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Intelligent Biomaterials Based on Langmuir-Blodgett Monolayer Films

Biotinylated Polymers-Streptavidin and Biotinylated Lipid-Streptavidin Recognition Incorporating Photodynamic Proteins

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Our aim is to use Langmuir-Blodgett (LB) trough technology in novel ways incorporating biological components to create new types of monolayer and multilayer materials possessing interesting electronic and optical properties. In a major focus of our research we have demonstrated an LB trough cassette approach to create ordered systems by using either a biotinylated lipid or a biotinylated polymer that first binds streptavidin protein. In a second step streptavidin binds added biotinylated phycoerythrin, a fluorescent antenna protein. For signal transduction possibilities, conducting polymers may be mixed with the biotinylated lipid in the LB monolayer film in this system. This cassette approach would allow the attachment and ordering of any biotinylated species. These techniques can yield ordered monolayer films possessing unusual optical and electronic properties for potential device applications.

THE CREATION OF A CLASS OF INTELLIGENT BIOMATERIALS with sensory, adaptation, or response capabilities comprised of ordered monolayer

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films for sensing applications is our intent. These systems share the LB trough technology as a basis for organizing them into thin-film layered structures. The thin film can be subsequently transferred onto desired substrates for further investigation and integration of their optical and electronic properties. We discuss here the systems we have been investigating that involve using biotinylated lipids or biotinylated polymer, in some cases mixed with conducting polymers, to create ordered monolayer films. This approach involves the utilization of bridging streptavidin or avidin proteins, both possessing four biotin binding sites. After binding to the film, unoccupied biotin binding sites are available in the subphase for further interaction. Other biotinylated biological macromolecules can then interact with the remaining sites on the bound streptavidin or on avidin protein. The result is an ordered monolayer in which the biochemical properties of the biomolecular component can be integrated with the LB monolayer optoelectronic signal transduction properties.

We use the inherently high specificity of the biotin-streptavidin interaction ($K_a = 10^{15}$) (which forms the basis of many assays in the field of biomedical research (1, 2) and has been demonstrated to function in an LB trough experiment) to bind biotinylated macromolecules with high specificity (3-6). The system of biotinylated lipid or polymer monolayers binding avidin or streptavidin, which then can bind and order other biotinylated components in a hierarchical structure forms a general "cassette system" for creating monolayer or multilayer sensing devices.

Biological macromolecules as materials possess superior "intelligent material" properties that have evolved over evolutionary time to function efficiently in integrated macromolecular arrays in cells in response to their environment. Another intelligent material is the ordered two-dimensional LB monolayer, in our approach comprised of mixed biotinylated lipids and electroactive and electrically conducting polymeric lipid materials (such as polyalkylpyrrole and polyalkylthiophene), and biotinylated polyalkylthiophene copolymer system. These systems possess interesting optical and electronic properties (7) that can be exploited for signal-transduction purposes.

A number of different classes of photodynamic proteins have been considered for this work, including algal phycobiliproteins and bacteriorhodopsin. We will focus on our work with the phycobiliprotein phycoerythrin as illustrative of the approaches possible. Phycobiliproteins are components of supraassemblies located on the thylakoid membranes of marine algal cells that function as light receptors to funnel ambient light into the photoreactive center to drive photosynthesis (8). The light receptor chromophores of these proteins are open-chain tetrapyrroles coupled to the protein residues through thioether linkages. The phycobiliproteins (phycoerythrin, phycocyanin, and allophycocyanin in se-

quence) are arrayed in vivo in an antenna-like structure called the phycobilisome, each with a region of maximum and relatively narrow wavelength of absorption in the visible spectrum. Light energy is transferred by a Forster-type mechanism with over 90% efficiency.

Some of these proteins are used commercially as biochemical and biotechnological probes (9). Additional interest centers on the regulatory control of these proteins' synthesis, because these systems are an example of light-regulated transcription, in that the amounts and types of the specific phycobiliproteins present in the phycobilisome in vivo appear to be regulated at the transcriptional level by the spectral features of the available light energy (10).

Methods

Conducting Polymers Synthesis and Characterization. In recent years, conducting polymers have constituted a growing field of science and engineering. One such class of compounds are polymers that have aromatic rings as repeat constituent units. Polythiophene, polypyrrole, and their derivatives are included in this group. These polymers exhibit excellent thermal and temporal stability and may be produced in many chemically modified forms. Although the pristine materials are insoluble because of their stiff macromolecular chains with strong interchain interactions, the materials can be made soluble by attaching long alkyl chains as the side groups of these conducting polymers. Appropriate molecular design can also make them amenable for manipulation on the LB trough. The poly(3-alkylthiophenes) with long alkyl side groups are readily soluble in common organic solvents and can subsequently be processed into uniform mixed monolayer films from their solutions.

Poly(3-hexylthiophene) can be synthesized by dehydrogenation condensation of 3-hexylthiophene by using FeCl_3 in an appropriate medium such as chloroform solution (Figure 1). In a similar manner, other poly(3-alkylthiophene)s have been synthesized from the corresponding monomers. For poly(3-hexylthiophene) the reaction proceeds as follows (11). In a three-necked round-bottomed flask anhydrous FeCl_3 (4.866 g, 0.06 mol) was put under dry nitrogen atmosphere. The reaction vessel was then connected to a vacuum line and evacuated at 100 °C prior to the reaction. Dry nitrogen was then reintroduced into the reaction vessel and dry distilled chloroform (100 mL) was added into the flask under dry nitrogen flow. 3-Hexylthiophene (1.164 g, 0.02 mol) was added dropwise to the metal-halogenide solution under vigorous agitation with a magnetic stirrer. After all the thiophene monomer was added, the reaction mixture was stirred for another 2 days at room temperature to ensure the completion of the reaction. The solution turned blue im-

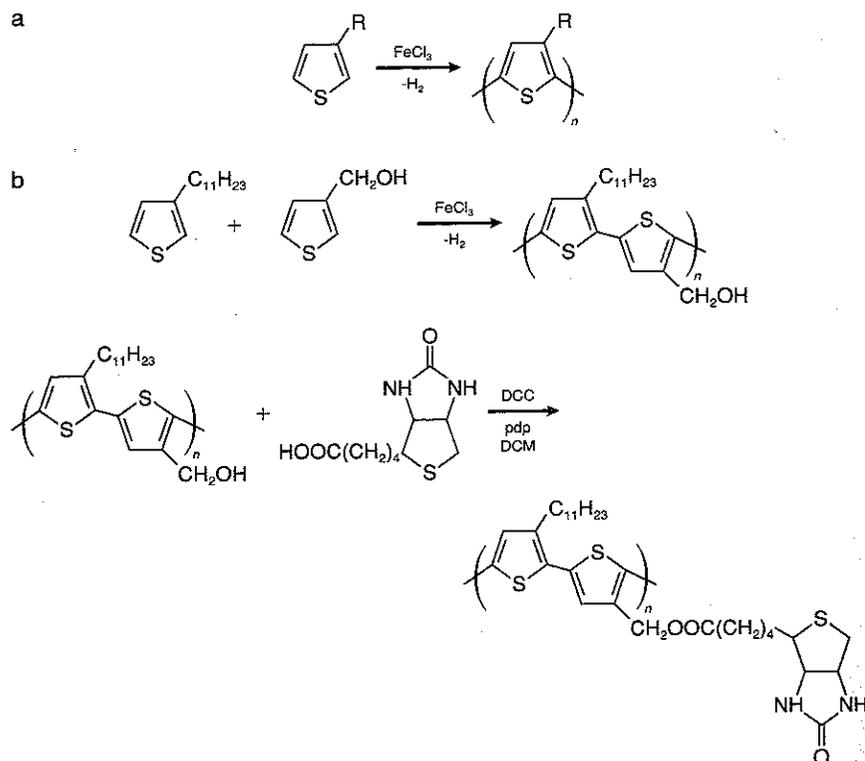


Figure 1. Synthetic schemes of (a) poly(3-alkylthiophene) and (b) B-PMUT.

mediately after addition of the thiophene monomer and took on a deeper color with time (oxidized form).

After completion of reaction, the reaction mixture was precipitated into methanol (500 mL). The resulting blue-black precipitate was collected by filtration. The precipitate turned dark red (neutral form) after being washed alternately with a large amount of methanol and water and was cleaned successively with methanol in a Soxhlet extractor for 20 h. It was further purified through reprecipitation into methanol (poor solvent) from an appropriate solution of good solvent such as 2-methyltetrahydrofuran.

Copolymers of 3-substituted thiophenes, such as poly(3-undecylthiophene-co-3-methanalthiophene) (PMUT), have been synthesized and subsequently biotinylated. Copolymer, PMUT, can be synthesized as follows. Synthetic grade anhydrous ferric chloride (Aldrich), 0.09 mol, was dried under vacuum at 100 °C prior to reaction. Then nitrogen was introduced along with 100 mL of dry chloroform (Aldrich). Next, 0.02 mol of 3-undecylthiophene (TCI America) and 0.01 mol of 3-metha-

nolthiophene (Aldrich) in 10 mL of chloroform was added dropwise with vigorous stirring. The reaction mixture was allowed to stir for 2 days until the reaction was complete. The reactant solution was precipitated into 500 mL methanol (Aldrich). The product was then purified with methanol in a Soxhlet extractor for 2 days.

Attachment of biotin to the PMUT copolymer, resulting in B-PMUT, can be accomplished by the following methods. A solution of 0.01 mol biotin (Biomed), 0.011 mol *N,N*-dicyclohexylcarbodiimide (Aldrich), 0.011 mol PMUT and 0.001 mol 4-pyrrolidinopyridine (Aldrich) in 50 mL dichloromethane (Aldrich) was stirred at room temperature until esterification was complete. The *N,N*-dicycloundecyl urea was filtered, and the filtrate was washed with water (3 × 50 mL), 5% acetic acid solution (3 × 50 mL), again with water (3 × 50 mL), dried (MgSO₄) and the solvent evaporated in a rotary evaporator under reduced pressure to give the biotinylated copolymer.

The UV-visible spectra of poly(3-hexylthiophene), PMUT, and B-PMUT thin film on a glass slide are shown in Figure 2.

An absorption maximum for poly(3-hexylthiophene) is located at 505 nm and two shoulders are noticeable around 550 and 600 nm. Both the copolymers showed an absorption maximum around 400 nm with absorption starting from 600 nm, indicating the presence of extended π -conjugation along the polymer backbone. B-PMUT exhibited a blue shift due to the breakage of π -conjugation by the introduction of biotin.

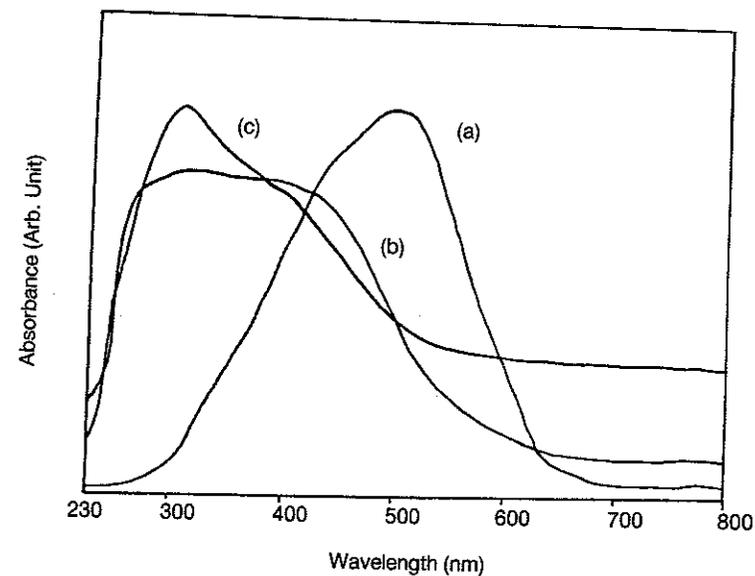


Figure 2. The UV-visible spectra of (a) poly(3-hexylthiophene), (b) PMUT, and (c) B-PMUT.

The Fourier-transform infrared (FTIR) spectrum of solution cast thin films of poly(3-hexylthiophene) showed a distinct peak around 845 cm^{-1} due to the C-H out-of-plane vibration of the 2,3,5-trisubstituted thiophene. FTIR measurements of both PMUT and B-PMUT showed a principal absorption peak at 780 cm^{-1} due to the C-H out-of-plane vibration of the 2,5-disubstituted thiophene, and a distinct peak around 810 cm^{-1} due to the C-H out-of-plane vibration of the 2,3,5-trisubstituted thiophene. B-PMUT exhibited new characteristic peaks at 1678 cm^{-1} due to the ester linkage, and a sharp peak at 3400 cm^{-1} from N-H stretching. Meanwhile, the broad O-H absorption peak at 3400 cm^{-1} shown in PMUT disappeared in B-PMUT (12).

The number average MW of poly(3-hexylthiophene) and B-PMUT were found to be 5×10^3 and 2.5×10^3 g/mol, respectively, through gel permeation chromatography.

3-Hexadecylpyrrole monomer was prepared at Brookhaven National Laboratory, and the synthetic procedure is described in an earlier publication (13).

LB Film Formation and Characterization. The biotinylated phospholipid, *N*-(biotinoyl)dipalmitoyl-L- α -phosphatidylethanolamine triethylammonium salt, (B-DPPE), was purchased from Molecular Probes (Eugene, OR) and used as received. L- α -Dipalmitoyl phosphatidylethanolamine (DPPE) was purchased from Avanti Polar Lipids (Pelham, AL) and was used as received. The unconjugated phycoerythrin (PE), biotinylated phycoerythrin (B-PE), and avidin- and streptavidin-conjugated R-phycoerythrin proteins used in our work were purchased from Biomedica Corporation (Foster City, CA) or from Molecular Probes.

Monolayer studies were carried out on Lauda MGW Filmwaage troughs with a surface area of approximately 930 cm^2 . The subphase was composed of an aqueous solution of 0.1 mM sodium phosphate and 0.1 M NaCl, pH 6.8. In the case of pressure-area isotherms, the lipid and the polymer were spread from a 0.5 mM chloroform solution, and 0.1 mg of the protein in 5 mL of the buffered subphase was injected under the spread film and left to incubate for 2 h at 30°C . Compression was then carried out at a speed of approximately $2\text{ mm}^2/\text{min}$, until collapse of the film was observed. For transfer studies, the lipid and the polymer were spread, followed by protein introduction and incubation in the expanded state for 2 h, and then were compressed to an annealing surface pressure of approximately 15 mN/m , for deposition. Monolayer films were then transferred onto glass solid supports for fluorescence spectroscopy.

Results

LB Trough. Our aim in this area is to create different lipid and polymer monolayers that are ordered and to provide a diverse array of

functional groups both to bind biological macromolecules (by biotin) in ordered arrays and subsequently to use the intelligent properties of the biological systems through signal-transduction mechanisms in the monolayer.

The LB technique has recently been used to prepare such oriented and spatially organized protein-molecular assemblies (3-6, 14). The LB technique is the method of choice because it allows direct manipulation of the molecular components that comprise the assembly. Furthermore, following monolayer formation, the ultrathin monolayer films may subsequently be transferred, a single monolayer at a time, for characterization and ultimately for device fabrication. In addition, judicious selection of the specific lipids incorporated in the monolayer should provide unique biomimetic environments to fully elicit the desired characteristics of the biological component. The initial approach involved the highly specific recognition of biotin on the LB trough subphase surface of biotinylated LB lipid monolayers by streptavidin-conjugated phycoerythrin (Str-PE) and avidin-conjugated phycoerythrin (Av-PE).

LB Trough Formation of Biotin Lipid-Str-PE Monolayers.

The biotinylated lipid B-DPPE forms a well-behaved LB isotherm. This is shown in Figure 3 along with the isotherms of B-DPPE from three experiments, where the following proteins were injected into the trough subphase: PE, Str-PE, and Av-PE. In all cases the isotherms displayed a relatively steep slope after a pressure of 15 mN/m , which corresponds to an area per molecule of approximately 100 \AA^2 . However, the biotin binding Str-PE and Av-PE protein monolayers, but not the PE control, exhibited much different behavior in the expanded state. A significant increase in surface pressure was observed with these monolayers at larger areas per molecule. This behavior suggests that the conjugated protein systems are in some way incorporating themselves into the B-DPPE monolayer in the expanded state. As compression is continued, the protein-injected monolayers reach a stage where they actually overlap the pure B-DPPE isotherm. This behavior differs from that observed in a previous study where fluorescein-labeled streptavidin was injected under the subphase of a biotinylated lipid monolayer using somewhat similar preparative conditions, but a significantly higher subphase ionic strength and without any phosphate buffer (3).

In the present case the large isotherm shift with binding Str-PE and Av-PE may be rationalized by the bulkiness of these conjugates. Phycoerythrin by itself is known to be disc-shaped, having dimensions of approximately $60 \times 120\text{ \AA}$ and a 240-kD MW (8). In the schematic presented in Figure 4, we indicate the effect of the large Str-PE conjugate on the expanded state pressure. When the Str-PE conjugates bind to biotin in the expanded state, a significantly greater pressure

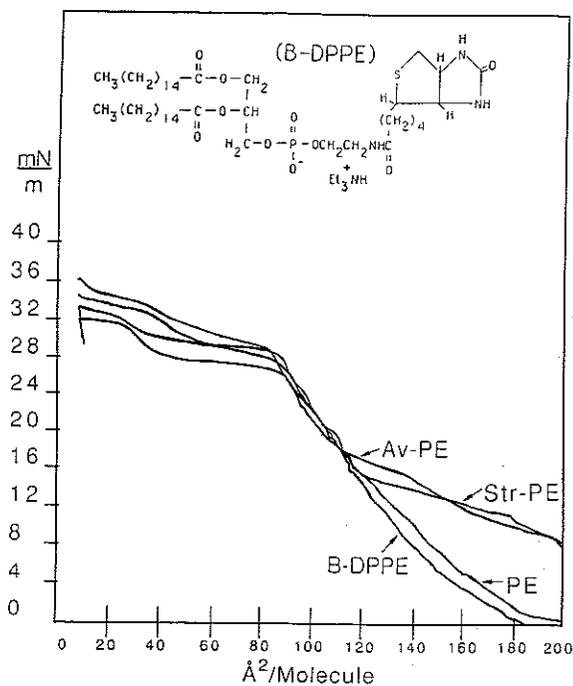


Figure 3. Structure of the biotinylated phospholipid (B-DPPE) and pressure-area isotherms of B-DPPE and B-DPPE with protein PE, Av-PE, and Str-PE injected into the subphase. (Reproduced with permission from reference 15. Copyright 1992.)

develops from protein-protein steric interactions than in the case of the small streptavidin (60 kD) by itself.

The overlapping of the isotherms for Str-PE and Av-PE with the others also fit this model because the bulky conjugates will swing down into the subphase to reorient as a monolayer as the barrier pressure is increased. The control experiment of PE protein only injected into the B-DPPE monolayer exhibits the same isotherm as B-DPPE, supporting the idea that biotin binding to the streptavidin and avidin binding sites are solely responsible for the monolayer isotherm film effects seen.

Formation of Polymer-Streptavidin-Biotinylated Protein Monolayers. We next demonstrated that the fluorescent PE protein could be attached to form a fluorescent monolayer film in a sequential fashion by using a modular or "cassette" approach, involving the streptavidin-biotinylated copolymer interaction. In this case, streptavidin is injected under the subphase of an expanded B-PMUT film. Then, in a subsequent step, biotinylated PE was injected under the film and allowed

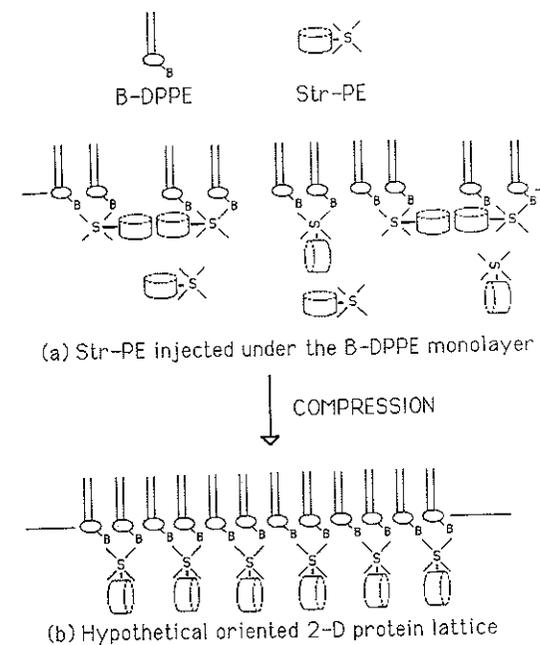


Figure 4. Idealized schematic of the binding and two-dimensional ordering of Streptavidin-derivatized protein monolayers onto a biotinylated lipid LB film. (Reproduced with permission from reference 15. Copyright 1992.)

to bind to the other available biotin binding sites on the bound streptavidin. The cassette approach is shown in schematic form in Figure 5. The isotherms for this sequence of experiments are displayed in Figure 6. Clearly, a different behavior is noted from our previous isotherm. In this case an increase in area throughout the entire compression cycle occurs not only in the expanded phase but also for the monolayer when B-PE was injected. This continuous expansion suggests that the streptavidin and B-PE are interacting with the monolayer film, but do not reorient upon compression in the same manner as the Str-PE system.

As already discussed, this difference may be due to the sizes of the protein systems interacting with the B-DPPE or B-PMUT. The relatively small streptavidin does not obviously create any significant pressure in the expanded state, as evidenced by its isotherm. However, the streptavidin followed by B-PE, a bulky system attached to the expanded film, does create a significant additional pressure noticeable in the isotherm. These results seem to compare well with the initial observations of Blankenburg et al. (3), and to suggest that the bulkiness of the protein initially interacting with the monolayer plays a significant role in the monolayer formation.

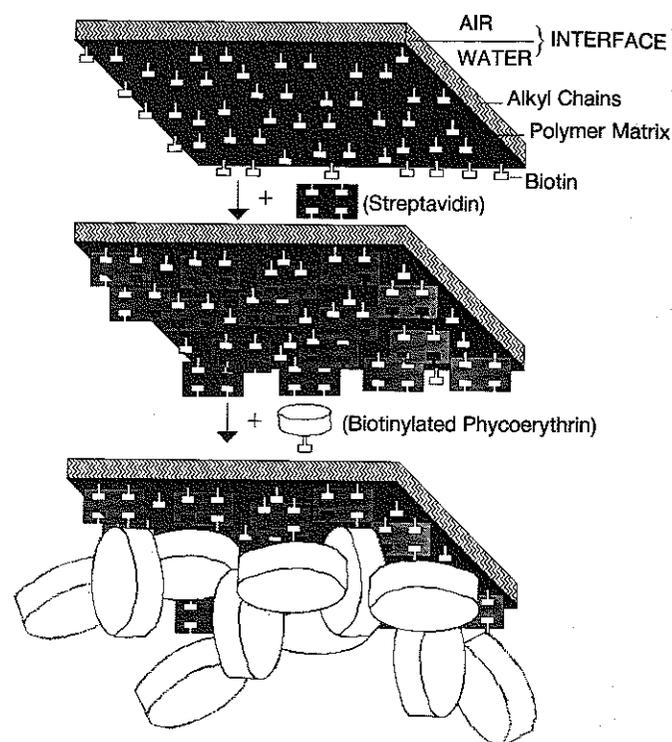


Figure 5. Schematic of the experimental sequence and hierarchical ordering of the protein on the subphase surface of the B-PMUT monolayer in the "cassette" approach.

Using the LB trough technique, we have shown that the mixed-monomer system of either substituted polyalkylthiophenes or polyalkylpyrroles and B-DPPE are capable of forming good monolayers as seen in their isotherms in Figure 7. Both B-DPPE and poly(hexadecylpyrrole) form good characteristic isotherms and their 1:1 mixture has an isotherm of intermediate character. Poly(octylthiophene) mixed with B-DPPE possesses a modified B-DPPE isotherm as shown in Figure 8. These film-forming abilities are important because our intention is to use the optical and electronic properties of the conducting polymers like alkylpolythiophenes, in signal transduction roles in the monolayers of systems such as those described previously. Bulk conductivity measurement of the poly(3-hexylthiophene) has been carried out by the four-point probe method and gives a value of 0.012 S/cm (16). LB films of these electroactive systems containing biomolecules have been successfully prepared, and the electrical measurements are currently under investigation.

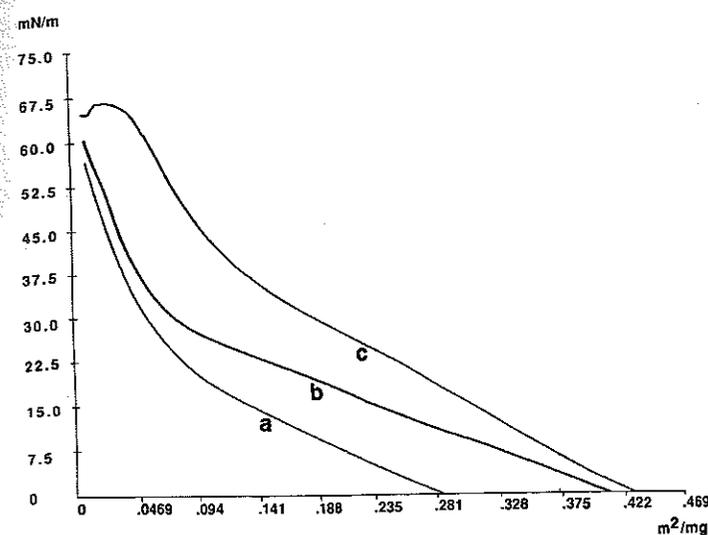


Figure 6. Pressure-area isotherms of (a) B-PMUT, (b) B-PMUT + streptavidin-injected, and (c) B-PMUT + streptavidin + B-PE injected, "cassette" system.

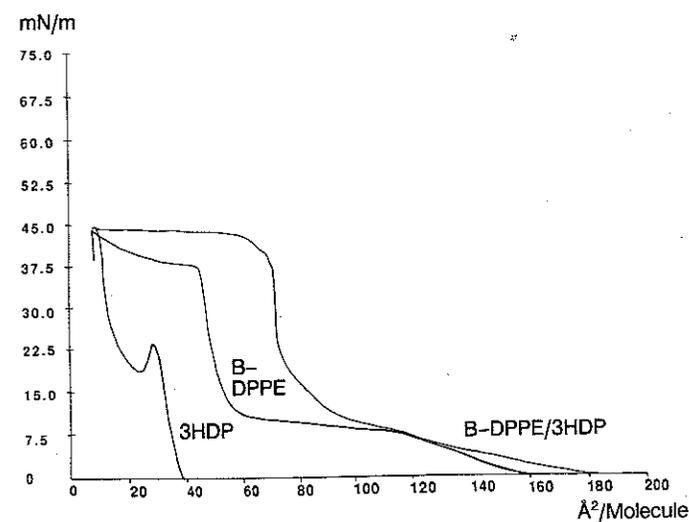


Figure 7. Pressure-area isotherms of B-DPPE, 3-hexadecylpyrrole and a 1:1 mixture of B-DPPE and 3-hexadecylpyrrole.

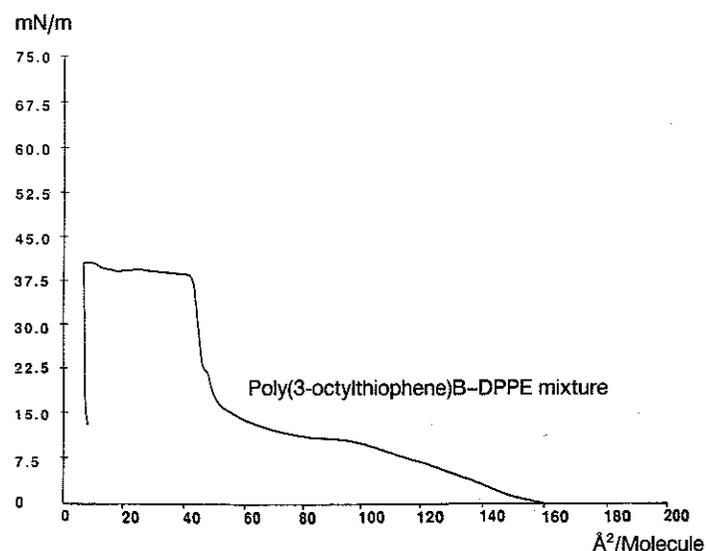


Figure 8. Pressure-area isotherm of a mixture of B-DPPE and poly(3-octylthiophene).

Fluorescence Spectroscopy. Following formation of the films, presented in the isotherms of Figures 3 and 6, we transferred the monolayer films for further characterization. For this transfer the film was compressed to an annealing surface pressure of approximately 15 mN/m. Monolayer films were then vertically transferred onto hydrophilic glass solid supports, with transfer ratios ranging from 100 to 150%. Vertical deposition results in transfer ratios ranging from 100 to 150% on the upstroke. This high value may be explained by possible disruption of this now very rigid film during transfer, which results in either a slight collapse of the film or reorganization of the molecules upon passage of the substrate through the film.

Transferred monolayer protein films were characterized by fluorescence spectroscopy. The measurement system is shown schematically in Figure 9. The pump light source was an argon ion laser, with 10-mW laser power. The excitation wavelength used for PE was 496.5 nm, which is close to the absorption peak of the native PE. The laser beam was collimated with a cylindrical lens and the illuminated area of the sample was imaged, with 1:1 ratio, onto the entrance slit of the monochromator. A 500- μm slit width of the monochromator provided a 6- \AA spectral resolution. Emission was scanned from 500 to 700 nm and the signal was detected with a photomultiplier tube (PMT) cooled to -20°C and then sent to a photon counter with an integration time of 1 s. Background correction for the glass solid support was performed. The photon counter

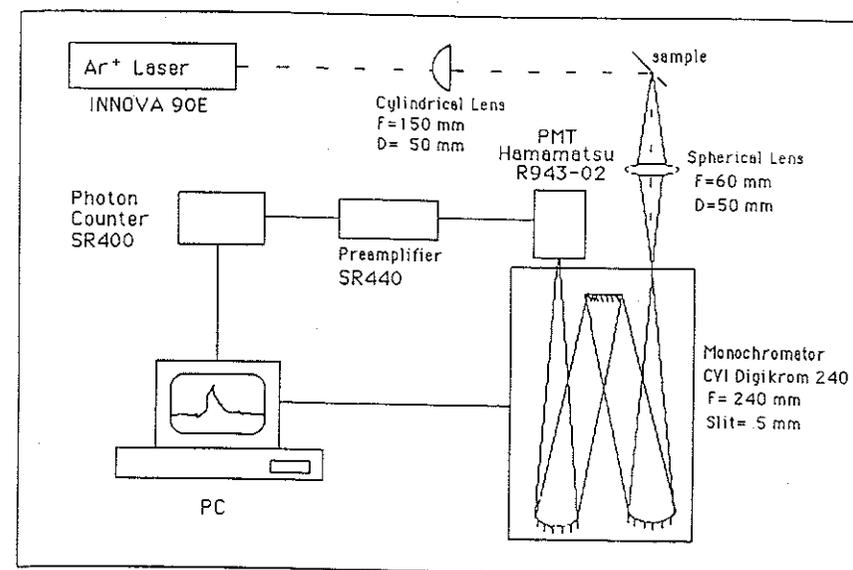


Figure 9. Schematic diagram of the fluorescence set-up. (Reproduced with permission from reference 17. Copyright 1992 Plenum.)

and monochromator were both interfaced to a personal computer. As the monochromator was scanned at 10- \AA increments, the computer recorded the counts from the photon counter in the set range.

Because of the intense fluorescence signals from the high quantum efficiency multiple chromophores of the phycobiliproteins, fluorescence spectroscopy provides a very sensitive probe for the monolayer protein film samples. Figure 10 displays the emission spectra for the films described in the Figure 3 isotherms. The native emission spectra for PE can be seen in the Str-PE-injected sample, which is bound to B-DPPE. However, no native fluorescence can be seen for the PE-injected B-DPPE film, or when Str-PE was injected under a DPPE lipid film, which lacks the biotin functionality. These controls establish that the highly specific biotin-streptavidin interaction is responsible for the protein binding to films observed here.

In Figure 11 a similar result can be seen with Av-PE injected under the subphase, resulting in binding and a native PE-emission spectrum. Two further negative controls were performed, as shown, to support the conclusion that the biotin-streptavidin interaction is responsible for the binding of PE in these monolayer films. Furthermore, the visual fluorescence from these films was continuous at the light microscope level of resolution. These data are not shown, because the low-fluorescence intensity observation could not be photographically imaged.

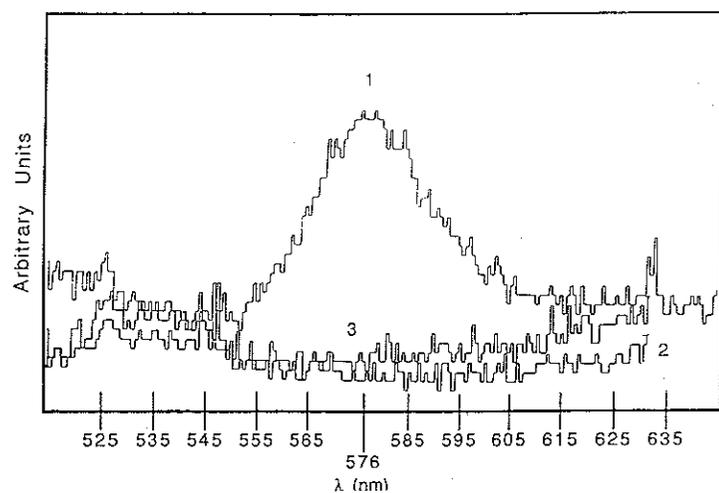


Figure 10. Fluorescence spectra of protein-adsorbed LB films. Subphase: 0.1 mM Na phosphate buffer, pH 6.8, and 0.1 M NaCl. 1. B-DPPE + Str-PE-injected, 2. B-DPPE + PE-injected (control) (2), and 3. DPPE + Str-PE-injected. (Reproduced with permission from reference 15. Copyright 1992.)

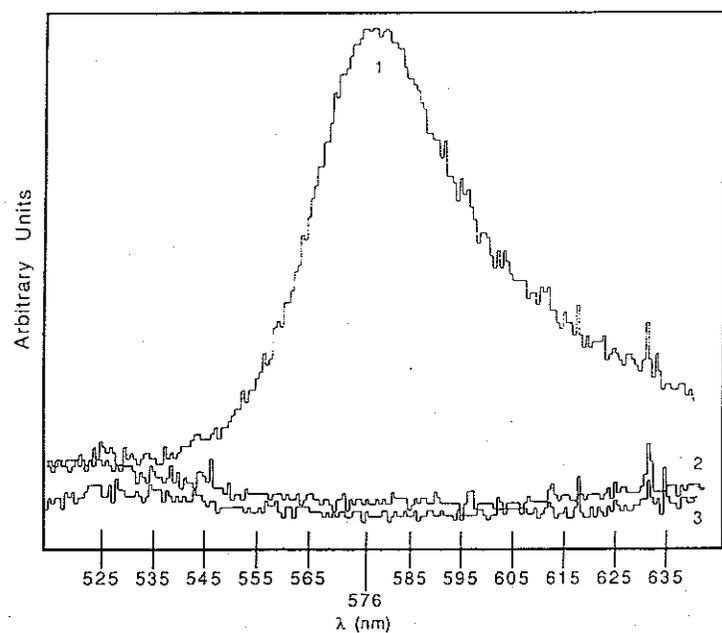


Figure 11. Fluorescence spectra of protein adsorbed LB films. Subphase: 0.1 mM Na phosphate buffer, pH 6.8, and 0.1 M NaCl. 1. B-DPPE + Av-PE-injected, 2. control (monolayer cleaned off the subphase) (2), and 3. control (slide down in subphase only).

Finally, in Figure 12 we demonstrate that monolayer films containing B-PMUT formed by the second LB method, using the cassette approach, also result in native PE emission spectra. These results argue that we are measuring a native protein chromophore environment for the tetrapyrrole moiety in PE. Thus, at least for this protein, the LB methodology yields an undistorted native structure bound to the LB monolayer. This may be an important point, because the significant advantage for the use of this technique in creating intelligent biomaterials would be its ability to stably incorporate labile biological components. This apparent stability may have to do with both the anchoring by biotin bound to its streptavidin binding site, as well as the potential for close protein packing known to occur for streptavidin in these monolayers (3).

Photoconductivity. Photocurrent from layered-photodynamic-protein systems with different chromophores may be used as a probe for sensing the incident radiation. Prior to measurements on multilayered structures, photoconductivity measurements were carried out on dried films of PE. About 20 μL of an aqueous solution of PE (concentration 1 mg/mL) was deposited on an interdigitated gold electrode and allowed to dry at room temperature. Contact was made to the electrode system through silver-plated copper wires attached with silver paint, and the photoconductivity as a function of direct current (dc) bias voltage was measured at a given laser-power level. The arrangement of the apparatus for these measurements is shown in Figure 13. The light source was

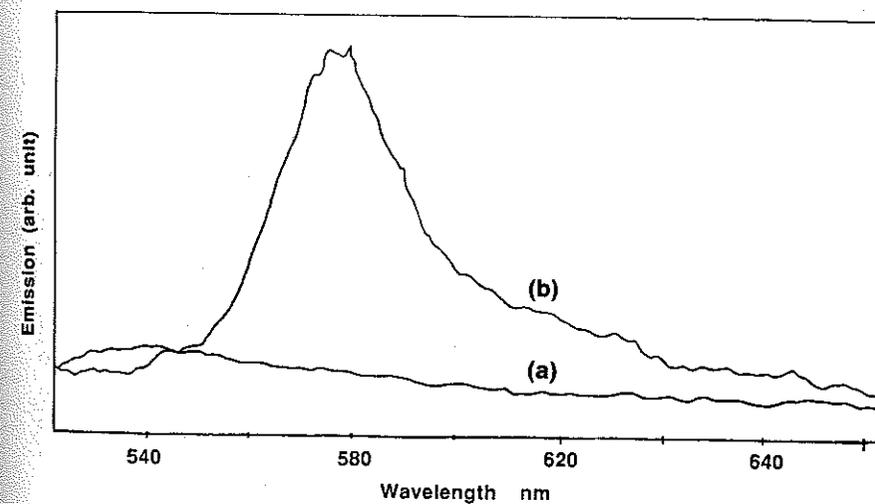


Figure 12. Fluorescence spectra of (a) B-PMUT (b) B-PMUT + Str + B-PE monolayers on glass substrates Subphase: 0.1 mM Na phosphate buffer, pH 6.8, and 0.1 M NaCl.

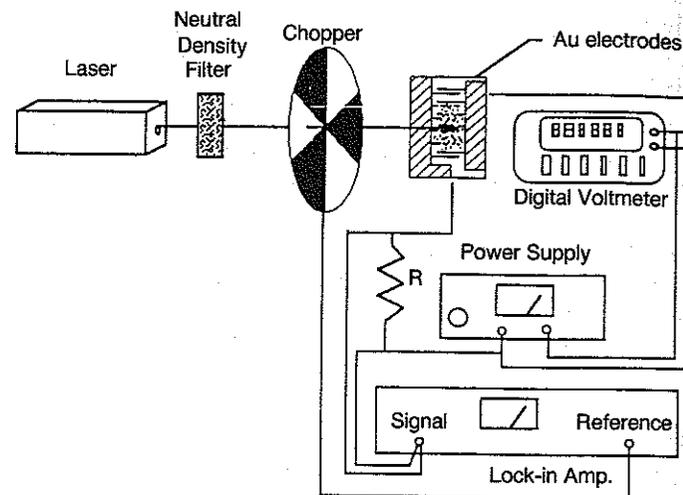


Figure 13. Schematic arrangement for the photoconductivity measurement experiment.

selected lines from an Ar⁺-ion laser (Coherent Innova 90), operating at a 10-mW level, further attenuated through a variable neutral density filter to the indicated value. The laser radiation was chopped, and the photocurrent was measured across a 1-M Ω resistor with a lock-in amplifier. The dc bias voltage was supplied by standard laboratory power supplies.

A typical measurement run at 496 nm is shown in Figure 14. The photocurrent saturates at higher bias voltages. The slight downward drop after saturation could be due to time-dependent effects, such as photodegradation of the protein, or polarization, and other electrochemical reactions at the electrodes, which would be both time- and voltage-dependent. The detailed conduction mechanism needs to be established, but involves charge generation at the chromophore, and transport assisted by the high external dc field applied (the interelectrode separation is only 15 mm, so the fields involved are quite large).

Measurements carried out on buffer sample blanks showed very small photocurrents, so that the photocurrent can be definitely assigned to the photodynamic protein system. Large amounts of electrolytes (as in buffers) interfere with the measurements, by forming solid layers on the electrodes and by creating excessive electrical noise, due to large electrolytic currents.

Discussion

Thin films of biologically derived materials have already been shown to possess interesting optical and electronic properties with potential future

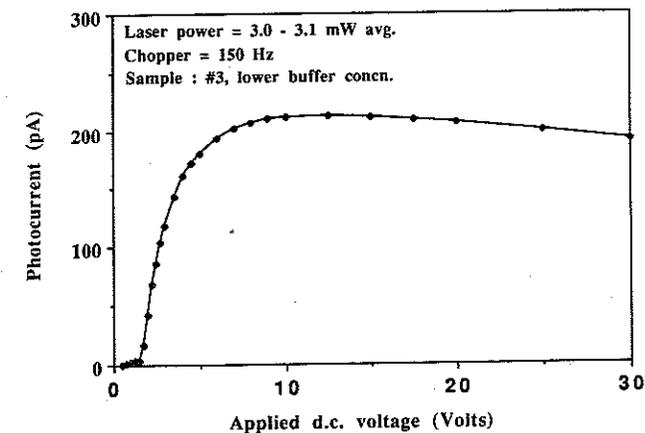


Figure 14. Photocurrent as a function of applied dc voltage in films of PE.

applications. LB assemblies of the kind described in this chapter further expand the role of biomaterials. Synthetic materials may be incorporated into the monolayers to tailor their properties for specific applications. Signal transduction in these materials may result in monitoring of fluorescence, chemiluminescence, photoconduction, absorption, or surface plasmon resonance signals.

Through combinations of photodynamic proteins, organized monolayers, and conducting polymers, a variety of materials and assemblies with intelligent properties can be designed and assembled. These systems would have the ability to respond in a highly specific fashion to optical and electronic inputs and to produce characteristic optical or electronic outputs. In addition, the systems should be stable, sensitive over a broad photodynamic range, and should provide opportunities to create conformal coatings. Most significantly, these approaches to assembling the individual components in the system are generic. Thus the cassette approach described allows for the incorporation of virtually any protein that can be derivatized with avidin, streptavidin, or biotin in virtually any hierarchical array desired based on the multiplicity of binding sites. Aside from the photodynamic proteins themselves, enzymes, antibodies, and nucleic acid probes could be incorporated into specific device designs based on the cassette technology and measurement methodologies described.

Acknowledgments

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Colloid Chemical Approach to Band-Gap Engineering and Quantum-Tailored Devices

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Silver-magnetic and semiconductor particulate films have been generated in situ at monolayer- and bilayer-lipid-membrane interfaces. The chemical method of preparation involved the attachment of one of the precursors to the membrane and the infusion of the other precursor across the membrane from the opposite side. Silver particulate films have also been generated by the electrochemical reduction of silver ions at monolayer interfaces. Evolution of the particulate film involved the initial formation of well-separated, 20-30-Å diameter and 5-6-Å high nanoclusters that grew in height to longer and more densely packed conical particles, which ultimately formed the interconnected arrays of the porous particulate film. Preparation, characterization, and potential applications of these systems are discussed.

RECENT ADVANCES IN MOLECULAR-BEAM EPITAXY have permitted the atom-by-atom generation of heterostructures. Such band-gap engineering has led to the formation of quantum-confined semiconductor particulates and particulate films that have enormous potential applications in memory storage and optical switching devices. This type of molecular-beam epitaxy requires ultrahigh-vacuum technologies, ultrapure facilities, and unique and expensive instrumentation. The approach has been limited to very few selected semiconductors, predominantly involving the GaAs-AlAs system. Furthermore, the approach primarily involved physicists. Realizing the opportunities of semiconductors we initiated a colloid- and surface-chemical approach to advanced materials in our laboratories. The approach was inspired by the organizational capability of the biological membrane, and the approach utilized monomolecular-

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