with graphite-monochromated $Mo_{K\alpha}$ radiation on a Siemens/Bruker SMART CCD single crystal diffraction system. Cell constants and an orientation matrix were obtained from least-squares refinement by using the measured positions of 4386 reflections with $I > 10\sigma$ in the range $3.00 < 2\theta < 45.00^\circ$. The Siemens/Bruker program SHELXTL-PC software package was used to solve the structure by direct methods. All stages of weighted full-matrix least-squares refinement were conducted with F_o^2 data with the SHELXTL-PC Version 5 software package and converged to give $R_1(F) = 0.062$ for 3300 independent absorption-corrected reflections with $2\Theta(Mo_{K\alpha}) < 49.5^\circ$ and $I > 2.50\sigma(I)$ and $wR_2(F^2) = 0.069$ with a GOF = 1.70. As a consequence of the twinning found in the crystal some reflections were from the major twin only, and some were measured from both components. At the end of the refinement 87 reflections which had $Fo > Fc$ and $w\Delta F^2 > 5$ were removed from the refinement to allow all of the Ge atoms to refine anisotropically. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-114519. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Induced Color Change of Conjugated Polymeric Vesicles by Interfacial Catalysis of Phospholipase A_2**

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Conjugated polymers (CPs) such as polydiacetylene (PDA), polythiophene, and polypyrrole display a remarkable array of color transitions that arise from thermal changes (thermochromism),[1] mechanical stress (mechanochromism),[2] or ion binding (ionochromism).[1, 3] The color changes can be ascribed to a change in the effective length of conjugation of the delocalized, π-conjugated polymer backbone.[4] The application of these "smart" materials for the detection of biological targets (biochromism)[5–11] is only just beginning to be exploited.

Interfacial catalysis on biomembranes plays a key role in extra- and intracellular processes and covers a range of enzyme classes such as lipolytic enzymes, acyltransferases, protein kinases, and glycosidases. In particular, lipolytic enzymes are involved in important biochemical processes, such as fat digestion and signal transduction. Recent interest in one such enzyme, phospholipase A_2 (PLA_2),[12, 13] is motivated by its role in the release of arachidonate and lysophospholipids from membranes. These compounds are the precursors for the biosynthesis of eicosanoids (for example, prostaglandins, leukotrienes) that have been implicated in a range of inflammatory diseases such as asthma, ischaemia, and rheumatoid arthritis.[14–16] Accordingly, the identification of PLA_2 inhibitors is an active area of current research that may lead to novel therapeutics and new biochemical insights into the mechanisms of enzyme activity.[16–18]

PLA_2 catalyzes the hydrolysis of an acyl ester bond exclusively at the 2-acyl position in glycerophospholipids to

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yield a free fatty acid and a lysophospholipid. Typical methods for measuring PLA_2 activity include discontinuous radiochemical,[19] fluorescent,[20] and spectrophotometric[21] techniques. Labeled acyl phospholipids are used as substrates in these measurements and enzyme activity is evaluated by the radioactivity, fluorescence, or absorbance of the cleaved fatty acids. Some procedures (particularly radiolabel methods) may require that the cleaved fatty acids be extracted and isolated from the unreacted substrate by thin-layer chromatography or HPLC. The extraction step and the need for synthetic labeled substrates are disadvantages when the rapid analysis of enzyme activity is considered, for example in high throughput assays that screen potential enzyme inhibitors. Furthermore, phospholipase catalysis is sensitive to the chemical structure of the phospholipid substrate,[22, 23] therefore the use of nonlabeled, naturally occuring substrates is highly desirable. The biochromic vesicles described herein offer a one-step approach to measuring enzyme activity through detection of a color change of PDA "signaling" lipids that surround the natural enzyme substrate. The strategy does not require additional chemical reagents or post-hydrolysis analytical methods. Furthermore, enzyme inhibitors can be rapidly identified by simply monitoring the color changes of aqueous vesicle suspensions in a standard 96-well microtiter plate.

PLA_2 activity has previously been studied in a variety of model membrane systems such as polymerized vesicles,[24, 27] micelles,[21] and monolayers.[22, 25] The biochromic vesicles[5, 6, 8] employed here are prepared by probe sonication of a mixture of the polymerizable matrix lipid 10,12-tricosandiynoic acid

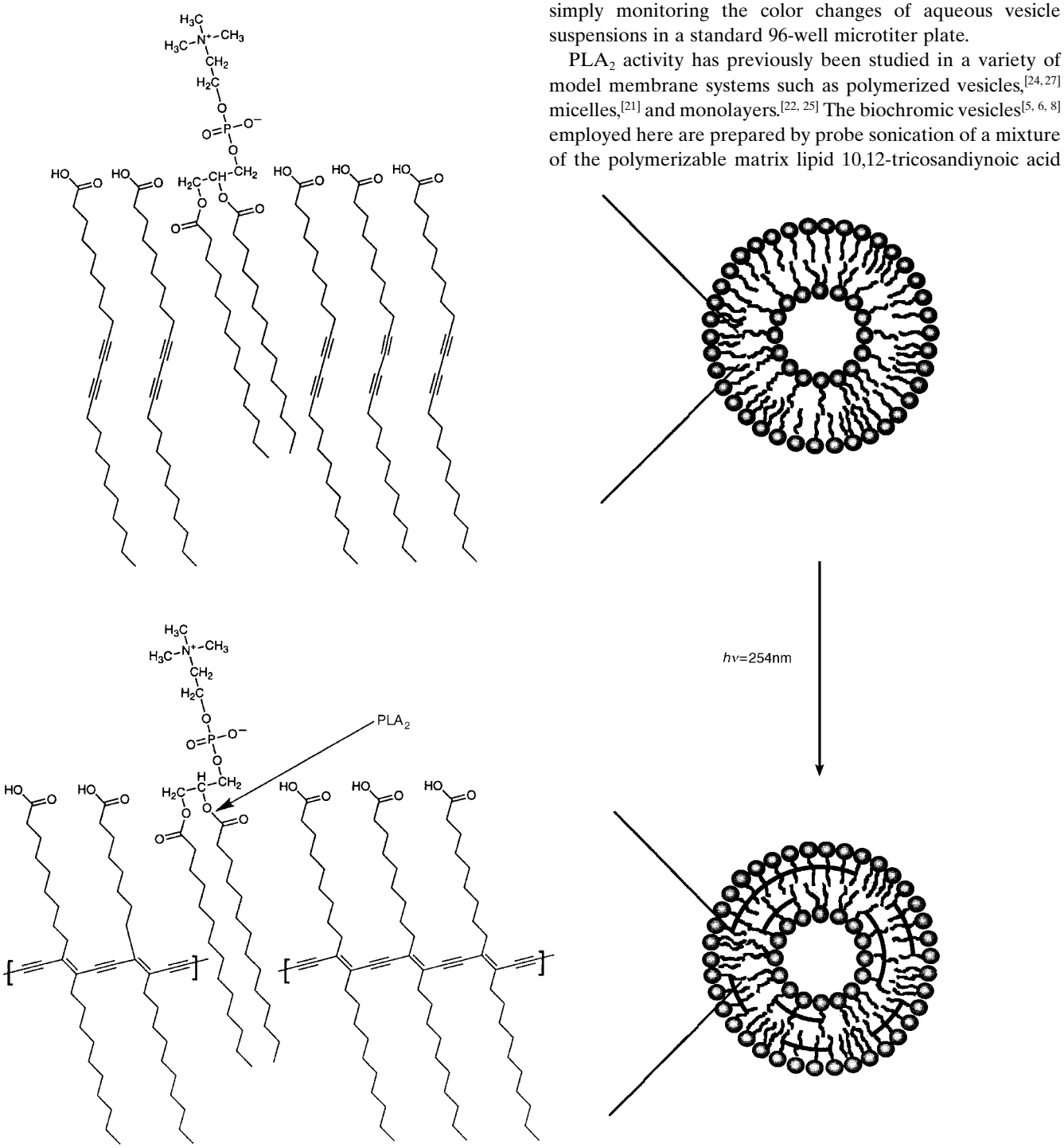

Figure 1. Preparation of biochromic vesicles from polymerizable signaling lipids and the natural lipid DMPC. Below is shown the site of attack by PLA_2. For further information see the text.

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and various mole amounts (0–40%) of dimyristoylphosphatidylcholine (DMPC) in water, followed by polymerization with 1.6 mJ cm^{-2} ultraviolet radiation at 254 nm (Figure 1). Analysis of the vesicle solution by transmission electron microscopy indicates an average vesicle size of 100 nm. In their initial state the vesicles appear deep blue to the naked eye and absorb maximally at around 620 nm (Figure 2a). The color of the vesicles arises from the ene–yne conjugated system that comprises the polymer backbone of the polymerized lipid–PDA matrix.[26] The suspension rapidly turns red (within minutes) upon addition of PLA_2, and exhibits a

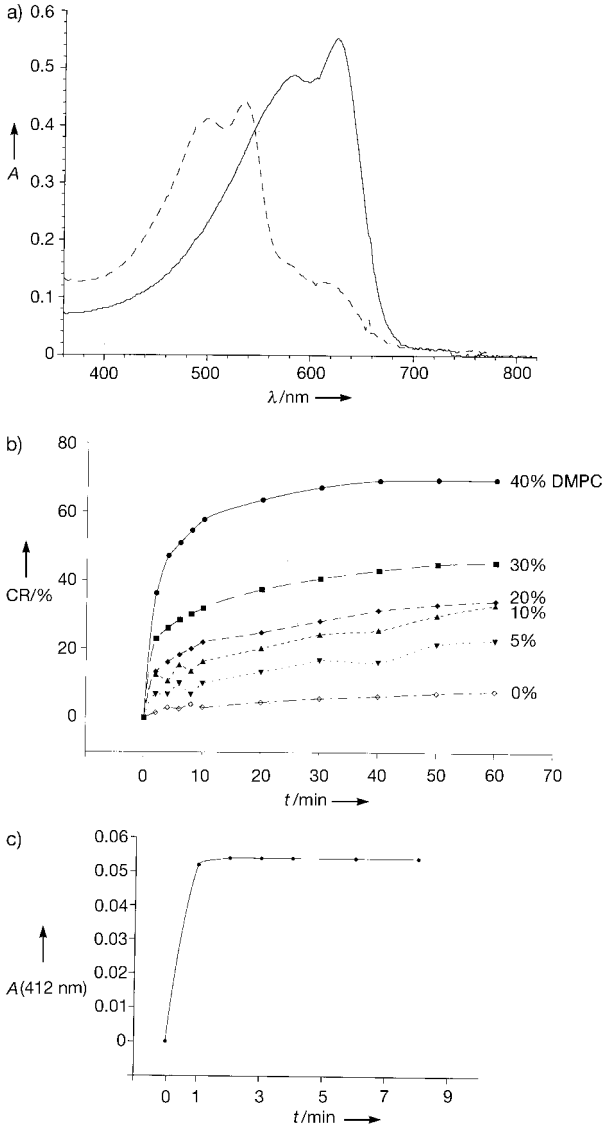

Figure 2. a) Visible absorption of the DMPC/PDA vesicles before (solid line) and after (dashed line) addition of PLA_2; b) colorimetric response curves of DMPC/PDA vesicles containing different molar ratios of DMPC upon incubation with PLA_2; c) verification of hydrolysis by PLA_2 by using DTPC and DTNB. For further information see the experimental section.

maximum absorption at approximately 540 nm (Figure 2a). A relative color change of 10% or more is observed clearly with the naked eye. The color change is modulated by altering the mole percentage of the natural lipid DMPC in the PDA vesicle and the vesicles that do not contain DMPC remain in their blue phase (Figure 2b).

Biochromic transitions of PDA vesicles and films have been proposed to arise from a perturbation of the extended π overlap of the conjugated polymer backbone. This structural rearrangement, induced in previous studies by multivalent receptor binding or penetration of peptide domains into the PDA matrix, results in absorption at shorter wavelengths (490–540 nm).[5, 8, 10] The intense color change observed upon the interaction between the enzyme PLA_2 and the mixed DMPC/PDA vesicles indicates, that in this case, chemical modification of the vesicles by interfacial catalysis provides an alternative pathway for inducing the biochromatic transitions.

It has been demonstrated previously that PLA_2 retains its activity at polymerized mixed vesicles composed of poly(dienoyl)lecithin and dipalmitoylphosphatidylcholine (DPPC).[22, 27] Since the PDA matrix represents a new environment for the enzyme substrate, the PLA_2 activity was independently measured by using a labeled lipid analogue incorporated into the PDA matrix, which allowed the simultaneous measurement of product formation and colorimetric response of the vesicles. The analogue used was the thioester 1,2-bis(*S*-decanoyl)-1,2-dithio-*sn*-glycero-3-phosphocholine (DTPC). Cleavage of DTPC by PLA_2 produces a soluble thiol-modified lipid that readily reacts with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) to produce a colored product that absorbs characteristically at 412 nm.[21] Indeed, when PLA_2 was added to mixed vesicles of 40% DTPC in PDA, the hydrolysis products reacted with DTNB to give rise to a significant absorption at 412 nm (Figure 2c). At the same time, the PDA vesicles also changed color, and the suspension exhibited a colorimetric response similar to that of vesicles that contain DMPC (Figure 2b). The differences in the rate of the response between the two methods most likely arises from the lag time in the response of the surrounding polymeric matrix. These results confirm that interfacial catalysis by PLA_2 occurs at the polymerized mixed vesicles.

NMR experiments further verified the occurrence of interfacial catalysis by PLA_2, and provided information on the fate of the enzymatic reaction products. Figure 3 shows

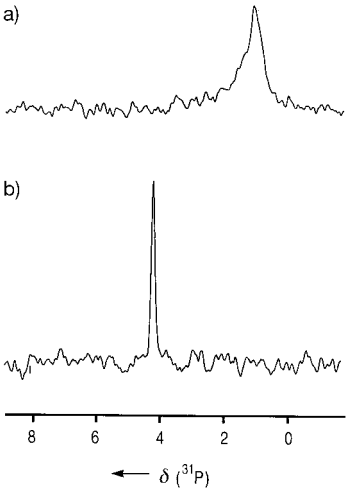

Figure 3. ^{13}P NMR spectra of DMPC/PDA vesicles (0.1 mM total lipid) a) before and b) after addition of PLA_2. Magnetic parameters: magnetic field: 11.7 Tesla, Bruker DMX500, Block-delay pulse sequence with 2048 acquisition data points, 20000 FIDs in each experiment with two second recycle delays, 0.1M phosphoric acid used as an external reference.

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the ^{31}P NMR spectra of the DMPC/PDA vesicles prior to the addition of PLA_2 (Figure 3a) and after the enzymatic reaction (Figure 3b). The relatively broad, anisotropic ^{31}P resonance from the intact vesicles (Figure 3a) corresponds to the choline head group of DMPC embedded in the PDA vesicles. The ^{31}P anisotropy indicates that the DMPC molecules are immobilized within the vesicle matrix. After addition of PLA_2 the ^{31}P signal is shifted downfield. The position of the ^{31}P resonance in Figure 3b coincides with the shift observed for the water-solubilized lyso-myristoylphosphatidylcholine, the hydrolysis product of DMPC. Furthermore, Figure 3 shows that the ^{31}P resonance observed in the suspension of the enzyme-treated vesicles becomes significantly narrower than the ^{31}P signal from the initial DMPC/PDA vesicles, which indicates a higher mobility of the phosphate groups after PLA_2 catalysis.[28] This result suggests that dissolution of the lysolipid reaction products occurs. Further support for this hypothesis comes from ^1H NMR data that indicate the appearance of dissolved lipid species after the reaction with PLA_2.[29]

The color change of the DMPC/PDA vesicles can be suppressed with known inhibitors to PLA_2. In the presence of the inhibitor 1-hexadecyl-3-trifluoroethylglycero-2-phosphomethanol (MJ33)[17, 30] the vesicles remain in their blue phase upon addition of PLA_2. Figure 4a depicts an image of the PLA_2/vesicle suspension in the presence (blue) and absence (red) of MJ33 in a 96-well microtiter plate. The absorbance of the wells quantitatively confirms the suppression of the colorimetric response (Figure 4b). The inhibition of the blue to red color change by MJ33 indicates that nonspecific adhesion does not play a role in the biochromic response, and PLA_2 activity is directly responsible for the color change. Inactivation of PLA_2 is also observed upon removal of Ca^{2+}, the catalytic cofactors for PLA_2,[17, 31] from the buffer solution: PLA_2 prepared in buffer containing Zn^{2+} instead of Ca^{2+} ions does not induce a blue to red color change of the vesicles (Figure 4b). The results also suggest that the degree of inhibition of the color change is dependent on the amount of Zn^{2+} added, which is in agreement with earlier viral inhibition experiments of PDA vesicles modified with sialic acid.[7] Finally, the effect of nonspecific adsorption on the color change was investigated. For example, the vesicles do not change color in the presence of other enzymes such as lysozyme and glucose oxidase, both of which only produce a colorimetric response below 5% after more than an hour of incubation with the 40% DMPC/PDA vesicles.

The vesicle color change is ascribed to lipid reorganization and destabilization of the vesicles by hydrolysis of the DMPC embedded in the PDA signaling matrix. In general, the color transitions of PDA-based materials (for example, single crystals, films) are affected by changes in the conformation, packing, and ordering of the pendant alkyl side groups.[4, 32, 33] Tomioka et al.[34, 35] demonstrated that the blue to red color change of pure amphiphilic PDA monolayers contained in a Langmuir trough can be induced directly by changes in lateral surface pressure and lipid packing. The effect is believed to arise from a reduction of the effective conjugated length of the ene–yne backbone that is induced by deformation of the alkyl side groups pendant to the polymer backbone. A similar perturbation is brought about in the DMPC/PDA vesicles by

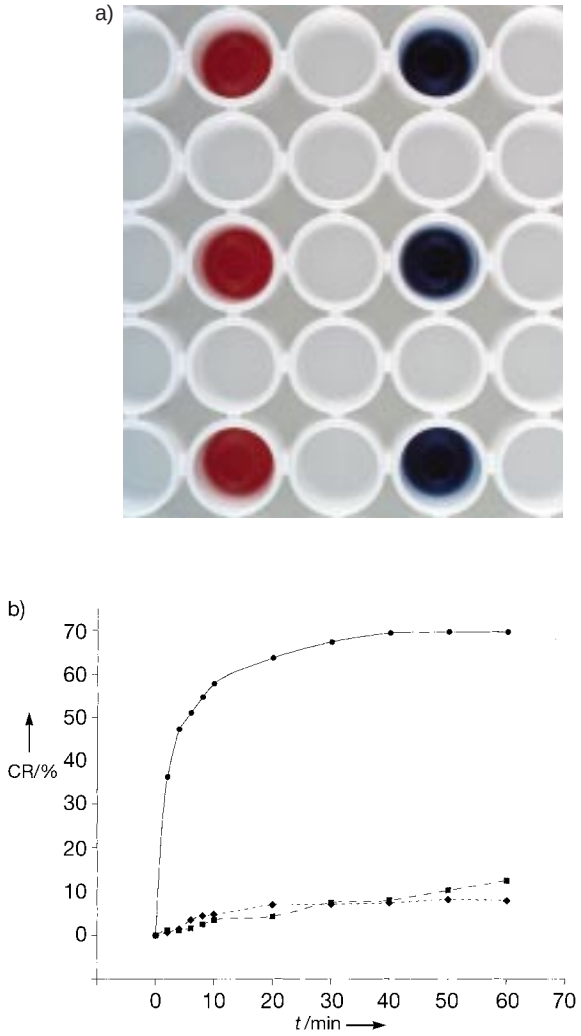

Figure 4. Inhibition of the color response (CR) by a known PLA_2 inhibitor. a) Image of microtiter wells containing a suspension of PLA_2/vesicles in the presence (blue) and the absence (red) of MJ33 (vesicles: 40% DMPC/PDA). b) Colorimetric response curves for DMPC/PDA vesicles in the absence (●, max error 1.9%) and presence of an inhibitor (■ MJ33, max error 6.9%; ◆, $ZnCl_2$, max error 6.5%). For further information see the experimental section.

alteration of the lipid–lipid packing that is induced by hydrolysis of DMPC. This is supported by previous studies that demonstrate hydrolysis by PLA_2 triggers a significant contraction in the area of Langmuir monolayers containing DMPC.[22] The extent of contraction reflects quantitative removal of the hydrolysis products from the monolayer. Fluorescence studies have also demonstrated independently that hydrolysis by PLA_2 destabilizes other DMPC-containing vesicles.[36]

Polymerized mixed vesicles are highly stable against chemical and physical degradation and offer a convenient, economical alternative to enzymatic assays that employ radiolabeled substrates. The vesicle stock solutions described herein have been stored for over six months without affecting the results of the assays. Colorimetric detection of interfacial catalysis by other enzymes such as phospholipase C (PLC) and phospholipase D (PLD) has been achieved also with substrate-modified PDA vesicles,[29] and suggests that the methodology described here is generally applicable. Both of

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these enzymes cleave at the hydrophilic head-group region (phosphate ester) of DMPC. The significance of the detection system described is that it both mimics the natural membrane interface, and also provides a visual reporting component (the conjugated polymer) for the rapid detection of biocatalysis.

The simple, one-step detection method for enzymatic catalysis and inhibition allows convenient adaptation to the high-throughput screening of catalytic inhibitors. In addition, this method may be applied to detect deadly neurotoxins that have enzyme-like activities (for example, β-bungarotoxin). Future efforts are geared towards the study of factors that affect enzyme recognition and activity, parameters that influence reorganization of the conjugated polymer membrane, and adaptation of the colorimetric method to other enzyme systems.

Experimental Section

Figure 2a: The polymerized vesicles composed of 40% DMPC/60% PDA, 1mM total lipid, were diluted 1:10 in 50mM Tris buffer (pH 7.0) to a final volume of 0.5 mL in a standard cuvette, and the spectra were recorded with a Hewlett Packard Spectrophotometer (model 9153C). Bee venom phospholipase A_2 (Sigma) was dissolved in a buffer (pH 8.9) of 10mM Tris, 150mM NaCl, and 5mM $CaCl_2$ to yield a final concentration of 1.4 mg mL^{-1}. 50 μL of this solution was added to the cuvette and the spectrum recorded after 60 min.

Figure 2b: 5 μL of the 1.4 mg mL^{-1} solution of PLA_2 was added to 50 μL of DMPC/DPA vesicles (0.1mM final total lipid concentration). The experiment was carried out in a standard 96-well plate with a UV_{max} kinetic microplate reader (Molecular Devices). The absorption of the vesicle solution was monitored as a function of time at 620 and 490 nm. The data was then plotted as colorimetric response (CR) versus time to yield the color response curves. Colorimetric response is defined here as the percentage change in the absorption at 620 nm relative to the total absorption maxima.[6]

Figure 2c: 5 μL of 40% DTPC/PDA vesicles diluted with 45 μL of 50mM Tris pH 7.0 and 5 μL of 6mM DTNB were incubated with 10 μL of 1.4 mg mL^{-1} PLA_2. The absorbance at 412 nm was monitored over time.

Figure 4b: MJ33 was added to 5 μL of unpolymerized 40% DMPC/PDA vesicles (0.015 mol ratio of MJ33 in the substrate interface) in 40 μL of 50mM Tris (pH 7.0) and 5 μL of a solution of 50mM Tris, 150mM NaCl, and 5mM $CaCl_2$ (pH 8.9). The mixture was incubated at room temperature for 20 min and polymerized prior to measuring the absorption at 490 and 620 nm. 5 μL of a 1.4 mg mL^{-1} solution of PLA_2 was added and the colorimetric response recorded as above. For Zn^{2+} inhibition the enzyme was dissolved in 10mM Tris, 150mM NaCl, and 0.1mM $ZnCl_2$ at pH 8.9.

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Practically Perfect Asymmetric Autocatalysis with (2-Alkynyl-5-pyrimidyl)alkanols

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Organic synthesis plays a central role in natural and technical sciences, and the development of organic reactions that proceed with perfect chemo- and stereoselectivity is an important goal for organic chemists.[1] Reactions that are catalyzed by enzymes in living organisms proceed with extremely high chemo- and stereoselectivities. Enzymes are, however, macromolecules that consist of thousands of amino

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