# Reconstitution of Apo-Glucose Oxidase on Nitrospiropyran and FAD Mixed Monolayers on Gold Electrodes: Photostimulation of Bioelectrocatalytic Features of the Biocatalyst

**Ron Blonder,[†] Itamar Willner,*[,†] and Andreas F. Bückmann[‡]**

*Contribution from the Institute of Chemistry and Farkas Center for Light-Induced Processes, The Hebrew University of Jerusalem, Jerusalem 91904, Israel, and Department of Molecular Structure, Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-38124 Braunschweig, Germany*

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**Abstract:** $N^6$-(2-Aminoethyl)-FAD is covalently linked to a thioctic acid monolayer associated with an Au-electrode. The surface coverage of the FAD units is $2.2 \times 10^{-11}$ mol cm$^{-2}$. Apo-glucose oxidase (apo-GOx) reconstituted onto the FAD monolayer, yields a bioelectrocatalytically active enzyme layer, GOx, at a surface coverage of $2 \times 10^{-12}$ mol cm$^{-2}$. In the presence of *N*-dimethyl-2-aminoethyl ferrocene (**I**) as diffusional electron mediator, the enzyme electrode stimulates the bioelectrocatalyzed oxidation of glucose. A photoisomerizable nitrospiropyran−FAD mixed monolayer is assembled on an Au-electrode. The surface coverage of the FAD units is $2 \times 10^{-11}$ mol cm$^{-2}$, and the molar ratio of the nitrospiropyran and FAD units is 3:4. Reconstitution of apo-GOx onto the FAD sites of the photoisomerizable nitrospiropyran−FAD mixed monolayer yields a photoswitchable enzyme electrode for the light-stimulated oxidation of glucose in the presence of **I** as electron mediator. The photoswitchable properties of the enzyme electrode originate from the electrostatic repulsion of the positively charged electron mediator from the electrode interface. The GOx-reconstituted-monolayer electrode represents an optobioelectronic assembly for the amplified amperometric transduction of photonic signals recorded by the photoisomerizable monolayer.

## Introduction

Photostimulated biomaterials provide the grounds for development of optobioelectronic devices.[1−3] The photochemical "on−off" control of the functions of biomaterials represents the optical recording event. The physical transduction of the recorded optical signal, in the form of a secondary electronic output, represents the complementary function for the integrated operation of an optobioelectronic device. Amperometric transduction of recorded photonic signals has been accomplished by photostimulation of the bioelectrocatalytic properties of redox proteins.[4] One method for photostimulating redox proteins involves the use of photoisomerizable monolayer-modified electrodes. The photoisomerizable monolayer acts as a command interface that controls by light the protein and monolayer associative/dissociative interactions; this results in photostimulated control of the protein−electrical communication with the electrode support. Light-controlled electrostatic interactions of cytochrome *c* with a nitrospiropyran/pyridine mixed monolayer associated with an Au-electrode have led to photostimulated amperometric transduction of optical signals recorded by the monolayer surface.[5] Similarly, a nitrospiropyran monolayer assembled on an Au-electrode has been used as a "command interface" for controlling the electrical contact between glucose oxidase (GOx) and the electrode.[6] Chemical modification of redox enzymes with photoisomerizable units[7] and reconstitution of an apo-flavoenzyme,[8] e.g., apo-GOx, with a photoisomerizable FAD cofactor have led to the generation of photoswitchable redox proteins that enable a cyclic light-induced activation and deactivation of bioelectrocatalytic processes.

Another method for photostimulating redox enzymes involves the application of photoisomerizable diffusional electron-transfer mediators. In one photoisomer state of the electron relay, a mediated electron transfer between the enzyme redox-center and the electrode occurs, and the biocatalyst is electrically activated. In the complementary photoisomer state of the electron relay, the mediated electrical communication of the enzyme and the electrode is perturbed, and the bioelectrocatalytic activity of the enzyme is blocked. Nitrospiropyran-ferrocene and nitrospiropyran-*N*,*N*′-bipyridinium photoisomerizable electron relays have been used to reversibly activate or inhibit the electrobiocatalytic activities of GOx and glutathione reductase, respectively.[9]

Recently, we reported the development of a novel method to introduce new functions into redox proteins via the reconstitution method. We showed that reconstitution of an apo-flavoenzyme, e.g., apo-GOx or apo-amino acid oxidase, with a semisynthetic

* To whom all correspondence should be addressed. Tel: 972-2-6585272. Fax: 972-2-6527715. E-mail: willnea@vms.huji.ac.il.
† The Hebrew University of Jerusalem.
‡ Gesellschaft für Biotechnologische Forschung.

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ferrocene-linked FAD cofactor, yields biocatalysts that exhibit direct electrical contact with electrode surfaces.[10] Reconstitution of apo-GOx with a semisynthetic photoisomerizable nitrospiropyran-linked FAD cofactor we reported generates a photoswitchable bioelectrocatalyst.[8] Specifically, the direction of the photoswitchable bioelectrocatalytic properties of GOx can be controlled in solution by the application of electron-transfer mediators of differing electrical charges.[8b]

Our laboratory has recently also reported novel means of generating aligned, electrically contacted, enzyme electrodes for bioelectronic applications.[11] We demonstrated that surface-reconstitution of apo-GOx on a pyrroloquinoline−quinone−FAD (PQQ-FAD) monolayer associated with an Au-electrode yields an aligned, bioelectrocatalytically active enzyme electrode of unprecedented efficient electrical contact.[12] This concept was further developed to yield electrically contacted $NAD(P)^+$−enzyme−electrodes via the cross-linking of affinity complexes generated between the $NAD(P)^+$-dependent enzyme and a PQQ-$NAD^+$ monolayer.[13] Also, cross-linking of the affinity complex layer formed between Co(II)−protoporphyrin IX−reconstituted myoglobin and microperoxidase-11 on an Au-electrode yielded a semisynthetic bioelectrocatalytic electrode for hydrogenation of acetylene dicarboxylic acid to form maleic acid.[14]

Here we report on a study that integrates our ability to assemble photoisomerizable monolayer-modified electrodes with the concept of generating aligned-enzyme electrodes via reconstitution to yield a photoswitchable enzyme−electrode. We demonstrate the light-stimulated activation of the bioelectrocatalytic functions of GOx that had been reconstituted on a nitrospiropyran−FAD-mixed monolayer assembled onto Au-electrodes.

## Experimental Section

**Materials.** The *N*-hydroxysuccinimide (NHS) ester of thioctic acid (**1**) was prepared by coupling thioctic acid and NHS. To a mixture of dry dichloromethane (5 mL) that contained thioctic acid (1 g) and NHS (0.7 g) was added 5 mL of a dichloromethane solution containing 1.7 g of dicyclohexyl carbodiimide, and 120 mg of 4-pyrrolidinopyridine. The resulting mixture was stirred overnight and filtered, after which the solution was washed three times with water, three times with 5% acetic acid solution, and again three times with water. The organic layer was dried with $MgSO_4$, filtered, and evaporated to yield 1 g of **1**. $N^6$-(2-Aminoethyl)-FAD (**2**),[15] and mercaptobutyl nitrospiropyran (**3**),[6] were synthesized and characterized as previously described. Apo-GOx was prepared according to the literature.[8b,16] All other materials were from commercial sources (Aldrich or Sigma).

**Chemical Modification of Electrodes. 1. Electrode Pretreatment.** To remove the previous organic layer and to regenerate a bare metal surface, we treated the Au-electrodes with a boiling 2 M solution of KOH for 1 h, rinsed with water, and stored them in concentrated sulfuric acid. Immediately before modification, we rinsed the electrodes with water, soaked them for 10 min in concentrated nitric acid, and then rinsed them again with water. Rough Au-electrodes were prepared by amalgamization of the cleaned Au-electrodes according to the literature.[17]

**2. Preparation of Electrochemically Active Monolayer-Modified Electrodes.** The FAD-short-spacer-monolayer electrode was prepared by soaking a clean, bare Au-electrode in a 0.02 M solution of 3,3′-dithiodipropionic acid-NHS ester, **4**, in dimethyl sulfoxide (DMSO) for 1.5 h. The resulting electrode was then soaked for 1 h in 0.01 M HEPES buffer, pH 7.4, that contained **2**, 5 mM. The resulting modified electrode was then rinsed thoroughly with water to remove any physically adsorbed components. The FAD-long-spacer-monolayer electrode was prepared by soaking a clean, bare Au-electrode in a 0.01 M solution of **1** in DMSO for 1.5 h. The resulting electrode was treated as described for the short-spacer-monolayer electrode.

The nitrospiropyran−FAD-mixed-monolayer electrode was prepared by treating the FAD-long-spacer-monolayer electrode with 50 mM of **3** in solution in DMSO for 1.5 h. Isomerization of the monolayers from the merocyanine-state ($MRH^+$-state) into the spiropyran-state (SP-state) and back was achieved by subjecting the electrodes to irradiation at appropriate wavelengths. The electrodes were illuminated in air outside the electrochemical cell.

The reconstituted GOx-monolayer electrodes were prepared by treating each of the FAD-containing-monolayer electrodes with apo-GOx, 4 mg $mL^{-1}$ in 0.1 M phosphate buffer, pH 7.0, for 4 h at 25 °C and 12 h at 4 °C. The electrodes were then rinsed by shaking the treated electrodes for 1 h in 0.1 M phosphate buffer, pH 7.0, at 4 °C to eliminate any nonspecific protein adsorbates.

**Electrode Characterization and Electrochemical Setup.** Gold electrodes (Au-wire 0.5 $mm^2$ diameter, geometric area ~0.2 $cm^2$, roughness coefficient ~1.3) were used for all the modifications and experiments that did not include the enzyme reconstitution. The modifications and the measurements of the bioelectrocatalytic system were done on rough Au-electrodes (0.5 $mm^2$ geometric area, $15 \pm 5$ roughness coefficient). A cyclic voltammogram for each electrode was recorded in 0.5 M $H_2SO_4$ to determine the purity of the electrode surface prior to the modification. The actual surface area and roughness coefficient for each electrode were estimated from the same cyclic voltammogram by integrating the cathodic peak corresponding to the electrochemical reduction of the oxide layer on the electrode surface. Electrochemical measurements were performed with a potentiostat (EG&G Versa Stat) linked to a personal computer equipped with an electrochemistry software (EG&G research electrochemistry software model 270/250). All measurements were carried out in a three-compartment electrochemical cell consisting of the chemically modified electrode as a working electrode, a glassy carbon auxiliary electrode isolated by a glass frit, and a saturated calomel electrode connected to the working volume with a Luggin capillary. All potentials are reported with respect to the reference electrode. Argon bubbling was used to remove oxygen from the solution in the electrochemical cell.

**Quartz Crystal Microbalance (QCM) Measurements.** Microgravimetric QCM measurements were performed with a quartz crystal analyzer (EG&G Model QCA917) connected to a personal computer. Quartz crystals, 9 MHz (AT-cut, EG&G), sandwiched between two Au-electrodes ($A = 0.186$ $cm^2$, roughness factor ~3.5) were used.

**Light-Induced Photoisomerization of Photoactive Monolayer Electrodes.** For generation of the nitrospiropyran photoisomer monolayer electrode (SP-state), the Au-electrode was irradiated at $\lambda > 475$ nm at ambient atmosphere with a 150-W xenon lamp (Oriel) equipped with a $\lambda > 475$ nm filter. For generation of the monolayer $MRH^+$-state, the surface was irradiated at 360 nm $< \lambda <$380 nm at ambient atmosphere with an Hg pencil lamp source (Oriel, 6042, long-wave filter) held 1 cm from the surface of the electrode.

## Results and Discussion

**Electrochemical Properties of FAD Monolayers.** The sequence of transformations to generate the FAD monolayers linked to the Au-electrode by two different space chains is outlined in Scheme 1. The primary generation of an NHS cysteic acid active ester monolayer or the assembly of the NHS active ester of thioctic acid on the Au-electrode is followed by covalent coupling of **2** to the base layer.

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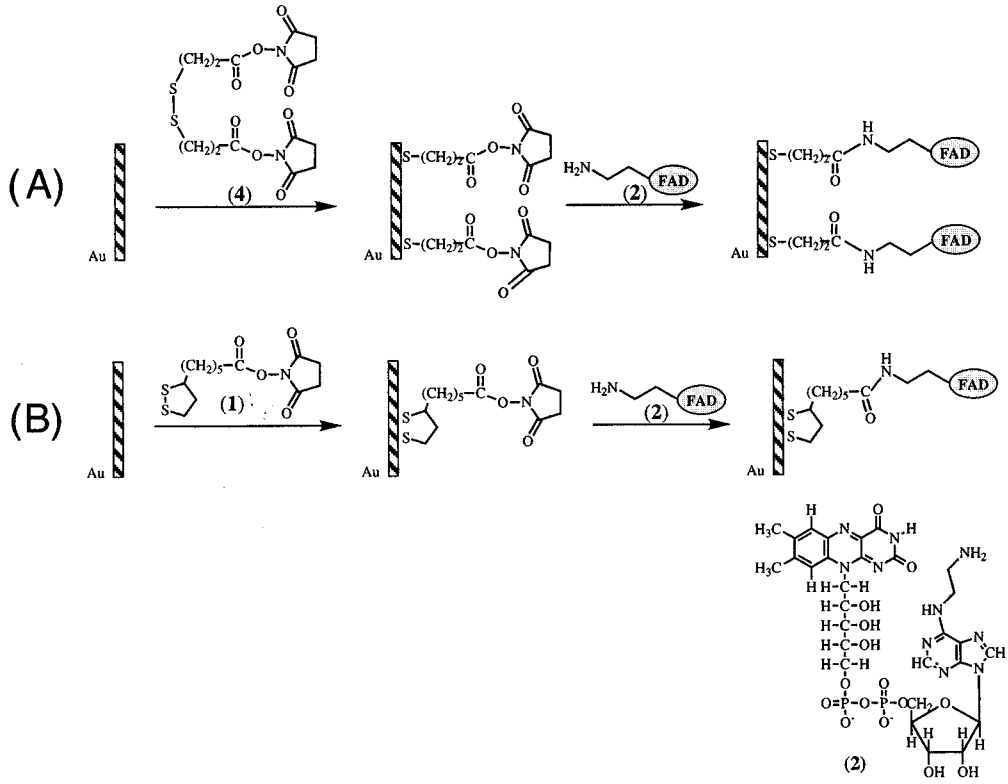
**Scheme 1.** Preparation of the FAD Monolayers by Using (A) a Short-Spacer Linker; (B) a Long-Spacer Linker



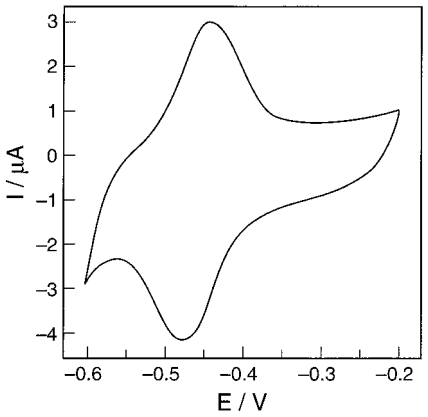




**Figure 1.** Cyclic voltammogram of an electrode modified with the long-spacer FAD monolayer in the background electrolyte of 0.1 M phosphate buffer, pH 7.0. Potential scan rate, 100 mV $s^{-1}$.

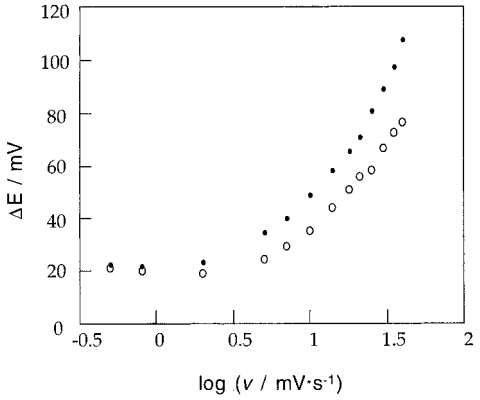



**Figure 2.** The peak-to-peak separation, $\Delta E$, of the long-spacer thioctic acid (solid circle) and the short-spacer cysteic acid (open circles) of FAD monolayer-modified-electrodes at different scan rates.

The cyclic voltammograms of the short-spacer FAD-monolayer electrode and the long-spacer FAD-monolayer electrode, taken against a background solution (pH 7.0) between 0 and −0.6 V show a reversible electrochemical process (Figure 1). The observed redox potential ($E^0 = -0.45$ V) corresponds to the reversible redox reaction of the FAD molecules shown in eq 1. Coulometric analysis of reduction (or oxidation wave) indicates surface coverages corresponding to $2.3 \times 10^{-11}$ mol $cm^{-2}$ and $2.2 \times 10^{-11}$ mol $cm^{-2}$ for the short- and long-spacer FAD-functionalized electrodes, respectively.

$$FAD + 2e^- + 2H^+ \rightleftarrows FADH_2 \qquad (1)$$

Both FAD monolayers are electrochemically stable, and no decrease in the electrochemical signal of the FAD was observed after 1000 cycles. The short-spacer FAD monolayer and the long-spacer FAD monolayer should have different distances between the FAD redox center and the electrode surface and hence should indicate a different electron-transfer rate constant. The interfacial electron-transfer rate constants for the short- and long-spacer FAD-functionalized electrodes were characterized by following the peak-to-peak separation ($\Delta E$) of the FAD-redox waves at different scan rates (Figure 2) and by applying Laviron's method.[18] The interfacial electron-transfer rate constants $k_{et} = 350$ $s^{-1}$ and $k_{et} = 230$ $s^{-1}$ are derived for the short-spacer and long-spacer FAD redox-active units, respectively. However, because the FAD-functionalized monolayer electrodes are nondensely packed assemblies, the spacer chains linking the FAD sites to the electrode are flexible, allowing the redox probe to reach close to the electrode surface. This prevents quantitative correlation between the interfacial electron-transfer rate and the lengths of the spacers linking the FAD sites to the electrode. The observed difference in the electron-transfer rates of the two FAD monolayers is consistent with an

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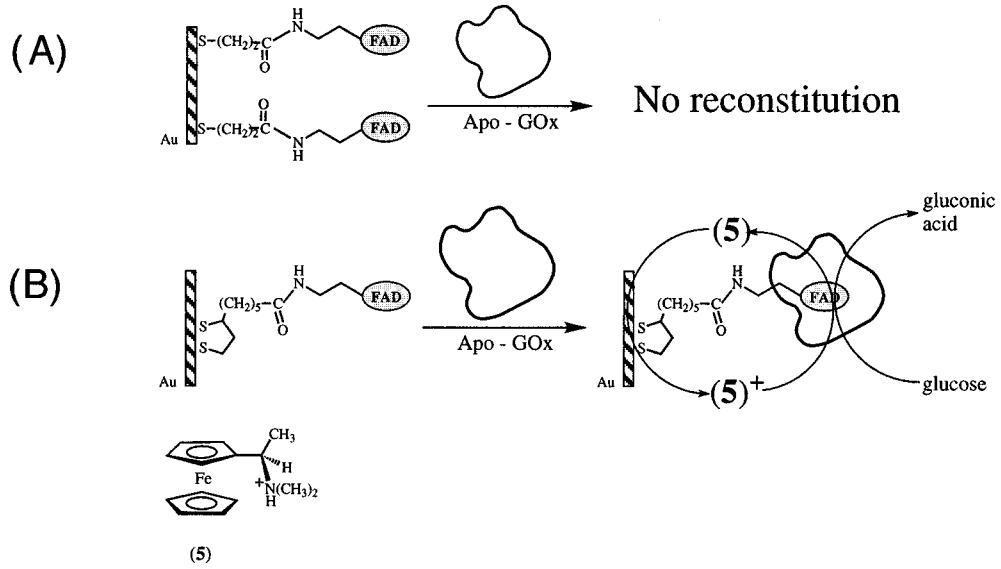
**Scheme 2.** Reconstitution of Apo-GOx onto (A) the Short-Spacer FAD Monolayer Electrode; (B) the Long-Spacer FAD Monolayer Electrode, and the Electrocatalyzed Oxidation of Glucose by the Modified Electrode




enhanced average spatial separation between the FAD component and the electrode generated by the longer tether chain.

**Bioelectrocatalytic Activities of Surface-Reconstituted GOx.** The FAD-monolayer-functionalized electrodes were interacted with apo-GOx to produce surface-reconstituted glucose oxidase, GOx. Previous studies have shown that synthetic electron-transfer mediators can electrically contact redox enzymes and the electrode and stimulate the bioelectrocatalytic activation of enzymes. For example, ferrocene derivatives,[19] quinones, ferricyanide,[20] and even $C_{60}$[21] act as electron-transfer relays that activate GOx. Thus, the reconstitution of apo-GOx onto the FAD monolayer can be probed by examining the bioelectrocatalyzed oxidation of glucose by the reconstituted enzyme electrode in the presence of a diffusional electron mediator, as shown in Scheme 2. The electron relay that was selected to probe the active reconstituted enzyme electrode is *N*-dimethyl-2-aminoethyl ferrocene (**5**). This specific electron mediator is positively charged at pH = 7.0 and was specifically selected to photoactivate at a later phase the surface-reconstituted GOx (see below). The long-spacer-bridged FAD-reconstituted GOx electrode reveals bioelectrocatalytic properties for the oxidation of glucose (Scheme 2B). Figure 3 shows the cyclic voltammograms of the reconstituted enzyme electrodes at different concentrations of the electron mediator **5**. The electrocatalytic anodic currents observed at the redox-potential of the ferrocene electron relay indicate that **5** indeed mediates electrical contact between the surface-reconstituted enzyme and the electrode. That is, oxidation of the ferrocene relay yields the oxidized electron mediator that oxidizes the flavin site of the enzyme by a diffusional route.

On the other hand, treatment of the short-spacer bridged FAD monolayer with apo-GOx does not produce an electrode that is bioelectrocatalytically active in the oxidation of glucose, even at high concentrations of the cosubstrates: glucose at 80 mM and **5** at 0.5 mM. We assume that the short chain that is bridging the FAD unit to the electrode does not provide sufficient flexibility for incorporation and reconstitution of the FAD cofactor into apo-GOx (see below and Scheme 2A). Control experiments reveal that no electrocatalyzed oxidation of glucose occurs in the presence of the FAD-monolayer electrodes plus **5** prior to interaction with apo-GOx. This indicates that the electrocatalytic anodic currents observed by the thioctic acid−FAD-reconstituted GOx originate from an active enzyme monolayer.

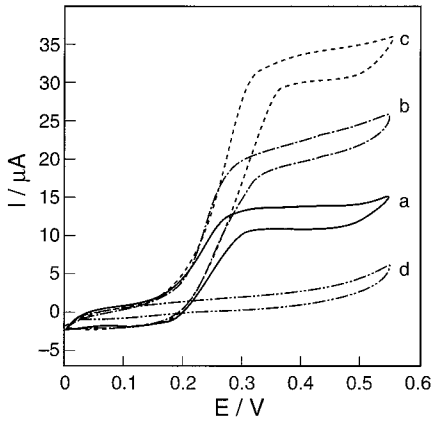



**Figure 3.** Cyclic voltammograms for the bioelectrocatalyzed oxidation of glucose, 80 mM, at different concentrations of **5**: (a) 0.1 mM; (b) 0.2 mM; (c) 0.5 mM (saturation concentration). Curve d was recorded against the background electrolyte solution. All experiments were recorded in a phosphate buffer, 0.1 M, pH 7.0, at 37 °C under argon. Scan rate 5 mV $s^{-1}$.

Microgravimetric QCM experiments allowed us to identify the formation of thin films at electrode supports.[22] In fact, we used QCM measurements to follow the dynamics of the assembly of thiolated monolayers on Au-electrodes associated with piezoelectric quartz supports[23] and to probe the association of antibodies[24] or glycoproteins[25] to antigen or to substrate−monolayer-functionalized Au/quartz crystals. The frequency change of the quartz crystal, $\Delta f$, upon a mass alteration of $\Delta m$

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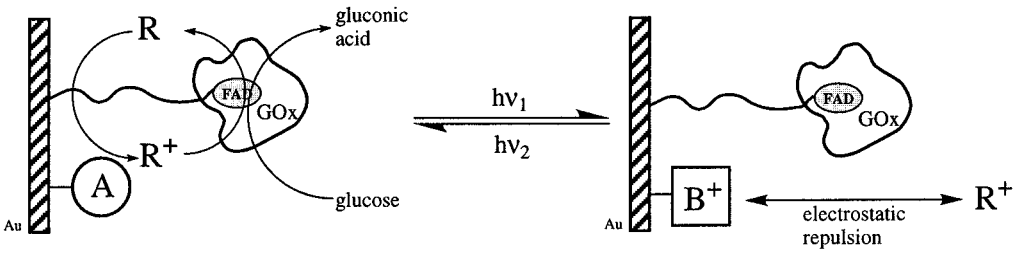
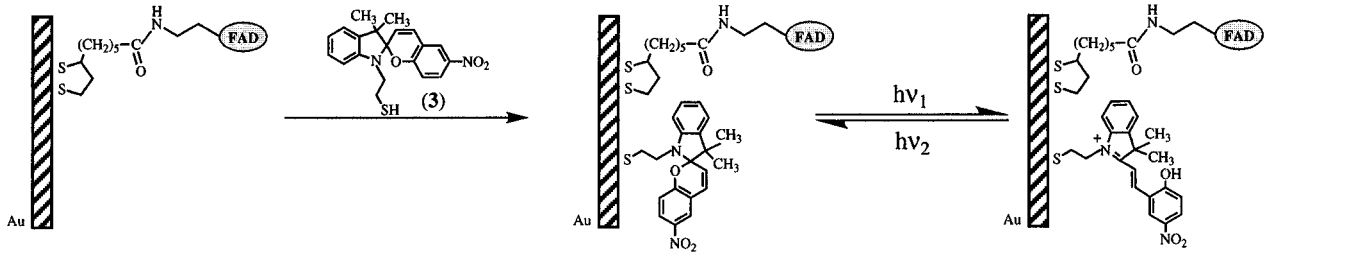
**Scheme 3.** Design of a Photoswitchable GOx Electrode




**Scheme 4.** Preparation of a Nitrospiropyran−FAD Mixed-Monolayer Electrode




occurring on the crystal, is given by

$$\Delta f = -\left[\frac{2nf_o^2}{(\mu_q r_q)^{1/2}}\right]\Delta m = -C_f \Delta m = -1.83 \times 10^8 \,(\text{Hz cm}^2\,\text{g}^{-1})\Delta m \quad (2)$$

where $f_o$ is the fundamental frequency of the crystal, $n$ is the overtone number ($n = 1$), $\rho_q$ is the density of the quartz (2.65 g cm$^{-2}$), and $\mu_q$ is the shear modulus of quartz ($2.95 \times 10^{11}$ dyn cm$^{-2}$). For the quartz crystals used in the present study (AT-cut, 9 MHz) $C_f = 1.83 \times 10^8$ Hz cm$^2$ g$^{-1}$. Thus, reconstitution of apo-GOx onto the FAD-monolayer electrodes could be quantitatively followed by QCM analyses. Formation of the reconstituted GOx electrode would be expected to increase the mass associated with the Au/quartz crystal, a process that would be reflected by a frequency decrease.

The reconstitution of apo-GOx onto the long-spacer-bridged thioctic−FAD monolayer electrode results in a frequency change ($\Delta f$) corresponding to −385 Hz. By applying eq 2 and taking into account the molecular mass of apo-GOx (180 kDa), we estimated the surface coverage of GOx to be $2 \times 10^{-12}$ mol cm$^{-2}$. No frequency change was observed after treating the short-spacer cysteic acid−FAD monolayer with the apo-GOx solution. Thus, the QCM experiments confirm the lack of reconstitution of apo-GOx onto the short-spacer cysteic acid−FAD monolayer.

The surface coverage of the FAD-reconstituted GOx layer, $2 \times 10^{-12}$ mol cm$^{-2}$, is close to the calculated value for a randomly densely packed GOx monolayer, $2.9 \times 10^{-12}$ mol cm$^{-2}$. This value is calculated from the reported dimensions of GOx,[26] which exhibits a footprint area of 58 Å$^2$. Realizing that a randomly densely packed enzyme monolayer showed only 60% of the coverage of an ordered two-dimensional densely packed array,[27] we find that the experimental biocatalyst surface coverage of the electrode coincides with the calculated value.

**Photostimulated Activation and Deactivation of Surface-Reconstituted GOx.** The concept of photoactivating GOx reconstituted onto a FAD monolayer electrode is schematically shown in Scheme 3. The apo-GOx is reconstituted on a mixed monolayer consisting of FAD and a photoisomerizable component. In state A, the photoisomerizable unit is neutral; hence the electron-transfer−mediated activation of the reconstituted biocatalyst is facilitated in the presence of the positively charged relay, $\mathbf{5}^+$. Photoisomerization of the monolayer to state B yields a positively charged isomer. This repels the positively charged electron mediator and perturbs the electrical contact between the enzyme and the electrode. Thus, co-immobilization of the photoisomerizable unit in the reconstituted GOx monolayer array yields a command surface for the electrical activation or inhibition of the biocatalyst. Since in an aqueous environment at pH = 7.0, the nitrospiropyran derivatives undergo photoisomerization between a neutral nitrospiropyran and a positively charged protonated nitromerocyanine,[28] we decided to assemble a mixed nitrospiropyran−FAD monolayer on an Au-support and to reconstitute apo-GOx on the functionalized monolayer to yield the photoswitchable electrobiocatalyst.

**Electrochemical Properties of Nitrospiropyran−FAD Mixed Monolayer.** The sequence of transformations to generate the photoisomerizable mixed monolayer consisting of nitrospiropyran (SP-state) and FAD, or the protonated MRH$^+$-state and FAD, is outlined in Scheme 4. The long-spacer FAD-monolayer electrode (Scheme 1B) was treated with **3**, 50 mM, for 1.5 h. The cyclic voltammogram of the resulting SP− FAD mixed-monolayer electrode, taken against a background solution at between 0 and −0.6 V, shows only the waves corresponding to the redox process of FAD/FADH$_2$ (Figure 4, curve a). If, however, a more-negative potential range is applied, a single irreversible cathodic peak appears in the first scan ($E_c = -0.73$ V). The observed peak corresponds to the irreversible electrochemical reduction of the nitro aromatic groups associated with

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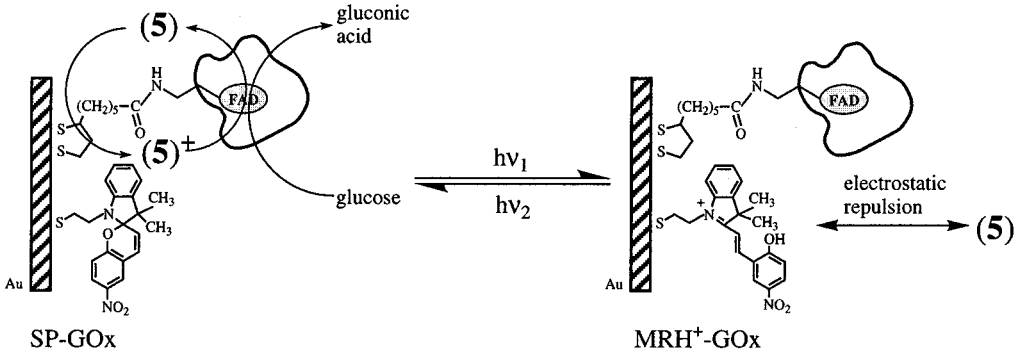
**Scheme 5.** Photoregulated Electrocatalyzed Oxidation of Glucose by the Reconstituted GOx onto the Photoisomerizable Nitrospiropyran−FAD Monolayer Electrode




the spiropyran component of the monolayer,[28b,29] eq 3, where Ar represents the aromatic system of the SP units.

$$ArNO_2 + 4H^+ + 4e^- \rightarrow ArNHOH + H_2O \qquad (3)$$

Three redox processes were recorded during the second scan (Figure 4, curve b). The more-positive redox process, which is quasi-reversible ($E^0 = -0.17$ V), is attributed to the reduction and the oxidation of the secondary product formed by the electrochemical reduction of the nitro group,[28b,29] as shown in eq 4. The second reversible redox process ($E^0 = -0.45$ V), corresponds to the FAD/$FADH_2$ electrochemical reaction.

$$Ar{-}NHOH + 2e^- \rightleftarrows Ar{-}NO \qquad (4)$$

The third irreversible cathodic peak, corresponding to the electroreduction of the nitro groups, decreased dramatically over several cycles and then disappeared completely. Thus, the electroactivity of the nitro substituents of the SP monolayer provides a means for characterizing the SP coverage on the electrode surface. If we assume a two-electron-transfer mechanism for the redox process of Ar−NHOH/Ar−NO (Figure 4, curve b), the estimated surface coverage of the immobilized SP will be $1.5 \times 10^{-11}$ mol cm$^{-2}$. Thus, the electroactivity of the SP monolayer provides a means for characterizing the monolayer coverage of the electrode surface. However, application of a potential more negative than −0.6 V destroyed the SP component in the monolayer; hence, to retain the photochemical properties of the photoisomerizable monolayer, we performed all further electrochemical experiments at potentials more positive than −0.6 V. The surface coverage of the FAD units by coulometric assay of the FAD/$FADH_2$ waves (Figure 4, curve b), was determined to be $2 \times 10^{-11}$ mol cm$^{-2}$. (After the FAD monolayer electrode was treated with **3**, only ∼10% of the FAD units were desorbed from the electrode surface.) The molar ratio of SP:FAD on the electrode surface was calculated to be 3:4.

**Photoswitchable Bioelectrocatalytic Activities of GOx Reconstituted onto the Photoisomerizable Nitrospiropyran−FAD Mixed Monolayer.** The photoisomerizable nitrospiropyran−FAD monolayer was interacted with apo-GOx to yield the reconstituted GOx on the electrode.

The electrocatalyzed oxidation of glucose by the reconstituted GOx electrode was recorded in the presence of **5** as a diffusional

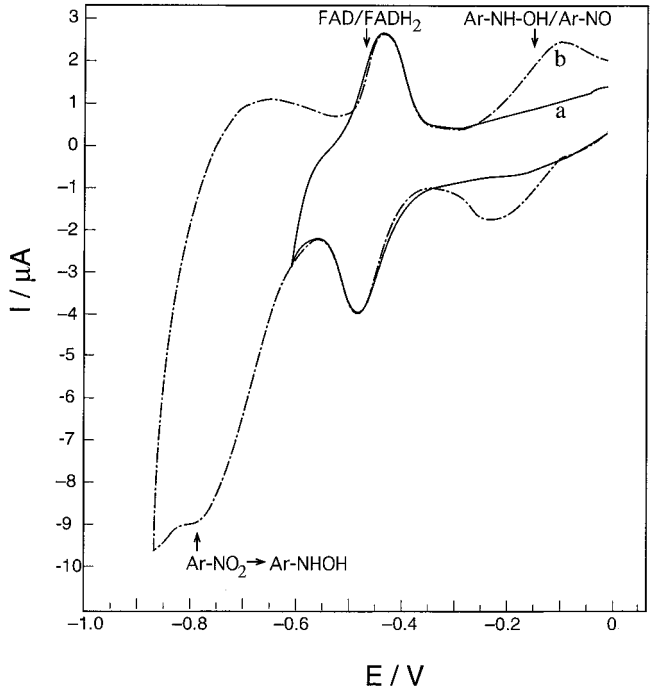



**Figure 4.** Cyclic voltammograms of an electrode modified with a nitrospiropyran−FAD mixed monolayer. (a) In the potential range from 0 to −0.6 V; (b) second cycle scanned in the region 0 to −0.85 V and after the application of a first cycle between 0 and −0.85 V. All voltammograms are recorded at the scan rate 100 mV s$^{-1}$.

electron mediator, as shown in Scheme 5, in two of the different photoisomer states of the monolayer, e.g., SP-GOx and $MRH^+$-GOx. The SP-GOx monolayer electrode responded with electrocatalytic activity (Figure 5, curve b). The electrocatalytic anodic current at the oxidation potential of the electron mediator indicates the bioelectrocatalyzed oxidation of glucose by the surface-reconstituted enzyme−electrode. In fact, the resulting anodic current is almost identical to the current observed with the reconstituted GOx on the pure FAD monolayer. Thus, the surface coverages of the enzyme in the single-component FAD monolayer and in the nitrospiropyran−FAD mixed monolayer are almost identical. This is consistent with the observation that the coulometric analysis of the FAD redox waves in the two monolayer configurations gives somewhat similar results. Thus, the nitrospiropyran units bind to the Au-electrode in surface-defect regions or in pinholes of the nondensely packed FAD-monolayer electrode. Upon photoisomerization to the $MRH^+$-GOx monolayer electrode (generated by UV irradiation at 360 nm $< \lambda <$ 380 nm), the electrocatalytic activity of the reconstituted GOx is inhibited (Figure 5, curve c) and only the

(29) The electrical responses of **5** are identical in the presence of either the SP- or $MRH^+$-monolayer electrodes, despite the positive charge associated with reduced **5**. This can be attributed to the presence of surface defects in the monolayer assembly, which enables electrical communication between **5** and the electrode. Upon oxidation of **5**, the electrostatic interactions are amplified as a result of the formation of a redox mediator bearing two positive charges.

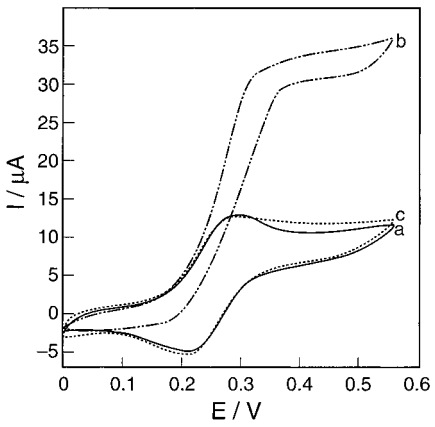



**Figure 5.** Cyclic voltammograms for the bioelectrocatalyzed oxidation of glucose in the presence of **5**, 0.5 mM. (a) SP-GOx or $MRH^+$-GOx monolayer electrode in the absence of glucose; (b) SP-GOx monolayer electrode in the presence of glucose, 80 mM; (c) $MRH^+$-GOx monolayer electrode in the presence of glucose, 80 mM.

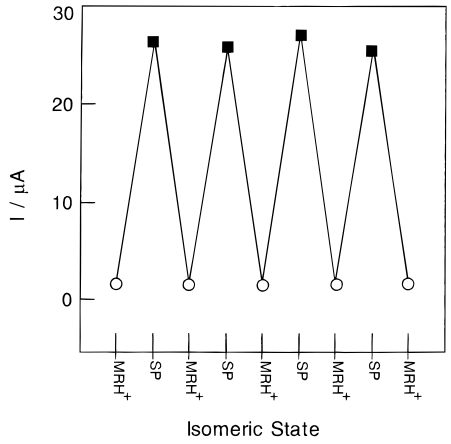



**Figure 6.** Cyclic amperometric transduction of optical signals recorded by the reconstituted photoisomerizable GOx electrode. Net electrocatalytic anodic currents at $E = 0.4$ V are presented. Circles, $MRH^+$; squares, SP.

background current of the electron mediator is observed. The $MRH^+$-GOx monolayer electrode is positively charged, so the positively charged oxidized electron mediator $\mathbf{5}^+$ is repelled by the functionalized monolayer.[29] Further isomerization of the $MRH^+$-GOx monolayer electrode (by irradiation at $\lambda > 475$ nm) to the SP-GOx monolayer restores the electrocatalytic anodic current. We applied the photoisomerizable GOx electrode to organize an optobioelectronic device: By cyclic photoisomerization of the enzyme monolayer electrode between the SP-GOx and the $MRH^+$-GOx states, reversible activation and deactivation of the electrobiocatalyzed oxidation of glucose are stimulated (Figure 6).

Further support for the electrostatic function of the composite monolayer in controlling the mediated electrical contact between the biocatalyst and the positively charged electron mediator is obtained by applying a negatively charged ferrocene electron mediator, i.e., 1,1′-ferrocene dicarboxylic acid, as electron mediator instead of **5** in the bioelectrocatalyzed oxidation of glucose in the presence of the photoisomerizable SP/$MRH^+$ and a surface-reconstituted GOx monolayer electrode. The ferrocene electron mediator includes two negative charges in its reduced form, turning into a single-negative-charge carrier upon oxidation. With this negatively charged electron mediator, the electrocatalytic anodic currents observed upon oxidation of glucose with the SP or $MRH^+$ states of the surface-reconstituted GOx-electrodes were almost identical, there being a slight (5%) enhancement of electrocatalytic current for the monolayer electrode in the $MRH^+$-state. This is consistent with the fact that the latter electron mediator is not repelled by any of the photoisomerizable states of the monolayer electrode, and the minute enhancement of the electrocatalytic current in the presence of the $MRH^+$-monolayer electrode can be attributed to the electrostatic attraction of the negatively charged electron mediator to the electrode. Thus reconstitution of the enzyme onto a photoisomerizable monolayer electrode provides a means for the amplified amperometric transduction of optical signals recorded by the monolayer. In other words, we have tailored an "on−off" photoswitchable enzyme-electrode for the bioelectrocatalyzed oxidation of glucose.

## Conclusions

In the present study we have addressed novel methods for generating FAD monolayers and nitrospiropyran−FAD mixed monolayers on Au-electrodes, and have detailed the assembly of biocatalytic GOx monolayer electrodes via the reconstitution of apo-GOx with FAD. The reconstituted enzyme electrodes yield bioelectrocatalytic interfaces for the electrooxidation of glucose. Reconstitution of apo-GOx onto the photoisomerizable nitrospiropyran−FAD mixed-monolayer electrode creates a photoswitchable bioelectrocatalytic electrode. The surface-reconstituted GOx on the photoisomerizable nitrospiropyran−FAD monolayer represents an organized array acting as a photocommand interface for controlling the bioelectrocatalytic activities of the enzyme. In the nitrospiropyran−FAD/GOx configuration of the monolayer, the ferrocene relay mediates electron transfer between the enzyme and the electrode and activates the bioelectrocatalyzed oxidation of glucose. In the protonated merocyanine−FAD/GOx configuration of the monolayer, the oxidized electron relay is electrostatically repelled from the electrode surface and the bioelectrocatalyzed oxidation of glucose is blocked. The reconstituted enzyme on the photoisomerizable electrode generates an optobioelectronic assembly that results in the amplified amperometric transduction of photonic information recorded by the monolayer interface.

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