes in the visible peaks corresponding to the *lesser* red color of the solution. The protein concentration was 0.1 μg/mL, and the receptor concentration was 1.1 μM.

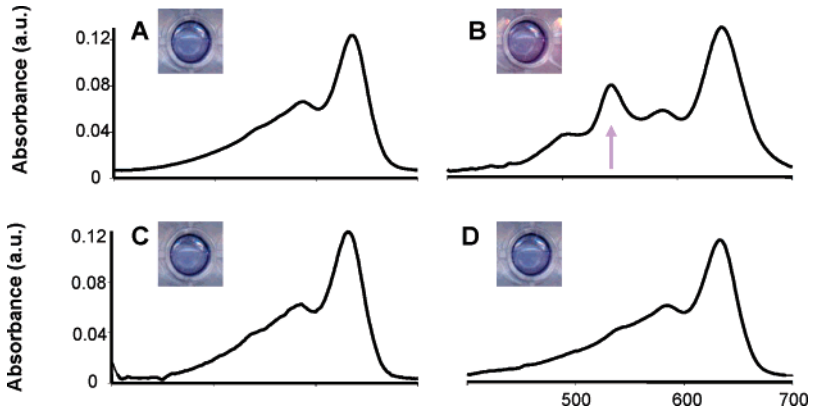

Figure 4. Pictures of DMPC/PDA vesicle solutions and corresponding visible spectra following addition of albumin (pI = 6.0) to (A) DMPC/PDA vesicles (no receptor added), (B) DMPC/PDA vesicles with **1** preadded, (C) DMPC/PDA vesicles with **2** preadded, and (D) DMPC/PDA vesicles with **3** preadded. The arrow indicates the increase of the visible peak corresponding to the blue−purple change. The peptide concentration was 0.1 μg/mL, and the receptor concentration was 1.1 μM.

to the DMPC/PDA vesicles (Figure 4A). Preaddition of **1** had a very small color effect (Figure 4B), much less pronounced compared to that of pepsin (Figure 2B).

The distinct color responses of the receptor/lipid/PDA vesicles to protein addition depicted in Figures 2−4 can be explained by the electrostatic interactions between the proteins and the embedded receptors, shown schematically in Figure 1B. Specifically, while pepsin by itself—being an acidic protein—does not bind to the negatively charged DMPC/PDA vesicles (and thus does not induce blue−red changes), the attractive electrostatic interactions between pepsin and the embedded cationic calixarene **1** induce the more significant blue−purple transformation depicted in Figure 2B. On the other hand, both the negatively charged calixarene **2** and receptor **3**, which, with a pK_a of 4.5, is hardly charged under the experimental conditions employed (pH 8), exhibit highly diminished electrostatic interactions with the protein, reflected in the insignificant effect on the color transition induced by pepsin (Figure 2C,D).

In the case of histone, the colorimetric effect of tetraphosphonate calixarene **2** depicted in Figure 3 was markedly different. Essentially, the affinity between histone, a highly basic protein, and receptor **2** weakens the binding of histone to the negatively charged PDA headgroups, thus reducing the structural perturbation of the polymer. In practice, the less pronounced perturbation of the polymer framework yields the observed blue−purple transition (Figure 3C), rather than the blue−red change recorded without preaddition of the receptor (Figure 3A). This interpretation was corroborated by the colorimetric data recorded after addition of histone to DMPC/PDA vesicles to which receptors **1** and **3** were preadded (parts B and D, respectively, of Figure 4); in both cases the receptors did not affect the blue−red transformations induced by histone since these receptors do not possess a negative charge.

The colorimetric data recorded in the case of albumin, a protein which is close to neutral (Figure 4), are consistent with the above description. Since at the mildly basic pH condition maintained in the vesicle solutions in the experiments the overall charge on the albumin surface is low, no significant electrostatic interactions occur when albumin is added to the vesicles. Similarly, the very weak electrostatic attraction between albumin and **1** explains the small colorimetric change induced by preaddition of this host to the vesicles. The absence of any colorimetric effect when **2** and **3** were preadded to the DMPC/PDA vesicles prior to albumin (Figure 4C,D) is similarly accounted for by the insignificant electrostatic attraction. Likewise, the low charge on calixarene **3** (pK_a = 4.5) corresponds to the observation that preaddition of this receptor did not alter

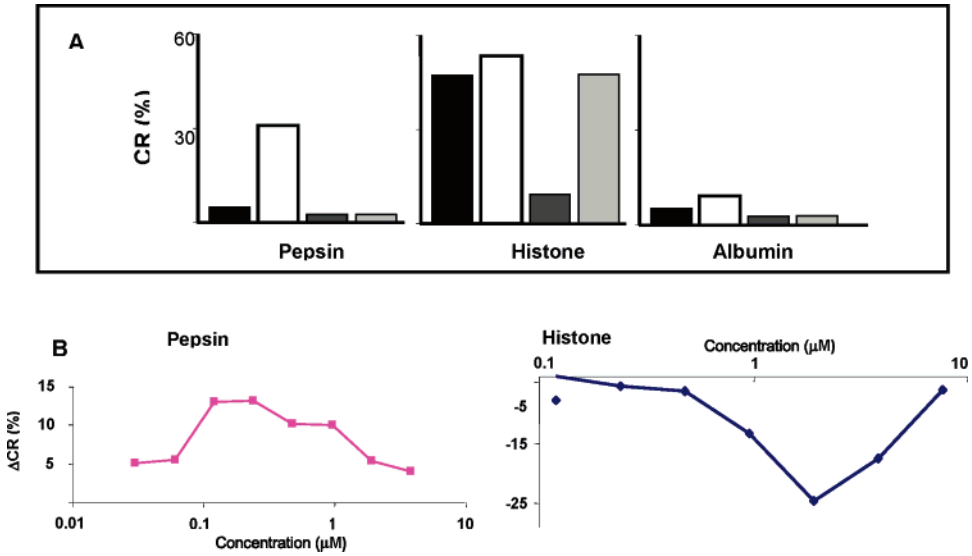

Figure 5. Quantitative colorimetric response of the receptor/DMPC/PDA system to protein addition. (A) Colorimetric response (CR, %; see the Experimental Section) of vesicle systems calculated from the visible spectra depicted in Figures 2−4. Key: black bars, no receptor preadded; white bars, **1** preadded to the vesicles; dark gray, **2** preadded; light gray, **3** preadded. (B) Concentration dependence of the net colorimetric response, Δ(CR). The receptor concentration was 1.1 μM.

the colorimetric phenomena for any of the three proteins tested in Figures 2−4.

Colorimetric Protein Fingerprinting. The colorimetric changes apparent upon receptor preaddition to the vesicles can be quantified, making the receptor/lipid/PDA system a useful tool for protein recognition. Figure 5A depicts representative percentage colorimetric response (CR, %) values, calculated for pepsin, histone, and albumin from the visible spectra recorded for these proteins in Figures 2−4. CR is a parameter reflecting the change in the visible spectrum after addition of a tested analyte to the colorimetric vesicles.[13] Essentially, a high CR value indicates a more red color of the vesicle solution compared to the initial blue, while a low CR corresponds to a less pronounced blue−red transformation (i.e., a more purple appearance of the solution; see the Experimental Section). For example, the significant red solution appearing after histone addition to the DMPC/PDA vesicles (Figure 3A) corresponds to a CR value of 47% (black bar in the histone diagram, Figure 5A), while the purple color recorded after histone addition to the DMPC/PDA vesicles to which **2** was preadded yielded a CR value of approximately 10% (dark gray bar in the histone diagram, Figure 5A). Indeed, the CR diagram in Figure 5A quantitatively portrays the significant color changes induced by addition of **1** to the vesicles prior to pepsin and addition of receptor **2** prior to histone.

Figure 5B depicts the concentration dependence of the net colorimetric response, Δ(CR), for pepsin and histone. Δ(CR) is defined as the difference between the CR after protein addition to the receptor/DMPC/PDA vesicles and CR induced by the protein in receptor-free DMPC/PDA vesicles. Figure 5B demonstrates that, for both proteins, Δ(CR) reaches a maximum value: the highest Δ(CR) was recorded for pepsin at a concentration of 0.24 μM, while for histone the optimum concentration was 1.92 μM. The maxima observed in the graphs in Figure 5B can be explained according to the protein−host interaction model: the greatest colorimetric effect is expected to occur when the added proteins bind to all available vesicle-embedded receptors.

Inspection of the Δ(CR) values of several proteins (at identical concentrations) in Figure 6A,B reveals a relationship between the protein p*I* values and the net colorimetric effects. In general, attractive electrostatic interactions between acidic proteins and embedded cationic calixarene **1** induced greater blue−red transformations (positive Δ(CR)), further increasing for proteins with smaller p*I* (Figure 6A, red data points). In contrast to the results obtained for receptor **1**, preaddition of **3** had negligible net colorimetric changes (blue data points, Figure 6A). As discussed above, calixarene **3** with a pK_a of 4.5 is much less charged under the experimental conditions employed (pH 8), thus resulting in weaker electrostatic interactions with the tested proteins.

Figure 6B compares the net colorimetric responses of calixarenes **1** and **2**. Figure 6B clearly shows that incorporation of the tetraphosphonate calixarene **2** had markedly different consequences on Δ(CR) as compared to that of **1**. As discussed above, receptor **2** weakens the strong affinity between basic proteins and the negatively charged PDA headgroups, thus giving rise to negative net color changes for proteins having high p*I* values (blue data points, Figure 6B). The differences between the Δ(CR) values recorded for **1** in parts A and B of Figure 6 are due to the use of different protein concentrations in the two measurements (see Figure 5B); the two concentrations depicted in the graphs were chosen to emphasize the differences between the colorimetric effects of the receptors. Overall, the changes in net colorimetric effects depicted in Figure 6A,B roughly follow the p*I* scale, confirming that the color changes arise from protein−receptor affinities at the vesicle interface, driven mainly by Coulomb interactions. The considerable dispersion of Δ(CR) values in Figure 6 around a linear p*I* dependence reflects that many other factors also influence the protein−receptor interactions, besides simple electrostatic interactions—such as overall protein polarity, location of charged amino acids at the protein surface (domain formation), surface topology, and protein size.[27]

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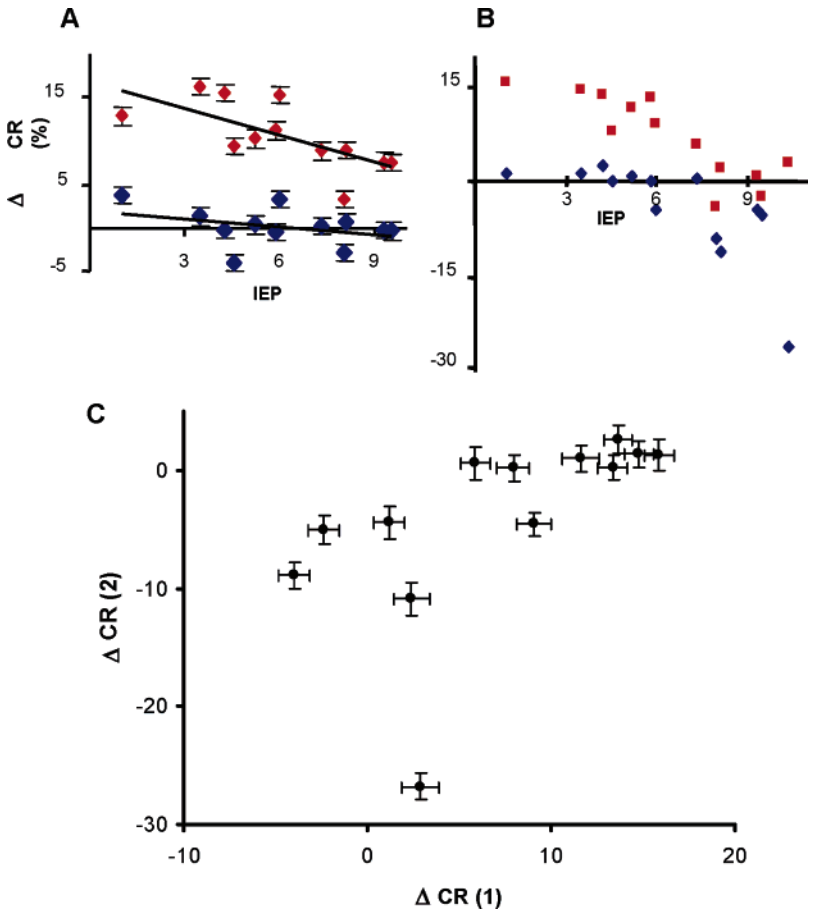

Figure 6. (A, B) Net colorimetric response (Δ(CR)) due to protein−receptor binding (calculated from the visible spectra of receptor/DMPC/PDA vesicles) versus the isoelectric point (IEP) of the proteins tested: (A) **1** (red), **3** (blue); (B) **1** (red), **2** (blue). Proteins: pepsin (pI = 1.0), amylglucosidase (3.5), glucose oxidase (4.25), trypsin inhibitor (4.55), lactoglobulin A (5.2), carbonic anhydrase (5.85), albumin (6.0), trypsin (7.3), chymotrypsin (8.0), proteinase K (8.1), trypsinogen (9.3), cytochrome C (9.5), histone (10.4). The protein concentrations were 0.24 μM (A) and 0.1 μg/mL (B). (C) Two-dimensional graph depicting the Δ(CR) values recorded for the proteins in (B). Δ(CR)(**1**)/(**2**) corresponds to the net colorimetric response obtained with embedded calixarene **1/2**. Each data point corresponds to a single protein and illustrates its "colorimetric fingerprint"; see the text.

An important observation that emerges from the Δ(CR) analysis is the feasibility for protein discrimination by the colorimetric receptor/vesicle assay. Specifically, proteins can be distinguished, in principle, by a combination of net colorimetric effects recorded by using different vesicle-embedded receptors. The protein "fingerprinting" concept is shown in Figure 6C. Each data point in the two-dimensional graph represents a protein for which Δ(CR) values were recorded by using calixarene **1** (*x* axis) or **2** (*y* axis). The dispersion of protein datapoints is particularly large for either acidic or basic proteins, which is an expected outcome since the platform relies on electrostatic interactions between the proteins and the calixarene hosts. The distribution map in Figure 6C indicates that, in principle, the construction of a sufficiently broad colorimetric protein database would allow identification of proteins by combining information on their molecular weights with the colorimetric assay.

Characterization of the Colorimetric Phenomena. Figure 7 depicts experimental evidence corroborating the proposed correlation between the observed colorimetric effects and protein binding onto the receptor/lipid/PDA vesicles. Representative tryptophan fluorescence experiments[28] shown in Figure 7A reveal that for acidic proteins such as pepsin the incorporation of receptor **1** in the DMPC/PDA vesicles clearly modified the overall hydrophobicity of its tryptophan environment (Figure 7Ai), most likely due to surface or structural modification of the protein following binding to the vesicle-embedded receptor. Such binding accounts for the high net color effects observed after addition of pepsin to **1**/DMPC/PDA vesicles (Figure 2B). In contrast to pepsin, the tryptophan fluorescence of the more basic protein chymotrypsin (pI = 8) hardly changed after mixing with **1**/lipid/PDA vesicles, reflecting the reduced protein−receptor affinity. This is also consistent with the smaller net colorimetric response apparent for the protein (Figure 6A,B).

The circular dichroism (CD) spectra shown in Figure 7B echo the tryptophan fluorescence analysis and further indicate that receptor recognition may indeed deeply affect protein structure. Specifically, Figure 7B demonstrates that the CD trace of pepsin was significantly altered—indicating the occurrence of a pronounced conformational change—if the benzylammonium calixarene **1** was preadded to the DMPC/PDA vesicles (solid line in Figure 7B). Preaddition of either **2** or **3** to the vesicles did not give rise to conformational changes of pepsin, yielding almost the same trace as depicted for the unstructured protein in Figure 7B (broken line).

Fluorescence quenching data shown in Figure 7C lend further evidence to the correlation between the colorimetric phenomena and interfacial protein−receptor binding. Figure 7C features

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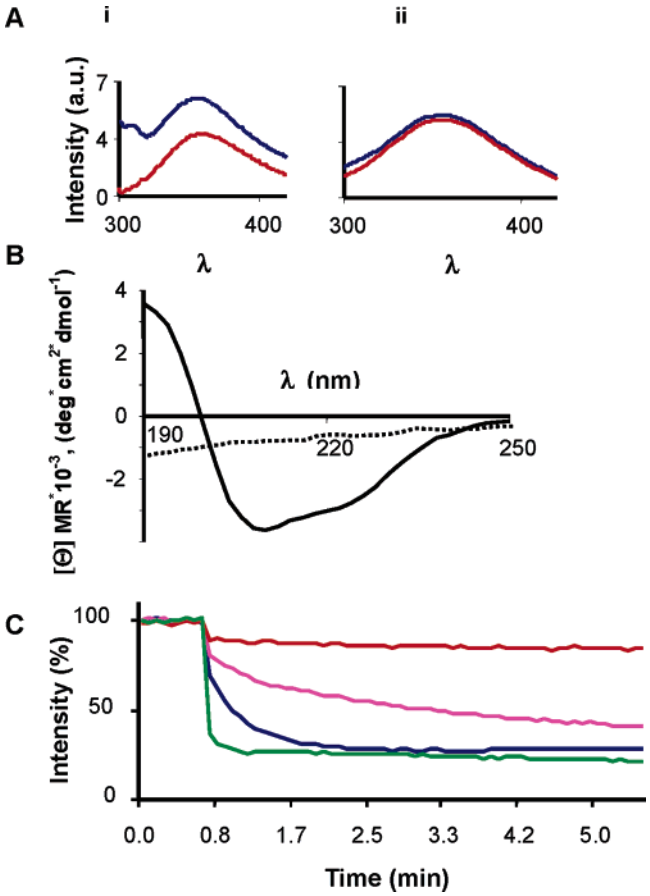

Figure 7. Spectroscopic characterization. (A) Fluorescence emission spectra (excitation 280 nm) of pepsin (pI = 1, i) and chymotrypsin (pI = 8, ii). Key: blue, in water; red, in a solution of **1**/DMPC/PDA vesicles (0.25 mM). (B) CD traces of pepsin added to DMPC/PDA vesicles (broken line) and pepsin mixed with DMPC/PDA to which receptor **1** was preadded (solid line). (C) Fluorescence quenching of NBD-PE embedded in **1**/DMPC/PDA vesicles by sodium dithionite after docking of proteins with varying pI values. Key: blue, control (no protein added); red, pepsin (pI = 1); purple, chymotrypsin (pI = 8); green, histone (pI = 10.4).

fluorescence quenching experiments utilizing DMPC/PDA vesicles that also incorporated the fluorescence dye NBD-PE.[29] Figure 7C demonstrates that the quenching rate of the lipid-incorporated NBD by water-soluble dithionite followed the order of protein pI. Specifically, very little quenching was observed when pepsin (pI = 1) was added to vesicles comprising calixarene **1**, NBD-PE, DMPC, and PDA. This reduced quenching arises from shielding of the NBD dye by the vesicle-bound protein.[29] In contrast, the lower affinity of proteins with higher pI to the vesicle-embedded receptor **1** resulted in reduced shielding, reflected in the faster fluorescence quenching depicted in Figure 7C. Together, the fluorescence and CD data depicted in Figure 7 provide compelling evidence that the distinctive colorimetric changes occurring after incorporation of the amphiphilic calixarene receptors into the lipid/PDA vesicles are directly related to specific electrostatic binding between the proteins and the vesicle-embedded receptors.

(29) McIntyre, J. C.; Sleight, R. G. *Biochemistry* **1991**, *30*, 11819.

Conclusions

We have shown that amphiphilic calixarene modules specific for basic or acidic amino acid residues can be embedded in chromatic vesicles constructed from phospholipids and polydiacetylene. Colorimetric data and application of complementary analytical techniques indicate that electrostatic interactions between the vesicle-embedded receptors and charged domains on protein surfaces result in a distinctive colorimetric response of the PDA matrix. Naked eye detection of nonmembrane protein is thus achieved at micromolar concentrations. The receptor/lipid/PDA assemblies constitute an efficient protein recognition platform, additionally facilitating protein fingerprinting through incorporating different receptors in the chromatic vesicles and recording their effects of the colorimetric transitions induced by the proteins. This generic and easily prepared platform could become a useful tool for protein identification in diagnostic applications, for example, following chromatography separation of unknown protein mixtures and protein overexpression systems, and for studying charge distribution on protein surfaces.

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Supporting Information Available: Table listing colorimetric data recorded for proteins and the effect of pre-insertion of the artificial receptors 1−3. This material is available free of charge via the Internet at http://pubs.acs.org.

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