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Photoswitchable Biomaterials: En Route to Optobioelectronic Systems

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Photostimulation of chemical or physical properties of molecules, macromolecules, and biopolymers provides the basis for the development of future optobioelectronic devices. A few potential applications of chemical assemblies exhibiting light-stimulated functionalities include optical signal recording and amplification, tailoring of reversible biosensors, microstructuring and patterning of surfaces, design of photosensitive chromatographic matrices, and the development of target-activated therapeutic substances.[1] For most of these applications, reversible photoswitchable functions of the chemical assemblies are desirable. Reversible light-stimulated control of physical or chemical properties of photoisomerizable polymers[2] and molecular host−guest complexes[3,4] is well documented. Cyclic volume changes, phase transitions, viscosity control, and wettability were reversibly controlled in photoisomerizable polymers.[5] The binding properties of host molecules such as crown ethers or cyclodextrins were regulated by covalent linkage of photoisomerizable units.[6,7] These photoisomerizable molecular receptors were applied in driving light-stimulated active transport of ions across liquid membranes and membrane mimetic systems.[8]

Photostimulation of the biocatalytic and highly specific recognition and binding functions of biomaterials would enable their application in optobioelectronic devices. Photoactivation of biomaterials, and specifically of enzymes, can be accomplished by two general approaches including a single-cycle activation of the biocatalyst and a reversible photostimulated activation−deactivation of bioactive materials.[1] Single-cycle photoactivated biomaterials were developed using photoprotected ("capped") materials,[9,10] and their potential applications as target-activated therapeutic substances were discussed.[1,11] Reversible photostimulation of enzymes was accomplished by the application of photoisomerizable enzyme inhibitors[12,] and, with partial success, by modification of the enzyme active site with photoisomerizable components.[13,14] This Account summarizes the research activities of our laboratory in developing novel means to reversibly photostimulate the activities of biomaterials and addresses the future perspectives of these substances in the development of optobioelectronic devices.

We address two general methods to photostimulate the functions of biomaterials. One approach includes the chemical modification of the biomaterial by photoactive units that yield a semisynthetic light-triggered biomaterial. The second approach applies an external photoactive interface that communicates with the biomaterial by information transfer that triggers "ON" and "OFF" the biological functions. These photoactive interfaces can be

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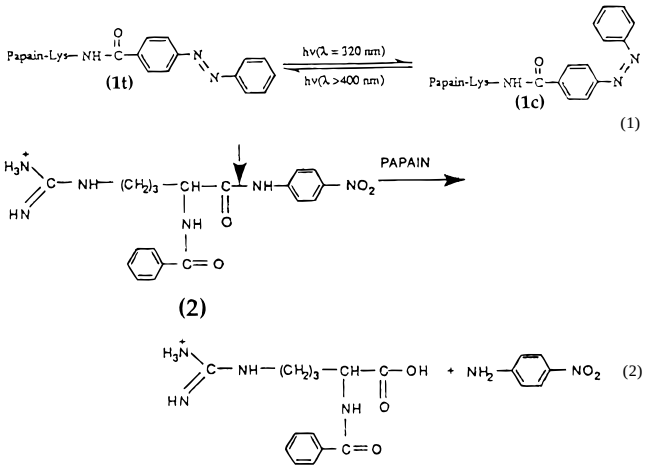
viewed as "command interfaces" for the biological substances. Activation and deactivation of biomaterials by external light signals represent the optical recording events. Physical, or electronic, transduction of the optical recorded optical signals provides the elements of a "write−store−read" biological optical memory. The present review is aimed to reveal the concepts of tailoring photoswitchable biomaterials and to demonstrate means to assemble these new substances into optobioelectronic systems.

Photostimulation of Proteins by Covalent Linkage of Photoisomerizable Components

It has been suggested that photostimulation of protein functions could be accomplished by covalent attachment of reversible photoisomerizable components to the biopolymers. In one photoisomer state, A, the tertiary structure of the protein and its active site configuration are retained and the biopolymer is activated for its biological function, namely, biocatalysis, binding, etc. Light-induced isomerization of the photoactive anchored components to the second photoisomer state B distorts the protein, and its active site is structurally perturbed. This leads to deactivation of the biomaterial, and its biological function is switched off. Back-isomerization of the structurally perturbed protein to state A restores its bioactive structure and regenerates its activity, switch "ON". Perturbation of the biomaterial could be effected by steric or electrostatic (attractive or repulsive) distortion introduced by the respective photoisomer state.

Papain was chemically modified by covalent linkage of 4-carboxy-*trans*-azobenzene to the protein lysine residues.[15] The *trans*-azobenzene papain, (**1t**), with a loading of 5 units, retained 80% of the native biocatalyst activity and exhibited reversible photoisomerizable properties, eq 1. The photoisomerizable enzyme exhibits reversible biocatalytic activities for hydrolysis of *N*-α-benzoyl-D,L-arginine-4-nitroanilide (**2**), eq 2. The *trans*-azobenzene

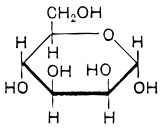


(1)

(2)

papain (**1t**) retained its biocatalytically active protein structure and effected the hydrolysis of **2** (v = 2.7 μM·min^{-1}). Photoisomerization to *cis*-azobenzene papin (**1c**) resulted in deactivation of the biocatalyst, and hydrolysis of **2** was inhibited (v = 1.0 μM·min^{-1}). Detailed kinetic analysis of the biocatalyzed hydrolysis of **2** effected by the two isomer states of papain, **1t** and **1c**, revealed that the two enzymes exhibit very similar V_{max} values (V_{max} = 19 mM·min^{-1}) but differ substantially in their K_m values (K_m = 2.2 and 6.5 mM for **1t** and **1c**, respectively). These results indicate that deactivation of the *cis*-azobenzene papain proceeds by a competitive inhibition pathway, where binding of the reaction substrate to the protein was perturbed by the *cis*-isomer photoactive units.

Photostimulation of the binding properties of proteins was demonstrated with concanavalin A, a lectin that specifically binds α-D-mannopyranose (**3**) and α-D-glucopyranose (**4**).[16] The protein was chemically modified



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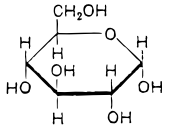

(4)

by photoisomerizable thiophenefulgide, eq 3, and nitrospiropyran, eq 4. The thiophenefulgide-modified concanavalin A exhibits reversible light-induced isomerization between the hexatriene state (**5E**) and the electrocyclized form (**5C**). Nitrospiropyran-modified concanavalin A (**6a**) photoisomerizes to the merocyanine isomer (**6b**), which photocyclizes to **6a** upon visible-light irradiation. The binding properties of the two photoisomerizable proteins were controlled by light. The isomer **5C** shows high affinity for the substrate **3**, where photoisomerization to **5E** effects dissociation of the complex. Similarly, the nitrospiropyran−concanavalin A (**6a**) revealed high affinity for binding the substrates **3** and **4**, while the merocyanine (**6b**) showed substantially lower binding affinities for these substrates.[17] Time-resolved light-scattering experiments have confirmed that photoisomerization of the light-

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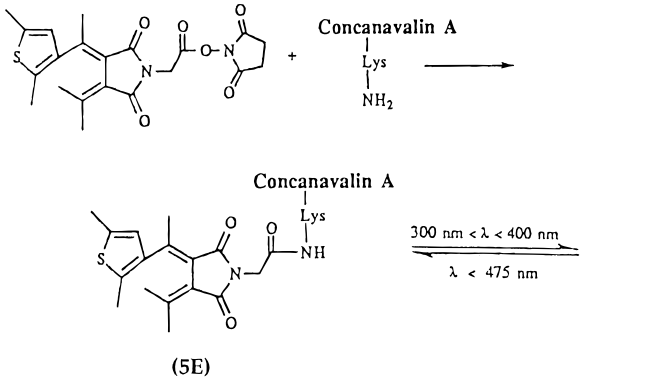
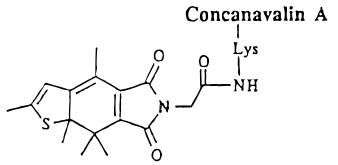
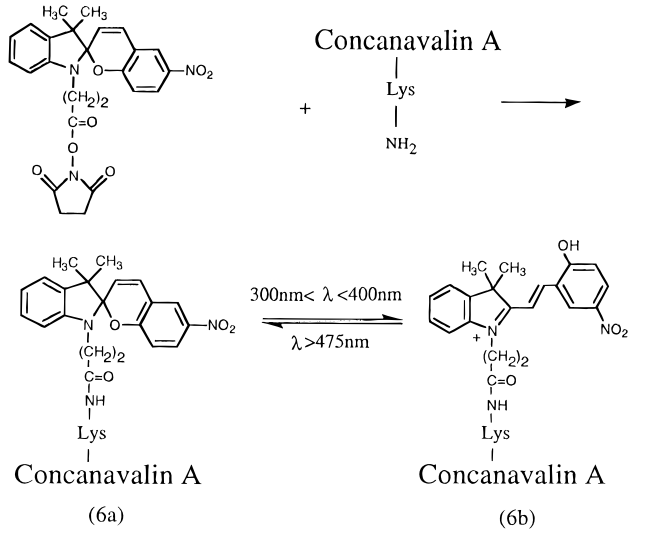


(5E)



(5C) (3)



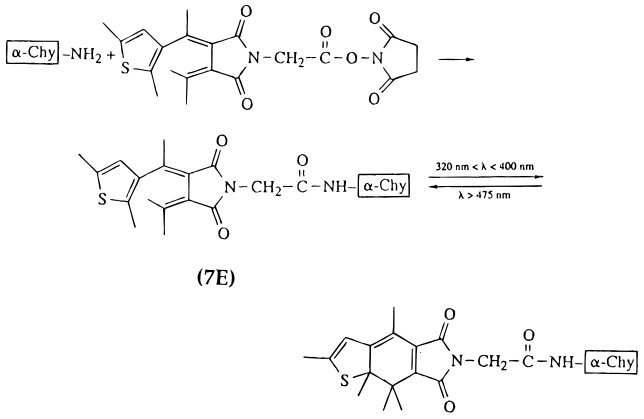
(6a) (6b)

(4)

sensitive groups is, indeed, associated with structural perturbation of the protein and enabled the dynamics of the protein distortion to be followed.[18] Since photoisomerization of spiropyran units occurs within ca. 10 ns, structural perturbations of the protein, proceeding with a longer time constant, are accompanied by a transient change in the intensity of scattered light by the biopolymer. The time-dependent changes in the scattered light intensity reflect, then, the dynamics of structural changes of the protein. The nitrospiropyran−concanavalin A (**6a**) was flashed with a third-harmonic pulsed Nd:YAG laser ($\lambda = 355$ nm, pulse width 2 ns) to induce the isomerization to **6b**. After the flash-induced isomerization to **6b**, the changes in the intensity of scattered light by the protein were monitored by exposing the sample to a CW Ar-laser ($\lambda = 455$ nm). A transient increase in the scattered light intensity, with a time constant corresponding to $\tau = 60$ μs, was detected for the nitrospiropyran− concanavalin A system, with a loading of 6. The increase in the scattered light intensity implies that photochemical transformation of **6a** to **6b** is associated with a shrinkage of the protein, a structural distortion that, presumably, leads to deactivation of the protein binding sites.

For all of the photoswitchable biomaterials an appropriate balance between the loading and the resulting switching efficiency must be maintained. Low loadings result in rather low switching efficiencies, where high loadings often deactivate the biomaterials in both of the isomer forms.

It was suggested that light-stimulated switching efficiencies of enzymes could be enhanced by the application of the photoisomerizable enzymes in organic solvents.[19] The protein structures are stabilized in organic solvents by intraprotein H-bonded water molecules, suggesting that structural photoisomerization of the linked components could enhance the steric distortion of the biopolymer due to perturbation of associated water molecules.[19] α-Chymotrypsin modified by thiophenefulgide units, eq 5, did not reveal photostimulated hydrolytic activities in an aqueous medium, although it exhibited reversible photoisomerizable properties between states **7C** and **7E**. Control of the biocatalyst activities was, however, accomplished in cyclohexane as the reaction medium. The photoisomerizable biocatalyst was examined in the organic solvent at two different configurations. One configuration included the crystallization of the protein **7C** in the presence of the enzyme substrate **8** from an aqueous system, followed by removal of the substrate from the solid protein. This procedure, acting as a general method to imprint the substrate configuration in the active site, is known to enhance the activities of enzymes in organic media.[20] The second configuration involved the use of precipitated **7C** without prior imprint of the substrate. Photostimulated biocatalyzed esterification of **9** by thiophenefulgide-modified α-chymotrypsin was observed in cyclohexane, eq 6. The photoswitchable esterification of **9** by the nonimprinted protein is shown in Figure 1A. The photoisomer state of α-chymotrypsin **7E** exhibits low biocatalytic activity, where photoisomerization to **7C** accelerates the biotransformation (v(**7C**)/v(**7E**) = 5). The cyclic photostimulated esterification of **9** by



(7E)

(7C)

(5)

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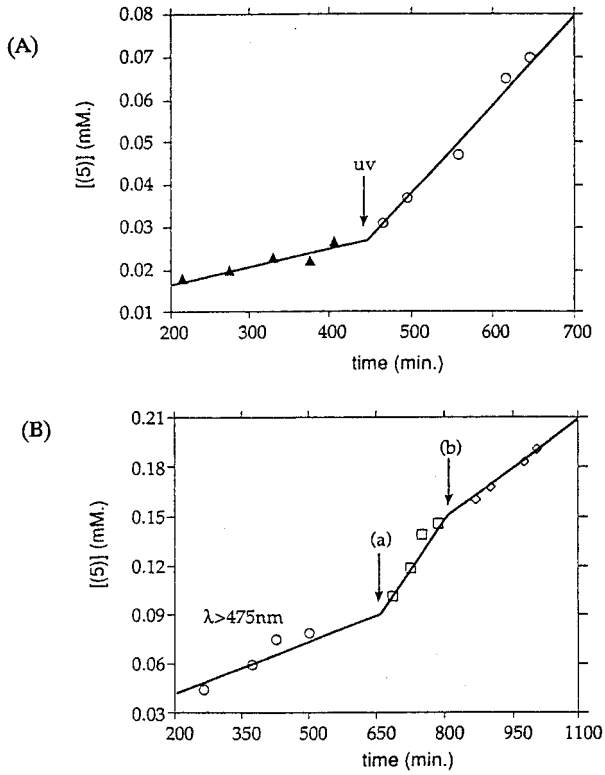
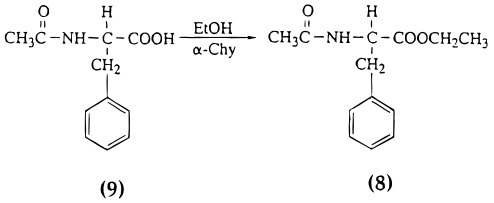


FIGURE 1. Photostimulated esterification of (9) by ethanol in cyclohexane (A) with nonimprinted (7E)-α-chymotrypsin, where the arrow indicates transformation of biocatalyst to (7C)-α-chymotrypsin state by UV irradiation (320 nm < λ < 380 nm), and (B) with imprinted (7E)-α-chymotrypsin, where arrows (a) and (b) represent photoisomerization to (7C)-α-chymotrypsin (320 nm < λ < 380 nm) and (7E)-α-chymotrypsin (λ > 475 nm), respectively.

the imprinted biocatalyst is shown in Figure 1B. The overall biocatalytic activity of the imprinted enzyme was substantially enhanced although the switching efficiency declined (v(**7C**)/v(**7E**) = 2). It was suggested that imprint of the substrate in the protein rigidified the active-site structure. As a result, its strucutral perturbation and subsequent deactivation by the photoisomerization process were limited.



(9) (8) (6)

In the different photostimulated biomaterials discussed to this point, the recorded optical signal was translated to a chemical biotransformation. Application of such photostimulated biomaterials in bioelectronic devices requires, however, the transduction of the recorded optical signal in the form of a viable physical response. Photostimulation of redox enzymes could transduce recorded optical signals as amperometric responses by their electrical interaction with electrode interfaces. Amperometric transduction of recorded optical signals was accomplished using nitrospiropyran-modified glucose oxidase as photoswitchable biomaterial.[21] The photoisomerizable nitrospiropyran flavoenzyme glucose oxidase, SP-GOD, was assembled as monolayer onto an Au electrode. The monolayer revealed reversible photoisomerizable properties, and illumination of the SP-GOD monolayer resulted in the protonated merocyanine-substituted enzyme monolayer, MRH^+-GOD (Scheme 1). In the presence of ferrocene carboxylic acid acting as electron-transfer mediator, electrical communication between the SP-GOD monolayer and electrode interface was attained. This led to bioelectrocatalyzed oxidation of glucose and the observation of an electrocatalytic anodic current (Figure 2). Upon photoisomerization to the MRH^+-GOD monolayer state, the enzyme was deactivated and the bioelectrocatalyzed oxidation of glucose was substantially inhibited. Figure 2 (inset) shows the reversible amperometric responses of the monolayer electrode upon cyclic optical switching of the monolayer between the SP-GOD and MR-GOD forms, respectively.

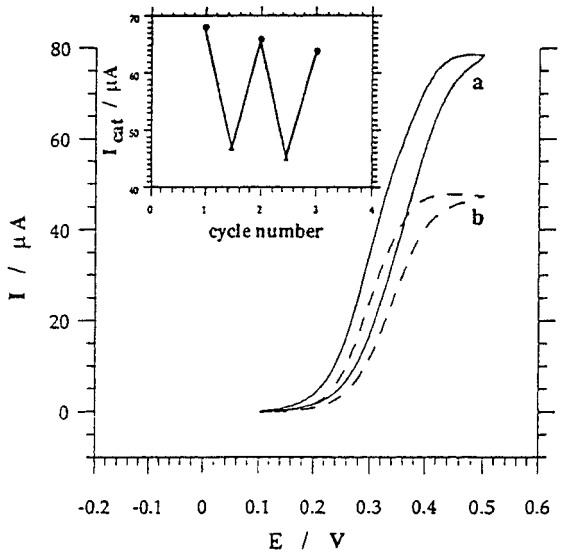


FIGURE 2. Photostimulated electrobiocatalyzed oxidation of glucose (2.5×10^{-2} M) using ferrocenecarboxylic acid as the diffusional electron mediator (5×10^{-3} M) in the presence of (a) SP-GOD (generated by irradiation, 320 nm < λ < 380 nm) and (b) MRH^+-GOD (formed by illumination, λ > 475 nm). Inset: Cyclic photostimulated amperometric transduction of optical signals that photoisomerize the enzyme monolayer between states SP-GOD (●) and MRH^+-GOD (▲).

The described method involves random modification of the biomaterial by the photoisomerizable units. Attachment of photoisomerizable units to protein positions remote from the active site perturbs only slightly the structure of the active-center microenvironment. As a result, incomplete photoswitchable properties of the resulting photoactive biomaterials are observed. Site-specific modification of a redox protein by a photoisomerizable component in the microenvironment of the redox center was recently demonstrated to yield a fully "ON−OFF" photoswitchable enzyme for the amperometric transduction of recorded optical signals.[22] The flavoen-

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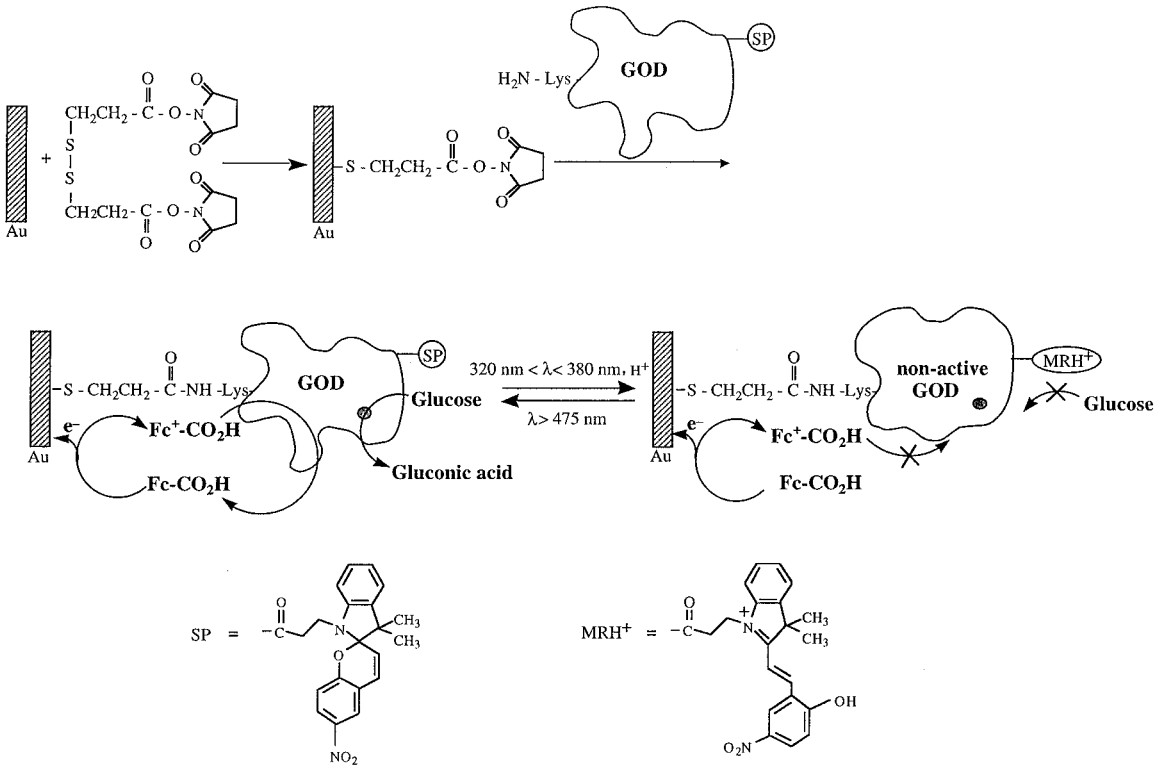
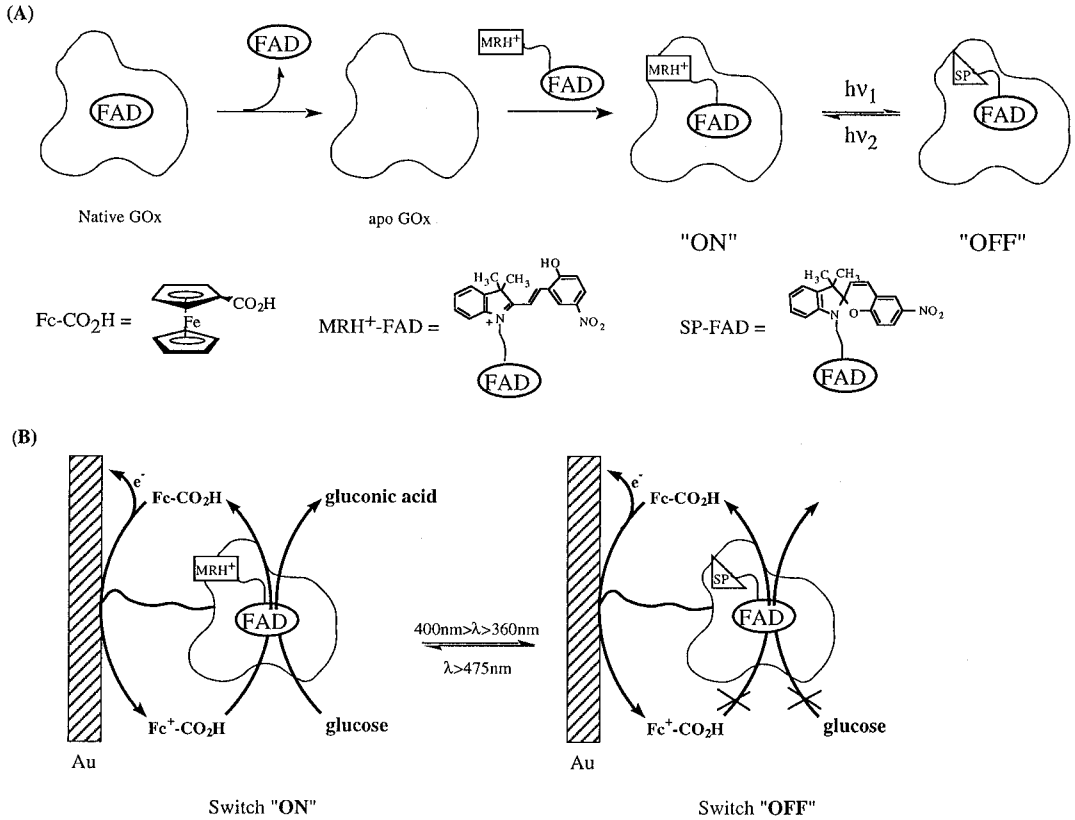
Scheme 1. Assembly of Photoisomerizable Nitrospiropyran-Functionalized Glucose Oxidase, SP-GOD, on a Au Electrode and Mediated Electrobiocatalyzed Oxidation of Glucose by the Two Photoisomers of the Enzyme



Scheme 2. (A) Reconstitution of Apo-glucose Oxidase with a Photoisomerizable Nitrospiropyran-FAD Synthetic Cofactor and (B) Assembly of SP—FAD Reconstituted GOD on a Au Electrode and Photoswitchable Amperometric Transduction of Optical Signals Recorded by the Photoactive Monolayers



zyme glucose oxidase, GOD, was transformed to the apo-GOD by extraction of the native FAD cofactor. The apo-enzyme was then reconstituted with a semisynthetic nitrospiropyran-FAD cofactor to yield the photoisomerizable GOD (Scheme 2A). The resulting reconstituted photoisomerizable enzyme was assembled as monolayer onto a Au electrode (Scheme 2B), and the resulting enzyme electrode was used as active biointerface for

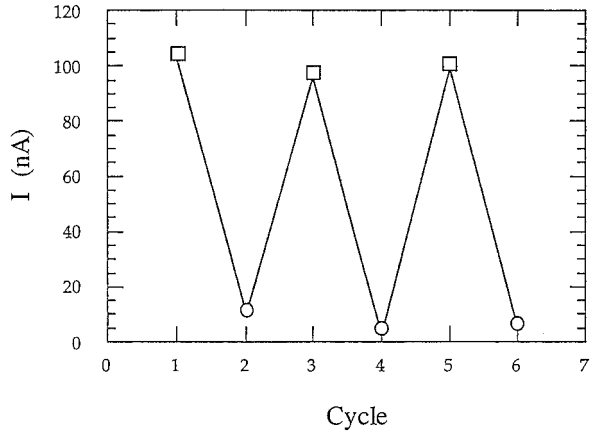


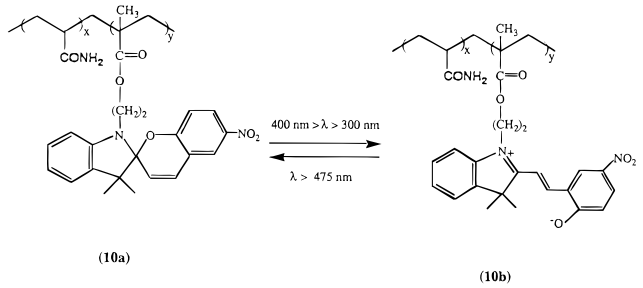
FIGURE 3. Photostimulated cyclic transduction of optical signals recorded by the photoisomerizable reconstituted glucose oxidase monolayer-electrode in the presence of (□) MRH^+-GOD (generated by irradiation, 360 nm < λ < 400 nm) and (○) SP-GOD (formed by illumination, λ > 475 nm).

reversible electrochemical transduction of recorded optical signals. In the presence of the nitrospiropyran-FAD-GOD monolayer, electrical communication between the diffusional electron mediator, ferrocene carboxylic acid, and the protein FAD redox center is blocked. This prohibits the bioelectrocatalyzed oxidation of glucose, yielding a zero electrochemical response of the system. Photochemical isomerization of the monolayer to the merocyanine-FAD-GOD, MRH^+-FAD-GOD, activates the electrical contact between the electron mediator and the FAD-redox sites. This yields the effective bioelectrocatalyzed oxidation of glucose, which is reflected by an amperometric response of the enzyme-electrode. By reversible photoisomerization of the reconstituted enzyme monolayer between the SP-FAD and MRH^+-FAD states, cyclic photochemical deactivation and activation of the bioelectrocatalyzed oxidation of glucose is stimulated (Figure 3). Kinetic analysis of the two enzyme states, SP-GOD and MRH^+-GOD at different concentrations of the electron mediator, revealed that the two enzyme states exhibited different K_m values (K_m(SP-GOD) = 2.6 mM, K_m(MRH^+-GOD) = 7.8 mM), while their I_{max} values were identical. This suggests that the penetration path of the ferrocene carboxylic acid electron mediator to the FAD redox site is perturbed in the presence of the SP-FAD state.

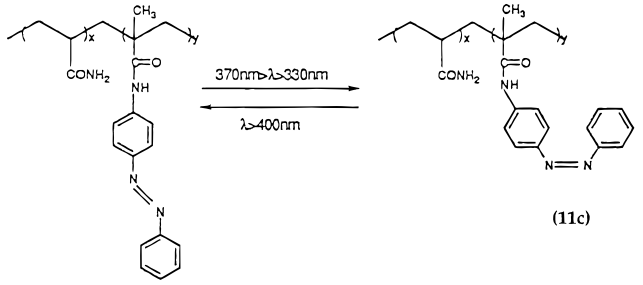
Photostimulation of Biomaterials by Photoisomerizable Assemblies

Interaction of biomaterials with photoisomerizable assemblies or interfaces is capable of triggering "ON" and "OFF" the functions of biomaterials. The permeability of molecular species across photoisomerizable polymer membranes is controlled by light.[23] By immobilization of an enzyme in photoisomerizable polymers and controlling the substrate permeability through the polymer matrices, the biocatalytic functions of the enzyme can be stimulated by light. In one photoisomer state of the membrane, it is nonpermeable toward the enzyme−substrate, and the entrapped biocatalyst is switched off for its characteristic biotransformation. Upon photoisomerization of the membrane it turns permeable to the substrate and the biocatalyst activity is triggered on, switch "ON". Other photostimulated physical parameters of surfaces or membranes such as electrostatic interactions, local pH alterations, or viscosity could, in principle, reversibly activate and deactivate biomaterials.

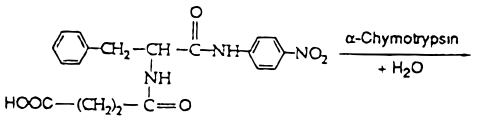
The enzyme α-chymotrypsin was immobilized in the photoisomerizable acrylamide−nitrospiropyran copolymer (**10a**)[24] and the acrylamide *trans*-azobenzene copolymer (**11t**). The polymer-embedded enzyme revealed photoregulated hydrolytic activities, eq 7. The enzyme



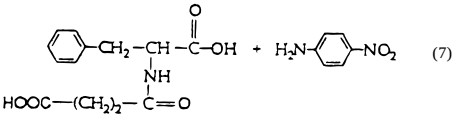
(10a) (10b)



(11t) (11c)



(12)



(7)

activity is entirely blocked in the polymer state **10a**, and photoisomerization to the polymer form **10b** induces the effective hydrolysis of the substrate (V_{max} = 1.5 μmol·min^{-1}). Similar "ON−OFF" cyclic activation and deactivation was observed in the azobenzene copolymer **11**. In this copolymer system, α-chymotrypsin is inactive in the *trans*-azobenzene copolymer **11t**, while it is triggered on for the hydrolytic biotransformation in the copolymer form **11c**. Flow dialysis experiments revealed that the photostimulated activities of the enzyme correlate with the permeability of the reaction substrate **12** through the polymeric membranes. The copolymers **10b** and **11c** were found to be permeable for **12**, while the substrate was nonpermeable through **10a** and **11t**. It was suggested that polar polymer membranes enhance the substrate permeation (polymer **10b** is charged, and the dipole moment of *cis*-azobenzene units comprising **11c** is ca. 3 D).

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The use of charged interfaces to photoregulate redox proteins was recently demonstrated with a photoisomerizable monolayer-modified electrode, acting as "command interface".[25] Amplified amperometric transduction of the recorded optical signals was also demonstrated using the photoisomerizable monolayer electrode and coupled redox enzymes. A mixed monolayer consisting of thiolpyridine and nitrospiropyran was assembled onto a Au electrode (Figure 4A). The photoisomerizable component undergoes reversible photoinduced isomerization between the nitrospiropyran state, SP, and the protonated merocyanine isomer form, MRH^+, that turns the monolayer to a positively charged interface. The pyridine monolayer components were included in the monolayer to associate cytochome *c*, Cyt *c*. Association of Cyt *c* to pyridine-modified electrodes orients the Fe(III)−heme center of Cyt *c* and facilitates the electrical contact between the redox protein and the electrode interface.[26,27] The mixed monolayer includes two functional units, the pyridine component that acts as binding site for Cyt *c* and the photoisomerizable sites that in their MRH^+ state repel the positively charged Cyt *c* and perturb the association of the protein to the monolayer interface. The mixed monolayer of pyridine and SP units allowed association of Cyt *c* to the electrode surface and electrical communication was attained. Light-stimulated transformation of the mixed pyridine-SP monolayer to the MRH^+ state resulted in electrostatic repulsion of positively charged Cyt *c*, and the electrical communication between Cyt *c* and the electrode was blocked. Cyclic control of the electrical contact between Cyt *c* and the electrode was stimulated by reversible photoisomerization of the mixed monolayer. This system enabled the electrical transduction of optical signals recorded by the monolayer assembly through the bioelectrochemical process.

The amplification of the amperometrically transduced signal, being the response to the optical recording event, was accomplished by coupling the nitrospiropyran/pyridine monolayer electrode, and the Cyt *c* as electron transfer mediator, to cytochrome *c* oxidase, COX, and molecular oxygen as terminal electron acceptor (Figure 4A). In the SP-pyridine monolayer state of the electrode, associated Cyt *c* mediates electrobiocatalyzed reduction of COX and subsequently the reduction of O_2. Recycling of the redox-active Cyt *c* component associated with the electrode via the biocatalyzed COX/O_2 reduction results in an electrocatalytic cathodic current that amplifies the primary electrical contact of Cyt *c* with the electrode. Figure 4B compares the amperometric response of the Cyt *c*/COX/O_2 assembly in the presence of the SP-pyridine and MRH^+-pyridine mixed monolayer-electrode. The electrocatalyzed reduction of O_2 is blocked in the presence of the MRH^+-pyridine monolayer electrode that lacks electrical communication with Cyt *c* whereas a high catalytic cathodic current is observed with the SP-pyridine monolayer. The cyclic amplified amperometric transduction of the photonic signals that activate the monolayer interface

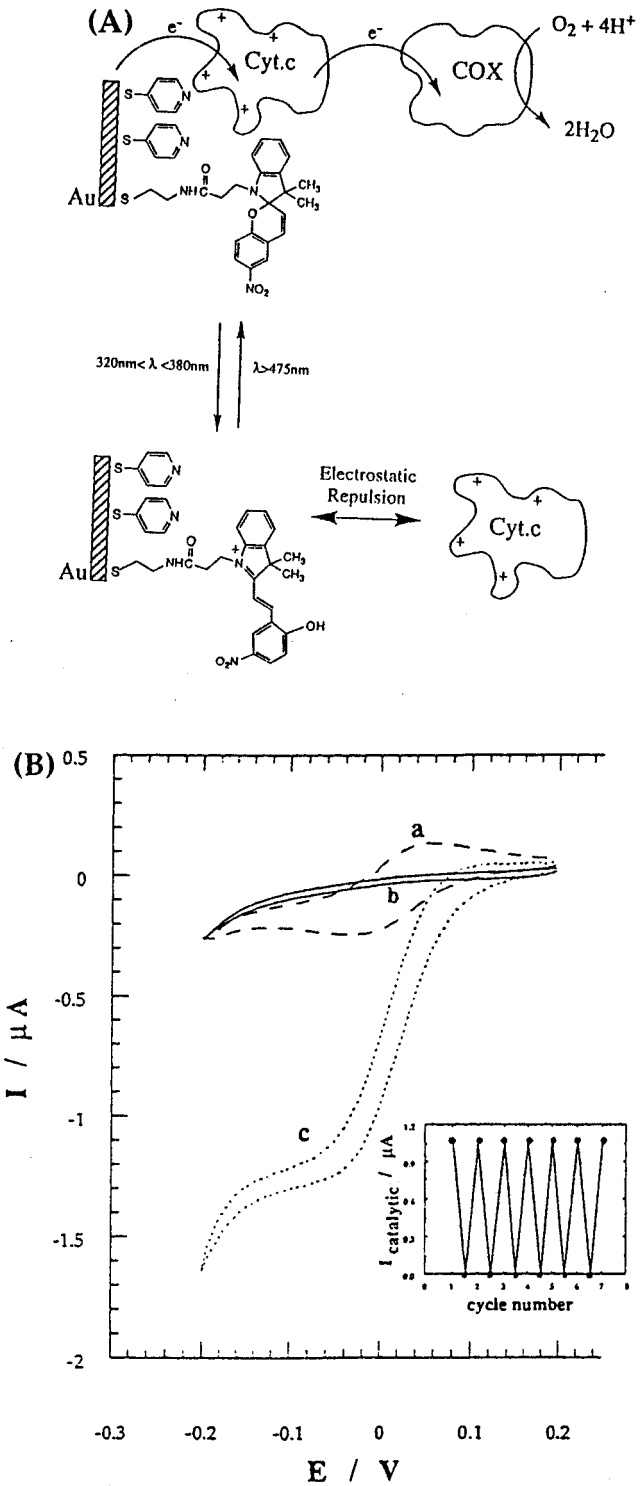

FIGURE 4. (A) Assembly of a thiol−pyridine−nitrospiropyran mixed monolayer on a Au electrode. Binding of Cyt *c* to the monolayer activates the inter-protein reduction of COX and the bioelectrocatalyzed reduction of oxygen. (B) Amperometric responses of the thiol−pyridine−photoisomerizable mixed monolayer (a) in the presence of SP-pyridine mixed monolayer and Cyt *c* alone (1×10^{-4} M); (b) in the presence of MRH^+-pyridine mixed monolayer and Cyt *c* (1×10^{-4} M)/COX (1×10^{-6} M)/O_2 and (c) in the presence of the SP-pyridine mixed monolayer and Cyt *c* (1×10^{-4} M)/COX (1×10^{-6} M)/O_2. Inset: Cyclic amplified amperometric transduction upon reversible photoisomerization of the mixed monolayer between states SP-pyridine formed by irradiation ($\lambda > 475$ nm) and MRH^+-pyridine generated by illumination (320 nm < λ < 360 nm).

and the electron-transfer cascade is shown in Figure 4B (inset). Another enzyme that was coupled to the photo-

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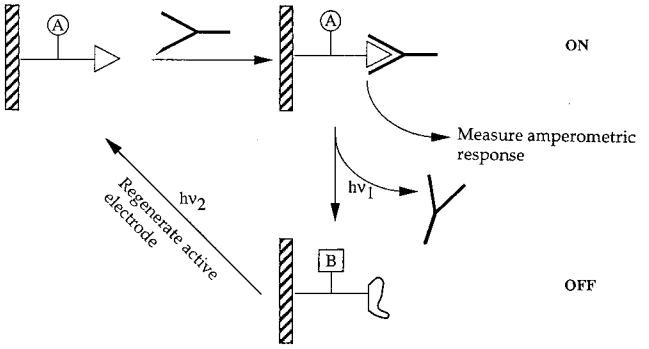

FIGURE 5. Schematic assembly of a reversible immunosensor electrode using a photoisomerizable antigen interface.

stimulated Cyt *c* system was lactate dehydrogenase that activated the bioelectrochemical oxidation of lactic acid to pyruvic acid.[21b]

The pyridine/nitrospiropyran monolayer electrode coupled to the Cyt *c*/COX system represents an artificial approach that duplicates functions of the vision process. In the vision process, photoisomerization of the retinal chromophore induces a conformational change in the surrounding protein that triggers on the binding of protein G. Association of protein G activates an enzyme cascade and the synthesis of c-GMP that stimulates the neural response. The enzyme cascade amplifies the primary event of the photonic signal recorded by the chromophore. In the present system, the photoisomerizable monolayer mimics the function of the vision process. Association and dissociation of Cyt *c* to and from the monolayer mimics the functions of protein G in activating the enzyme cascade. By a similar approach, a nitrospiropyran monolayer assembled onto a Au electrode was applied to photostimulate the electrical contact of glucose oxidase with the electrode surface.[28]

Photoisomerizable monolayers of antigens on electrode surfaces provide the basis for the development of reversible amperometric immunosensors.[29,30] We have developed a general approach to tailor amperometric immunosensors.[31] A Au electrode modified by a self-assembled monolayer of the antigen acts as sensing interface for the antibody, Ab. The antigen-functionalized electrode yields an amperometric signal or an amplified amperometric response in the presence of a redox probe (R^+/R) or a redox-labeled enzyme in the electrolyte solution, respectively. Challenging of the antigen monolayer electrode with the antibody results in the association of the Ab to the monolayer and the electrode insulation toward the solubilized redox probe. The extent of electrode insulation is controlled by the Ab concentration in the analyte sample, and this can be applied for quantitative analysis of the Ab. This method has been successfully applied to sense various antibodies such as the anti-DNP-Ab, fluorescein Ab, and others.[31] Reversible redox couples such as $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ or redox-labeled enzymes were applied to probe the Ab association to the antigen monolayer electrode. These antigen monolayer electrodes, as well as most other immunosensor devices, are limited to a single-cycle analysis due to the strong antigen−antibody interaction. The tight antigen−Ab interactions eliminate the regeneration of the active antigen interface. Photoregulated binding and dissociation of antigens to and from antibodies, could provide a general principle for tailoring reversible amperometric

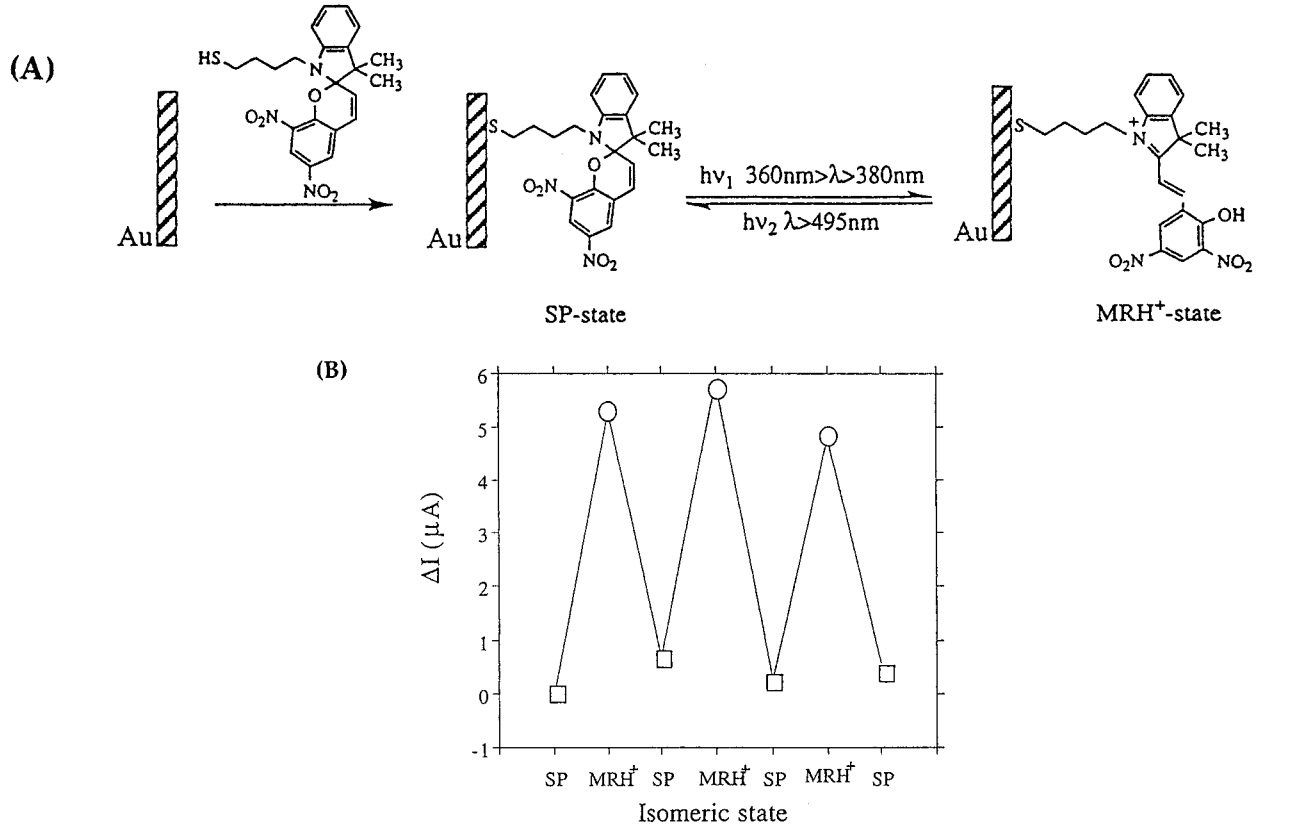

FIGURE 6. (A) Assembly of a thiolated dinitrospiropyran photoisomerizable monolayer on a Au electrode. (B) Cyclic amperometric analysis of DNP-Ab by the photoisomerizable antigen monolayer interfaces using a ferrocene-functionalized glucose oxidase (Fc-GOD, 5 mg·mL^{-1}) and glucose (50 mM) as redox-labeled biocatalyst that amplifies the antigen−DNP-Ab binding.

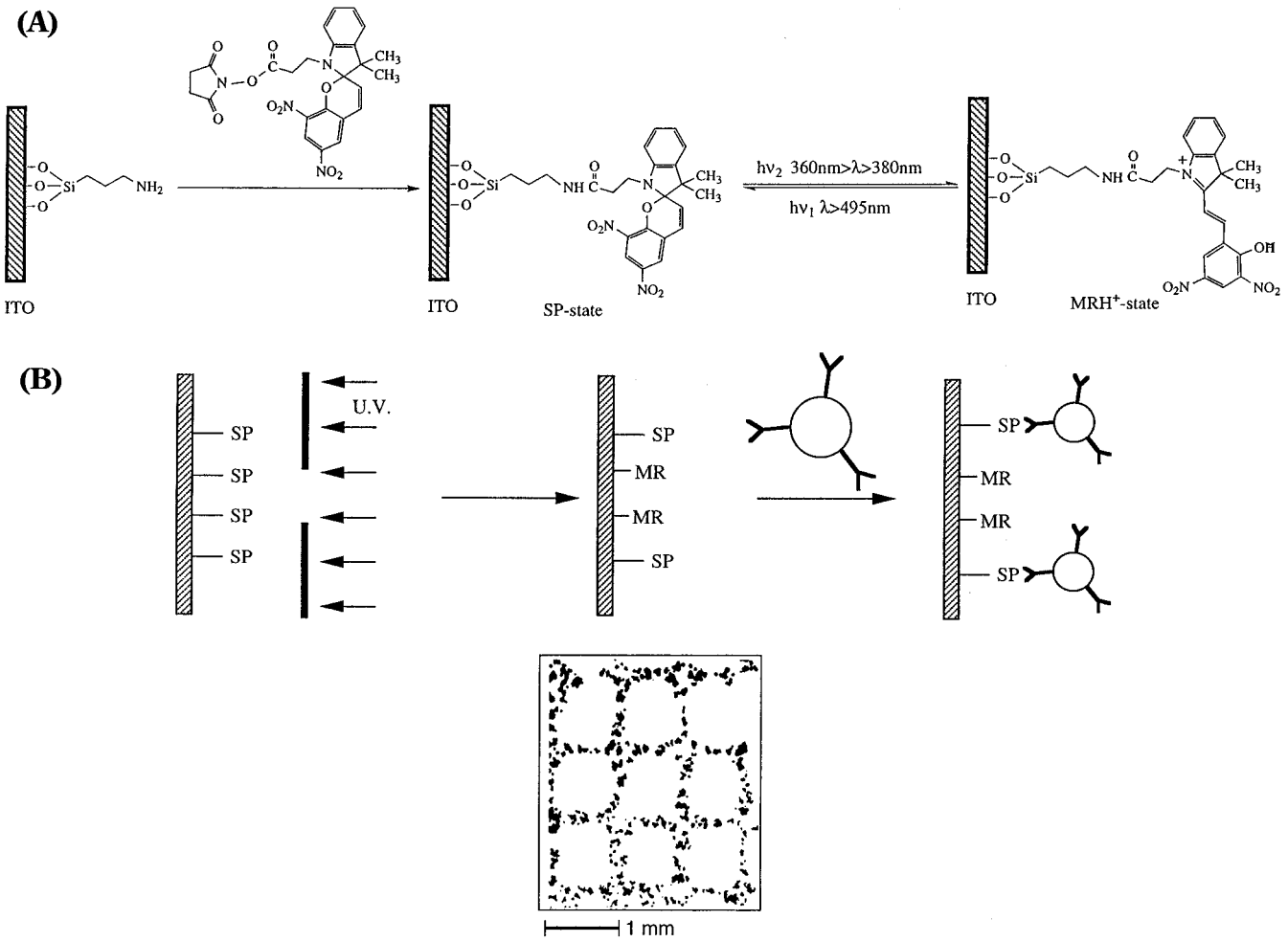

FIGURE 7. (A) Assembly of a dinitrospiropyran photoisomerizable layer on a ITO glass surface. (B) Photolithographic patterning of the photoisomerizable nitrospiropyran monolayer by primary irradiation through a mask and generation of the antigen pattern followed by the selective association of DNP-Ab linked to agarose beads to the patterned monolayer. Specific example shows the association of agarose beads/DNP-Ab to a pattern of SP-antigen formed upon irradiation (360 nm < λ < 380 nm) through a grid.

immunosensor devices (Figure 5). The antigen is chemically modified by a photoisomerizable component (A) and is organized as monolayer on the electrode. In this isomer state, the antigen is recognized by the Ab and the modified electrode is active for the sensing of the antibody in the presence of a solubilized redox probe. Upon completion of the analysis, the antigen monolayer is photoisomerized to state B and the antigenic properties of the monolayer are perturbed. The interface lacks affinity for the Ab, and the antibody can be rinsed off. Reverse isomerization of the monolayer to the original state A regenerates the active antigen monolayer electrode and establishes a reversible amperometric immunosensor. This methodology was successfully applied to develop a reversible monolayer electrode for the anti-DNP antibody.[29,30] A dinitrospiropyran monolayer is organized on a Au electrode, and the monolayer undergoes reversible photoisomerization between the dinitrospiropyran state, SP state, and the protonated dinitromerocyanine isomer, MRH^+ state (Figure 6A). The SP-monolayer state acts as an active interface for the anti-DNP-Ab, whereas the MRH^+-monolayer interface lacks affinity for the antibody.[29] The different properties of the monolayer allowed reuse of the electrode as an immunosensor device for the anti-DNP-Ab (Figure 6B). In the merocyanine state, the monolayer electrode lacks antigenic properties and the functionalized electrode is not insulated by the Ab. Photoisomerization of the monolayer to the SP state generates the active antigen interface that associates the Ab, as reflected by the insulation of the electrode. Further photoisomerization of the monolayer to the MRH^+ state results in dissociation of the Ab, and the exposure of the monolayer electrode to the redox probe is indicated by the enhanced amperometric response. Reisomerization of the monolayer to the SP state regenerates the active interface for the association of the Ab, and the electrode is re-insulated.

The different affinities of the photoisomerizable antigen monolayer for DNP-Ab can be used to photochemically generate patterns of the Ab on a surface.[32] A dinitrospiropyran photoisomerizable monolayer was assembled onto a ITO glass surface (Figure 7A). By irradiation of the dinitrospiropyran monolayer-modified surface, SP state, through a mask, the surface domains exposed to light were

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isomerized to the dinitromerocyanine configuration, MRH^+ state, that lacks affinity for the DNP-Ab. The pattern of the SP-monolayer sites on the surface was then treated with DNP-Ab-modified agarose beads (50 μm). The colloid particles selectively associate to the SP sites. Figure 7B shows the resulting DNP-Ab agarose colloid microstructure on the glass surface obtained upon photolithographic patterning of the dinitrospiropyran monolayer through a grid acting as a mask. The photochemically controlled site-specific modification of surface sites of the photoisomerizable antigen monolayer opens a novel method to tailor microstructures of different biomaterials on a single surface. Covalent linkage of the DNP-Ab to different biomaterials introduces a directing probe for the antigen target sites on the surface. Such ordered arrays of biomaterials could be applied to investigate inter-protein or cell−cell interactions or eventually to generate neural networks.

Conclusions and Perspectives

We addressed recent developments in tailoring photoswitchable biomaterials. Photostimulation of biological functions enables to reversibly record optical signals. This has been exemplified with the photochemically controlled association of substrate−receptor and antigen−antibody pairs and with the design of light-triggered enzymes. Although our report has emphasized the methods to photostimulate protein functions, one could envisage similar methods to photostimulate other biomaterials, i.e., double-helix formation of DNA/oligonucleotide structures.

Integration of photoswitchable biomaterials on conductive interfaces and solid supports opens new venues in material science and in the field of molecular devices. The amperometric transduction and amplification of recorded optical signals by photoswitchable biomaterials integrated with electrode surfaces represent the grounds for optoelectronic systems directed for information storage and data processing, signal amplification, and sensor devices.

Patterning of surfaces by antibodies using photoisomerizable antigen monolayers provides a basic means to organize ordered arrays of different biomaterials on surfaces. The photostimulated antigen−antibody complex formation on the surface can be used as a method to target by light a two-dimensional structure of various biomaterials. This could allow the generation of multifunctional sensing interfaces as well as the organization of complex ordered arrays that enable the investigation of inter-protein or protein−DNA interactions.

The recent progress in the miniaturization of chemical assemblies as nanoscale microstructures suggests that photoswitchable materials could be organized as nanostructured arrays. Densely ordered light-responsive assemblies could provide novel interfaces for optoelectronic devices, selective binding of biomaterials (antibodies, cells), and multifunctional biosensors. Clearly, photoswitchable biomaterials are envisaged to offer exciting perspectives at the frontiers of chemistry, biology, physics, medicine, and material science.

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