

# Rapid Colorimetric Detection of Antibody–Epitope Recognition at a Biomimetic Membrane Interface

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**Abstract:** Biomolecular recognition of antigens and epitopes by antibodies is a fundamental event in the initiation of immune response and plays a central role in a variety of biochemical processes. Peptide binding requires, in many cases, presentation of the peptides at interfaces, such as protein surfaces, cellular membranes, and synthetic polymer surfaces. We describe a novel molecular system in which interactions between antibodies and peptide epitopes displayed at a biomimetic membrane interface can be detected through induction of visible, rapid color transitions. The colorimetric assembly consists of a phospholipid/polydiacetylene matrix anchoring a hydrophobic peptide displaying the epitope at its N-terminus. The colorimetric transitions observed in the assembly, corresponding to perturbation of the polydiacetylene framework, are induced only upon recognition of the displayed epitope by its specific antibody present in the aqueous solution. Significantly, the color changes occur after a single mixing step, without further chemical reactions or enzymatic processing. The new molecular system could be utilized for studying antigen–antibody interactions and peptide–protein recognition, epitope mapping, and rapid screening of biological and chemical libraries.

## Introduction

Biomolecular recognition of peptides and antigens is central to a variety of immunological, biochemical, and diagnostic processes.<sup>1–3</sup> Interactions between peptides and antibodies are detected through various biochemical techniques, such as enzyme-linked immunosorbant assays (ELISA), Western blotting, immunoprecipitation, affinity chromatography, and others.<sup>4</sup> These bioanalytical techniques require, in general, that the peptides would be presented to the binding macromolecules at larger molecular interfaces. Peptide presentation on surfaces is also apparent in many biological systems, such as phage-display libraries<sup>5</sup> and antigen presentation by MHC molecules.<sup>6</sup> Most of the techniques utilized for detection of biomolecular recognition include several preparation and processing steps,<sup>4</sup> which usually require several hours until results are obtained.

We have developed a novel molecular assembly which facilitates rapid biomolecular recognition of peptides, displayed at a biomimetic membrane interface, by antibodies in aqueous solutions. Furthermore, the new system is capable of reporting the recognition event through visible, spectroscopically quantified, colorimetric transitions. The colorimetric sensor we have designed is based upon vesicular particles of conjugated polydiacetylene (PDA), incorporating membrane-like phospholipid domains, as well as the epitopes, displayed at the N-termini of hydrophobic amino acid sequences. Polymerized diacetylenes

exhibit unique chromatic properties. In particular, it has been shown that PDA aggregates readily undergo blue–red color changes, induced by a variety of external factors, such as temperature,<sup>7</sup> changes in pH and salt concentration,<sup>8</sup> and interfacial ligand–receptor binding.<sup>9</sup> Recent studies have demonstrated that phospholipids embedded within the PDA framework essentially mimic lipid bilayer environments, thus allowing colorimetric detection of peptide–membrane interactions<sup>10</sup> and ion transport.<sup>11</sup> We show here that the combined epitope–peptide/phospholipid/polydiacetylene molecular aggregates undergo rapid colorimetric transitions when they come in contact with antibodies that specifically recognize the epitopes.

## Experimental Section

**Sample Preparation.** All epitope-displaying peptides (except the p8 protein of fd filamentous bacteriophage) were synthesized by solid-phase peptide synthesis (Alpha Diagnostic Inc., San Antonio, TX), and their purity (>98%) and consistency were verified by HPLC and mass spectroscopy. The p8 protein was isolated and purified according to established protocols.<sup>12</sup> The colorimetric particles were produced by sonication of the mixed components (peptides, dimyristoylphosphatidylcholine (DMPC, Avanti Polar Lipids, Alabaster, AL), and 10,12-tricosadiynoic acid (GFS Chemicals, Powell, OH)), at 70 °C (3–4 min, 100-W Sonix vibracell probe sonicator), followed by overnight cooling at 4 °C. Polymerization was carried out by 30-s irradiation at 254 nm (0.8 J/cm<sup>2</sup>).

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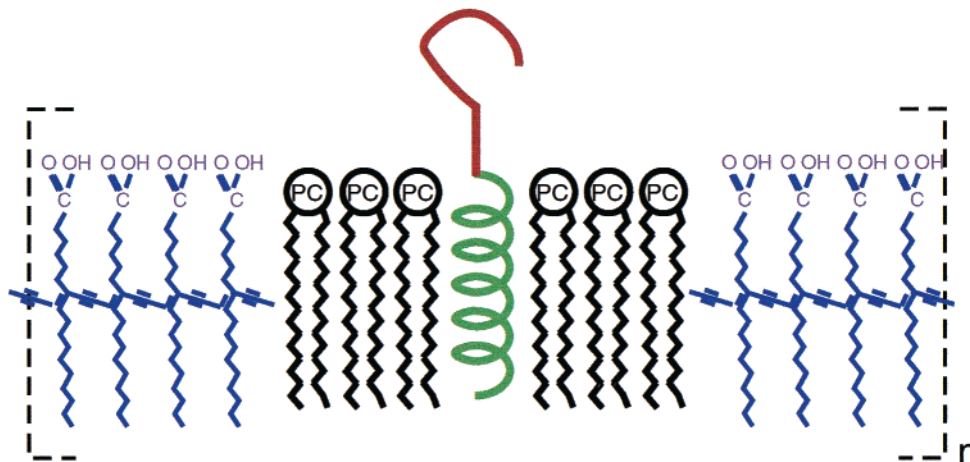
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**Figure 1.** Schematic diagram showing part of the interface of the colorimetric assembly (molecular components are not to scale). Blue, conjugated polydiacetylene composed of 10,12-tricosadiynoic acid; black, DMPC (PC denotes the phosphatidylcholine headgroup of the lipid molecules); red, epitope displayed at the N-terminus of a helical membrane-associated peptide sequence (green), see text.

**Colorimetric Measurements.** UV-vis spectroscopy measurements were carried out at 27 °C on a Hewlett-Packard 8452A diode array spectrophotometer, using a 1-cm optical path cell. Spectra were acquired at wavelengths between 400 and 700 nm.

A quantitative value for the extent of the blue-red color transitions within the solutions is given by the colorimetric response (CR), which is defined as follows:<sup>10</sup>

$$CR = (PB_0 - PB_1)/PB_0$$

where

$$PB = A_{\text{blue}}/(A_{\text{blue}} + A_{\text{red}})$$

$A$  is the absorbance either at the “blue” component in the UV-vis spectrum (~640 nm) or at the “red” component (~500 nm). (Note: “blue” and “red” refer to the visual appearance of the material, not its actual absorbance).  $PB_0$  is the red/blue ratio of the control sample (before induction of a color change), while  $PB_1$  is the value obtained after the colorimetric transition occurs.

**ELISA Assay.** The ELISA assay was carried out using Polysorp surface multiwell plates (96 flat-bottom wells, Nunc, Denmark). The epitope/DMPC/PDA particles were suspended in 0.01 M phosphate-buffered saline (PBS), pH 7.4, and placed in the wells. Aliquots (0.35 mL) of a 5% solution of bovine serum albumin (BSA, Sigma) in PBS were added to each well, and the plates were incubated for 2 h at 37 °C. This procedure was followed by four washing steps with PBS. After addition of the primary antibodies diluted in PBS (2 μg/mL), the plates were incubated overnight at 4 °C. Following incubation, the secondary antibody (peroxidase-conjugated, diluted by 1:1000) was added, the solutions were then incubated for 2 h at 37 °C and washed four times with PBS, and the substrate *o*-phenylenediamine dihydrochloride [OPD] was added. Absorbance values were recorded at 450 nm in a UV-vis microplate reader (Dynatec MR 5000).

**Transmission Electron Microscopy.** Samples were placed on carbon-stabilized copper grids for 1 min and after removal of excess solution were stained with 1% uranyl acetate. Dried samples were viewed under a Philips CM-12 transmission electron microscope at 100 kV accelerating voltage.

## Results and Discussion

The design of a new system for rapid detection of interfacial biomolecular interactions in general, and antibody-epitope recognition in particular, has to fulfill two main objectives. First, the chemical construct should allow physical access and binding between the epitope and the antibody in an aqueous solution. The second requirement is that specific interactions between the antibody and the epitope should be reported through easily detected chemical or physical transformations within the system.

The molecular assembly we have designed, shown schematically in Figure 1, adheres to the above requirements.

The recognition element in the colorimetric sensor depicted in Figure 1 consists of the epitope (red) displayed at the N-terminus of a lipophilic helical amino acid sequence (green). The helical peptide, which is anchored within a phospholipid domain (black), serves as a vehicle for displaying the epitope at the lipid-water interface. Two types of helical peptides have been successfully employed for presenting the epitopes at the phospholipid interface; in principle, other hydrophobic anchors could be utilized as well. Specifically, we have used the generic sequence GKKLALALALALALALKKA (single-letter codes), denoted L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G, which has been designed to span lipid bilayers in a helical conformation.<sup>13</sup> The alanine-leucine repeat in the L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G sequence stabilizes the lipid-associated helical structure of the peptide, while the role of the lysine residues close to the two termini is to anchor the helical domain at the lipid-water interfaces.<sup>14</sup> The second sequence examined in this work was the 50-residue major coat protein (p8) of the fd filamentous bacteriophage.<sup>15</sup> The p8 protein is a mostly helical membrane protein, which has been widely used in phage-display libraries as a vehicle for presenting epitopes, proteins, and antibodies at the phage surface.<sup>16</sup>

The phospholipid scaffold depicted in Figure 1 is incorporated within a framework of conjugated polydiacetylene (PDA, shown in blue), which is the chromatic component in the sensor system. Polymerized diacetylene assemblies appear blue to the naked eye due to their alternating triple-bond/double-bond backbone structure.<sup>17</sup> Distinct blue-red colorimetric transitions observed in PDA systems have been ascribed to structural rearrangements of the pendant side chains of the polymer.<sup>17,18</sup> Significantly, it has been demonstrated recently that structural perturbations, occurring locally within phospholipid domains incorporated within PDA vesicles, could also disrupt the surrounding PDA network, thus inducing colorimetric transitions.<sup>10,11</sup> The colorimetric sensor particles have been prepared by probe sonication of an aqueous solution of the three components (peptide,

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phospholipid, diacetylenic lipid) at an elevated temperature, followed by polymerization.

Figures 2–5 present data which demonstrate that epitope–antibody recognition is directly responsible for induction of color changes within the epitope/phospholipid/PDA aggregates depicted schematically in Figure 1. Figure 2, for example, features colorimetric data obtained for a representative epitope–antibody system. Figure 2A shows a photograph of the solutions placed in a subsection of a 96-well plate. The cells shown in Figure 2A contain an aqueous solution of particles consisting of PDA, DMPC, and the 10-residue c-myc epitope, EQKLISEEDL (derived from the C-terminus of the human c-myc protein<sup>19</sup>), displayed at the N-terminal of the generic L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G helical peptide. The control well, containing the c-myc-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particles immersed in a buffer solution, appears blue (Figure 2A, i). However, a few minutes after addition of the anti-c-myc monoclonal antibody (mAb), the solution turns red-purple (Figure 2A, ii). In contrast to the purple color induced by the anti-c-myc mAb, no blue–red color transition is detected when an antibody raised against a *different* epitope is added to the c-myc-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particle solution (Figure 2A, iii).

Figure 2B depicts the UV–vis absorbance spectra recorded for the blue control solution (without antibody) and the purple-red solution formed after addition of the anti-c-myc mAb, respectively. Comparison of the relative intensities of the absorbance at 500 nm (the “red” band) and the absorbance at 640 nm (the “blue” band) allows a quantitative determination of the extent of the blue–red colorimetric transition, through calculation of a “colorimetric response” (CR).<sup>10</sup> In principle, a higher CR value indicates a stronger reddish appearance of the solution, compared to the blue control sample. For example, the CR calculated for the red-purple sample in the cell shown in Figure 2A, ii, is 35% (the initial blue solution yields, by definition, a 0% CR).

Figure 2C describes the change of the CR as a function of antibody and protein concentrations in the solution containing c-myc-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particles. The titration curves depicted in Figure 2C confirm that the colorimetric transition indeed depends on a specific epitope–antibody interaction. In particular, the solid curve in Figure 2C indicates that the red-purple appearance of the solution intensifies as the concentration of the anti-c-myc mAb is increased. In contrast, the flat dashed curves shown in Figure 2C provide clear evidence that nonspecific antibodies, as well as soluble proteins such as albumin, induce much smaller, or negligible, color changes in the system. Figure 2C further illuminates the dynamic range of the colorimetric assay. Specifically, the titration curve of the anti-c-myc mAb indicates that the system can distinguish a signal arising from a specific antibody–epitope interaction at an antibody concentration of around 10  $\mu\text{g/mL}$ . Parameters that affect the sensitivity of the system are discussed in detail below.

Figure 2D features data obtained in a competition experiment using the c-myc-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particle solution. Figure 2D indicates that addition of the 10-residue c-myc peptide (EQKLISEEDL) to the anti-myc mAb solution significantly reduces the colorimetric response, i.e., the blue-red transition, of the PDA particles (solid line). This result is consistent with conventional competition experiments, because the free peptide binds to the antibody and diminishes its activity.<sup>1,4</sup> Figure 2D also shows that a nonspecific peptide epitope reduces the colorimetric transition of the solution to a much lesser extent (dashed line) compared to the c-myc peptide. The results shown

in Figure 2D further confirm the selectivity of the colorimetric system and point to potential applications of the new method in rapid screening of antagonists, agonists, and epitope mimics.

Figure 3 displays diagrams showing the colorimetric responses of DMPC/PDA assemblies incorporating different epitopes, following interactions with various antibodies. Representative sequences that were displayed at the N-terminus of the L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G peptide include the c-myc epitope (Figure 3A), the FLAG epitope (amino acid sequence DYKDDDDK<sup>20</sup>), which is widely used in epitope tagging experiments (Figure 3B), and the HA epitope (YPYDVPDYA), derived from the human influenza virus hemagglutinin protein<sup>21</sup> (Figure 3C). We have also examined DMPC/PDA particles containing the major coat protein (p8) of the fd filamentous bacteriophage, in which the primary epitope recognized by the anti-fd antibody has been mapped to the N-terminus<sup>22</sup> (Figure 3D).

The colorimetric data shown in Figure 3 demonstrate the general applicability of the system for detection of epitope–antibody recognition. The CR values recorded for each epitope-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA assembly indicate that pronounced colorimetric transitions occur *only* when the displayed epitopes are recognized by their specific antibodies. The color changes induced by nonspecific interactions between the antibodies and the lipid–polymer particles are considerably smaller than the real signal. The colorimetric changes depicted in Figure 3 have been generally recorded short times (seconds to minutes) after mixing the PDA particle solutions with the antibodies.

The colorimetric response of the epitope-peptide/DMPC/PDA assembly depends on the composition of the particles. A direct correlation exists, for example, between the extent of color transition and the concentration of the epitope within the lipid/PDA assembly. However, it has been observed that incorporation of a high peptide concentration induces aggregation and adversely affect the functionality of the assay. Accordingly, the approximately 2  $\mu\text{M}$  concentration of immobilized epitope reported here has been selected in order to magnify the sensitivity of the system, on one hand, and maintain the integrity and stability of the lipid/PDA particles, on the other hand.

The data presented in Figures 2 and 3 indicate that the epitope-peptide/DMPC/PDA assemblies exhibit colorimetric responses to antibody concentrations in the range of 100–700  $\mu\text{g/mL}$ , which are considered high in conventional ELISA experiments.<sup>4</sup> The sensitivity of the technique, however, could be further improved by optimization of several experimental parameters. These include increasing the concentration of the lipid within the PDA matrix, as well as that of the immobilized epitope. In addition, the time duration between mixing the PDA assembly with the antibody and making the colorimetric measurements could be modified in order to obtain the highest signal-to-noise ratio. Additional parameters that could contribute to higher colorimetric signals are the type and pH of buffer employed in the experiments. In principle, as is the case with other biochemical and analytical techniques, the experimental protocols should be optimized for each epitope–antibody system examined. In addition, it should be emphasized that the results presented here have been acquired using a conventional UV–vis spectrophotometer. Application of a dedicated, high-sensitivity instrument (such as a microplate ELISA reader) would, most likely, significantly increase the sensitivity and the dynamic range of the assay.

To corroborate the specificity and the biochemical reliability of the new assembly, we have carried out ELISA-type experi-

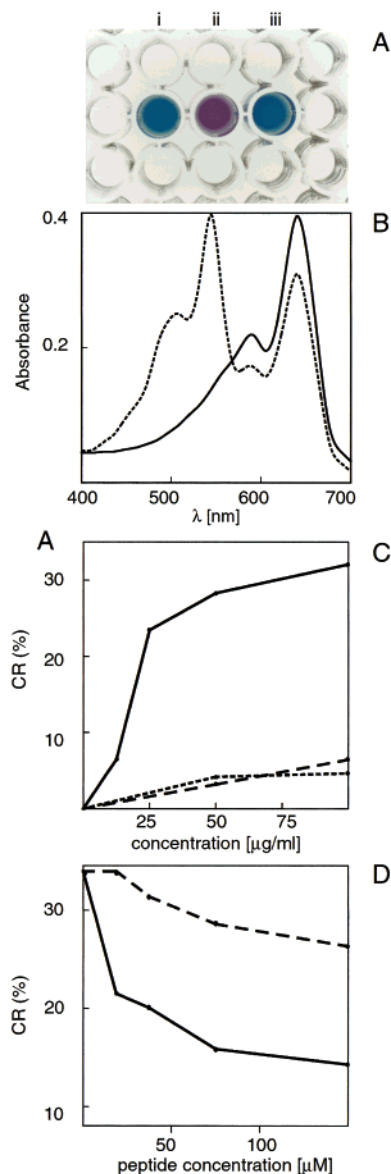
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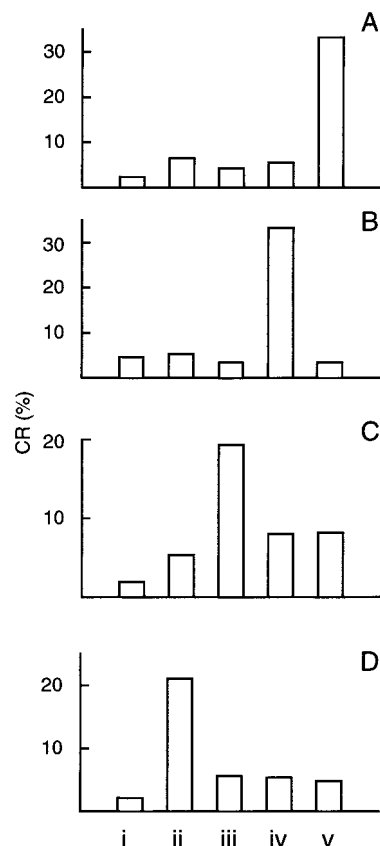
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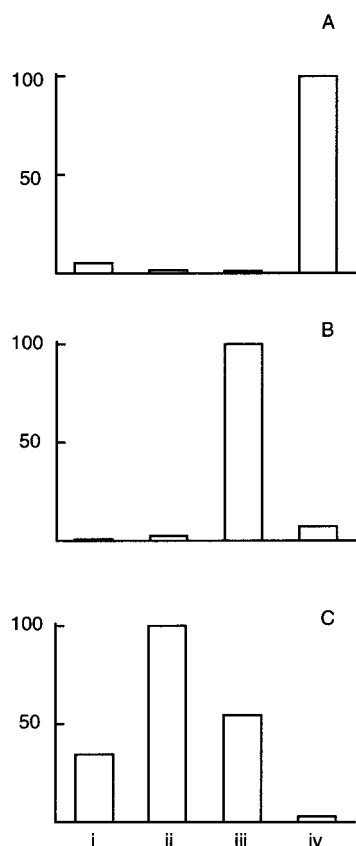


**Figure 2.** (A) Photograph of a subsection of a 96-well plate containing a phosphate-buffered saline (PBS) solution of mixed particles consisting of PDA, DMPC, and a peptide with the sequence EQKLISEEDL-GKKLALALALALALALKKA (composed of the c-myc epitope displayed at the N-terminus of the L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G transmembrane peptide). The total volume in each cell was 50  $\mu$ L (composed of equal volumes PBS buffer and aqueous particle solutions). Concentrations of the constituents: PDA, 0.6 mM; DMPC, 0.4 mM; epitope, 2  $\mu$ M. (i) Control (no antibody added); (ii) anti-c-myc mAb (Boehringer-Manheim) added (final concentration, 100  $\mu$ g/mL); (iii) nonspecific antibody added (anti-FLAG mAb (raised against the FLAG epitope) (Research Diagnostic Inc., Flanders, NJ); final concentration, 400  $\mu$ g/mL). (B) UV-vis absorbance spectra of (solid line) PBS solution of mixed particles consisting of PDA, DMPC, and the peptide c-myc-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G and (dashed line) the same solution after addition of anti-c-myc mAb (final concentration, 100  $\mu$ g/mL). (C) Titration curves depicting the colorimetric response (CR) of a PBS solution of mixed particles consisting of PDA, DMPC, and the peptide c-myc-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G, as a function of protein concentration. (Solid line) anti c-myc mAb added; (long dashed line) anti FLAG mAb added; (short dashed line) bovine serum albumin (Sigma) added. (D) Graph depicting the %CR of a PBS solution of c-myc-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particles, to which anti c-myc mAb has been added (100  $\mu$ g/mL) incubated with (solid line) the c-myc epitope EQKLISEEDL (Boehringer Manheim) and (dashed line) the FLAG epitope DYKDDDDK (Research Diagnostic Inc., Flanders, NJ). The peptides have been separately added to the antibody solution prior to mixing with the PDA particles.



**Figure 3.** Colorimetric transitions induced by epitope-antibody interactions. Diagrams depicting the %CR recorded following addition of antibodies to solutions containing DMPC/PDA particles incorporating different displayed epitopes: (A) c-myc-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G (c-myc epitope, EQKLISEEDL, displayed at the N-terminus of the L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G peptide); (B) HA-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G (YPYDVPDYA); (C) FLAG-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G (DYKD-DDDK); (D) p8 coat protein of fd filamentous bacteriophage. Antibodies, separately added to the particle solutions: (i) control, PBS buffer solution (no antibody added); (ii) anti-fd (Sigma), 700  $\mu$ g/mL; (iii) anti-FLAG mAb, 400  $\mu$ g/mL; (iv) anti-HA mAb (Boehringer-Manheim), 200  $\mu$ g/mL; (v) anti-c-myc mAb, 100  $\mu$ g/mL. Antibodies were dialyzed in 0.01 M PBS, 70 mM sodium chloride, pH 7.4, prior to mixing with the particles.

ments. ELISA assays, in general, detect interactions between bound peptides and soluble antibodies.<sup>1</sup> In conducting the ELISA assay we have modified the conventional procedures.<sup>4</sup> Here, the *entire* epitope/phospholipid/PDA particles have been attached to the cells of the 96-well plate, instead of only the peptide epitopes (which is the procedure commonly used). Figure 4 presents the results of the ELISA experiments obtained for three antibodies examined in this work (ELISA measurements for the anti-FLAG antibody could not be carried out, due to the absence of attachment of the FLAG-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particles to the plastic walls). The ELISA data show that significantly higher absorbances are recorded in cells in which the primary antibodies have been raised against the displayed epitopes. Similar to the colorimetric data shown in Figure 3, the nonspecific background absorbances are low; the relatively high background observed for the anti-fd antibody (Figure 4C) might be related to the fact that this antibody is polyclonal. Overall, the results of the ELISA assay again confirm the occurrence of specific interfacial binding between the antibodies and the epitopes displayed at the surface of the lipid-polymer particles. It should be emphasized that, while the ELISA protocols involve several mixing, washing, and processing steps conducted over several hours, the colorimetric approach we have

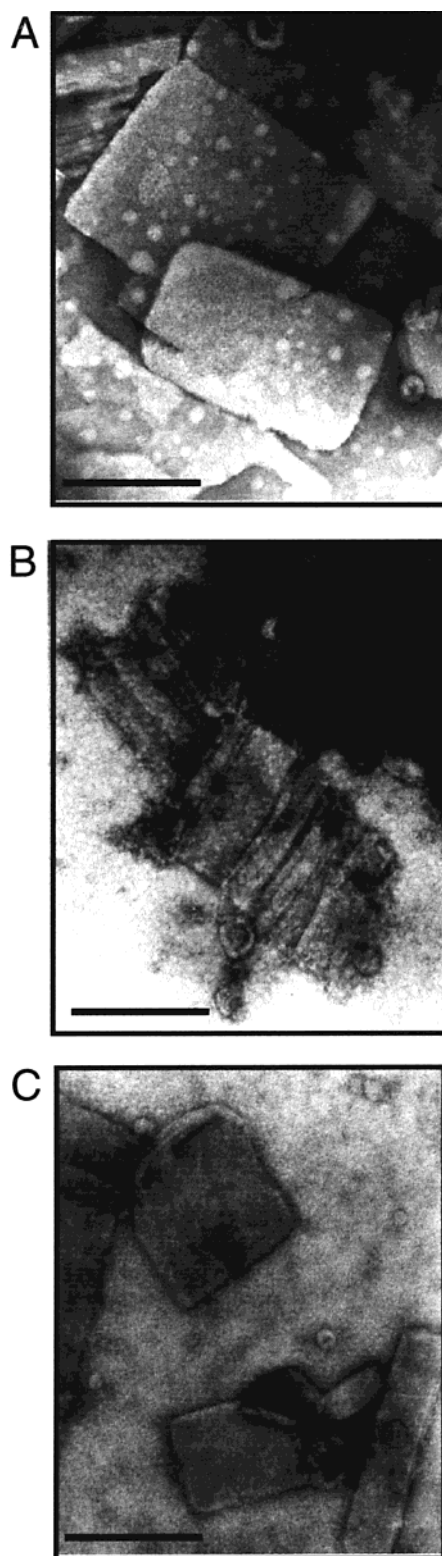


**Figure 4.** Diagrams depicting the results of an ELISA-type assay using the epitope/DMPC/PDA aggregates. The absorbance data have been normalized for each of the antibodies examined. Primary antibodies examined: (A) anti-c-myc mAb; (B) anti-HA mAb; (C) anti-fd. Particles cross-reacted with the antibodies: (i) control (DMPC/PDA aggregates, no epitope incorporated); (ii) p8/DMPC/PDA; (iii) HA-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA; (iv) c-myc-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA.

developed requires only a single mixing step between the epitope–PDA assemblies and the antibody, and results are obtained significantly faster.

Blue–red chromatic transitions observed in PDA-based materials have been ascribed to reorganization of the conjugated network of the polymer backbone, induced by external structural effects.<sup>17,18</sup> Structural disruption is also most likely responsible for the colorimetric transitions occurring in the epitope/phospholipid/PDA system developed here. In particular, binding between the antibodies, which are relatively big macromolecules, and the displayed epitopes at the lipid–water interface is expected to result in significant perturbations of the particle surface, giving rise to the observed color changes within the PDA matrix.

Transmission electron microscopy (TEM) images, presented in Figure 5, support this proposal. Figure 5A shows DMPC/PDA particles, containing the HA epitope displayed at the N-terminus of the L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G peptide. The HA-epitope-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA assemblies form submicrometer-size particles in the aqueous solution. The particles resemble rectangular sheets, which are similar to other modified PDA matrixes previously observed in microscopy experiments.<sup>23,24</sup> Figure 5B depicts the TEM image of the HA-epitope-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particles, following mixing with the anti-HA mAb. The addition of the



**Figure 5.** Negative-stained transmission electron microscopy (TEM) images of epitope/DMPC/PDA particles. (A) HA-epitope-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particles (PDA, 0.6 mM; DMPC, 0.4 mM; epitope-peptide, 2  $\mu$ M) suspended in PBS buffer; (B) HA-epitope-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particles after addition of anti-HA-mAb (antibody concentration, 200  $\mu$ g/mL); (C) HA-epitope-L<sub>7</sub>A<sub>7</sub>K<sub>4</sub>G/DMPC/PDA particles after addition of anti-FLAG-mAb (antibody concentration, 400  $\mu$ g/mL). Scale bar, 200 nm.

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anti-HA antibody clearly affects the morphology of the particles; specifically, the particle surface becomes highly fissured, with an appearance of abundant cracks or grooves. In contrast to the

substantial effect of the anti-HA antibody, interaction with a *nonspecific* antibody hardly affects the smooth appearance of the particles' surface, as well as their shapes and morphologies (Figure 5C). Similar TEM data have been obtained for other epitope/DMPC/PDA aggregates.

### Conclusions

This work introduces a new epitope/phospholipid/polydiacetylene assembly, which facilitates rapid colorimetric detection of interfacial antibody–epitope interactions. The peptide epitopes are displayed at the N-terminus of generic membrane-spanning peptides, which are anchored within the phospholipid moieties incorporated in polydiacetylene matrixes. The scope of the method presented here could be broad. The colorimetric technique could be applied for studying processes occurring at cellular membrane surfaces, as well as general biological recognition events, such as antibody–antigen interactions, agonist/antagonist–receptor binding, and others.

The epitope-display assembly described here requires covalent attachment of the epitope to a lipophilic moiety. This requirement could be easily accommodated when the epitope is derived

synthetically; however, this requisite might limit the applicability of the colorimetric assay if the assay is used to examine epitopes from natural sources. This limitation might be overcome by designing the lipid/PDA interface to allow immobilization of epitopes anchored to non-hydrophobic residues as well. Such a manipulation might be possible, for example, through modification of the PDA headgroups.

The colorimetric assay is robust and easy to carry out. The new method yields results significantly faster compared to commonly used biochemical techniques, such as ELISA. The system is compatible with 96-well formats and could be utilized as a platform for various biochemical and immunological applications, including rapid screening of biological and chemical epitope libraries, phage-display techniques, and disease diagnostics.

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