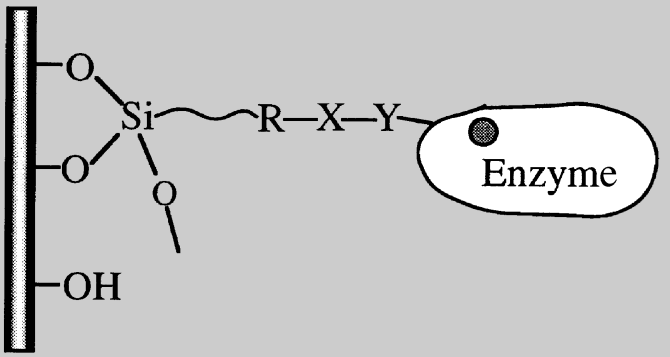
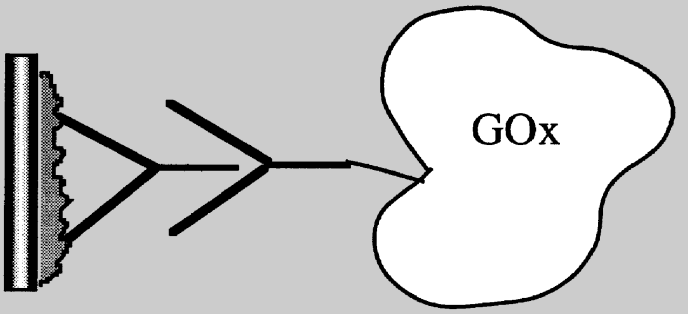
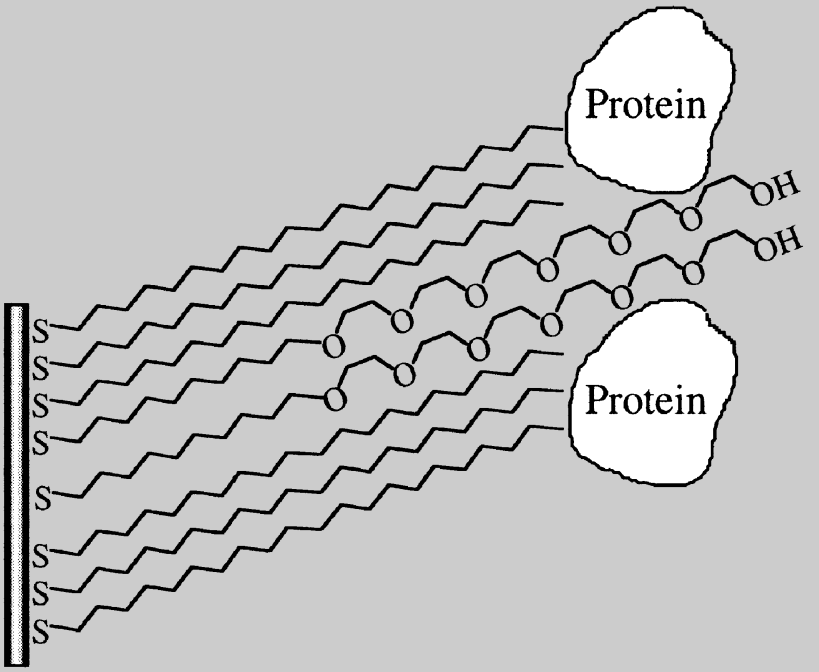
Enzyme electrodes allow the production of more types of products:



through building covalent bonds



with help from affinity interactions



through the use of hydrophilic-/hydrophobic-type interactions

Integration of Layered Redox Proteins and Conductive Supports for Bioelectronic Applications

Itamar Willner* and Eugenii Katz

Integration of redox enzymes with an electrode support and formation of an electrical contact between the biocatalysts and the electrode is the fundamental subject of bioelectronics and optobioelectronics. This review addresses the recent advances and the scientific progress in electrically contacted, layered enzyme electrodes, and discusses the future applications of the systems in various bioelectronic devices, for example, amperometric biosensors, sensoric arrays, logic gates, and optical memories. This review presents the methods for the immobilization of redox enzymes on electrodes and discusses the covalent linkage of proteins, the use of supramolecular affinity complexes, and the reconstitution of apo-redox enzymes for the nanoengineering of electrodes with protein monolayers of electrodes with protein monolayers and multilayers. Electrical contact in the layered enzyme electrode is achieved by the application of diffusional electron mediators, such as ferrocene derivatives, ferricyanide, quinones, and bipyridinium salts. Covalent tethering of electron relay units to layered enzyme electrodes, the cross-linking of affinity complexes formed between redox proteins and electrodes functionalized with relay-cofactor units, or surface reconstitution of apo-enzymes on relay-cofactor-functionalized electrodes yield bioelectrocatalytic electrodes. The application of the functionalized electrodes as biosensor devices is addressed and further application of electrically "wired" enzymes as catalytic interfaces in biofuel cells is discussed. The organization of sensor arrays, self-calibrated biosensors, or gated bioelectronic devices requires the microstructuring of biomaterials on solid supports in the form of ordered micro-patterns. For example, light-sensitive layers composed of azides, benzophenone, or diazine derivatives associated with solid supports can be irradiated through masks to enable the patterned covalent linkage of biomaterials to surfaces. Alternatively, patterning of biomaterials can be accomplished by noncovalent interactions (such as in affinity complexes between avidin and a photolabeled biotin, or between an antibody and a photoisomerizable antigen layer) to provide a means of organizing protein microstructures on surfaces. The organization of patterned hydrophilic/hydrophobic domains on surfaces, by using photolithography, stamping, or micromachining methods, allows the selective patterning of surfaces by hydrophobic, noncovalent interactions. Photoactivated layered enzyme electrodes act as light-switchable optobioelectronic systems for the amperometric transduction of recorded photonic information. These systems can act as optical memories, biomolecular amplifiers, or logic gates. The photoswitchable enzyme electrodes are generated by the tethering of photoisomerizable groups to the protein, the reconstitution of apo-enzymes with semisynthetic photoisomerizable cofactor units, or the coupling of photoisomerizable electron relay units.

Keywords: bioelectronics · biosensors · electron transfer · enzyme electrodes · monolayers

1. Introduction

Bioelectronics is a rapidly progressing field at the junction of chemistry, biochemistry, and physics.[1] The basic feature of a bioelectronic device is the immobilization of a biomaterial onto a conductive or semi-conductive support, and the electronic transduction of the biological functions associated with the biological matrices. Biomaterials that can be thus assembled include proteins, that is, enzymes,[2] receptors,[2a, 3] antibodies or antigens,[4] oligonucleotides or DNA fragments,[5] or low molecular weight molecules exhibiting affinity interactions with other biomaterials such as cofactors, namely, $NAD(P)^+$,[6] biotin,[7] or, for example, saccharides exhibiting affinity interactions with lectins.[8] Different electronic signals were employed to transduce the biological functions occurring at the support element.[9a] These include electrical

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transduction, namely, current,[9] potential changes,[9a, 10] capacitance,[11] and impedance,[12] piezoelectric transduction,[9a, 13] and field effect transistor (FET) transduction[14] and others.[9a, 15]

Systems that employ enzymes as bioactive interfaces represent the most extensively studied assemblies in bioelectronics. Electronic transduction of the enzyme–substrate interactions provides a general analytical means to detect the respective substrate. The high specificity of enzyme–substrate interactions, and the usually high turnover rates of biocatalysts, open the way to tailor sensitive and specific enzyme-based biosensor devices.[16] Activation of enzymes at conductive surfaces allows the application of biocatalysts in electrically driven biotransformations (electrobiosynthesis).[17] Recently, photosensitive enzymes[18] and proteins[19] assembled on electrode surfaces were activated by light, and the recorded optical signals were electronically transduced by the support. The high turnover rates of the enzymes allowed the amplified electronic transduction of weak optical signals.[18b] The potential application of such systems in optical memories, optical bioswitches, electronic gates, and biological actinometers has been addressed.[20]

Bioelectronic systems based on other biointerfaces, for example, DNA sensors or immunosensors, often make use of enzymes as biological probes of the recognition elements for biological affinity.[21] The high turnover rates of enzymes provide a sensitive means for the amplified transduction of biointeractions between the affinity recognition pairs that control the enzyme functions and their electrical contact with electrode supports.

The organization of an enzyme-based bioelectronic system requires the integration of the biocatalyst with the conductive supports to the extent that the biocatalytic transformation is electronically transduced (Figure 1 A). Any electrical changes at the support element as a result of the biocatalytic process, that is, depletion of the reactant or formation of the product,

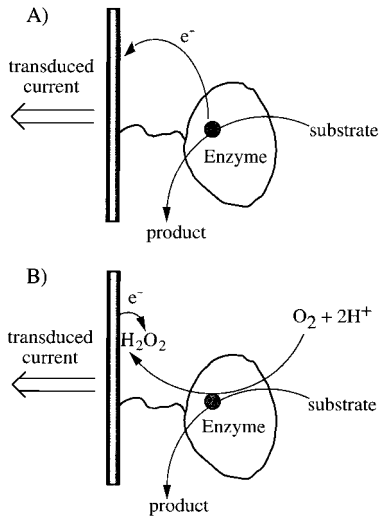

Figure 1. Integrated enzyme electrodes for bioelectronic applications: A) bioelectrocatalyzed oxidation of a substrate, B) electrochemical reduction of H_2O_2, which is formed upon the O_2-biocatalyzed oxidation of the substrate.

provide routes for the electronic transduction of the biological processes occurring at the electrode surface. For example, the changes in potential at conductive supports as a result of biocatalyzed O_2 depletion,[22] NH_3 formation, or CO_2 evolution,[23] represent electronic signals responding to the respective biocatalyzed transformations. Amperometric detection of enzyme-generated products, such as H_2O_2, was often used in the development of biosensor devices (Figure 1 B).[24]

Direct electrical activation of enzymes, and particularly redox enzymes, represents a general approach to stimulate the bioelectrocatalyzed oxidation (or reduction) of the enzyme substrates.[25] Provided electron transfer between the electrode and the redox enzyme is fast, the resulting current

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corresponds to the turnover rate of the electron exchange between the substrate and the biocatalyst. Hence, the transduced current reflects the substrate concentration in the system. Direct electrical communication between redox proteins, and specifically redox enzymes, and electrodes is, however, usually prohibited.[9] Electron-transfer theory[26] defines the rate of electron transfer between a donor–acceptor pair by Equation (1), where ΔG° is the change in free

$$k_{et} \propto e^{-\beta(d-d_o)} e^{\frac{-(\Delta G^{\circ}+\lambda)^2}{4RT\lambda}} \quad (1)$$

energy associated with electron transfer and the generation of the redox products; λ is the reorganization energy; d_o is the van-der-Waals distance and d the actual distance separating the donor and acceptor; and β is the electronic coupling coefficient. This relation emphasizes that the donor–acceptor separation distance is a major factor that controls the electron transfer rates. In fact, for most redox proteins (diameter 80–150 Å) the redox centers are deeply embedded in the protein and therefore electrically insulated. That is, redox enzymes (or proteins) that form a donor–acceptor pair with an electrode support lack electrical contact with this support.

The topic of bioelectronics relies on this basic feature of electrical contact between biomaterials and electrode surfaces. Various bioelectronic devices are based on the design of an artificial electrical contact between redox proteins and the electrode interfaces. In these systems the biofunctions of the proteins are electrochemically driven and stimulated by electron transfer.[25] In other bioelectronic systems the perturbation of the electrical contact between redox proteins and the conductive support is used as a transduction signal of biochemical events.[2, 16]

The tailoring of bioelectronic systems requires the assembly of biomaterials on solid conductive supports and the design of the appropriate electronic communication between the biological matrices and the support element. The chemical means to support the biomaterials include the immobilization of proteins and redox enzymes on electrodes by means of polymers,[27] membranes,[28] carbon paste,[29] and sol–gel materials.[30] The electrical contact with conductive supports was accomplished by the application of redox polymers[31] or conductive polymers.[32] Functionalization of solid surfaces with monolayers of organic compounds and biomaterials has been a subject of extensive research for more than two decades.[33] It is the aim of this review article to address the chemical methods for integrating biomaterials and electronic transducing elements. We discuss in particular the assembly of redox enzymes, redox proteins, and semisynthetic redox biocatalysts of natural origin as monolayers or multilayers on solid supports. We address recent advances involved in the integration and electronic coupling of redox proteins with conductive surfaces. Special emphasis is given to summarize the state-of-the-art methods of spatially patterning surfaces with biomaterials, and of generating ordered nanostructures of proteins on solid supports. Finally, we discuss the organization of optobioelectronic systems, and present the activation of redox proteins associated as layers on electrode supports by external light stimuli. We have tried to emphasize the present and future applications of such integrated assemblies consisting of proteins and electronic transducer elements within the broad context of bioelectronics and optobioelectronics. It is anticipated that the rapid progress in the area will lead to novel biosensor devices, biofuel cell elements, and biomaterial-based computing systems and logic gates.

2. Surface Functionalization of Electrodes for Covalent Linkage of Enzymes

Covalent linkage of proteins to conductive or semiconductive supports often utilizes the availability of functional groups on the surface of the solid support. For example, metal-oxide materials such as TiO_2, SnO_2, contain surface hydroxyl groups that are synthetically useful for the coupling of organic materials.[34] Noble metals (Au, Pt) were chemically[35] or electrochemically[36] pre-treated to generate such surface hydroxyl functions.[37] Carbon electrodes, after appropriate chemical treatment, contain different surface-associated functional groups (carboxylic, carbonyl, lactone, hydroxyl, etc.) capable of attachment to proteins.[38] To control the surface density of functional groups on a graphite surface and their chemical activity, O_2-plasma,[39] strong oxidants,[40] or electrochemical oxidation[41] were applied. Amino functions were introduced onto graphite electrodes by their treatment with NH_3-plasma.[42] Some other electrode materials, for example, p-InP, were functionalized by applying special chemical treatment.[43] The following section will summarize different routes used for the activation of solid surfaces for the covalent attachment of proteins.

Cyanuryl chloride (2,4,6-trichlorotriazine, **1**), is a versatile reagent for the activation of surfaces and subsequent immobilization of enzyme layers.[44] Electrode surfaces functionalized by hydroxyl groups, such as glassy carbon, pyrographite, or metal oxides, such as SnO_2, In_2O_3, TiO_2, react with **1** to form oxy-substituted mono- and dichlorotriazine layers (Figure 2). These residues react with amino or hydroxyl groups on the lysine and tyrosine residues of the protein. The resulting protein monolayer is linked to the electrode by a single anchoring site or by multiple sites involving several amino acid residues and triazine units. Table 1 summarizes several enzymes and proteins that were anchored as monolayers to different electrode supports using the triazine as a linking unit.

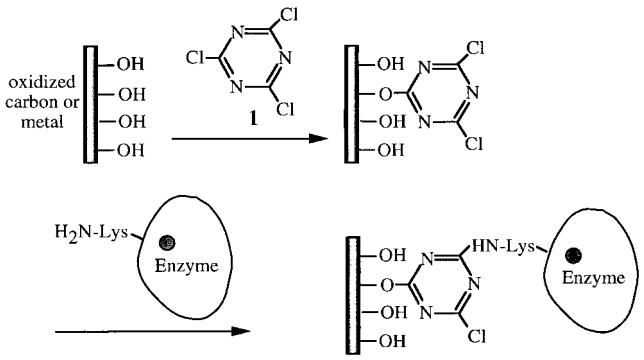


Figure 2. Assembly of enzyme-layered electrodes by the functionalization of the conductive support with cyanuryl chloride (**1**).

Table 1. Enzymes linked to electrode surfaces through cyanuryl chloride (**1**).

Enzyme	Electrode material	Enzyme loading [mol cm^{-2}]	Electrochemical response	Substrate	Analyzed concentration range [M]	Response time [s]	Optimal pH	Electrode stability	Ref.
glucose oxidase (EC 1.1.3.4) from *Aspergillus niger*	pyrographite	–	amperometric	glucose	$2\times10^{-5}-5\times10^{-2}$	25	7.0	20 d	[45a]
	pyrographite	–	differential pulse voltammetry	glucose	–	–	–	–	[45b]
	pyrographite	–	amperometric	glucose	–	–	–	–	[45c]
L-amino acid oxidase (EC 1.4.3.2) from *Crotalus atrox* venom	pyrographite	–	amperometric	L-phenylalanine	$1\times10^{-4}-5\times10^{-2}$	25	7.5	30 d	[45a]
	pyrographite	–	potentiometric	L-phenylalanine, L-methionine, L-leucine	$1\times10^{-5}-1\times10^{-2}$	2–2.5	7.0–7.5	75 d	[46]
alkaline phosphatase (EC 3.1.3.1) from *E.Coli*	glassy carbon	$(1.4-9.7)\times10^{-12}$	amperometric, RDE	catechol phosphate	$1\times10^{-3}-1\times10^{-2}$	30	–	–	[47]
α-chymotrypsin (EC 3.4.21.1)	IrO_2	–	potentiometric, flow-injection	*N*-benzoyl-L-tyrosine ethyl ester	–	12	7.8	–	[48]
ferritin (type 1) from horse spleen	pyrographite	$(7.7-20)\times10^{-11}$	–	–	–	–	–	–	[49]
urease (EC 3.5.1.5)	IrO_2	–	potentiometric	urea	$5\times10^{-5}-1\times10^{-3}$	100	7.0	12 d	[50]

Carboxylic acid groups or amine functions, directly attached to the conducting support can be used for the covalent coupling of complementary amino groups on the lysine residues or carboxylic acid groups of aspartic/glutaric acid residues, respectively. Oxidation of carbon surfaces yields surface-associated carboxylic acid functions.[38a] The carboxylic acid residues are functionalized to an active ester using carbodiimide reagents,[51] to acyl halides using thionyl chloride,[52] or to a mixed anhydride by the reaction with an anhydride[53] prior to the coupling of the functionalized monolayer associated with the electrode to the protein (Figure 3A). The surface density of carboxylic acid residues on the electrode is an important parameter in controlling the effectiveness of covalent attachment of the protein. It was suggested that the electrode area equivalent to the footprint of the protein molecule should include at least ten carboxylic acid residues to achieve an efficient coupling of the protein.[24b] The average distance, about 5 Å, between surface carboxylic groups was determined[54] by labeling experiments and X-ray analysis. The deposition of albumin on the electrode surface as a submonolayer resulted in an increase in the amount of the peroxidase linked to it as a secondary monolayer.[55] This effect was attributed to the increase in the number of functional groups available for the peroxidase coupling. It was reported that enzymes also link to carbon surfaces functionalized with carboxylic acid groups in the absence of appropriate activation reagents.[52, 53, 56] This was attributed to the possible availability of anhydride groups on the carbon surface, which were formed during the oxidation of the support.[38a] Amino-functionalized graphite electrodes[43] were used for covalent coupling with carboxylic groups of enzymes (Figure 3B).[51] Table 2 summarizes some of the enzymes immobilized onto carbon electrodes through amide bonds.

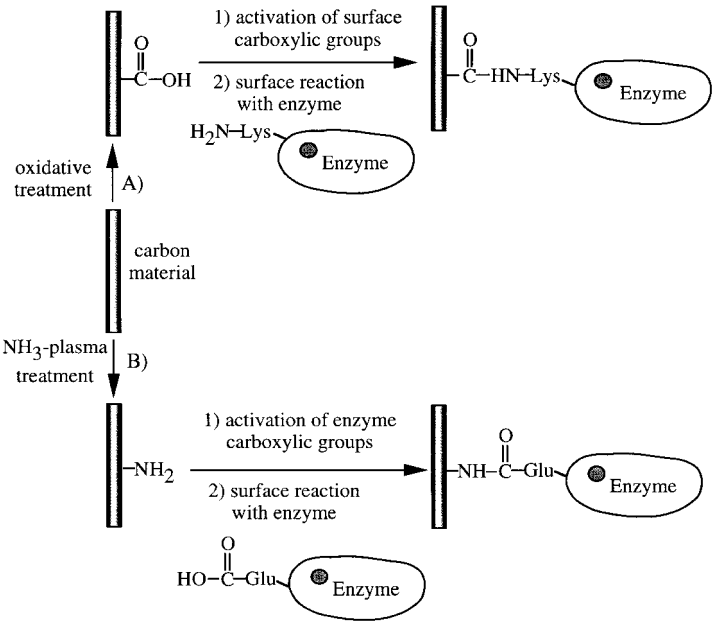


Figure 3. Covalent linkage of enzyme layers to carbon electrodes by coupling of amino and carboxylic functions.

The attachment of functionalized olefins or acetylenes to Pt surfaces[61] or carbon electrodes[62] allows the activation of the support for the secondary coupling of organic molecules, particularly enzymes.[51] Pretreatment of the electrodes yields radical sites that react with π bonds and result in their attachment to the electrode surface. Different redox groups were immobilized at Pt and graphite electrode surfaces by this method, and the technique was also applied for the immobilization of enzymes (for example, glucose oxidase[51]). Figure 4 shows such an immobilization by the primary activation of the surface with $(\omega-1)$-vinylcarboxylic acids or amines and the subsequent coupling of the enzyme to the base layer.[51]

The strong π-π interactions between the hydrophobic basal surface of graphite electrodes (highly oriented pyrographite monocrystals) and polycyclic aromatic systems allow the stable immobilization of functionalized aromatic systems on the conductive support.[63] The functionalized surfaces were used for the covalent immobilization of many organic and bioorganic materials (Figure 5).[64]

Table 2. Enzymes linked to carbon electrodes through amide bonds.

Enzyme	Enzyme loading [mol cm^{-2}]	Electrochemical response	Substrate	Analyzed concentration range [M]	Optimal pH	Electrode stability	Ref.
glucose oxidase (EC 1.1.3.4) from *Aspergillus niger*	$(0.4-3.5)\times10^{-12}$	amperometric	glucose	–	4.5–6.0	12–15 d	[24a–c, 41a, 51, 57–59]
xanthine oxidase (EC 1.2.3.2) from buttermilk	–	amperometric, potentiometric	xanthine	$7\times10^{-6}-6\times10^{-6}$	–	7 d	[10, 38b]
horseradish peroxidase (EC 1.11.1.7)	–	amperometric	H_2O_2	–	3.8	10 d	[38b]
lactoperoxidase (EC 1.11.1.7) from bovine milk	–	amperometric	lactate	–	4.5	10 d	[38b]
α-Chymotrypsin (EC 3.4.21.1) from bovine pancreas	–	–	acetyl-L-tyrosine ethyl ester	–	–	–	[41a]
lactate dehydrogenase (EC 1.1.1.27) from rabbit skeletal muscle	1.4×10^{-13}	amperometric	lactate		6.0–7.5	21 d	[60]

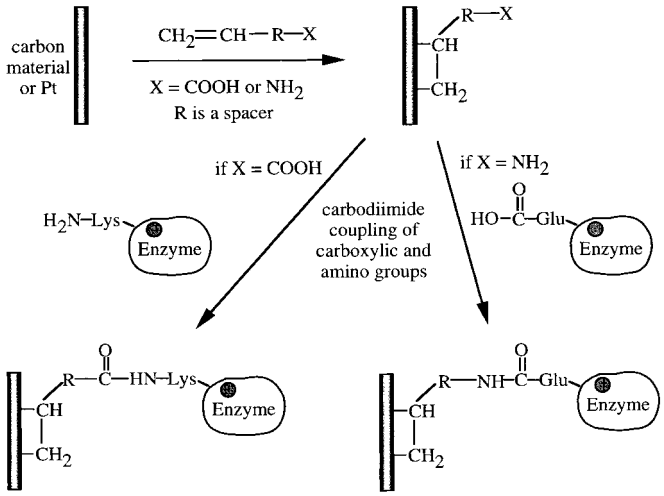


Figure 4. Modification of electrodes with functionalized π adsorbates for the covalent linkage of enzymes. $R=(CH_2)_n$, $n=0-8$.

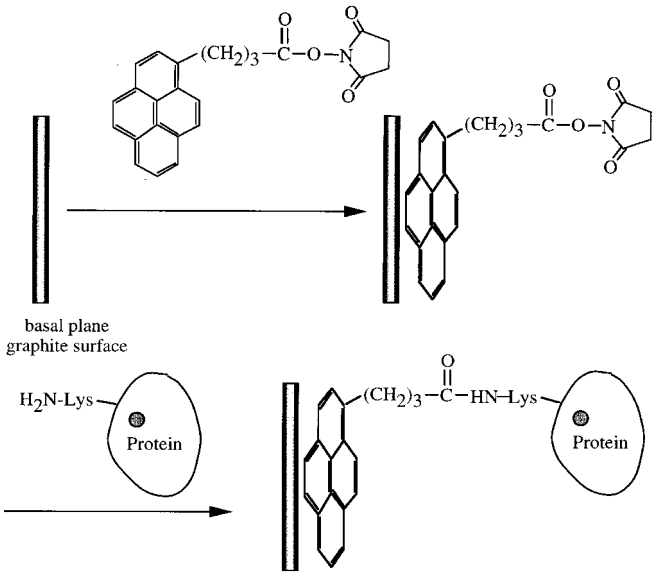


Figure 5. Functionalization of graphite electrodes with polyaromatic π-electron modifiers for protein immobilization.

Alkoxy- or halosilanes are reactive substances for the derivatization of hydroxy-functionalized supports, and their hydrolysis by the hydroxyl groups yields siloxane monolayers or multilayers on the conductive or semiconductive surfaces. Metallo-oxide electrodes, such as SnO_2,[65] TiO_2,[66] RuO_2,[67] and $In_xSn_yO_z$,[68] represent suitable hydroxylated supports. Silanization of metal electrodes, such as Pt, requires electrochemical oxidation of the surface to an oxide monolayer that provides hydroxyl functions.[37] Non-metal oxidized electrode surfaces (for example, glassy carbon electrodes) were also successfully silanized.[69] Different functional groups (usually amino, but also thiol, ester, etc.) can be introduced onto hydroxylated electrode surfaces by the application of functionalized silane reagents. These modified electrode surfaces were used for immobilization of numerous organic and bioorganic compounds with electrochemical, photochemical, catalytic, and biocatalytic properties.[70] Several of the enzymes (proteins) assembled by this method (Figure 6) are summarized in Table 3.

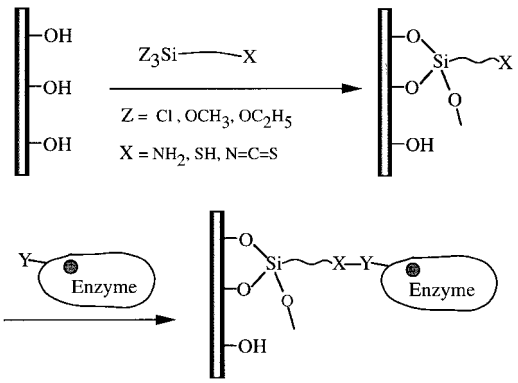


Figure 6. Assembly of functionalized, layered siloxane assemblies on electrodes for the immobilization of enzymes. Y is a complementary functional group for X.

The self-assembly of thiol[73] or disulfide[74] (and less commonly sulfide,[75] thione,[76] S-heterocycle,[77] and isothiocyanate[78]) monolayers on various conductive supports, mainly Au[79, 80] but also Pt,[81] Ag,[82] Cu[82e–g] (and less frequently Ir,[83] Fe,[84] Hg,[85] amalgamated Pt,[86] etc.) or semiconductive materials such as GaAs[87] and InP[88] have been the subject of extensive recent research efforts.[2, 3, 79, 89] Thiolated monolayers on Au surfaces were extensively characterized in respect to their structural features[79] and dynamics of formation[90] by using various physical methods such as XPS (X-ray photoelectron spectroscopy),[91] FTIR

Table 3. Enzymes (proteins) covalently linked to electrode surfaces through functionalized silanes.

Enzyme/protein	Electrode material	Protein loading [mol cm^{-2}]	Electrochemical response	Substrate	Analyzed concentration range	Response time	Optimal pH	Electrode stability	Ref.
glucose oxidase (EC 1.1.3.4) from *Aspergillus niger*	Pt	–	amperometric	glucose	1 – 4 mM	40 s	–	9 months	[24d]
horseradish peroxidase (EC 1.11.1.7)	SnO_2	–	amperometric	H_2O_2	–	–	–	–	[71]
acetylcholinesterase (EC 3.1.1.7), electric eel, type VI-S	Pt	–	–	acetylcholine	–	–	7.2 – 7.8	–	[72]
Photosynthetic reaction centers from bacteria *Rhodobacter sphaeroides* R-26	Pt	1.5×10^{-12}	amperometric or potentiometric	light and reduced cytochrome c	–	3 min	8.0	7 d	[19a – e]

spectroscopy,[92] contact-angle determination,[93] ellipsometry,[94] surface plasmon resonance,[95] quartz-crystal microbalance,[96] electrochemical methods,[97] STM (scanning tunneling microscopy),[98] AFM (atomic force microscopy),[99] and others.[100] Formation of Au-thiolate monolayers from thiols was attributed to oxidation of the Au surface by the thiol [Eq. (2)].[73, 79a] The reduction of an oxide layer associated with the Au surface by the thiol was suggested as an alternative route [Eq. (3)].[101] Formation of the thiolate monolayer on Au surfaces from the disulfide precursor was attributed to the reduction of the disulfide bond by the Au surface [Eq. (4)].[74, 79a]

$$Au_n + RSH \longrightarrow Au_{n-1}Au{-}SR + \tfrac{1}{2}H_2 \quad (2)$$

$$Au_{n-2}Au_2O + 2\,R{-}SH \longrightarrow Au_{n-2}(Au{-}SR)_2 + H_2O \quad (3)$$

$$Au_n + RSSR \longrightarrow Au_{n-2}(Au{-}SR)_2 \quad (4)$$

A variety of redox species, for example, ferrocenes,[102] *N*,*N*′-dialkyl-4,4′-bipyridinium,[75b, 103] quinones,[81e, 86, 104] porphyrins and phthalocyanines,[86, 105] heterocyclic dyes,[106] metal complexes,[107] molecular receptors such as cyclodextrins,[108] calixarenes,[75c, 109] supramolecular complexes,[110] and bulky organic molecules,[111] such as fullerene (C_{60})[111a–c] and dendrimers,[111d] were anchored to Au supports by thiolate bridging groups. Thiolate monolayers associated with metal surfaces or semiconductor surfaces (GaAs, CdS) were used to generate nanostructures of colloids and clusters.[112] The self-assembly of thiolate monolayers on Au supports was used to generate microscopic patterns[113] and to tailor interfaces of controllable hydrophobic/hydrophilic domains.[114] The assembly of photoisomerizable monolayers on Au surfaces through thiolate bonds was used to organize surfaces for the amperometric transduction of optical signals recorded by the monolayer interface.[115] Different aspects of the assembly of thiolate monolayers have been reviewed.[2, 3, 79, 89]

Amino- or carboxylic acid-functionalized thiolate monolayers associated with Au electrodes were covalently coupled to complementary carboxylic or amino groups of glutaric/aspartic or lysine residues of enzymes and redox proteins, respectively (Figure 7 A).[116] To prevent the enzyme deactivating upon linkage to short thiolate spacers, through interaction of the protein with bare Au domains, stepwise construction of thiolate layers with long spacers on electrode supports was performed.[117] For example, coupling of glutaric dialdehyde to a cystamine monolayer associated with an Au electrode, followed by the coupling of lysine residues of the protein through formation of a Schiff base, yields a long-spaced protein monolayer.[18c] Similarly, linkage of diisothiocyanatostilbene disulfonate to a cystamine monolayer, followed by

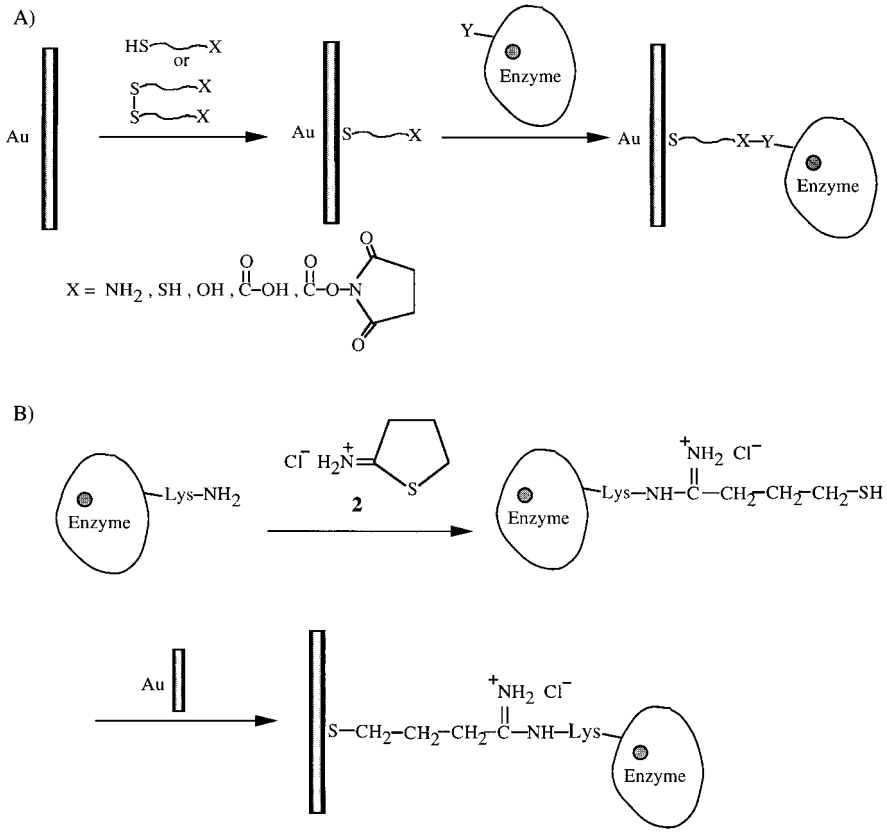


Figure 7. A) Self-assembly of functionalized, thiolated monolayers on Au electrodes for the covalent linkage of enzymes. X is a functional group for coupling with a protein. Y is a complementary functional group to X. B) Modification of proteins with thiol functionalities for self-assembly on Au supports.

the coupling of the protein glutathione reductase to the thiocyanate function by a thiourea bond, generates an enzyme monolayer separated by a stilbene unit.[118] Table 4 summarizes different proteins that were assembled onto Au electrodes as monolayers through thiolate bridging spacers. The surface coverage of the protein on the electrode is controlled by the footprint of the protein, and in the case of the nondensely packed protein monolayers, by the density of the sublayer associated with the conductive solid support.

Direct modification of solid supports by proteins is often associated with the denaturation of the protein in the primary adsorbate layer associated with the surface. Chemical modification of the abundant lysine residues associated with proteins with thiol tethers allows the selective association of the thiol groups to gold surfaces without denaturation of the biomaterial. Reaction of the lysine residues with Traut's salt (**2**) yields the thiol-tethered protein that binds spontaneously to Au supports.[126b] Catalase was functionalized and linked to a gold electrode by using this method (Figure 7B).

An enzyme base layer associated with a conductive support was used as an active interface for the stepwise construction of three-dimensional, multilayer protein arrays. Figure 8 shows a method to organize a multilayer network of glucose oxidase (GOx) on a gold electrode through a primary thiolate bridging unit.[118] The primary enzyme layer is treated by a two-step synthesis with 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (**3**) and the enzyme GOx. The number of repeated steps controls the number of layers associated with the electrode. It was shown that the surface density of the enzyme in each layer is almost identical, approximately 5×10^{-11} mol cm^{-2} per layer up to a network of 12 layers. A similar approach was used to assemble layered protein electrodes composed of two enzymes in an ordered manner.[119b] Choline oxidase was assembled in the form of two consecutive protein layers and acetylcholine esterase was covalently linked to the base enzyme array in a further two-layered structure (Figure 9). Other multilayer enzyme networks on a base-thiolated bridging monolayer were also reported.[119d, 120] A nonorganized enzyme multilayer asembly of glucose oxidase (GOx) was produced by cross-linking the enzyme in the presence of an amino-functionalized electron-transfer mediator on top of a primary thiolated monolayer associated with the electrode, using glutaric dialdehyde as the cross-linker.[119d]

3. Chemical Modification of Surfaces and Proteins to Yield Layered Enzyme Electrodes

The previous section emphasized the nature of chemical bonds between the monolayer spacer units and the electrode that led to the linkage of proteins to the solid support. The surface functions associated with different supports, or with the monolayer(s) assembled on the electrodes, can be further modified by a variety of chemical functions that allow subsequent covalent coupling of proteins. Also, chemical

Table 4. Enzymes (proteins) covalently linked to Au electrodes by functionalized thiolated monolayers.

Enzyme/protein	Enzyme/protein loading [mol cm^{-2}]	Electrochemical response	Substrate	Analyzed concentration range	Response time	Optimal pH	Electrode stability	Ref.
glucose oxidase (EC 1.1.3.4) from *Aspergillus niger*	–	amperometric	glucose	–	–	7.0	–	[18c]
	7.5×10^{-12}	amperometric	glucose	1–20 mM	–	7.3	14 d	[118]
	$(1-4) \times 10^{-11}$	amperometric	glucose	1–25 mM	–	7.0	20 d	[119a,b]
	–	amperometric	glucose	1×10^{-5}–6×10^{-3} M	10 s	–	–	[119c]
	–	amperometric	glucose	–	–	–	–	[119e]
glucose dehydrogenase (EC 1.1.99.17) from *Acinetobacter calcoaceticus*	–	amperometric	glucose	–	10 s	6.0–9.5	60 d	[120]
bilirubin oxidase (EC 1.3.3.5) from *Myrothecium verrucaria*	6×10^{-12}	amperometric	bilirubin	10–120 μM	20 s	8.0	75 d	[121]
malic enzyme (EC 1.1.1.40) from chicken liver	4×10^{-12}	amperometric	malate	1×10^{-7}–1×10^{-3} M	–	7.2	–	[122]
diaphorase (EC 1.6.99) from *Bacillus stearothermophilus*	6×10^{-12}	amperometric	NADH	1×10^{-5}–5×10^{-4} M	–	7.5	–	[123]
glutathione reductase (EC 1.6.4.2)	2×10^{-11}	–[a]	glutathione oxidized	–	30 min	7.2	–	[116]
microperoxidase 11	$(0.8-2.4) \times 10^{-10}$	amperometric	H_2O_2	–	–	7.0	30 d	[125a]
			organic peroxides	5×10^{-5}–3×10^{-3} M		non-aqueous		[125b]
			hemoproteins	5×10^{-5}–1×10^{-3} M		7.0		[125c]
catalase (EC 1.11.1.6) from bovine liver	–	–[b]	–	–	–	–	–	[126]
acetylcholinesterase (EC 3.1.1.8)	–	amperometric	acetyl choline	8–80 mM	–	7.8	–	[119b]
choline oxidase (EC 1.1.3.17)	–	amperometric	choline	10–60 mM	–	7.8	–	[119b]

[a] Spectral detection of the product. [b] Scanning tunneling microscopy (STM) study.

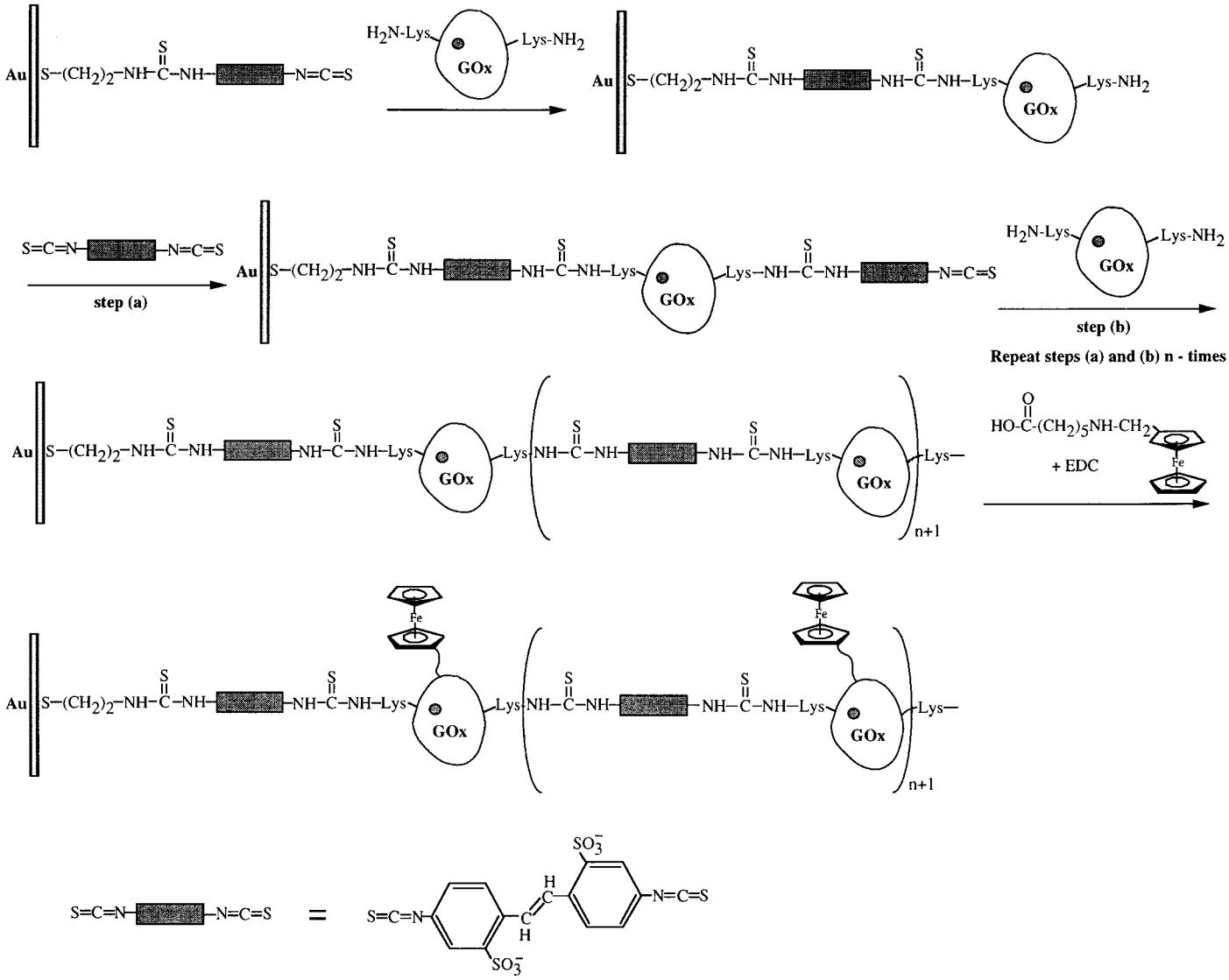


Figure 8. Stepwise assembly and electrical contacting of a cross-linked multilayer array of glucose oxidase (GOx) on a Au electrode.

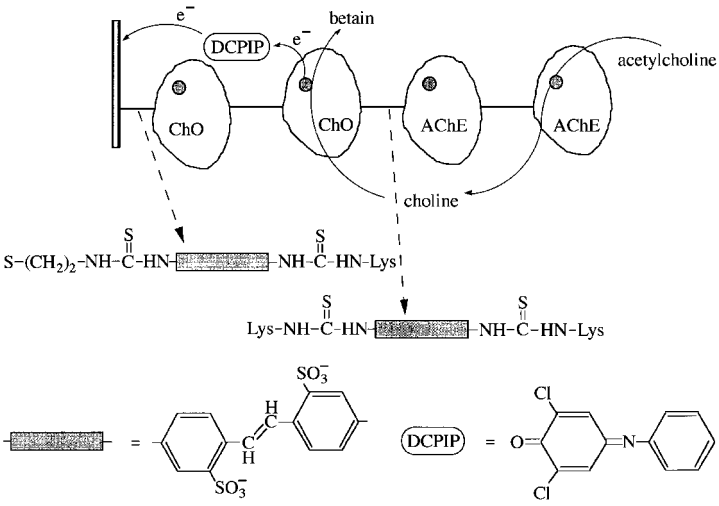


Figure 9. Assembly of a layered array of organized multienzymes for the amperometric sensing of acetylcholine: ChO = choline oxidase; AChE = acetylcholine esterase.

modification of the protein allows the generation of chemical functions that can be linked to the activated monolayer.[126a] Figure 10 lists a series of functional units used for the modification of conductive supports and applied to the secondary coupling of proteins. The amino functions of lysine residues on the proteins can be linked to surface carboxylic groups generating amide bonds (Figure 10a) or to surface aldehyde functions to yield imine bonds[24a,b, 124a] (Figure 10b).[71, 127] Alternative means for attaching proteins to conductive surfaces include nucleophilic substitution of surfaces functionalized with cyanuric chloride (Figure 10c),[45–50] incorporation of the isothiocyanate functionalities (Figure 10d),[116c, 118] and Michael additions to quinone functions linked to electrodes (Figure 10e). The latter quinone–enzyme monolayer assembly represents an enzyme layer that is bridged to the electrode by a redox-active component.[128] Aromatic amines linked to surfaces enable the generation of the surface-bound diazonium salt, which reacts with tyrosine or histidine residues to form proteins linked to

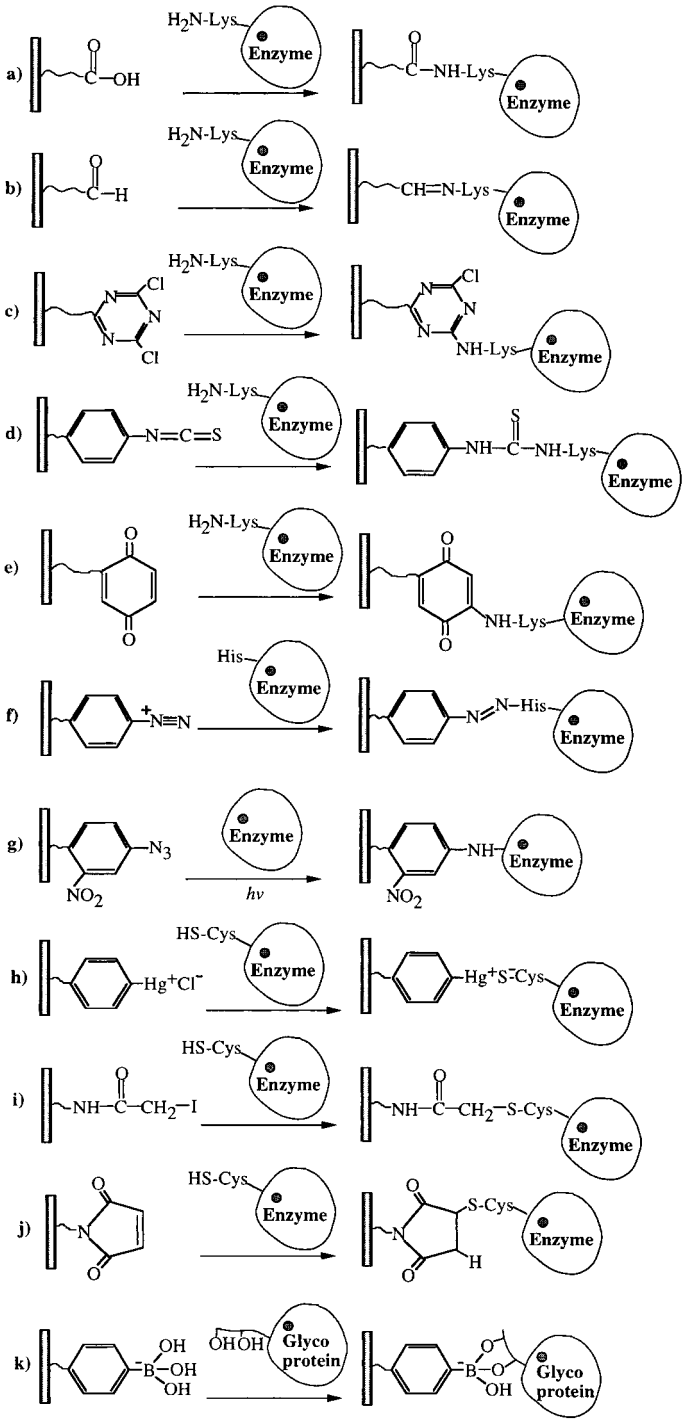


Figure 10. a)–k) Different monolayer-functionalized electrodes for the covalent linkage of enzymes.

the solid support through azo groups (Figure 10f).[127c] Arylazides are frequently used as photoactivated labels of proteins. Accordingly, the photochemical activation of a phenylazide monolayer yields the respective nitrene, which reacts with the amino group of lysine residues (Figure 10g).[129]

The linkage of proteins to solid supports through lysine (or glutamic/aspartic residues) is limited by the fact that proteins usually include many of these hydrophilic residues on the protein periphery. As a result, a heterogeneity in the modes of protein linkage and orientation in respect to the surface is obtained. The structural alignment of the redox protein relative to the conductive support is essential for many bioelectronic applications, and hence the development of methods for specific binding and alignment of proteins on surfaces is important. Cysteine is usually an uncommon amino acid residue in proteins. A single cysteine residue on the protein periphery, or a genetically engineered cysteine component in the protein, allows the linkage of proteins to surfaces at a single point. Solid supports functionalized by *p*-mercurybenzoate,[19c] iodoacetamide layers,[64] or maleimide,[130] were applied for the covalent linkage of cysteine residues as monolayers on surfaces (Figure 10h–j). As examples, the cysteine site of the photosynthetic reaction center (from *Rhodobacter sphaeroides* R-26) was covalently linked to an iodoacetamide monolayer immobilized onto a pyrolytic graphite electrode.[19c] Genetically engineered cytochrome b_5 (in which threonine 8 or threonine 65 were replaced with cystein units) was immobilized onto glass by a siloxane bridging layer.[72, 130] The bifunctional reagent, *N*-succinimidyl-3-maleimidopropionate, was covalently linked to an aminosiloxane layer associated with a glass surface, and the cysteine-modified proteins were bound to the maleimide sites by a nucleophilic Michael addition. Similarly, genetically engineered myoglobin was bound to a haloalkylsilylated glass support.[130c] A similar approach was used to immobilize a synthetic de novo protein consisting of 128 amino acids (14728 Da) in a four-helix-bundle configuration, in which their flexible Gly-Gly-Cys units were attached to the surface maleimido groups.[131] An oriented genetically engineered cytochrome b_5 was generated on an electrode[132] by treating the the thiol groups of the cysteine residue with terminal thiol groups of the monolayer to produce disulfide bonds. Glycoproteins (for example, GOx[133]) exhibiting protein-associated sugar sites allow the binding of *cis*-diol sugar sites to a surface functionalized with phenyl boronic acid groups (Figure 10k).

Potential-induced covalent attachment of proteins was suggested as a means to align and orient redox proteins on conductive supports.[124a] A glassy carbon electrode was activated with a water-soluble carbodiimide, and cytochrome c was coupled to the functionalized electrode with an applied potential. Cytochrome c includes positively charged sites at the position where the edge of the heme is associated with the protein and where electron tunneling proceeds at a maximum rate.[124b] Coupling of cytochrome c to the electrode at potentials more negative in respect to the zero charge potential (0.31 V versus the standard hydrogen elecrode) was found to yield a cytochrome c-modified electrode that exhibits an effective electrical contact. Covalent attachment of the hemoprotein at potentials more positive than the potential of zero charge, generates a hemoprotein electrode that lacks direct electrical communication with the conductive support. The enhanced electrical contact of cytochrome c with the electrodes, upon the application of a negative potential in respect to the zero-charge value, was attributed to the alignment of the monolayer and the orientation of the hemoprotein by facing the heme site relative to the electrode

4. Noncovalent Coupling of Proteins to Solid Supports by Electrostatic, Hydrophobic, and Hydrophilic Interactions

Hydrophobic and hydrophilic interactions of enzymes with membranelike layered assemblies, or electrostatic interactions between charged interfaces and proteins, can lead to organized, supramolecular layered protein interfaces. A mixed, self-assembled monolayer consisting of octadecylthiol and dodecyl thiol was assembled on Au electrodes. The short-chain, charged thiols provide defect sites (or "pockets") for the association of the enzyme fumarate reductase.[134] The resulting enzyme electrode exhibits direct electrical contact between the redox site and the electrode surface, and the bioelectrocatalyzed reduction of fumarate enables the application of the modified electrode as an amperometric fumaric acid sensor.

Hydrophobic membrane proteins incorporate into a lipid layer supported on surfaces only if an aqueous layer separates the lipid layer from the substrate. This separation was achieved by transferring lipids to hydrophilic surfaces either by the Langmuir–Blodgett technique[135] or by the fusion of liposomes.[136] Incorporation of the membrane protein ATPase into a lipid layer associated with an Au surface was accomplished by the fusion of ATPase-containing liposomes with a functionalized lipid layer (Figure 11).[137] A thiol-functionalized, helix-forming polypeptide was assembled onto

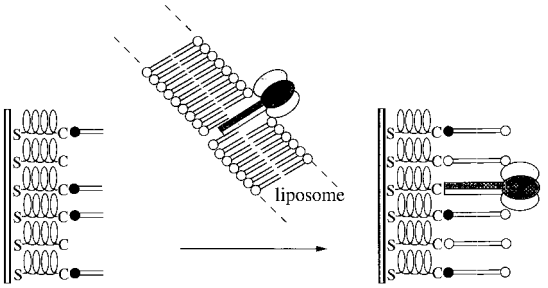


Figure 11. Hydrophobic association of liposome-bound ATPase onto thiolated, amphiphilic, helical oligopeptides.

an Au surface and the lipid DMPE (dimyristoylphosphatidyl ethanolamine) was covalently coupled to the carboxylic acid terminus of the peptide. The resulting lipid layer was mixed with liposomes that contain ATPase and spontaneous fusion of the liposomes with the lipid layer resulted in the incorporation of ATPase into the bilayer. The ATPase bilayer assembly was used as a semisynthetic proton-pump system.

Hydrophobic proteins such as fibrinogen, lysozyme, pyruvate kinase, or RNAase were adsorbed onto alkylthiolates associated with Au surfaces.[138] Mixed monolayers consisting of the oligo(ethylene oxide) and alkane thiols were found to resist nonspecific hydrophobic adsorption of the proteins when a sufficiently high mole fraction of the ethylene oxide was included in the monolayer.

Electrostatic interactions combined with hydrophilic adhesion may lead to irreversible adsorption and alignment of proteins on monolayers terminated with carboxylic acids.[139] Cytochrome c was adsorbed onto a 16-sulfanylhexadecanoic acid ($HS(CH_2)_{15}CO_2H$) monolayer associated with an Au surface. The resulting protein-functionalized electrode exhibits electrical contact with the electrode support, and from the rate constant for the electron transfer at the interface, $k_{et}^{\circ} = 0.1\ s^{-1}$, a distance between the heme center of cytochrome c and the electrode of 31 Å was estimated.

5. Noncovalent Coupling of Proteins to Surfaces through Affinity Interactions

Affinity interactions between an enzyme and its substrate, a receptor protein and its recognition pair, or antigen–antibody pairs are often characterized by high association constants of the resulting complexes, which has enabled the use of specific recognition interactions to construct protein layers on solid supports. Furthermore, as the native receptor proteins often include more than one binding site for the substrates, and antibodies can be coupled to anti-antibodies, complex arrays of proteins can be tailored, and, specifically, multilayers of enzymes can be organized in a controllable order and structure.

Antigen–antibody interactions were used to organize layers and multilayers of glucose oxidase on glassy carbon electrodes (Figure 12).[4] A glassy carbon electrode was coated with gelatin, and rabbit IgG antibodies were adsorbed onto the gelatin. A conjugate consisting of glucose oxidase linked to the anti-rabbit IgG antibody derived from goats was linked to the antibody associated with the gelatin to yield an enzyme-layered electrode (Figure 12A). This concept was further developed to construct multilayers of the enzyme on electrodes

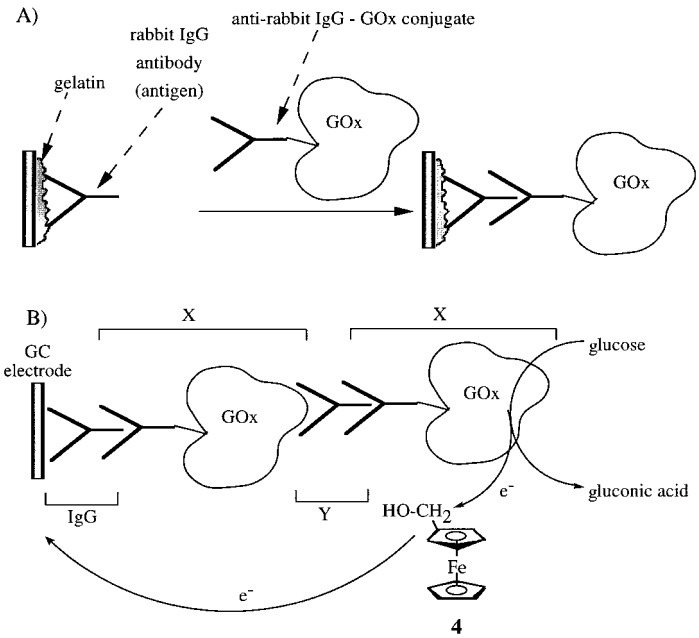


Figure 12. Assembly of enzyme layers on electrodes by noncovalent antigen–antibody interactions: A) Association of an antibody–enzyme conjugate to an antigen-monolayer electrode. B) Generation of an enzyme multilayer electrode (GOx multilayer glassy carbon electrode) by the application of an anti-IgG-GOx conjugate (X) and an IgG and anti-GOx antibody (Y) as linking components (>–). The enzyme electrode is electrically contacted by the diffusional electron mediator ferrocenylmethanol (**4**).

(Figure 12B): Mouse IgG antibodies were adsorbed onto a gelatin layer associated with a glassy carbon electrode. The conjugate X consisting of glucose oxidase and an anti-mouse IgG antibody derived from goats was linked to the primary mouse antibody that acts as the antigen. A monoclonal mouse anti-GOx IgG antibody (Y), was then linked to the primary GOx layer. By the repeated interaction of the electrode with X and Y, a controllable number of layers could be deposited on the electrode. The resulting enzyme multilayer electrode was electrically contacted with the electrode, using ferrocenylmethanol (**4**) as a diffusional electron mediator. The effectiveness of the bioelectrocatalyzed oxidation of glucose is controlled by the number of layers deposited onto the electrode support. The transduced currents are enhanced as the number of enzyme layers associated with the electrode are increased, which implies that the sensitivity of the sensing electrode can be tuned by the number of enzyme layers assembled onto the electrode.

Biotin–avidin affinity interactions were used to assemble monolayer and multilayer enzyme electrodes (Figure 13).[7] From the covalent immobilization of avidin on a glassy carbon electrode was studied and the importance of a long spacer to preserve the affinity properties was demonstrated.[140] A monolayer of horseradish peroxidase (HRP) was deposited onto a carbon electrode using biotin-avidin affinity ineractions[7a] (Figure 13A). Poly(oxyalkylene)diamine (Jeffamine ED-600) was coupled to carboxylic acid residues associated with the carbon surface as a hydrophilic spacer, and the resulting surface amino functionalities were biotinylated. Biotinylated HRP was linked to the modified surface using avidin as a polydentate linker for the biotin units (Figure 13B). The resulting HRP monolayer electrode stimulated the bioelectrocatalyzed reduction of H_2O_2 and the oxidation of *ortho*-hydrobenzoquinone. Biotin–avidin interactions were also used to assemble monolayer and multilayer enzyme arrays on other conductive supports.[7b,c] Avidin was adsorbed onto a Pt electrode and biotinylated alcohol oxidase (AOx) linked to the avidin layer. By the sequential reaction of the enzyme layer with avidin and biotinylated AOx, a controllable number of enzyme layers was deposited onto the electrode. The enzyme electrode biocatalyzes the oxidation of ethanol to acetaldehyde [Eq. (5)]. The amperometric

$$CH_3CH_2OH + O_2 \xrightarrow{AOx} CH_3CHO + H_2O_2 \quad (5)$$

response of the electrode as a result of the oxidation of H_2O_2 was used to quantitatively sense ethanol in blood and liquor samples in the concentration range 10^{-4}–10^{-1} mg mL^{-1}. The sensitivity of the enzyme electrode was controlled by the number of enzyme layers associated with the electrode.

A thin layer of poly(L-lysine) (PL) assembled on gold surfaces was found to control the specific adsorption of proteins:[141] PL functionalized with biotin units was electrostatically associated with a base thiolate monolayer of 11-sulfanylundecanoic acid. Avidin was found to bind selectively to the biotin sites. By using surface plasmon resonance measurements and fluorescence-probe labeling the surface coverage of lysine residues was found to be 4×10^{14} lysine residues per cm^2, and that of avidin molecules 3×10^{12} molecules per cm^2. The thickness of an avidin monolayer was estimated to be 41 Å. Affinity interactions in biotin/avidin/enzyme systems were studied using nanotechniques (STM and AFM) and used to prepare nanostructures with maskless photolithography.[142] Affinity interactions of glycoproteins and the respective lectins were similarly used to organize protein layers on solid supports.[143] The glycoprotein glucose oxidase forms an affinity complex with concanavalin A. Accordingly, a cross-linked glucose oxidase/peroxidase monolayer was assembled on the lectin layer (concanavalin A) associated with a fluoride-sensitive field-effect-transistor (FET; Si/SiO_2/Si_3N_4/LaF_3 layers) to sense glucose.[143a] The oxygen-mediated biocatalyzed oxidation of glucose generates H_2O_2, which is transformed in the presence of peroxidase and *p*-fluoroaniline to fluoride ions [Eq. (6)] that are sensed by the FET device.

$$H_2O_2 + \text{4-fluoroaniline} \xrightarrow{\text{peroxidase}} F^- + \text{polyaniline} + H_2O \quad (6)$$

Upon the deactivation of the enzyme, the sensing interface associated with the FET device can be replaced and reloaded by interaction of the sensing matrix with α-D-mannopyranose. This sugar exhibits a high affinity for concanavalin A, and thus the

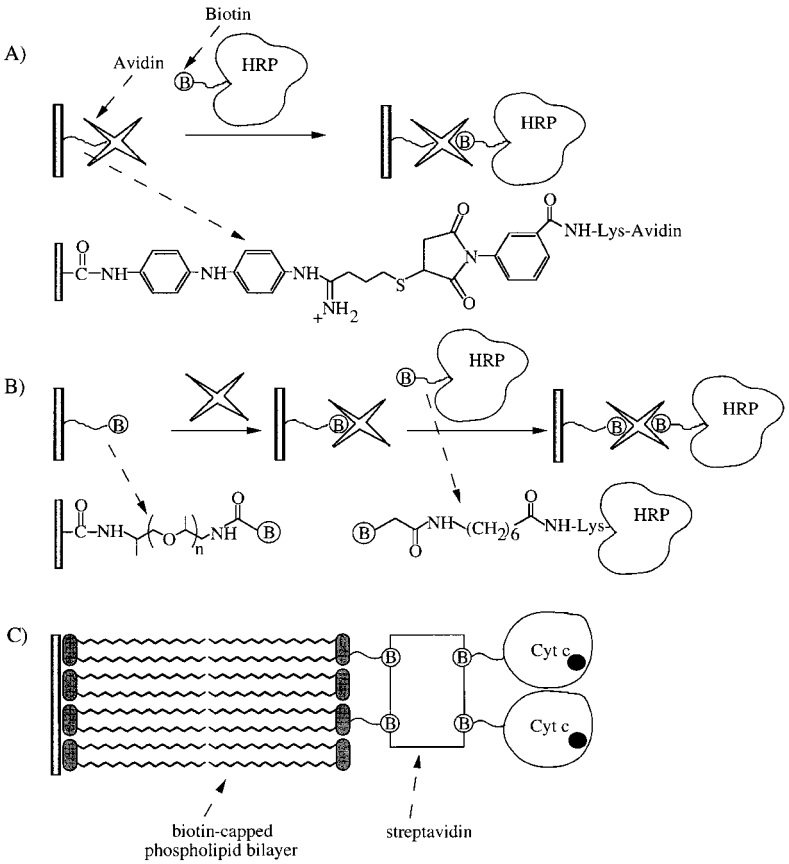


Figure 13. Organization of enzyme electrodes based on avidin–biotin interactions: A) Using a biotinylated enzyme and an avidin-monolayer electrode, B) using a biotinylated enzyme, a biotinylated monolayer electrode, and avidin as a cross-linker, C) aligned deposition of a biotin site-specific functionalized cytochrome c onto a biotin-capped phospholipid bilayer through a streptavidin linker.

original glycoprotein is removed from the sensing interface.

Biotin–avidin (or streptavidin) interactions were used to align and orient redox proteins (Figure 13C).[143b] A bilayer consisting of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[biotinyl] and 1,2-di[*cis*-9-octadecenoyl]-*sn*-glycero-3-phosphocholine was deposited onto a glass substrate. Avidin or streptavidin were used as cross-linkers for the alignment of the cytochrome c after the site-specific biotinylization at the cysteine residue 102 with *N*-[6-(biotinamido)hexyl]-3′-(2′-pyridyldithio)propionamide. Linear dichroism and fluorescence anisotropy measurements enabled one to conclude that the cytochrome c is oriented, and its tilt-angle is $(41 \pm 11)^\circ$ relative to the bilayer matrix.

Choline-functionalized monolayers assembled on a gold electrode demonstrated affinity for a chimera protein made by the fusion of the β-galactosidase and the choline-binding domain.[144] The enzyme immobilized through affinity interactions was biocatalytically active for the hydrolysis of (*p*-aminophenyl)-β-D-galactopyranoside, a synthetic substrate of β-galactosidase, and the released *p*-aminophenol was detected electrochemically.

Cofactor–protein interactions can be used to assemble enzymes on solid supports. Redox cofactors such as flavin adenine dinucleotide phosphate (FAD) or pyrroloquinoline quinone (PQQ, **5**) are tightly associated with the protein by noncovalent interactions. Other redox cofactors, such as nicotinamide adenine dinucleotide (NAD^+, **6**), and nicotinamide adenine dinucleotide phosphate ($NADP^+$, **7**), operate by a diffusional route and form temporary affinity complexes with the respective $NAD(P)^+$-dependent enzymes that enable cofactor-mediated electron transfer. The affinity interactions between cofactors and proteins enable the assembly of redox-enzyme electrodes (Figure 14A). A monolayer of **5**, covalently linked to the amino-derivatized flavin adenine dinucleotide **8**, was assembled on an Au surface. Apo-glucose oxidase (apo-GOx), which lacks its native FAD cofactor, was reconstituted onto the semisynthetic FAD cofactor associated with the electrode.[145] The PQQ component was found to mediate the electron transfer from the FAD unit to the electrode surface, and the reconstituted enzyme revealed an unprecendentally high bioelectrocatalytic activity for the oxidation of glucose (see Section 8). A PQQ monolayer assembled onto an Au electrode was similarly employed to reconstitute the PQQ-dependent apo-glucose dehydrogenase.[2c, 146] The electrochemical oxidation of glucose was stimulated by the reconstituted biocatalyst in the presence of a diffusional electron-transfer mediator.

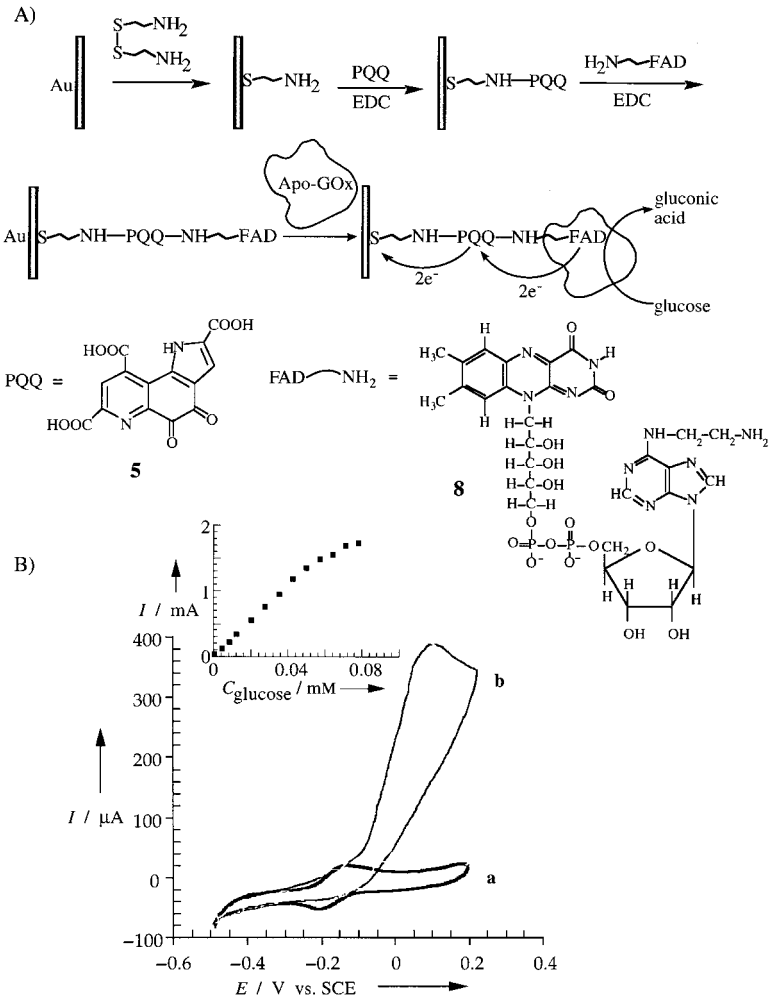


Figure 14. A) Reconstitution of apo-glucose oxidase on a PQQ-FAD monolayer assembled on a Au electrode. B) Cyclic voltammograms of the reconstituted glucose oxidase on a Au-electrode a) in the absence of glucose and b) with glucose (80 mM); potential scan rate: $5\,mV\,s^{-1}$. Inset: Calibration curve corresponding to the amperometric responses (measured by chronoamperometry) of the enzyme electrode at different concentrations of glucose.

Redox proteins such as myoglobin were also reconstituted on conductive supports.[147] For example, a Fe^{III}-protoporphyrin IX complex was assembled as a monolayer on an Au electrode and apo-myoglobin was reconstituted with this heme-cofactor monolayer. Although native myoglobin usually lacks direct electrical communication with electrode supports because of insulation of the heme center, electrical contact between the surface-reconstituted myoglobin and the electrode was evident. This property was attributed to the alignment of the heme center on the electrode surface in a structural orientation that facilitates electron transfer to and from the electrode.

Artificial compounds capable of substituting for natural materials and exhibiting affinity interactions to proteins were immobilized as monolayers on electrodes and used as "instruction matrices" for assembling further protein layers. Artificial dyes exhibiting affinity interactions to $NAD(P)^+$-dependent enzymes (for example, cibacron blue F3G-A) were assembled as monolayers on Au electrodes, and used for the anchoring of NAD^+-dependent enzymes.[148] The affinity complexes consisting of the layered dye–enzyme matrices exist in a bioactive configuration, but lack electrical contact with the electrode. Addition of the diffusional NAD^+-cofactor was found to trigger the biocatalytic activities of the enzyme electrode.

6. Cross-Linking of Protein (Enzyme) Layers Stabilized by Affinity Interactions on Electrodes

Electrically contacted biocatalytic electrodes of NAD^+-dependent enzymes were organized by generating affinity complexes between a catalyst/NAD^+ monolayer and the respective enzymes.[6] A PQQ monolayer covalently linked to an amino-functionalized nicotinamide adenine dinucleotide (**9**) was assembled onto an Au electrode. The resulting monolayer-functionalized electrode binds NAD^+-dependent enzymes such as lactate dehydrogenase (LDH) or alcohol dehydrogenase (AlcDH) by affinity interactions between the cofactor and biocatalyst (Figure 15A). These enzyme electrodes electrocatalyze the oxidation of the respective substrates,

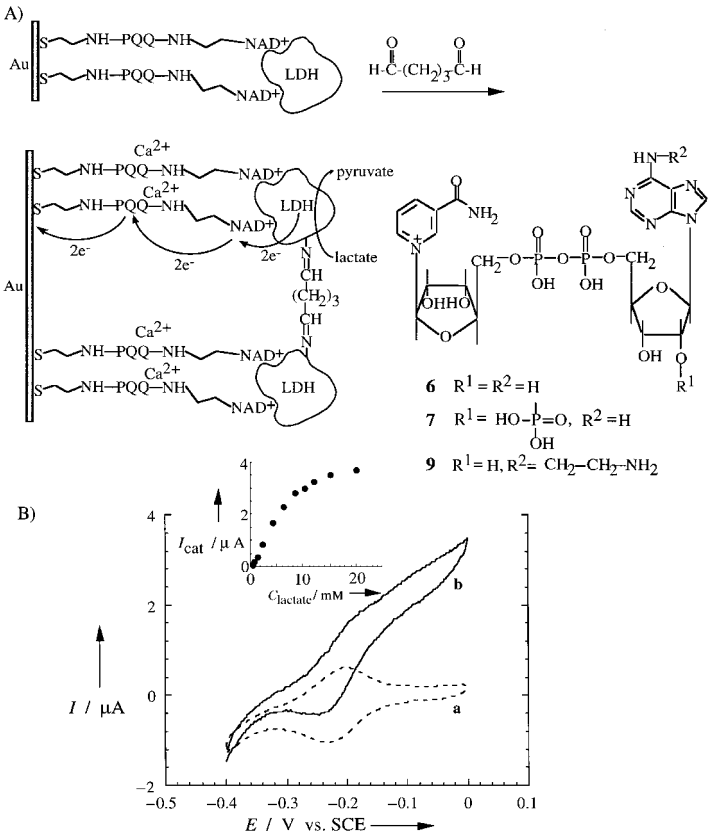


Figure 15. A) Assembly of an integrated LDH-monolayer electrode by cross-linking an affinity complex formed between the enzyme and a PQQ-NAD^+ monolayer Au electrode. B) Cyclic voltammograms of this electrode: a) in the absence of lactate and b) with lactate (20 mM); potential scan rate: 2 $mV s^{-1}$. Inset: amperometric responses of the integrated LDH layered electrode at different concentrations of lactate.

lactic acid or ethanol. The bioelectrocatalytic transformations proceed via the biocatalyzed substrate-induced formation of NADH and its oxidation by the PQQ catalytic site. The resulting enzyme electrode revealed only temporary stability and approximately 25% of the biocatalyst dissociated from the monolayer to the electrolyte solution within 30 min. Cross-linking the enzyme layer associated with the PQQ/NAD^+-cofactor monolayer with glutaric dialdehyde generated a stable, integrated, electrically contacted, cofactor–enzyme electrode (Figure 15A).

Protein–protein affinity interactions play a major role in biological electron transfer. For example, cytochrome c mediates electron transfer to different redox enzymes, such as cytochrome oxidase, lactate dehydrogenase, or copper enzymes.[149] These mediated electron transfer reactions proceed within the protein–protein affinity complexes.[150] Microperoxidase-11 (MP-11, **10**, see Figure 16) is a heme-containing oligopeptide consisting of 11 amino acid residues that compose the microenvironment of the active site of cytochrome c.[151] It is obtained by digestion of the native cytochrome c with trypsin. Despite the structural similarities between MP-11 and cytochrome c, the heme sites of the oligopeptide and the native protein differ substantially in their redox potentials, $E^\circ = -0.40$ V for MP-11[125a] and $E^\circ = +0.012$ V (versus SCE) for cytochrome c.[149] Microperoxidase-11 was assembled as a monolayer on an Au electrode, [152] and this functionalized electrode stimulated electron transfer to hemoproteins such as myoglobin, hemoglobin, and cytochrome c.[125c] The mediated electron transfer was attributed to the formation of affinity complexes between the proteins and MP-11 at the electrode interface. The affinity interactions between MP-11 and hemoproteins or cytochrome-dependent enzymes were used to assemble a nitrate reductase enzyme electrode (Figure 16).[152] The cytochrome-dependent nitrate reductase (NR, E.C. 1.9.6.1 from *Escherichia coli*) forms an affinity complex with the MP-11 monolayer associated with the electrode. Cross-linking of the enzyme-MP-11 affinity complex layer with glutaric dialdehyde generated a stable, enzyme-layered electrode that effected the bioelectrocatalyzed reduction of nitrate to nitrite. The bioelectrocatalyzed transformation proceeds with a current effificiency of approximately 85%, and the electrode was applied as an amperometric nitrate sensor.

Cobalt(II)–protoporphyrin IX reconstituted myoglobin was also found to form an affinity complex with the MP-11 monolayer electrode (association constant $K_a = 1.6 \times 10^5\ M^{-1}$, electron-transfer rate constant in the resulting supramolecular complex $k_{et} = 0.3\ s^{-1}$).[153] The MP-11 mediates the electrocatalyzed reduction of Co^{II}-myoglobin and the resulting cobalt hydride hydrogenates alkynes, for example, acetylene dicarboxylic acid to maleic acid. The cross-linking of the Co^{II}-reconstituted myoglobin affinity complex at the MP-11 monolayer with glutaric dialdehyde generated an integrated, stable, layered electrode for the electrocatalytic hydrogenation of acetylene dicarboxylic acid with a current yield of approximately 80%.

Recently, de novo synthesized four-helix polypeptides were applied to mimic functions of cytochrome b and to tailor layered cross-linked electrocatalytic electrodes. One such protein (14728 Da) that includes four histidine units in the respective "A"-helices was assembled onto Au electrodes (Figure 17). Two units of Fe^{III}-protoporphyrin IX were reconstituted into the assembly to yield a vectorial electron-transfer cascade.[131] The affinity complexes formed between the de novo synthesized protein and the cytochrome-dependent nitrate reductase (NR) or with Co^{II}-protoporphyrin IX-reconstituted myoglobin[154] were cross-linked with glutaric dialdehyde to yield electrically contacted electrocatalytic electrodes. The Fe^{III}-de novo protein/NR-electrode assembly

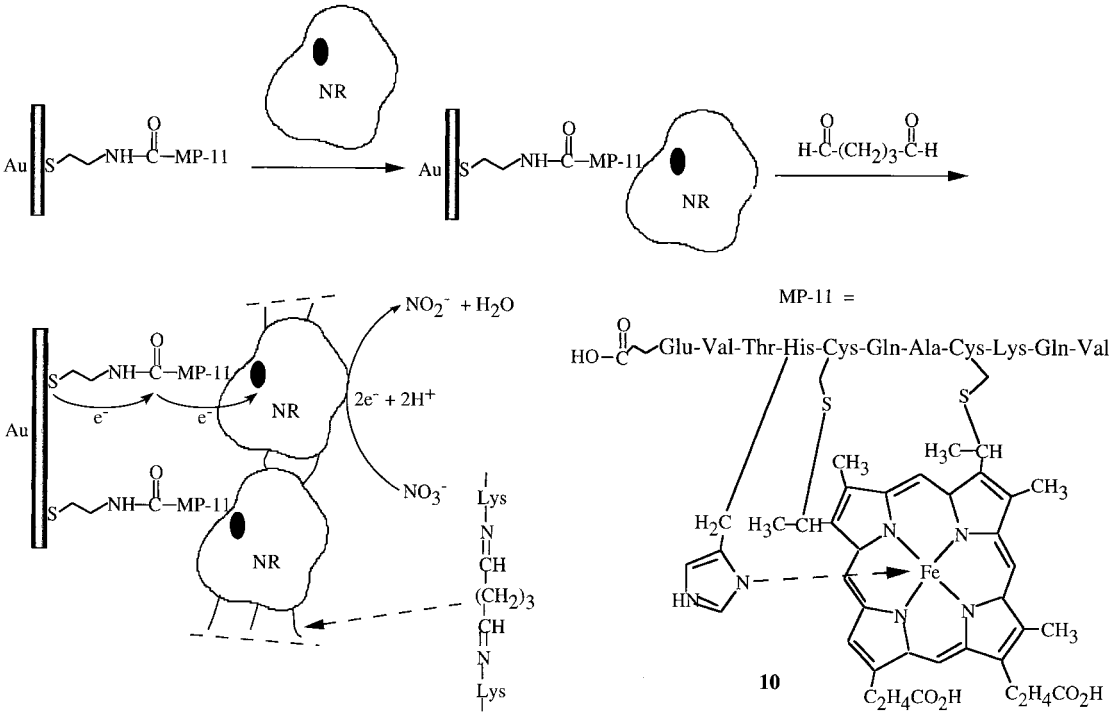


Figure 16. Assembly of an integrated nitrate sensor electrode by cross-linking of an affinity complex formed between MP-11 and nitrate reductase on a Au electrode.

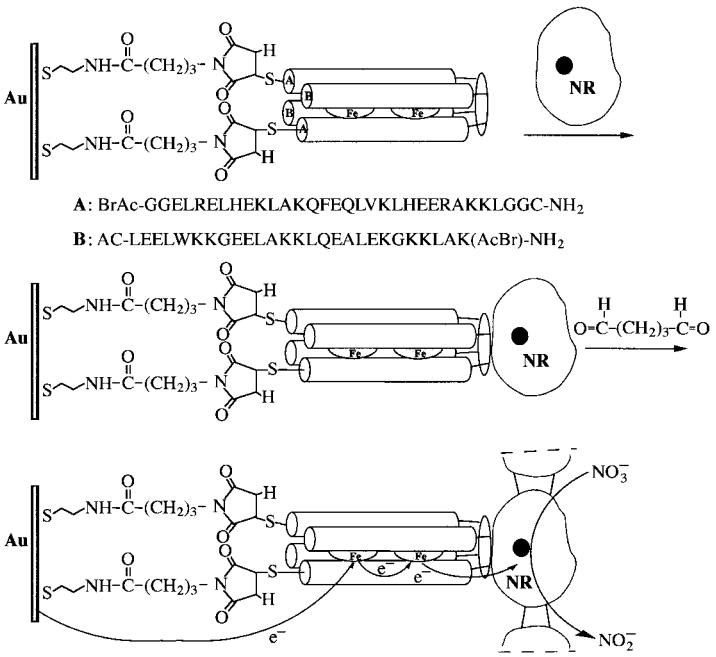


Figure 17. Assembly of a nitrate-sensing electrode by the cross-linking of an affinity complex formed between nitrate reductase and a de novo four helix-bundle protein reconstituted with Fe^{III}-protoporphyrin.

was applied to the electrocatalyzed reduction of NO_3^- to NO_2^- and acted as an amperometric NO_3^- sensor. The Fe^{III}-de novo protein/Co^{II} reconstituted myoglobin integrated electrode stimulated the electrocatalyzed hydrogenation of acetylene dicarboxylic acid to maleic acid.

7. Electron Transfer in Layered Enzyme Electrode Assemblies

The electrical contacting of redox proteins and the electrode supports is the essence of any biosensor or bioelectronic device. The direct electron transfer between redox-active sites of proteins and the electrode is usually prohibited since the redox center is sterically insulated by the protein matrices (see Introduction). Early studies have demonstrated that certain enzymes or redox proteins exhibit electrical communication with electrode supports, and biocatalyzed electrically stimulated transformations are driven by these enzymes.[155] The direct electroreduction of H_2O_2 by horseradish peroxidase (HRP [Eq. (7)]) was demonstrated,[156] and related heme-

$$H_2O_2 + 2H^+ + 2e^- \xrightarrow{HRP} 2H_2O \quad (7)$$

containing oxidases, for example, cytochrome c peroxidase,[157] lactoperoxidase,[158] chloroperoxidase,[159] showed similar direct electron transfer with electrode surfaces. Laccase (a copper-containing polyphenol oxidase) was found to electrocatalyze the reduction of molecular oxygen [Eq. (8)].[160] The direct electron transfer in these enzymes is

$$O_2 + 4H^+ + 4e^- \xrightarrow{laccase} 2H_2O \quad (8)$$

attributed to the location of the redox-active center at the protein periphery. For example, a detailed kinetic study[161] of the peroxidase-catalyzed reduction of H_2O_2 revealed that 42% of the total enzyme molecules are aligned on the electrode surface in a configuration where the redox heme site is accessible for electron transfer. Some of the enzymes include two redox sites within the protein, and electron

transfer proceeds vectorially from a peripheral redox site to an inner redox-active component. For example, *p*-cresolmethyl hydroxylase (PCMH), an FAD- and heme-containing redox enzyme, effects the direct oxidation of *p*-cresol to *p*-hydroxybenzaldehyde [Eq. (9)].[162] Similarly, flavocytochrome c_{552}

$$p\text{-cresol} + H_2O \xrightarrow{\text{PCMH}} p\text{-hydroxybenzaldehyde} + 4H^+ + 4e^- \quad (9)$$

(sulfide cytochrome c oxidoreductase), which contains a FAD and two covalently linked heme groups, is bioelectrocatalytically active in the oxidation of sulfide to sulfur [Eq. (10)].[163]

$$HS^- \xrightarrow{\text{flavocytochrome c}} S^0 + H^+ + 2e^- \quad (10)$$

The direct electron transfer of this series of redox enzymes allows their application as bioactive sensing interfaces in amperometric biosensor devices. Detection of *p*-cresol,[162] methylamine,[164] and fructose[165] was reported in the presence of *p*-cresolmethyl hydroxylase, methylamine dehydrogenase, and fructose dehydrogenase, respectively.

The direct electrical contact of certain enzymes with electrodes can be used to tailor bioelectrocatalytic assemblies for sensor applications. A variety of oxidases utilize molecular oxygen as the electron acceptor for the oxidation of their substrates (Sub). Enzymes such as catechol oxidase, amino acid oxidase, glucose oxidase, lactate oxidase, pyruvate oxidase, alcohol oxidase, xanthine oxidase, and cholesterol oxidase catalyze the O_2-oxidation of the respective substrates with the concomitant formation of H_2O_2 [Eq. (11)]. The

$$Sub_{red} + O_2 + 2H^+ \xrightarrow{\text{oxidase}} Sub_{ox} + H_2O_2 \quad (11)$$

direct electron transfer between HRP and electrodes enables the electroreduction of H_2O_2 in the range -0.2 to 0 V (versus SCE) and allows for the coupling of HRP to these oxidases to yield amperometric biosensors. The amount of generated H_2O_2 is controlled by the substrate concentration and, hence the resulting current provides a quantitative measurement of the analyzed substrate. Accordingly, amperometric biosensors based on HRP coupled with glucose oxidase for glucose analysis,[166] with alcohol oxidase for alcohol detection,[167] with choline oxidase for choline analysis,[166a] and with amino acid oxidase for amino acids determination[168] were constructed. Various conducting materials such as carbon black,[156] pyrolytic graphite,[169] platinized carbon particles,[170] and colloidal gold[171] were used to support the enzymes, and carbon paste[172] or polypyrole[166e, 172a] were used to entrap the biocatalysts.

Electrical contact of redox enzymes that lack direct electrical communication with electrodes can be established by mediated electron transfer using synthetic or biologically active charge carriers. Mediated electron transfer (MET) can be effected by a diffusional mechanism (Figure 18 A). The electron relay is oxidized at the electrode surface (in an oxidative biotransformation) or reduced at the electrode (in a reductive biotransformation). Diffusional penetration of the reduced or oxidized relays into the protein yields sufficiently short electron-transfer distances in respect to the active redox center for MET and the electrical activation of the biocatalyst. Ferrocene derivatives, ferricyanide, quinones, *N*,*N*′-dialkyl-4,4′-bipyridinium, and other electroactive molecular substrates were employed for MET and electrical activation of redox enzymes.[9b] Table 5 gives a representative summary of bioelectrocatalytic transformations stimulated by diffusional MET.

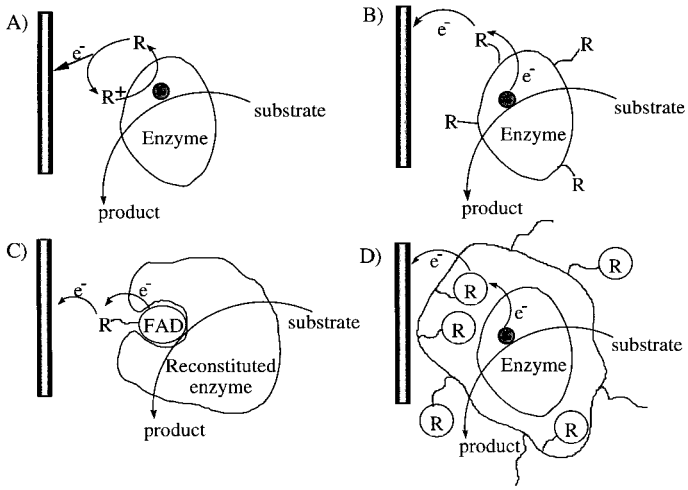


Figure 18. Electrical contacting of redox enzymes and electrode supports (R is an electron relay): A) Using a diffusional electron relay, B) by chemical modification of the protein with electron relay units, C) by reconstitution of an apo-flavoenzyme with a synthetic electron-relay-FAD dyad, D) by immobilization of the biocatalyst into a redox polymer.

Chiroselective mediated electron transfer has been addressed in a series of studies using diffusional chiral electron mediators.[185] The chiral electron relays (*S*)- or (*R*)-*N*,*N*-dimethyl-1-ferrocenyl-ethylamine [(*S*)- or (*R*)-(Fc)] were reported to stimulate chiroselective bioelectrocatalyzed oxidation of glucose in the presence of glucose oxidase,[185a] whereby the oxidation in the presence of (*S*)-(Fc) was double that in the presence of (*R*)-(Fc). Kinetic analysis of the bioelectrocatalyzed oxidation of glucose by the two enantiomeric electron relays suggests that the protein induces chiral discrimination through diastereoisomeric interaction with the penetration–dissociation routes of the electron mediator, to and from the biocatalyst. Chiral discrimination in the MET, and the subsequent bioelectrocatalyzed transformations, were observed using different diffusional electron relays and various enzymes.[185c, 185d]

The concept of enantioselective electrical contacting of redox enzymes and electrode surfaces was further developed by the organization of a chiral electron mediator monolayer on an electrode.[186] (*S*)- or (*R*)-2-Methylferrocene carboxylic acid was assembled as a monolayer on an Au electrode (Figure 19). The monolayer-mediated chiroselective oxidation of glucose in the presence of glucose oxidase and an approximate 1.9-fold enhanced biocatalytic oxidation of glucose was observed in the presence of the monolayer electrode functionalized with the (*S*)-relay enantiomer.

Chemical modification of redox enzymes with electron-relay groups enables the mediated electron transfer and the electrical wiring of the proteins (Figure 18 B).[187] Covalent attachment of electron-transfer units at the protein periphery, as well as inner sites, yields short inter-relay electron-transfer distances. Electron "hopping" or tunneling from the periph-

Table 5. Diffusional electron-transfer mediators applied to the immobilized enzymes.

Enzyme	Mediator	Mediator redox potential [mV versus SCE]	Ref.
glucose oxidase (EC 1.1.3.4) from *Aspergillus niger*	1,4-benzoquinone	39	[173]
	1,1-dimethylferrocene	100	[174]
	ferrocenecarboxylic acid	275	[175]
	phenazine methosulphate	− 161	[176]
	tetrathiofulvalene	300	[177]
	tetracyanoquinodimethane	127	[178]
xanthine oxidase (EC 1.2.3.3) from buttermilk	$[Fe(CN)_6]^{4-}$	180	[10a]
cholesterol oxidase (EC 1.1.3.6) from *Pseudomonas sp.*	ferrocenecarboxylic acid	275	[179]
hydrogenase (Fe/S type)	dimethylviologen	− 681	[180]
horseradish peroxidase (EC 1.11.1.7)	$[Fe(CN)_6]^{4-}$	180	[181]
lactate dehydrogenase (EC 1.1.2.3) from yeast	$[Fe(CN)_6]^{4-}$	180	[173a]
glucose dehydrogenase (EC 1.1.99.17) from *A. Calcoaceticus*	1,1′-dimethylferrocene	100	[182]
	phenazine ethosulfate	− 172	[183]
NADH dehydrogenase (EC 1.6.99) from *Bacillus stearothermophilus*	ferrocenylmethanol	185	[184]
	ferrocenecarboxylic acid	275	[184]

ery to the active site, or vice versa, enables electron transfer between the redox enzyme and its environment. Glucose oxidase was covalently modified with ferrocene electron-relay groups by carbodiimide coupling of ferrocenecarboxylic acid to the amino groups of the lysine functions of the protein backbone.[187a,b] Some other chemical procedures were also used to produce relay groups peripherally bound to proteins. For example, carbonyl groups, generated in glycoproteins (for example, glucose oxidase) by oxidative treatment with periodate were coupled to the amino-functionalized ferrocene through the formation of Schiff bases.[187c] A ruthenium complex was assembled on the glucose oxidase backbone that was pre-modified with pyridine units.[187b] The relay groups randomly distributed on the protein provided electrical contact between the diffusional enzyme and the unmodified electrode surface. It has been demonstrated that longer spacer groups bridging the electron-relay groups and the enzyme provide higher mobility, shorten the electron-transfer distance, and enhance the enzyme bioelectrocatalytic activity.[187c] Partial and reversible unfolding of the enzyme by urea during the covalent modification of the protein with the relay is also important since it allows the attachment of the electron mediator to inter-protein positions close to the enzyme active site.[188] The electron-relay-tethered enzymes were also cross-linked on an electrode surface, producing a nonorganized multi-layer,[119d] or incorporated into a conductive polymer on electrode supports.[189] The immobilized enzymes were electrically contacted through the electron-relay groups to provide electron propagation through the layer.

Chemical modification of redox proteins with synthetic electron mediators is always accompanied by the partial degradation of the native biocatalyst. The effectiveness of electrical contacting is enhanced upon increasing the loading of the electron mediator on the protein, since electron-transfer distances are shortened. The increase of the loading, however, partially deactivates of the enzyme. With glucose oxidase, the optimal MET was found at a loading corresponding to 12 – 13 ferrocene units per molecule.[188] The rate constant of the electron transfer between the enzyme FAD site and the nearest electron-relay group was found to be

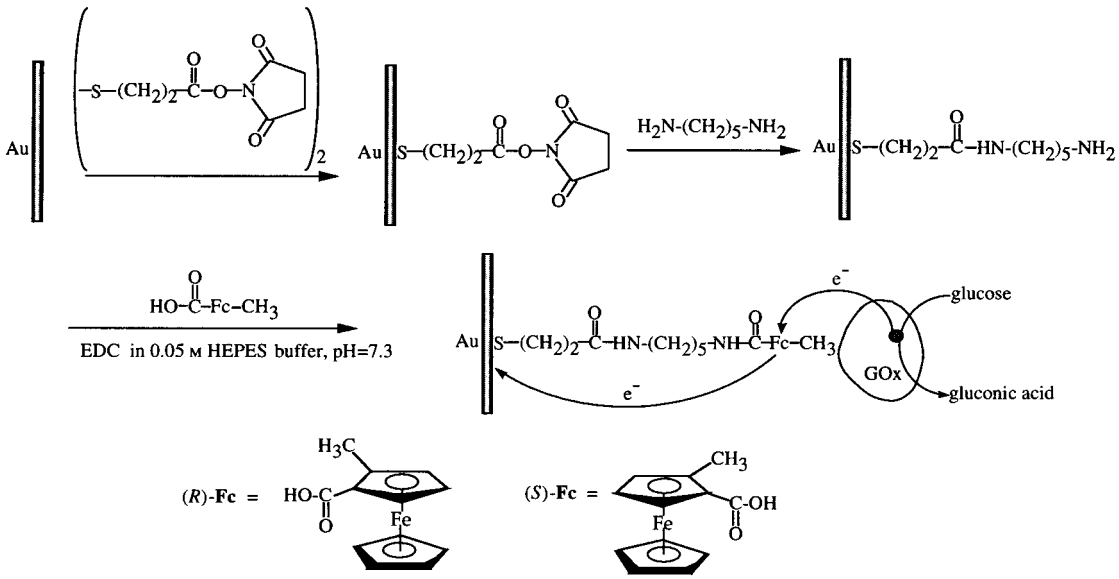


Figure 19. Organization of a chiral ferrocene monolayer on an electrode.

about 0.9 s^{-1}, that is, substantially lower than the electron transfer to the native dioxygen electron acceptor of the enzyme, which proceeds with a rate constant of about 5×10^3 s^{-1}.[188] Improvement of the electrical contact between the active center of the enzyme and the electrode can be envisaged by two complementary modes: 1) modification of the redox protein with relay units at optimal positions for mediated electron transfer, and 2) alignment of the relay-functionalized bioelectrocatalyst in respect to the electrode surface.

A novel means for the electrical wiring of redox enzymes and, specifically, flavo-redox-proteins was reported by the site-specific modification of the redox enzyme with the electron-relay group, and by using a reconstitution method (Figure 18C).[190] The native cofactor, for example, flavin adenine dinucleotide phosphate (FAD) is substituted with a semisynthetic FAD cofactor tethered to an electron relay group to yield an electrically contacted biocatalyst. The semisynthetic cofactor composed of *N*-(ferrocenylmethyl)aminohexanoic acid (**11**) covalently linked to N^6-(2-aminoethyl)-FAD (**8**; Figure 20) was used to reconstitute apo-glucose oxidase or apo-D-amino acid oxidase. The resulting reconstituted enzymes revealed direct electrical contact with electrode surfaces and acted as "electroenzymes" for the electroinduced biocatalyzed oxidation of glucose or D-alanine.[190]

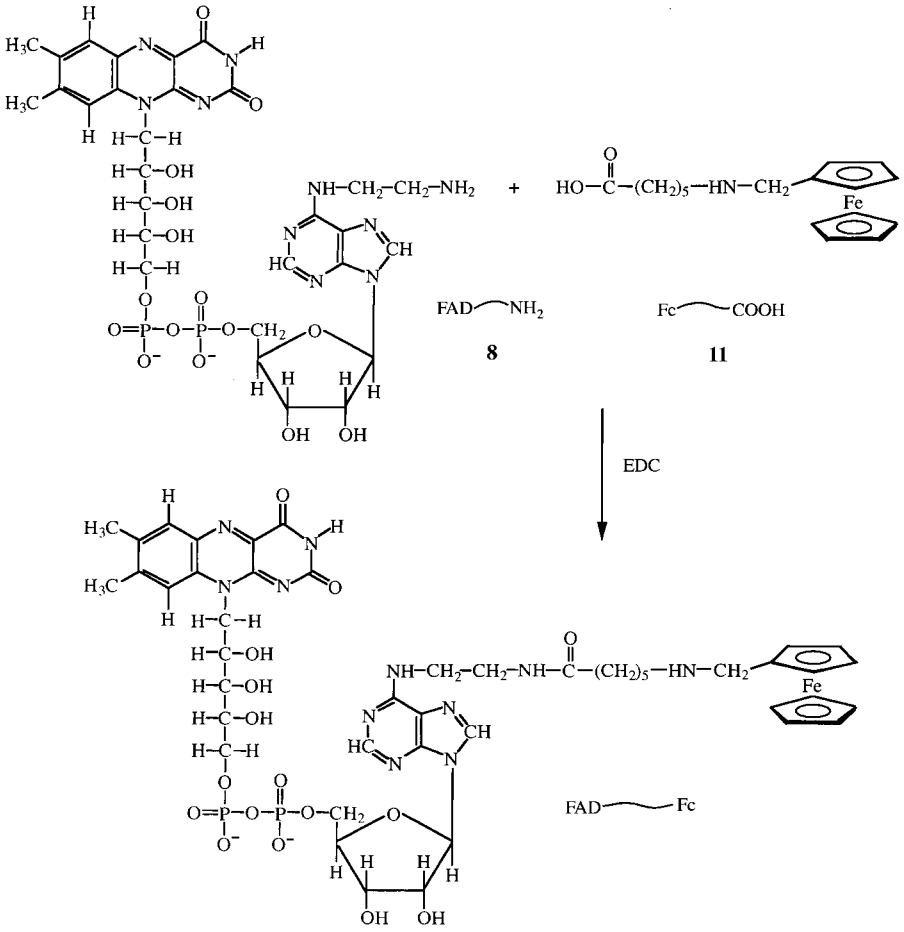


Figure 20. Synthesis of the FAD-ferrocene dyad.

A further approach for bringing redox enzymes in contact with electrode supports is the entrapment of the protein in redox-tethered polymers or conductive polymers (Figure 18D).[25, 31c, 191] The redox relay groups tethered to the polymer penetrate into the redox protein and reach appropriate distances to mediate electron transfer. It has been shown that the chain lengths tethering the redox mediator to the polymer control the efficiency of mediated electron transfer.[192] Polylysine was functionalized with *N*-carboxyalkyl-*N*′-methyl-4,4′-bipyridinium (**12**) redox-active groups. Mediated electron transfer to glutathione reductase and the bioelectrocatalyzed reduction of oxidized glutathione (GSSG) were examined with different chain lengths that tether the bipyridinium group to the polymer (Figure 21). Mediated electron transfer was found to be enhanced when the tethering chain is longer. The bioelectrocatalyzed reduction of GSSG in the presence of the enzyme and the functionalized polymer was attributed to the formation of a complex between the positively charged polylysine and the negatively charged glutathione reductase (GR), where long tethering bridges attain short distances of the redox relay in respect to the protein active center, thereby enhancing the MET.

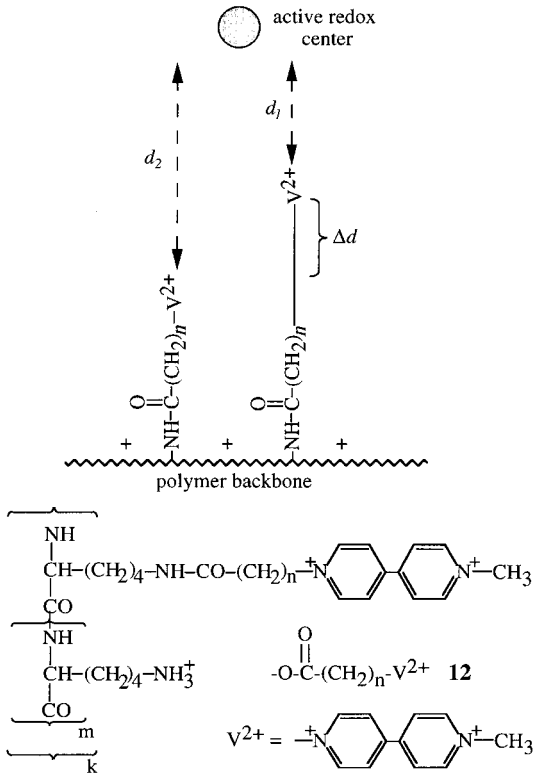


Figure 21. Schematic model for the spatial orientation of a redox center in a protein relative to a positively charged redox-tethered polymer.

Many examples of thin-film bioelectrocatalytic electrodes with the enzyme embedded in the polymer exist.[31c] The amphiphilic polymers for the complementary immobilization and electrical wiring of enzymes are usually based on polypyrroles,[193] polythiophenes,[31d] siloxanes,[29d, 31g] polyamines,[31h] and copolymers of 4-vinylpyridine and 4-aminostyrene.[25] The polymeric backbone was modified with ferrocene groups,[31g, 193a, 194] $Os(bpy)_2$ complexes,[25] or quinones[195] to establish electrical contact with oxidative enzymes, for example, glucose oxidase, glutamate oxidase, or polyphenol oxidase. Viologens (*N*,*N*′-dialkyl-4,4′-bipyridinium salts) tethered to the polymer matrices were applied to electrically contact reductive enzymes, such as nitrate reductase[31d, 193c] and pyridine nucleotide oxidoreductase.[196]

8. Electrical Contact of Redox Proteins at Monolayer-Functionalized Electrodes

Functionalization of electrodes with low molecular weight, electrochemically inactive compounds was found to affect the electrical communication between redox proteins or redox enzymes and electrodes. Reversible electrochemistry of cytochrome c, which includes a partially exposed heme site,[197] was observed at Au electrodes (in some investigations at Pt or Ag electrodes) modified with monolayers of different promoters.[149c, 198] These promoters usually include a sulfur group as an anchor on the surface (thiol or disulfide) and an organic functional unit that interacts with the cytochrome c protein backbone. The promoter–monolayer prohibits the direct contact between the protein and the metal electrode surface, thus preventing irreversible unfolding and denaturation of the protein. The interactions between the promoter and the protein molecules are based on hydrogen bonds and/or attractive electrostatic association, which result in the specific alignment of the protein at the electrode surface. In the aligned configuration the proteins exhibit short electron-transfer distances that allow efficient electrical contact and electrochemical processes. The most common promoter for the activation of the interfacial electrochemistry of cytochrome c is bis(4-pyridyl)disulfide.[199] Several other thiol- or disulfide-derivatized molecules also act as efficient promoters.[200] Amino acid and oligopeptide monolayers were used as promoters for interfacial electrochemistry with cytochrome c[201] as well as for reversible electrochemical activation of other cytochromes.[202] Other promoters, such as imidazole,[203] thiophene,[204] and iodide[205] deposited as monolayers onto Au or Ag electrodes were also applied to facilitate the electrochemistry of cytochrome c. A monolayer of colloidal Au particles with diameters of 12 nm on a SnO_2 electrode was demonstrated to be an efficient promoter for cytochrome c electrochemistry.[206] The nanometer-scale morphology of the metal particles plays a key role in the electrical contacting of the protein. A similar methodology was applied to activate the interfacial electrochemistry of other redox proteins and enzymes such as ferritin, blue copper proteins, azurin, pseudoazurin, umecyanin, stellacyanin, plantacyanin, plastocyanin, and cucumber ascorbate oxidase[207] on Au electrodes modified with 4-pyridinethiol (or some other thiol and disulfide derivatives) monolayers. Protonated aminosiloxane-modified indium oxide electrodes were reported to be efficient interfaces for stimulating electron transfer to the negatively charged ferredoxin.[208] These promoters apparently align the protein molecules on the electrode surface to an orientation that facilitates electron transfer.

Low molecular weight, redox-active oligopeptides containing redox groups derived from native biomaterials may establish direct electron-transfer communication with electrodes. Microperoxidase-11 (**10;** see Figure 16) is a heme-undecapeptide, which constitutes the active-site microenvironment of cytochrome c.[151] Since the active site of MP-11 is not sufficiently isolated by the oligopeptide chain the direct electrochemistry of MP-11 was observed in aqueous[209a] and nonaqueous[209b] solutions at unmodified electrodes. Microperoxidase-11 was covalently linked to amino-[125a] and carboxyl-functionalized[125d] Au electrodes through carboxylic or amino groups of MP-11, respectively. The MP-11 monolayer revealed electrocatalytic activities for H_2O_2 reduction[125a] and organic peroxide reduction in nonaqueous solutions.[125b]

The MP-11 monolayer forms affinity complexes with hemoproteins such as cytochrome c, myoglobin, and hemoglobin.[125c] Microgravimetric analysis enabled the association constant of myoglobin to the MP-11 monolayer to be determined as $K_a = 3.9 \times 10^2\,\text{M}^{-1}$. The MP-11 and hemoprotein affinity complexes enable the mediated reduction of the heme sites in the different proteins (Figure 22 A). The MP-11 monolayer was found to yield an affinity complex with the native cytochrome b_5-dependent nitrate reductase (NR, E.C. 1.9.6.1 from *Escherichia coli*).[152] The association constant of the MP-11/NR complex was determined by quartz-crystal-microbalance measurements to be $K_a = 3.7 \times 10^3\,\text{M}^{-1}$, and the surface coverage of the enzyme on the surface was estimated to be $3.8 \times 10^{-12}\,\text{mol cm}^{-2}$. The layered affinity complex was cross-linked to yield an integrated, stable, electrically contacted layered electrode (see Figure 16). Figure 22 B shows the electrocatalytic currents transduced by the MP-11/NR layered-electrode in the presence of nitrate. The transduced currents are controlled by the nitrate concentration in the electrolyte sample, (Figure 22 B, inset), and thus the functionalized electrode acts as an amperometric biosensor for nitrate.

Low molecular weight native cofactors such as PQQ (**5**) were reported to form an electrical contact between $NAD(P)^+$-dependent enzymes and electrode supports. The PQQ cofactor was linked to a cystamine monolayer[104b, 122] and was found to act as an electrocatalyst for the electrooxidation of NAD(P)H, especially in the presence of Ca^{2+} ions.[210a] Accordingly, a PQQ monolayer covalently linked to malate dehydrogenase (MalDH; Figure 23) was used to electrically activate the enzyme.[122] The biocatalyzed oxidation of malic acid by MalDH generates diffusional NADPH that is oxidized by the PQQ electrocatalyst. The transduced current relates to the concentration of NADPH (or malic acid) and thus the layered enzyme electrode exhibits elements of an amperometric biosensor. The use of $NAD(P)^+$-dependent enzymes as bioactive materials for amperometric biosensor devices requires, however, the integration of the cofactor and

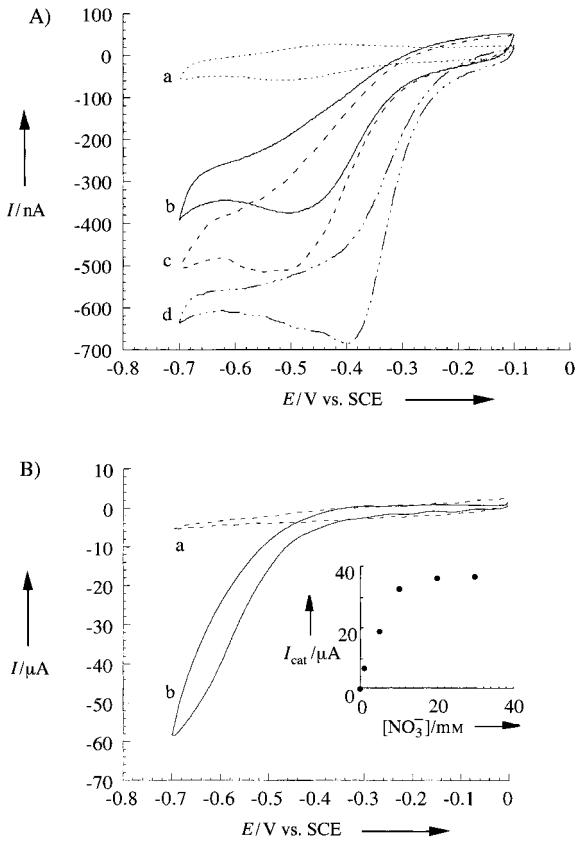


Figure 22. A) Cyclic voltammograms of a MP-11-monolayer electrode: a) in the absence and b) with hemoglobin (6×10^{-5} M); c) with myoglobin (2×10^{-4} M); d) with cytochrome c (2×10^{-4} M); scan-rate: 5 mV s^{-1}. B) Cyclic voltammograms of the electrode with a cross-linked MP-11/nitrate reductase monolayer: a) in the absence of nitrate, b) with nitrate (20 mM); potential scan rate: 5 mV s^{-1}. Inset: amperometric responses of the integrated electrode at different concentrations of nitrate.

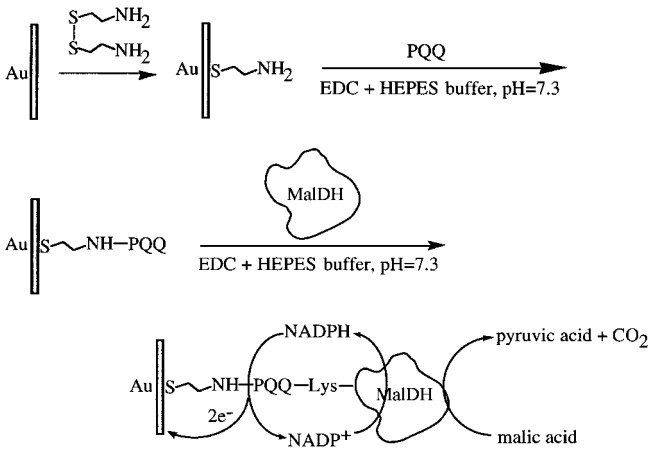
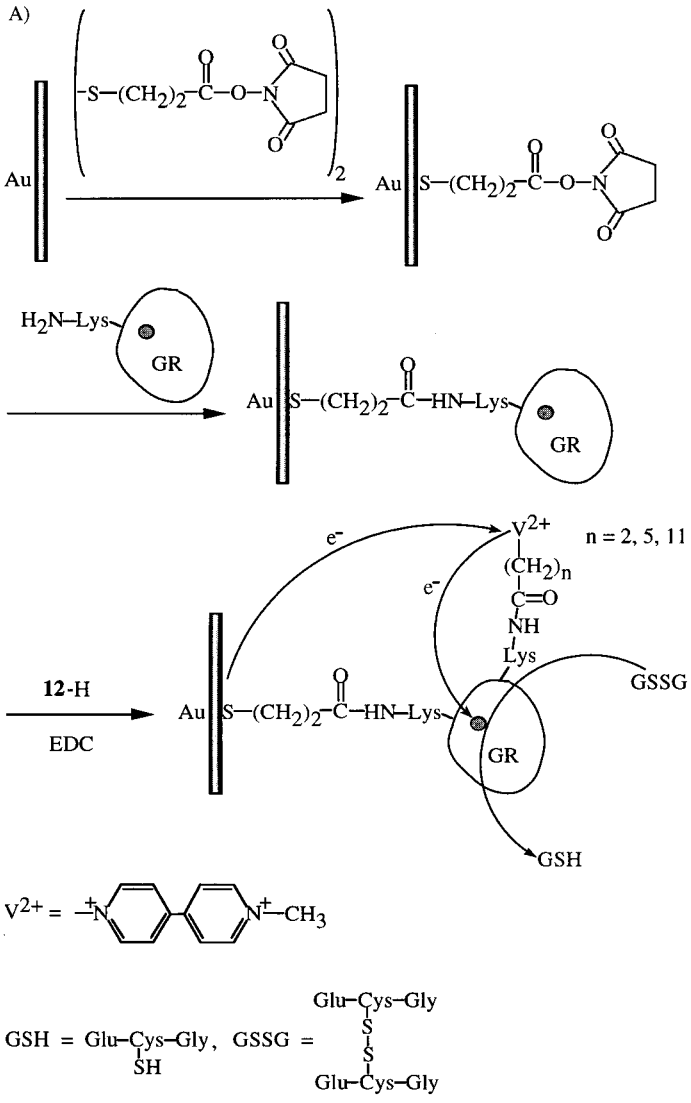


Figure 23. Assembly of a PQQ-MalDH monolayer electrode, and the amperometric sensing of malic acid using $NADP^+$ as a diffusional cofactor.

the enzyme in a rigid configuration, as a result of the high cost of the $NAD(P)^+$-cofactors, which prevents their use as diffusional components. The $NAD(P)^+$-dependent enzymes operate in nature by a diffusional route in which the oxidized cofactor penetrates into the protein, accepts the electrons (as a hydride equivalent), and diffuses from the protein matrices. Further oxidation of the NAD(P)H by the electrode requires a catalyst.[210] The affinity interactions between the NAD^+-cofactor and the respective cofactor-dependent enzymes were used to assemble integrated electrodes with layers of cofactors and enzymes exhibiting electrical communication.[6] The semisynthetic NAD^+-cofactor **9** was covalently linked to a PQQ monolayer (see Figure 15A). The NAD^+-dependent enzymes lactate hydrogenase (LDH) or alcohol dehydrogenase (AlcDH) bind to the PQQ-NAD^+ monolayer by affinity interactions. Microgravimetric, quartz-crystal-microbalance studies revealed the coverage of LDH to be 3.5×10^{-12} mol cm^{-2}. The cross-linking of the enzyme layer with glutaric dialdehyde yields a stable, layered cofactor–enzyme electrode that stimulates the electrooxidation of lactic acid (see Figure 15B). The biocatalyzed oxidation of lactate proceeds by the integrated cofactor–enzyme assembly where the enzyme-generated NADH is oxidized by PQQ in the presence of Ca^{2+} ions. The amount of reduced NADH cofactor is controlled by the concentration of lactate, and thus the transduced currents correlate with the concentration of lactate. The electrode thus provides a general configuration for biosensor electrodes employing NAD^+-dependent enzymes.[6]

The covalent attachment of electron relays to layered enzyme electrodes provides a means to facilitate electrical communication between the enzyme layer(s) and the electrode, and to electrically activate the biocatalyst.[116] A glutathione reductase monolayer was assembled onto Au electrodes through a thiolate bridging unit (Figure 24A).[116a,b] The enzyme layer was functionalized with **12**-H, where the chain length tethering the electron relay was systematically lengthened. Electroreduction of the bipyridinium unit was found to activate the enzyme for the reduction of oxidized glutathione (GSSG). The effectiveness of GSSG reduction was controlled by the length of the chain tethering the electron relay to the protein, with longer bridging chains enhancing the bioelectrocatalyzed reduction of glutathione (Figure 24B). This result was rationalized in terms of shorter electron-transfer distances between the electron-relay sites and the enzyme redox center for the systems with bipyridinium units tethered by long chains. It was found that it is important to modify the protein with the bipyridinium components in the presence of urea to attain electron transfer between the enzyme redox center and the electrode. Added urea partially unfolds the protein and enables the substitution of inner-protein lysine residues with the bipyridinium electroactive sites (see Section 7).[116a,b]

A multilayer of glucose oxidase (GOx, see Figure 8) was similarly modified with a carboxyferrocene derivative (**11**).[118] The electroactive ferrocene relay units are oxidized by the electrode, and these then oxidize the redox sites of the enzyme. The amperometric response of the layered electrodes was found to be controlled by the number of protein layers,[119b] implying that all of the enzyme layers are electrically contacted with the electrode by the ferrocene units. The rate-limiting process in the oxidation of glucose is the electron transfer between the substrate and the active center. This results in different amperometric responses of the electrode at variable glucose concentrations, and allows the application of the layered enzyme electrode as an amperometric glucose



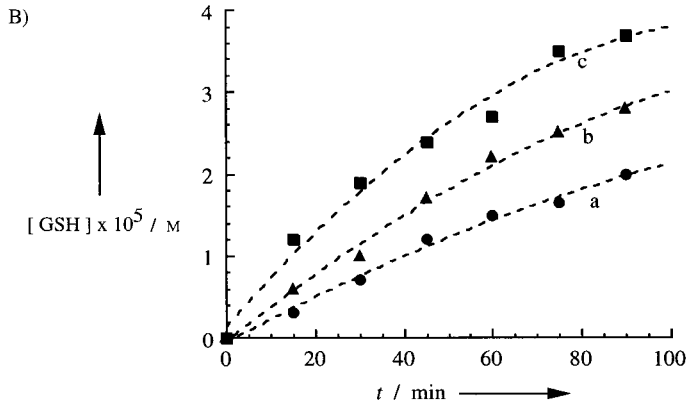


Figure 24. A) Assembly of an electrically contacted glutathione reductase electrode. B) Rate of bioelectrocatalyzed reduction of oxidized glutathione (GSSG) by this electrode using different tethering chain lengths of the contacting relay units: a) $n=2$, b) $n=5$, c) $n=11$.

biosensor. A related thin layer of glucose oxidase, electrically contacted by ferrocene units, was assembled on an Au electrode by the cross-linking of GOx, 2-aminoferrocene, and a monolayer of *p*-mercaptoaniline with glutaric dialdehyde.[17b]

The reconstitution of apo-GOx on the PPQ-FAD monolayer (see Figure 14A) yields a stable, electrically contacted, bioelectrocatalytically active layer.[145] The current densities transduced by the electrode appear to be unprecendentally high in the area of bioelectronic devices: A current density of 300 $\mu A\,cm^{-2}$ at a glucose concentration corresponding to 80 mM and at 35 °C was found. The theoretical turnover rate of GOx at 35 °C in the presence of molecular oxygen as an electron acceptor is approximately $900\,s^{-1}$,[145] while the calculated value of the current to be transduced by a densely packed GOx monolayer ($2.9\times10^{-10}\,mol\,cm^{-2}$) operating at this maximal electron transfer turnover rate is approximately 300 $\mu A\,cm^{-2}$, which is in excellent agreement with the experimental value. It was concluded that the reconstituted biocatalyst reveals the theoretical value for MET as a result of the alignment of the enzyme onto the electrode surface. The aligned enzyme allows the effective oxidation of the reduced FAD cofactor by the PQQ electron-relay unit and the oxidation of glucose by MET. The high current densities transduced by the GOx reconstituted onto the PQQ-FAD monolayer, and the insensitivity of the enzyme electrode to oxygen and other biological interferrants, led to the suggestion that these functionalized enzyme electrodes could be miniaturized into implanted invasive microelectrodes for in vivo continuous monitoring of glucose levels in blood.

9. Biomaterial-Based Electrodes for Biofuel Cells

One of the attractive applications of bioelectrocatalytic electrodes is the development of biofuel cell elements. The biofuel cell utilizes biocatalysts for the conversion of chemical energy into electrical energy.[211] Since many organic substrates undergo combustion in oxygen or are oxidized with the release of energy, the biocatalyzed oxidation of the organic material at two compartmentalized electrode interfaces could lead to the conversion of chemical energy into electrical energy. Abundant raw materials, such as methanol or glucose, can be used as fuel substrates for the oxidation, and molecular oxygen or H_2O_2 can act as the oxidizer. The extractable power (P) of a fuel cell is the product of the cell voltage (V_{cell}) and the cell current (I_{cell}). Although the ideal cell voltage is affected by the difference in the formal potential of the fuel and oxidizer substrates, irreversible losses in the cell voltage originate from kinetic limitations associated with the electron transfer, ohmic resistances, and concentration gradients. Similarly, the cell current is controlled by the size of the electrodes, the ion permeability, and transport across the membrane separating the cathodic and anodic compartments, and specifically, the rates of electron transfer at the anode and cathode interfaces. All of these parameters collectively influence the resulting cell power, and the V_{cell} and I_{cell} values should be optimized to obtain improved efficiencies.

The fact that layered biomaterial-functionalized electrodes catalyze the oxidation of substrates such as glucose, methanol, and lactic acid, or catalyze the reduction of substrates such as H_2O_2, enables these electrodes to be used as key elements of biofuel cells. Accordingly, a biofuel cell element based on the bioelectrocatalytic oxidation of glucose by H_2O_2 was con-

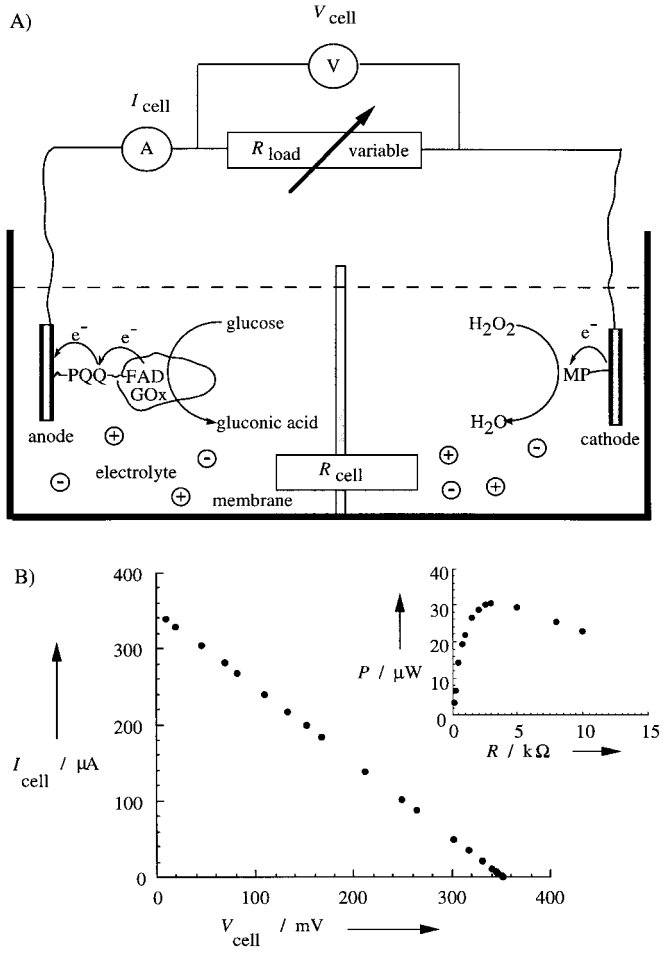


Figure 25. A) Schematic configuration of a biofuel cell employing glucose and H_2O_2 as a fuel and an oxidizer, respectively, and PQQ-FAD/reconstituted GOx MP-11-functionalized electrodes as biocatalytic anode and cathode, respectively. B) Current–voltage behavior of the biofuel cell at different external loads. Inset: electrical power P extracted from the biofuel cell at different external loads.

structed (Figure 25A).[212a] An electrically contacted glucose oxidase monolayer assembled on an electrode by the reconstitution of apo-glucose oxidase on a PQQ-FAD layer (see Figure 14A) was used as the anode, whereas a MP-11-functionalized electrode acted as the cathode in the system. The bioelectrocatalyzed oxidation of glucose proceeds at the anode, while the MP-11-catalyzed reduction of H_2O_2 occurs in the cathodic compartment. The current–voltage behavior of the biofuel cell and the power extracted from the cell at different external loads and at optimized concentrations of the oxidizer and fuel substrates are shown in Figure 25B. The maximum extracted power corresponds to 32 μW at an external load of 3 kΩ (inset in Figure 25B). The configuration of this biofuel cell represents a very general method for developing biofuel cell elements, and it can be extended to other oxidative enzymes and fuel substrates such as methanol oxidase or lactate oxidase and the respective alcohol or lactic acid fuel substances.

A different general approach to tailor biofuel cells is based on the bioelectrocatalyzed oxidation of 1,4-dihydronicotinamide cofactors. Various substrates, for example, alcohols, hydroxy acids, or sugars, undergo biocatalyzed oxidation by enzymes dependent on the $NAD(P)^+$ cofactor. The bioelectrocatalytic oxidation of the resulting dihydronicotinamide cofactors (NAD(P)H) at the anode, with the concomitant reduction of the oxidizer (H_2O_2) at the cathode, could provide a general configuration for a biofuel cell (Figure 26A).[212b] The bioelectrocatalyzed oxidation of NADH by the PQQ-functionalized electrode represents the electrochemical transformation in the anodic compartment, while the MP-11-catalyzed reduction of H_2O_2 is the complementary process at the cathode interface. Figure 26B shows the current–voltage behavior and the electrical power extracted from the cell at different external loads. The maximum extractable power from the cell was estimated to be 8 μW at an external load of 3 kΩ (inset of Figure 26B). Although the system represents a general methodology that utilizes NAD(P)H cofactors as the fuel of a biofuel cell, it is essential to further develop this system for future practical use. The cost of $NAD(P)^+$ cofactors prohibits their use as the primary fuels of the biofuel cell. It is essential to immobilize the cofactor on the anode interface and to couple it to a primary abundant fuel, for example, an alcohol, and the respective enzyme, namely

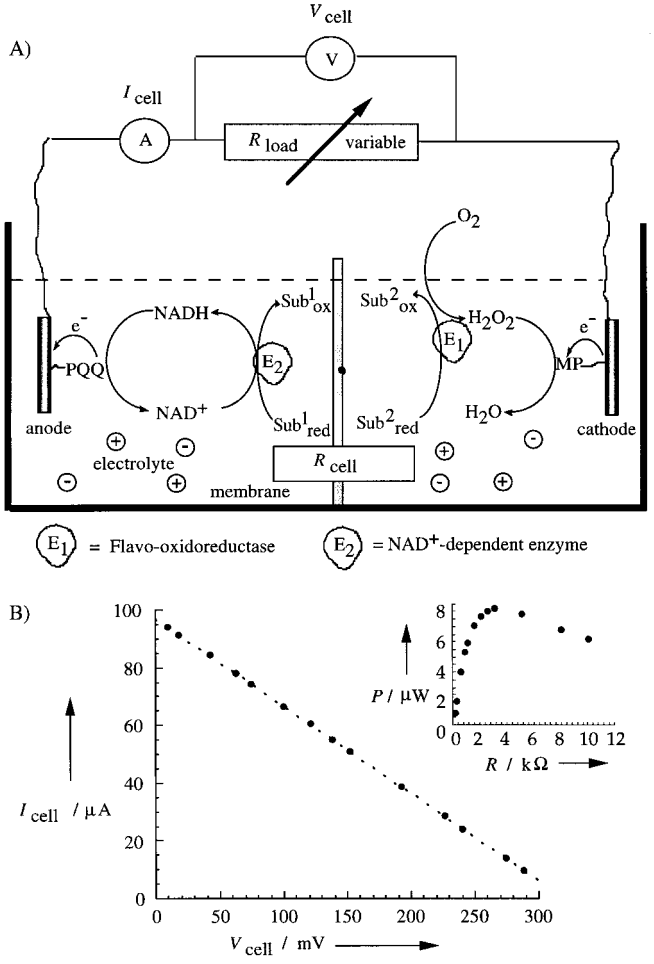


Figure 26. A) Schematic configuration of a biofuel cell employing NADH and H_2O_2 as a fuel and an oxidizer, respectively, and a PQQ-functionalized anode and a MP-11-functionalized cathode. B) Current–voltage behavior and electrical power P (inset) of the biofuel cell at different external loads.

alcohol dehydrogenase, for the in situ generation of the NAD(P)H cofactor. The progress in the organization of integrated, electrically contacted, bioelectrocatalytic $NAD(P)^+$ electrodes (see Figure 15) paves the way toward engineering new layered enzyme electrodes for biofuel cells.

10. Microscale Patterning and Structuring of Biomaterials on Surfaces

The potential applications of biosensors and bioelectronic devices could be expanded to novel horizons if biomolecules, and specifically, enzymes, antigens, antibodies, and receptors, could be organized in ordered microstructured arrays on surfaces.[213] The tailoring of multisensing surfaces (sensor batteries) for the simultaneous addressable detection of several analytes or the analysis of spatial distributions and gradients of analytes in samples could be envisaged by microstructured arrays of bioactive sensing materials. Also, patterned sensor arrays of spatially resolved biomaterials could act as self-calibrated sensor arrays, where one sensor domain is used to calibrate a secondary sensing interface. A further application of structurally ordered domains of biomaterials could include the organization of interacting arrays that respond to external physical or chemical stimuli. A series of enzymes in the appropriate ordering could lead to the transformation of the analyte substrate to a redox-active species that is electronically transduced by the conductive support. Similarly, a chemical reaction of two species generated by spatially separated biocatalysts could generate a product that activates a third spatially organized electroactive enzyme (gated bioelectronics).

The generation of microstructures consisting of conductive materials is a mature research field that has led to tremendous developments in microelectronics and information storage. Microscale lithography using UV irradiation, electron beams, and X-rays are common technologies for surface patterning.[214] The addressing of biomaterials to target surface domains and their specific chemical or physical deposition in biologically active forms on these defined areas on the support represent scientific challenges of interdisciplinary interests. Substantial research efforts have recently been directed to the microscale-patterning of monolayers[215a,b] and functionalized thin films on solid supports.[215c,d]

Photolithography[214] and micromachining of monolayers,[216] microstamping of solid supports,[215a,b, 217] and microwriting on surfaces[218] were often applied to generate patterned monolayers of controlled wettability,[216b–d, 219] or chemical and electrical properties.[220] The controlled etching of metals[218] or the selective deposition of metals[221] enabled the microscopic structuring of surfaces by a variety of polymeric, organic, conductive, and inorganic materials.

Several of the concepts of patterning surfaces with microscale chemical functions were used to generate arrays of biomaterials. Some of these methods were used to pattern oligonucleotide arrays[213c, 222] or antigen–antibody structures.[223] Other studies have used redox-inactive or model proteins to demonstrate the strategies of assembling protein structures on solid supports.[224] Although the present review emphasizes the use of redox proteins for bioelectronic applications, we take the liberty of surveying recent advances in the patterning of proteins on surfaces. We believe that these approaches may find central roles in tailoring future bioelectronic systems of different functions.

10.1. Patterning of Surfaces with Biomaterials by Covalent Bonding

Modification of the surface of solid supports with photoactivable groups allows the light-induced generation of reactive intermediates, for example, radicals, carbenes, nitrenes, that react with proteins to yield covalent bonds.[129] Photoirradiation of the photosensitive monolayer on the solid support through a mask yields patterned domains of the reactive intermediates, which allow the covalent attachment of biomaterials to the activated surfaces. Figure 27 summarizes several photochemical routes to generate reactive

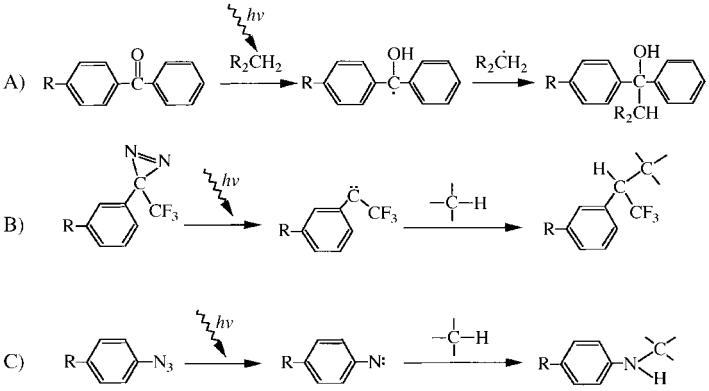


Figure 27. Photochemical generation of: A) benzhydrol radicals. B) carbenes, C) nitrenes, for covalent linkage to proteins.

intermediates. Benzophenone undergoes photochemical activation in the presence of a proton source to form the ketyl radical, and the recombination of the two radicals permits the formation of a new bond. Photolysis of aryldiazine generates the carbene and its insertion into a C–H bond allows proteins to be linked to the reactive intermediate. Irradiation of azides produces the aryl nitrene that reacts with C–H bonds to form the stable amine. Accordingly, Si chips were silylated by aminopropyltriethoxysilane to yield the amino-functionalized Si surface, which was then treated with benzophenone-4-isothiocyanate (**13**) or with 3-(*m*-isothiocyanatophenyl)-3-trifluoromethyldiazirine (**14**) to yield the thiourea-linked, photosensitive benzophenone or diazirine layers, respectively (Figure 28).[225] Photolysis of the respective monolayers through masks produces the ketyl or carbene species that link biomaterials (proteins) to the Si surface. Aryl azides were used for the photochemical patterning of polymer-functionalized surfaces with proteins.[226] By one approach, an aryl azide functionalized bovine serum albumin (BSA) was prepared by the reaction of BSA with *N*-(4-azidobenzoyloxy)-succinimide (**15**; Figure 29 A) and deposited onto a polyvinyl alcohol film. Irradiation of the resulting assembly

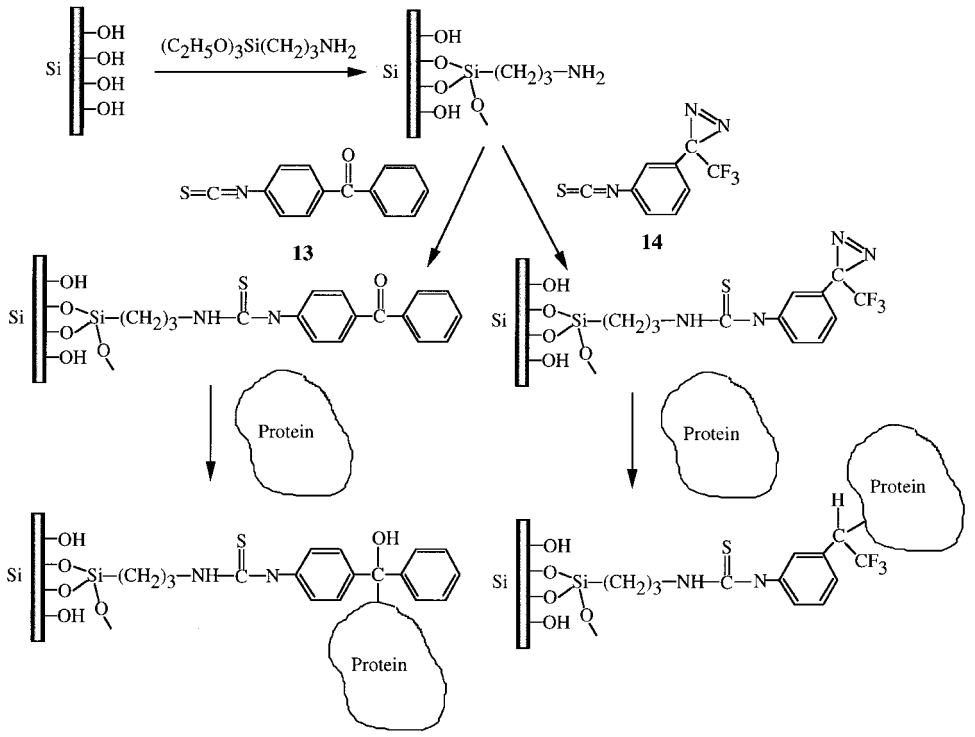


Figure 28. Photostimulated covalent attachment of proteins to a benzophenone- or a diazidine-functionalized layered support.

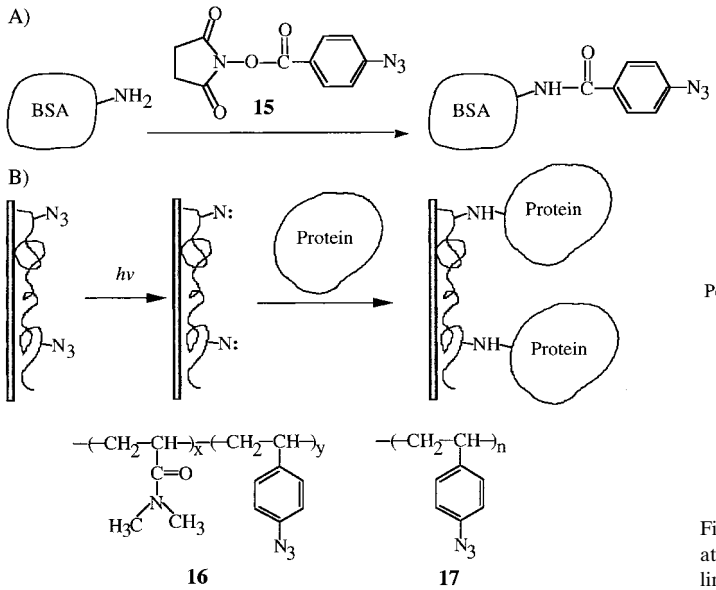


Figure 29. A) Preparation of a photolabile azido-functionalized protein. B) Photoinduced covalent linkage of a protein to an azido-functionalized polymer film.

through a mask results in the covalent attachment of the protein to the base polymer layer, and the BSA from the areas that were not exposed to light was washed off. A related approach used aryl azide functionalized polymers as photoactive matrices for the covalent attachment of the protein at irradiated surface domains (Figure 29B). Two polymers, for example, the copolymer poly(*N*,*N*-dimethylacrylamide-*co*-4-azidostyrene) (**16**) and poly(3-azidostyrene) (**17**) were deposited as thin films on solid supports, and the protein was spread on the respective polymer films. Irradiation of the assemblies through a mask results in the covalent linkage of the protein to the polymer via the intermediate generation of phenylnitrene species. Using a similar approach, an aryl azide functionalized layer that includes an active ester moiety was photolithographically deposited onto a polystyrene (PS) film (Figure 30). *N*-(4-azido-2,3,5,6-tetrafluorobenzoyloxy)succinimide (**18**) was deposited on the polymer. The film irradiated through a mask yielded a pattern of the reactive *N*-hydroxysuccinimide groups that was subsequently linked to horse radish peroxidase (HRP) or avidin.[227] Alternatively, *N*-(5-aminopentyl) biotinamide was treated with the patterned active ester and fluorescein-labeled avidin was associated to the biotin domains. Figure 31A shows the micrograph of the fluorescein–avidin pattern on

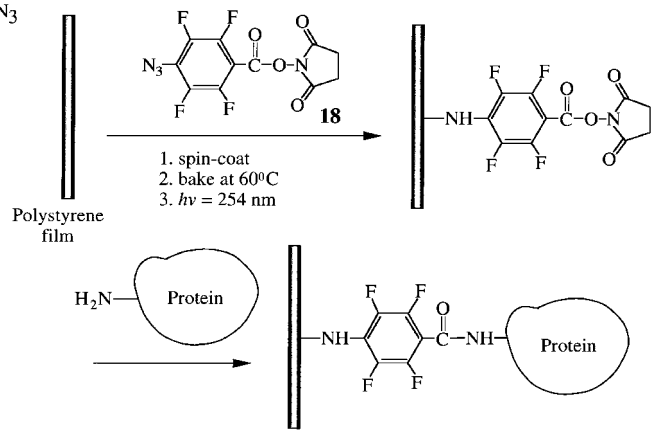


Figure 30. Photochemical patterning of a polystyrene film by the irradiation of an azido-photolabeled active ester through a mask. The protein is linked to the domains functionalized with the active ester.

a glass support. Note that the avidin pattern enables the secondary attachment of any enzyme by using biotinylated proteins (see Section 10.2). Photochemical deprotection of functional groups that can be linked to proteins allows the micropatterning of surfaces with enzymes or proteins. The *ortho*-nitrobenzyl ester unit is one of the most studied photosensitive protective groups for functional groups, particularly carboxylic acids.[228] A copolymer consisting of protected acrylic acid, *co*-poly-*ortho*-nitrobenzyl acrylate poly-*N*,*N*-dimethylacrylamide (**19**) was used as an active matrix for microdeposition of protein structures.[226] Irradiation of the polymer layer through a mask results in the

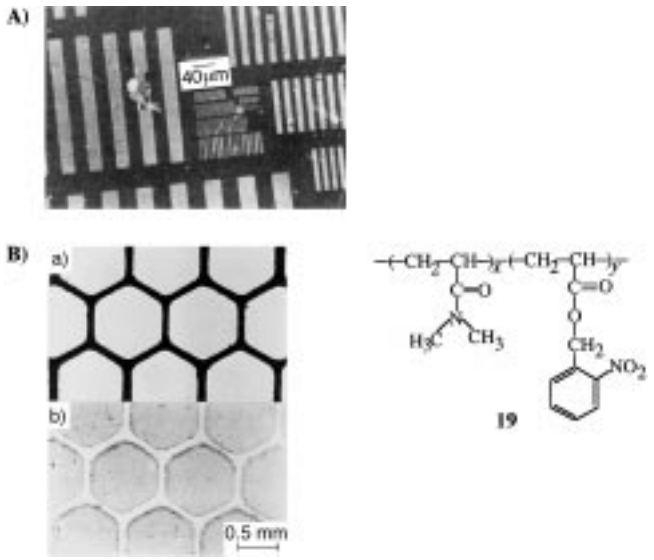


Figure 31. A) Micrograph of a fluorescein-labeled avidin attached to a patterned active ester monolayer generated by the irradiation of polystyrene through a mask in the presence of an azido-labeled active ester (see Figure 30, from ref. [227]. B) Photolithographic activation of a polymer film and site-specific covalent attachment of BSA to the patterned polymer (**19**). a) The structure of the mask, b) Patterned BSA/polymer surface obtained upon irradiation of a thin film of **19** on glass in the presence of BSA. The image (from ref. [226]) was recorded by the staining of the resulting pattern with the respective enzyme-labeled antibody using Vectastain ABC-AP (Vector Laboratories, Burlingame).

photodeprotection of acrylic acid residues. Covalent attachment of bovine serum albumin to the carboxylic acid residues using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as the coupling reagent yields the protein pattern shown in Figure 31 B. This method can be extended to immobilize any redox protein in a patterned, well-defined, configuration.

A different approach involves "negative photolithography", namely, the photochemical deactivation of functional groups associated with a monolayer for the secondary covalent immobilization of proteins.[223b] A silylated, sulfuranylpropyl-functionalized surface was irradiated under oxygen through a mask, and the areas exposed to light were oxidized to the sulfonated functionalized monolayer. The resulting patterned monolayer was then treated with the bifunctional reagent *N*-4-maleimidobutyryloxysuccinimide ester (GMBS, **20**), which allows the covalent linkage of proteins to the patterned interface (Figure 32). Different antibodies, for example, anti-goat IgG from rabbits or phycoerythrin, were linked to the patterned interfaces, and protein microstructures exhibiting a resolution of approximately 25 μm were tailored. Note that the patterning of antibodies on surfaces enables the organization of any protein (enzyme) pattern by the secondary noncovalent association of antigen-labeled proteins to the base antibody pattern.

10.2. Patterning of Surfaces by Layered Biomaterials through Noncovalent Interactions

The patterning of surfaces with molecular components that allow the formation of host–guest complexes with biomaterial receptors provides a general means of generating bioactive microstructures. Of specific interest is the patterning of addressable multidentate bio-receptors such as avidin, concanavalin A, and antibodies, that allows the secondary association of any appropriately labeled biomaterial to the patterned layered assembly. Biotin/avidin affinity interactions were used to generate micropatterns of proteins and, specifically, enzyme patterns.[229]

A layer of deglycosylated avidin (avidin D) was covalently linked[229a] to Au or SiO_2 surfaces, and the photoactive azide-functionalized biotin (photobiotin, **21**) was bound to the avidin layer by noncovalent interactions (Figure 33). Irradiation of the monolayer through a mask in the presence of a protein yields the nitrene biotin that reacts with the protein through nitrene insertion. By the two-step irradiation of two addressable domains in the presence of two different proteins, for example, rabbit IgG and rat IgG, the antibodies were assembled on the respective spatially addressed surfaces. By the further irradiation of the resulting protein-functionalized layer, the nonmodified photobiotin sites were activated towards the reaction with casein, which resulted in the

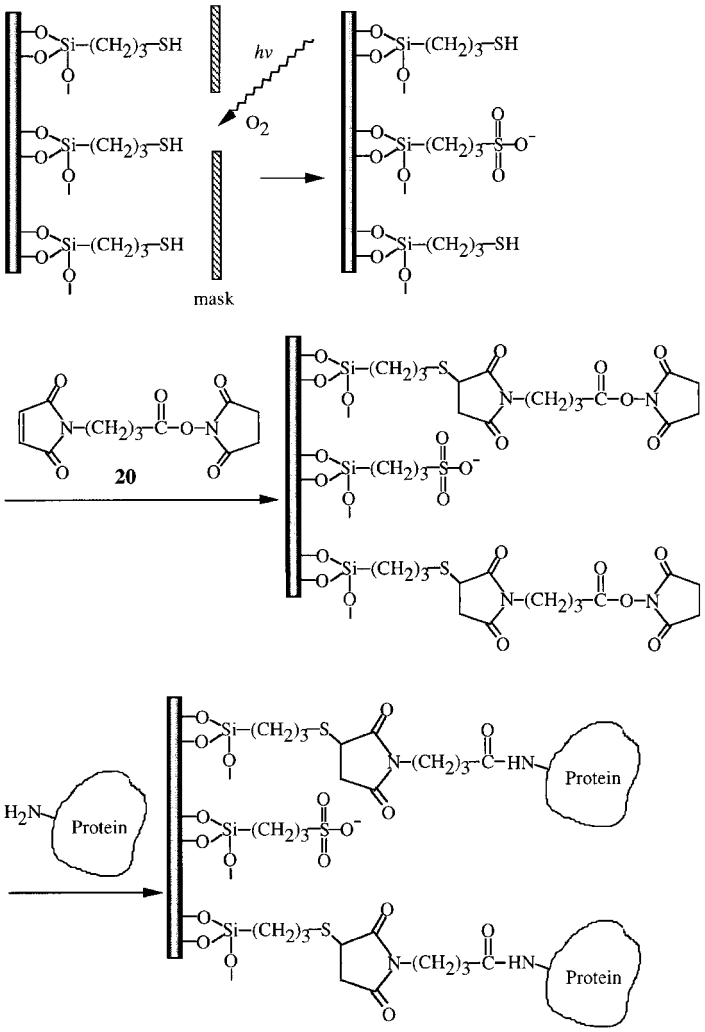


Figure 32. Photolithographic patterning of a thiol-functionalized surface with a protein by the light-stimulated deactivation of surface functionalities by irradiation through a mask.

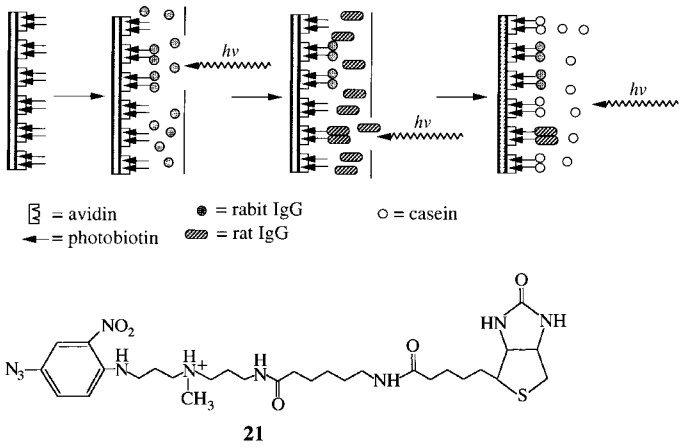


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Figure 33. Sequential photochemical patterning of a surface with different proteins by site-specific irradiation of surface domains functionalized with an avidin/photolabeled biotin layer.

assembly of an ordered three-protein composite. The resulting antibody-patterned surface was imaged by the reaction with rhodamine-labeled anti-rabbit IgG derived from goats and fluorescein-labeled anti-rat IgG derived from rabbits, respectively. By using this method lines of biomaterials with a resolution corresponding to 1.5 μm could be written. A related method of patterning polymers with biomaterials using photobiotin (as the acetate salt) as the active component was reported.[229b] Photobiotin was physically adsorbed onto a nitrocellulose membrane or a polystyrene film. The polymer was irradiated through a mask, and the photoactivation of biotin generated a polymer-linked biotin pattern. The binding of the multidentate avidin to the biotin binding sites linked to the polymer matrices enabled the secondary association of biotinylated alkaline phosphatase or biotinylated HRP.

A similar approach was used to generate patterns of enzymes on glassy carbon surfaces (Figure 34).[142b] Photosensitive biotin (**21**) was cast onto the glassy carbon surface. The pattern of biotin on the surface was generated by an interference pattern on the substrate rather than the irradiation through the mask. Subsequent interaction of the biotin-

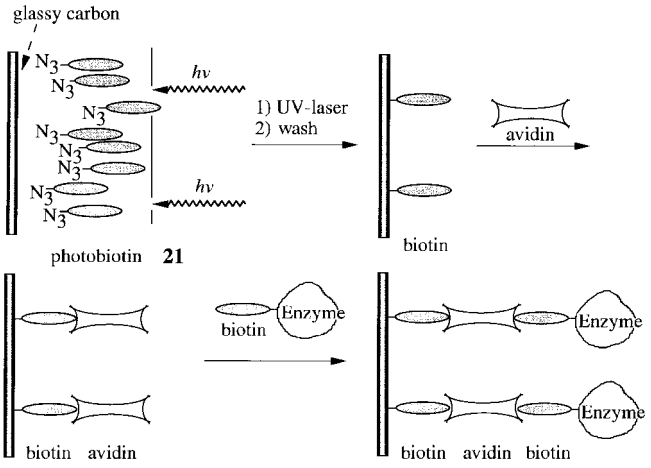

Figure 34. Photochemical patterning of a glassy carbon electrode with biotin and a secondary avidin/biotinylated enzyme layer by the irradiation of the surface through a mask in the presence of photolabeled biotin.

layer pattern with avidin yields the protein pattern. The microstructured avidin layer was imaged by fluorescence microscopy by the application of fluorescein-labeled avidin or avidin labeled with Texas Red. The tetramer ligation ability of avidin allows the secondary binding of biotinylated enzymes (for example, alkaline phosphatase) to the base avidin pattern.

Photoisomerizable antigens were used to tailor reversible immunosensors by controlling the antigen–antibody interactions with light.[230] In one photoisomer state the molecular substrate exhibits antigen features for the antibody, while in the secondary photoisomer configuration a molecular species lacking antigen properties is generated (Figure 35A). A cyclic, light-induced association and dissociation of the

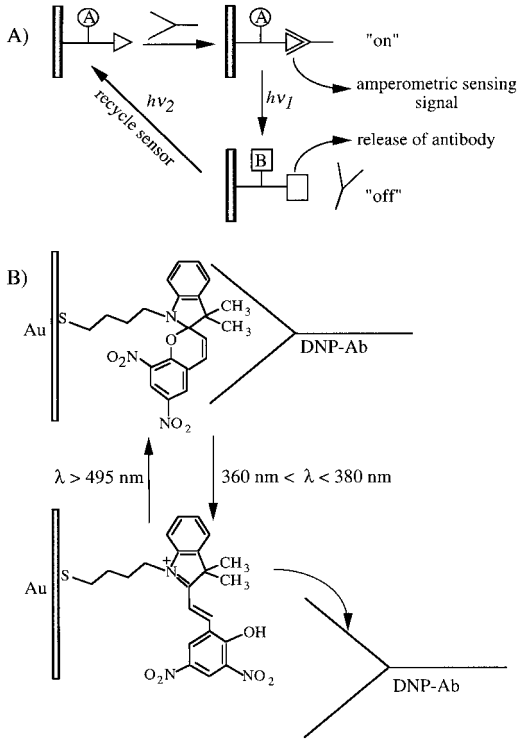

Figure 35. A) Photocontrolled association and dissociation of an antibody to and from an electrode with an photoisomerizable antigen monolayer. This is a model for a reversible immunosensor electrode, and the light-induced regeneration of an immunosensor. B) Photoinduced binding and dissociation of DNP-Ab to and from an electrode with a photoisomerizable dinitrospiropyran/protonated dinitromerocyanine monolayer.

antigen–antibody complex was stimulated by the reversible photoisomerization of the photoactive molecular substrate between the two states. It was shown that a dinitrospiropyran monolayer associated with an Au electrode or a conductive ITO electrode reveals photochemically controlled binding and dissociation of the dinitrophenyl antibody (DNP-Ab, Figure 35B).[230] The dinitrospiropyran monolayer acts as an antigen for the DNP-Ab, whereas the protonated dinitromerocyanine lacks antigen features. This difference enabled the photolithographic patterning of surfaces with DNP-Ab.[230b,c, 231] A glass surface was silylated with aminopropyltriethoxysilane, and dinitrospiropyran (SP) **22a** was covalently coupled to the surface (Figure 36A). The resulting antigen

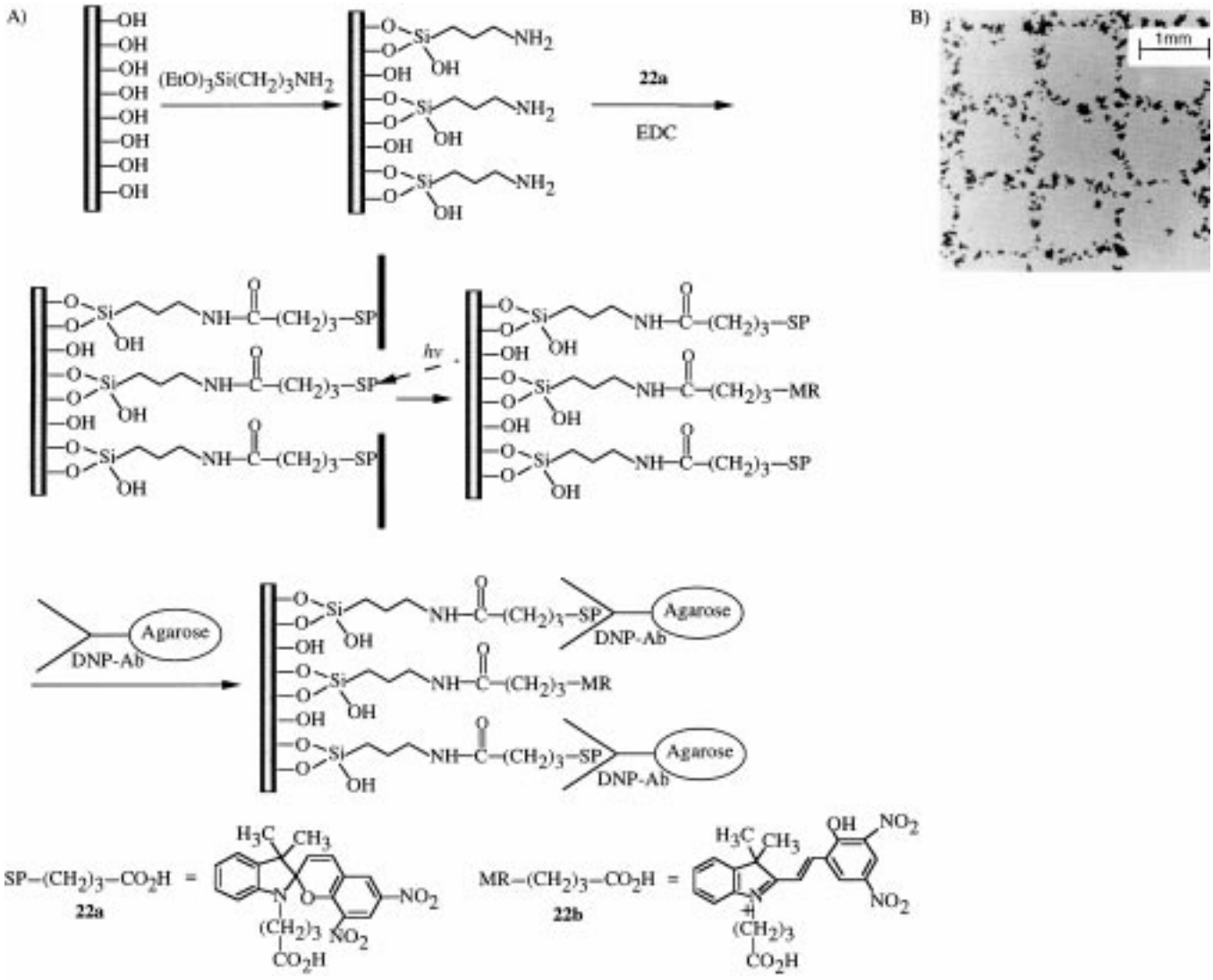

Figure 36. A) Assembly of a photoisomerizable dinitrospiropyran (SP) antigen monolayer on a glass support, the photolithographic patterning of the monolayer by irradiation through a mask, and the selective binding of DNP-Ab-functionalized agarose beads to the patterned surface. B) The pattern of DNP-Ab covalently linked to agarose beads formed upon the selective deposition of the particles to a photolithographic patterned dinitrospyropyran monolayer. Particles are deposited onto the dinitrospyropyran sites, whereas the vacant domains are in the dinitromerocyanine (MR) state (from ref. [231]).

monolayer was irradiated through a mask and all of the illuminated domains were transformed to protonated dinitromerocyanine (**22b**) areas lacking antigen features. The resulting patterned antigen layer was then interacted with DNP-Ab-functionalized agarose particles. The domains that include the dinitrospiropyran antigen units associate with the antibody–agarose particles. Figure 36B shows the pattern of the antibody–agarose particles formed upon irradiation of the base dinitrospiropyran layer through a grid. This system enables, in principle, the secondary microstructuring of surfaces with any enzyme, and the organization of a three-dimensional array of different biomaterials. For example, any dinitrophenyl-functionalized enzyme will link by noncovalent interactions to the base DNP-Ab-patterned microstructure since the agarose beads include vacant DNP-Ab sites.

Proteins may be spatially patterned by the organization of patterned monolayers on solid supports that have the appropriate hydrophilic/hydrophobic balance for the specific adsorption of the biomaterial. Monolayers consisting of oligo(ethylene glycol) terminated thiols ($HS(CH_2)_{11}(OCH_2CH_2)_6OH$) resist the adsorption of proteins, whereas hydrophobic monolayers (for example, $HS(CH_2)_{15}CH_3$) adsorb proteins such as avidin, hexokinase, pyruvate kinase, ribonuclease A, lysozyme, and fibrinogen effectively.[232] Several techniques, including microwriting,[232c, 233] micromachining,[216d] stamping,[215b] and UV photolithography[100d] were used to generate the respective patterns of oligo(ethylene glycol) and hexadecylthiol.[224] Adsorption of the proteins to the patterned hydrophobic domains was imaged by scanning electron microscopy (SEM). The relative brightness of a micrograph area relates to the secondary electron emission

from that domain. The association of the protein to the hydrophobic monolayer is reflected by a decrease in the brightness of the domain in the micrograph.[215b] A pattern of lines was written on an Au film using $HS(CH_2)_{15}CH_3$ as the ink solution, and the background surface was treated with the oligo(ethylene glycol).[232c]

Irradiation of the hexadecylthiol monolayer through a mask (UV light, under oxygen) results in the oxidation of the thiolate to the sulfonate. Desorption of the latter product and specific association of $HS(CH_2)_{11}(OCH_2CH_2)_6OH$ with the bare Au domains (Figure 37) enabled the specific adsorption of pyruvate kinase to the patterned hydrophobic domains.[224] The stamping of an Au surface with a stamp molded from

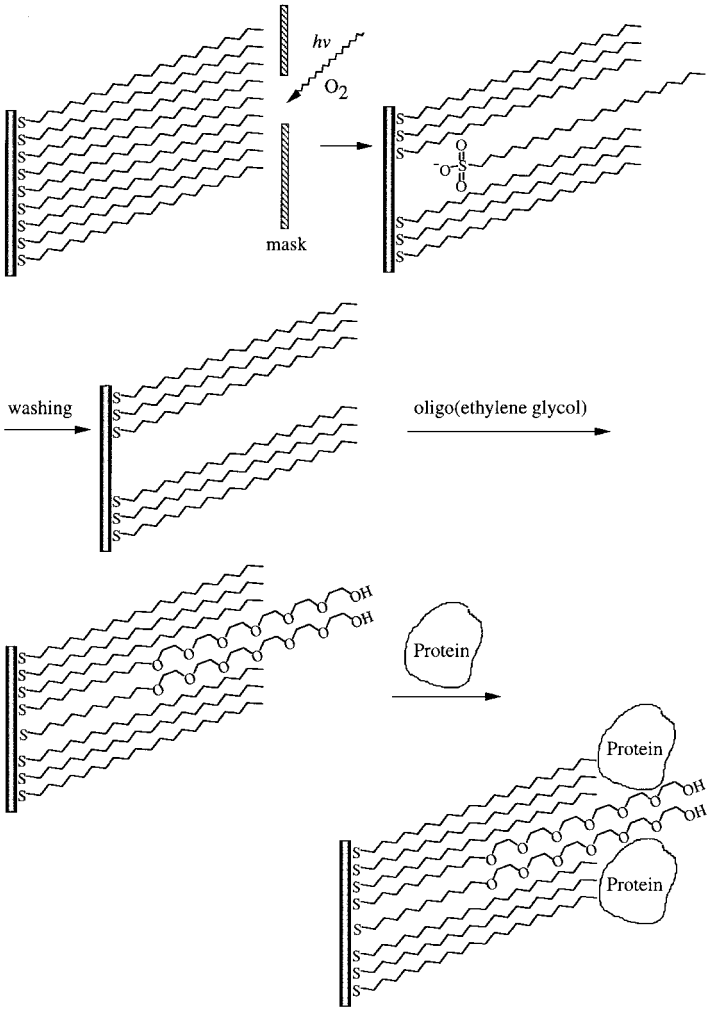

Figure 37. Photolithographic oxidative desorption of a thiol monolayer followed by the filling of empty domains with a hydrophilic oligo(ethylene glycol) terminated thiol for the noncovalent patterning of proteins on the surface. The alkyl chains are only representative.

poly(dimethylsiloxane) and functionalized with the hydrophobic thiol was used to generate a pattern of hexadecylthiol, and the remainder of the base surface was derivatized with the sulfanyl oligo(ethylene glycol). Selective association of pyruvate kinase to the hydrophobic domains resulted in the protein pattern.[224]

A micromachining approach[215a, 216, 218] was similarly applied to generate patterns of pyruvate kinase. An Au surface was functionalized with the hydrophilic sulfanyl oligo(ethylene glycol). The resulting monolayer was scratched with a scalpel blade and the scratched domain was then treated with the hydrophobic thiol to which pyruvate kinase was specifically associated. Different proteins such as fibronectin, streptavidin, and antibodies were selectively associated on the patterned hydrophobic layer. It was possible to distinguish between proteins associated with stamped hydrophobic domains by the relative intensities of the SEM images. The larger the protein, the darker is the SEM image.

11. Photoactivated Layered Enzyme Electrodes for Optobioelectronics: Amperometric Transduction of Recorded Optical Signals

Photoactivated layered redox enzymes on conductive supports enable the amperometric transduction of optical signals recorded by the bioactive interfaces.[20, 234] Figure 38A shows the application of a layered, photoswitchable redox enzyme on an electrode as an optobioelectronic assembly for the amplified amperometric transduction of recorded optical signals. The enzyme is functionalized by a photoisomerizable unit (**A**) that distorts the protein structure and prohibits the bioelectrocatalyzed oxidation (or reduction) of the respective substrate. Photoisomerization of the light-active groups to state **B** restores the bioactive tertiary structure of the enzyme. This process activates the bioelectrocatalytic transformation and the photonic information recorded is transduced as an amperometric output. As the optical signal activates an electrocatalytic process the resulting current represents an amplified response to the recorded photonic signal. Such systems can be used as optical memories (write-read-erase), where the optical signal represents the writing process, the electrocatalytic reaction the reading mechanism, and the reversible photoisomerization the erasure step. Similarly, the use of photoactivated redox enzymes on electrodes as actinometric devices, biomolecular amplifiers of weak light signals, and logic gates,[115l] were recently addressed.

The enzyme glucose oxidase (GOx) was modified chemically with nitrospiropyran photoisomerizable units (**23a**) and assembled as a monolayer onto an Au electrode (Figure 38B).[18a] In the presence of the diffusional electron mediator, ferrocenecarboxylic acid (**24**) the nitrospiropyran-functionalized GOx is activated for the oxidation of glucose, as evidenced by the resulting high electrocatalytic current (Figure 39). Photoisomerization of the monolayer to the protonated merocyanine (**23b**) results in the distortion of the protein that perturbs the electrical contact by the electron mediator. This process results in a substantially lower amperometric response. Reversible "high" and "low" amperometric responses of the functionalized electrode are transduced upon cyclic photoisomerization of the enzyme monolayer between the two states. The photoisomerizable enzyme electrode revealed photoswitchable transduction of recorded optical signals (Figure 39). However, the "switched-off" state does not reveal complete blocking of the

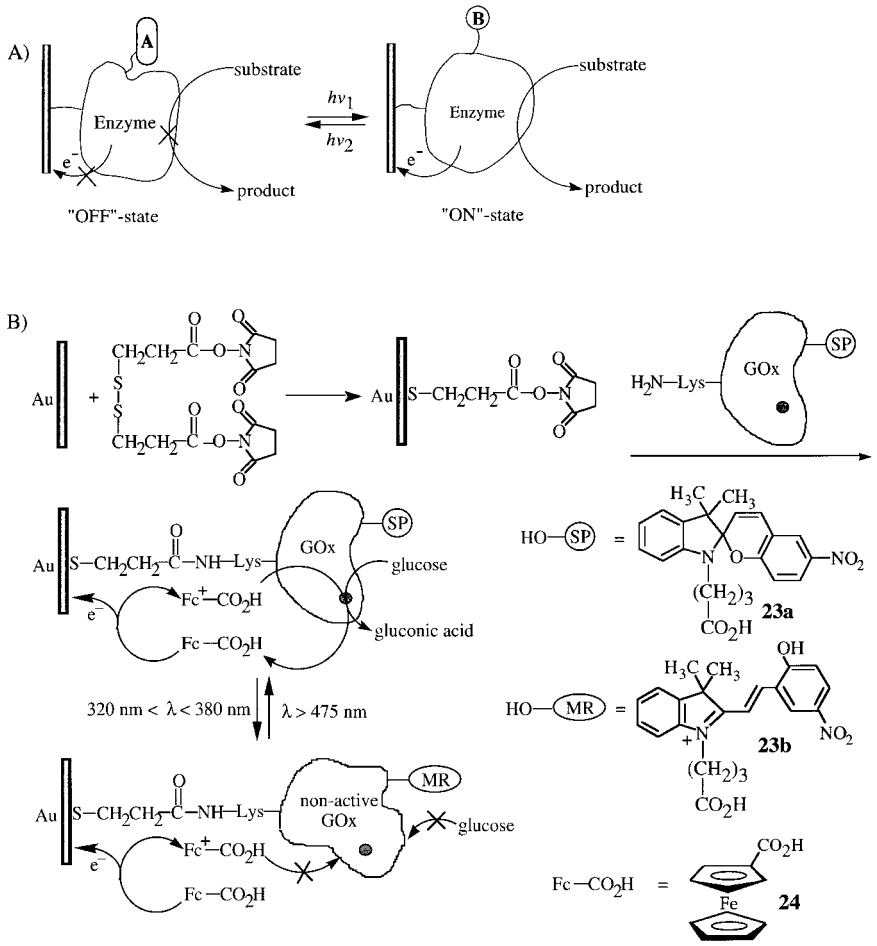

Figure 38. A) Assembly of an optobioelectronic system by the organization of a layer of photoisomerizable redox enzyme on an electrode support. B) Assembly of a nitrospiropyran-tethered glucose oxidase layer on a Au electrode for the photoswitchable, bioelectrocatalyzed oxidation of glucose. Ferrocenecarboxylic acid is used as a diffusional electron mediator to provide an electrical contact between the redox enzyme and the electrode support.

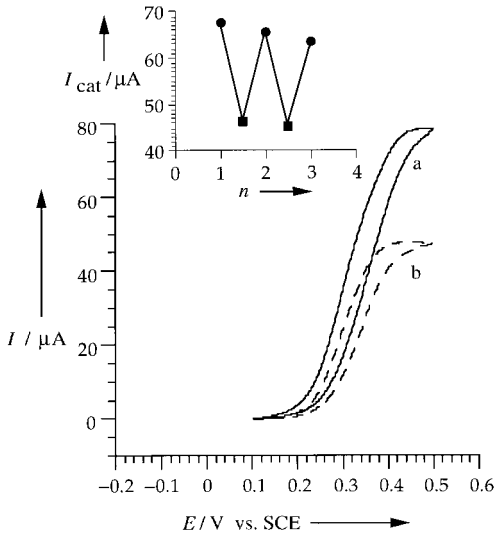

Figure 39. Cyclic voltammograms of the photoswitchable bioelectrocatalyzed oxidation of glucose (2.5×10^{-2} M) as described in Figure 38B using a photoisomerizable GOx monolayer electrode (in the presence of **24**, 5×10^{-3} M): a) in the SP form, b) in the MR form; potential scan rate: 5 mV s^{-1}. Inset: Cyclic amperometric responses of the electrode with a photoisomerizable enzyme monolayer upon reversible light-induced isomerization of the protein layer between the SP (●) and MR form (■).

bioelectrocatalytic functions of the enzyme. This was attributed to the insufficient structural distortion of the protein by the covalent attachment of the photoisomerizable units. That is, attachment of photoisomerizable components to amino acid residues far from the enzyme redox center, or the channel pathway of the electron mediator, is not sufficient to effectively perturb the electrical contact between the enzyme and the electrode.

One approach of improving the photoswitchable functions of a redox enzyme to complete "ON" and "OFF" states involved the introduction of the photoisomerizable unit close to the biocatalytic redox active site by a reconstitution process (Figure 40). The native FAD-cofactor associated with GOx was excluded from the enzyme and substituted with the semisynthetic nitrospiropyran-FAD cofactor (**25a**).[18d, 235] The reconstituted photoisomerizable enzyme was then assembled as a monolayer on an Au electrode. In the presence of the electron mediator **24** the bioelectrocatalyzed oxidation of glucose was fully switched to "ON" or "OFF" positions (Figure 41). In the nitrospiropyran state of the enzyme (**25a**-GOx) the mediated electron transfer by **24** is blocked. Upon photoisomerization of the enzyme monolayer, **25b**-GOx state, the diffusion path of the electron mediator is opened, and the bioelectrocatalytic oxidation of glucose is activated (Figure 42).

The partially photostimulated properties of glucose oxidase that had been substituted randomly by photoisomerizable groups were used to design a three-state switch by the coupling of the photoisomerizable enzyme with an antibody (Figure 43).[18c] Glucose oxidase was modified with dinitrospiropyran units, and the resulting photoisomerizable enzyme was assembled as a monolayer on an Au electrode. The resulting monolayer enzyme electrode was activated for the bioelectrocatalyzed oxidation of glucose in the dinitrospiropyran photoisomer state (SP-GOx) and using ferrocenecarboxylic acid (**24**) as an electron transfer mediator (switch "ON"). Photoisomerization of the monolayer to the protonated dinitromerocyanine state (MR-GOx) results in the partial deactivation of the bioelectrocatalytic features of the enzyme electrode (partial switch "OFF"). The photoisomerizable units of dinitrospiropyran and protonated dinitromerocyanine, however, exhibit light-stimulated binding affinities to the dinitrophenyl-antibody (DNP-Ab, see Section 10.2). The SP state acts as an antigen for DNP-Ab, while the MR state lacks antigen features for DNP-Ab. The binding of the DNP-Ab to the SP-GOx insulates the diffusion path of the electron-transfer mediator, and the electrical contact of the enzyme and the electrode is blocked. This process yields the

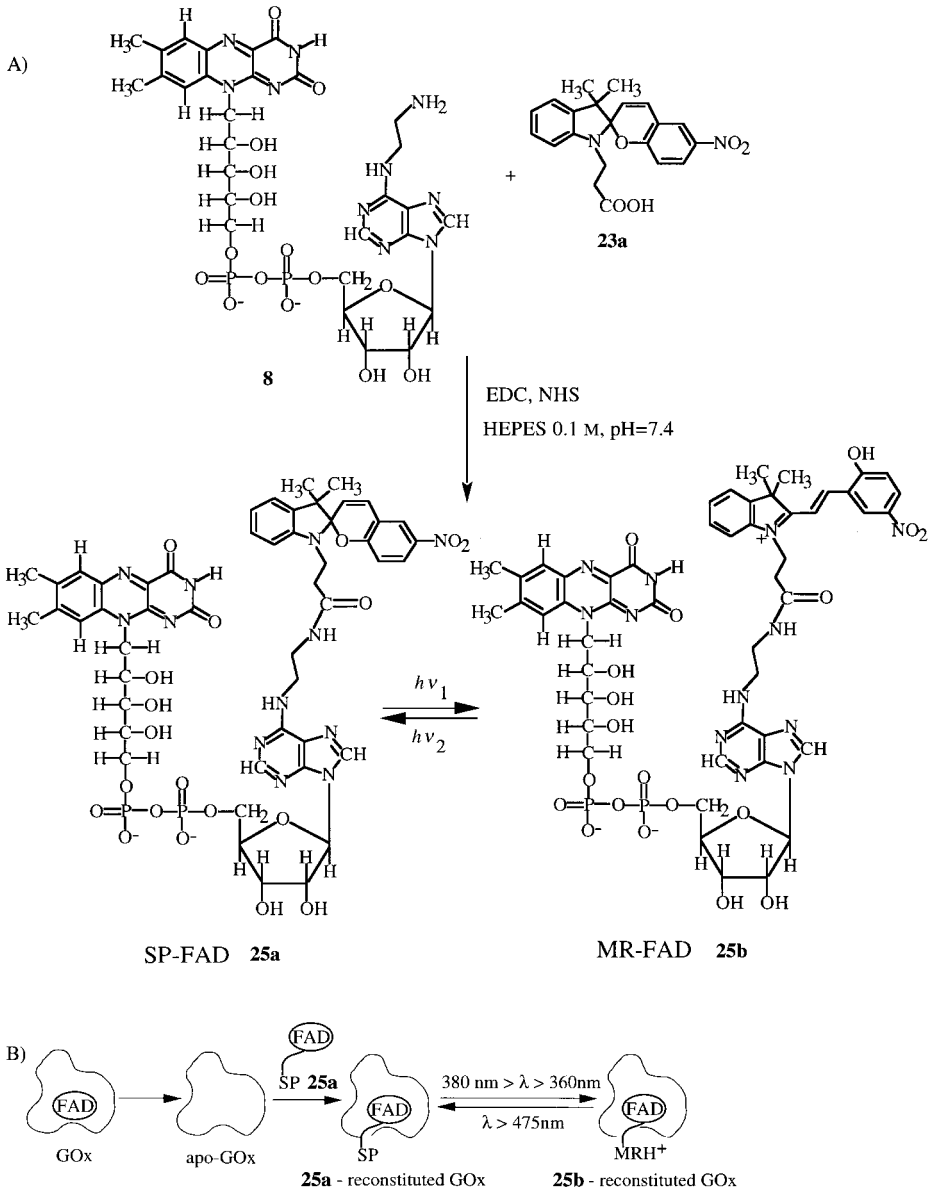

Figure 40. A) Synthesis of a semisynthetic nitrospiropyran-FAD cofactor. B) Reconstitution of apo-GOx with the photoisomerizable cofactor.

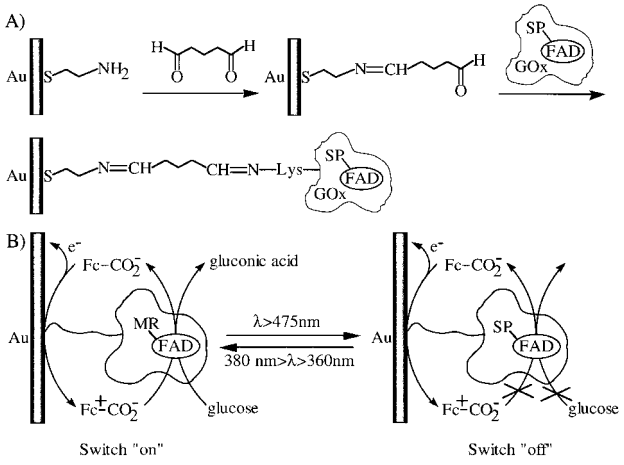

Figure 41. A) Assembly of the photoisomerizable enzyme layer in accordance with Figure 40B on an Au electrode. B) Photoswitchable bioelectrocatalyzed oxidation of glucose by this electrode in the presence of ferrocenecarboxylic acid as a diffusional electron mediator.

complete switching-off of the electrobiocatalytic properties of the enzyme electrode. The MR-GOx photoisomer state of the monolayer is not affected by DNP-Ab, since the photoisomer sites lack antigen features for the antibody. By reversible photoisomerization of the enzyme monolayer between the SP-GOx and MR-GOx states in the presence or absence of DNP-Ab, the system is cycled through the switch-ON, switch-OFF, and switch partial-ON states (Figure 44). The layered enzyme–antibody and electrode assembly mimics functions of an electronic logic gate of "AND" characteristics. The amperometric response of the photoisomerizable monolayer-electrode is turned to the switched-off position when two conditions are met: The monolayer is photochemically transformed to the SP-GOx state *and* the monolayer interacts with the DNP-Ab to yield the complex layer on the electrode.

The electrical contact of the layered enzyme assembly and the conductive support is driven by a diffusional electron mediator, which enables the photostimulation of the bioelectrocatalytic functions of the enzyme layer by the application of a photoisomerizable electron mediator (Figure 45; p. 1211).[127b] In one photoisomer state (SP) of the electron mediator the relay establishes the electrical contact between the enzyme layer and the electrode support and activates the bioelectrocatalyzed transformation. In the complementary photoisomerizable state (MR) the electron transfer between the biocatalyst layer and the conductive support is blocked, as a result of steric or electrostatic barriers for diffusion of the relay into the protein. Two photoisomerizable relays, the covalently linked SP-ferrocene derivative **26** and the SP-bipyridinium salt **27**, were reported to photostimulate the activities of glucose oxidase (GOx) and glutathione reductase (GR), respectively.[127b] The enzyme GOx was assembled as a five-layer array on an Au electrode. The SP-ferrocene **26a** forms an electrical contact to the enzyme multilayer and the electrode surface, and results in the bioelectrocatalyzed oxidation of glucose. The protonated MR-ferrocene **26b** does not mediate the electron transfer, and its diffusion to the protein is sterically (or electrostatically) hindered. By the reversible photoisomerization of the electron relay between **26a** and **26b**, the cyclic activation and deactivation of the enzyme was induced by light. Similarly, the

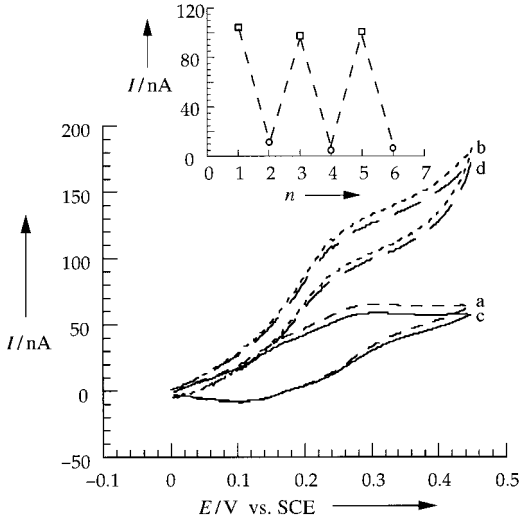

Figure 42. Cyclic voltammograms corresponding to the bioelectrocatalyzed oxidation of glucose described in Figure 41 B by the photoisomerizable GOx-monolayer electrode a) and c) in the SP state, b) and d) in the MR state. Inset: Cyclic amperometric responses of the electrode upon reversible photoisomerization of the monolayer between the protonated MR (□) and SP state (○).

photoisomerizable SP-bipyridinium relay **27** allows the photochemical activation and deactivation of glutathione reductase assembled in a layer on an Au electrode.

12. Conclusion and Perspectives

This review has addressed recent advances in the organization of redox proteins as monolayer or multilayer assemblies on solid conductive supports for bioelectronic applications. Specifically, the electrical contacting of the protein layers with the electrode supports and the spatially controlled deposition of proteins as integrated micropatterned arrays were discussed. The potential use of electrically contacted redox proteins as future biosensor devices was addressed. In

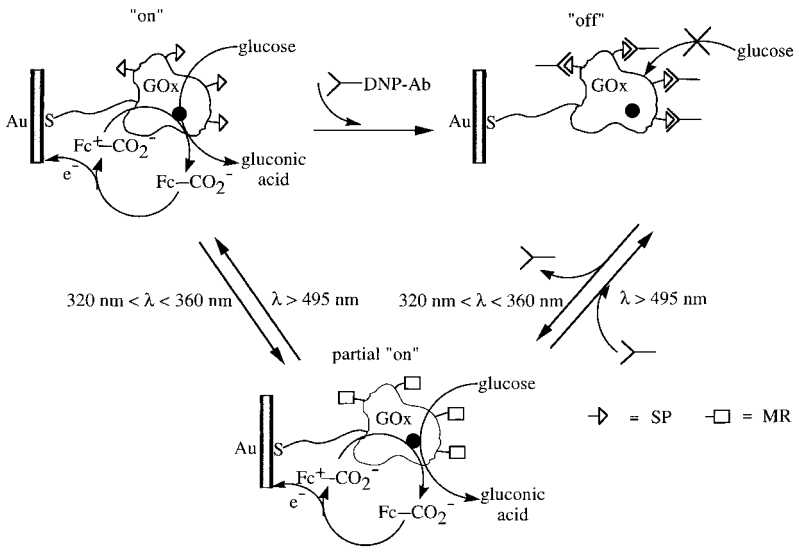

Figure 43. Schematic operation of a biphasic photobiological switch based on a SP-GOx-monolayer electrode and DNP-Ab.

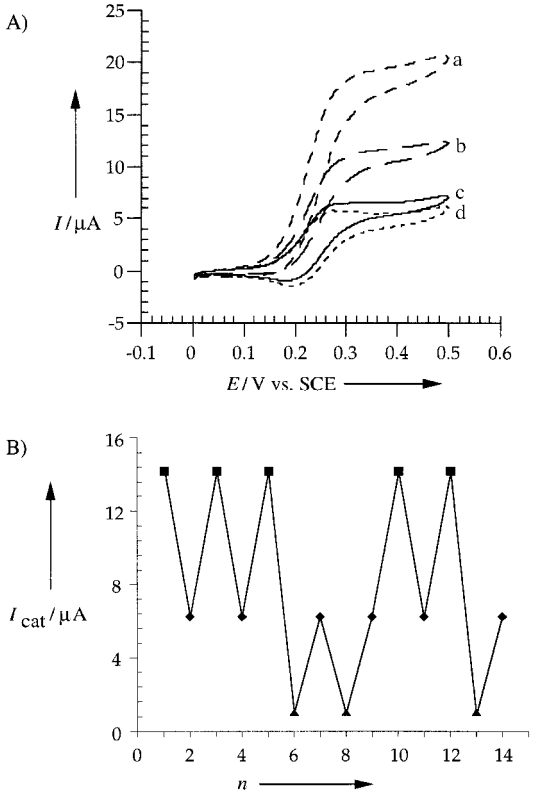

Figure 44. A) Cyclic voltammograms of the two-phase switch described in Figure 43 in the presence of ferrocenecarboxylic acid (4×10^{-4} M) a) in the SP state and in the presence of glucose (5×10^{-2} M), b) in the MR state, in the presence of glucose (5×10^{-2} M), and with (20 mg mL^{-1}) or absence of DNP-Ab, c) in the presence of the SP state and DNP-Ab (20 mg mL^{-1}), d) in the SP or MR state and in the absence of glucose. B) Cyclic amperometric transduction of the biphasic switch composed as a result of the reversible light-induced isomerization of the protein layer between the SP and MR states in the absence and presence of DNP-Ab: (■) SP-GOx monolayer without DNP-Ab, (◆) MR-GOx monolayer with or without DNP-Ab, (▲) SP-GOx monolayer in the presence of DNP-Ab.

contrast to the previous methods that used random, non-organized, deposition of proteins onto surfaces, for example, encapsulation or immobilization in polymer matrices, we have emphasized the basic scientific interests and the practical implications of tailoring organized protein architectures in ordered and defined nanostructures. The key elements in the integration of redox proteins with electronic transducers include the physical or chemical deposition of the proteins on the solid supports, the electrical communication between the biomaterials and the transducers, and the electronic transduction of the biocatalyzed transformation that occurs at the biomaterial/transducer interfaces. Monolayer and multilayer arrays of proteins can be organized on transducers using covalent bonds, affinity interactions, or hydrophobic/hydrophilic interactions. Theoretical understanding of electron-transfer processes in proteins and the availability of chemical and biological means to modify biomaterials have enabled us to improve the electronic coupling between the redox proteins and the transducer

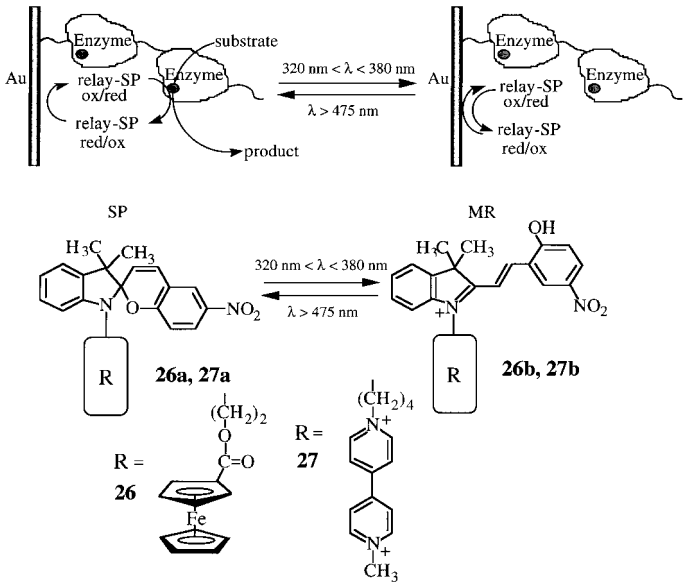

Figure 45. Photostimulation of redox-enzyme electrodes by photoisomerizable electron relays.

element. Site-specific modification of redox proteins, genetic engineering of protein structures, and surface-reconstitution of proteins, represent novel and attractive means to align and orient redox proteins on surfaces. The effective electrical contact of aligned proteins with electrodes suggests that future efforts should be directed at engineering further structural mutations in proteins in order to further develop ordered nanostructures of proteins on surfaces. Although our report has emphasized the electronic coupling of redox proteins and transducer elements, the concept can be further adopted to organize monolayer and multilayer systems using other biomaterials, such as antigens, antibodies,[236] oligonucleotides (or DNA),[5, 237] or bioreceptors.[8, 238] Indeed, recent reports have demonstrated the electronic coupling of antibody–antigen complexes with electrode supports[21, 239] or with piezoelectric quartz crystals.[13b, 240] Also, the electronic transduction of photoswitchable formation and dissociation of antigen–antibody complexes using photoisomerizable antigen monolayers was reported.[230] The formation of oligonucleotide–DNA double-stranded assemblies in monolayer assemblies was electronically[21c, 241] and microgravimetrically[242] transduced. These systems were suggested as future bioelectronic devices for the detection of genetic disorders (for example, Tay–Sachs).[243] The continuous interest in the development of antigen/antibody-based sensors (immunosensors) and the growing demand for sensitive electronic DNA sensors suggest that integration of these biomaterials as monolayer/multilayer assemblies on transducer elements will expand and will be utilized as a general immobilization strategy.

Another aspect that will require future development involves the integration of the biomaterial–monolayer assemblies with other transducers capable of signaling the biological events at the biointerface by other electronic, photoelectric, or optical means. In this context the organization of the bioactive materials on field-effect-transistor devices (FET),[14] the use of impedance spectroscopy,[12, 244] or surface plasmon resonance,[245] to probe biointeractions at the monolayer interface seems to be attractive.

The electrical contacting of protein layers associated with electrodes for sensing applications and for the tailoring of optoelectronic assemblies are already mature research areas of solid scientific frames. The concept of integrating biomaterial monolayers with solid supports leads, however, to new bioelectronic applications. The effective electrical contact of surface-reconstituted flavoenzyme monolayers on electrodes, and the ability to assemble electrocatalytic monolayer electrodes, led to the use of the functionalized electrodes as active components in biofuel cells.[212]

The concepts of monolayer assembly on solid supports were extended to the functionalization of surfaces with metal and semiconductor nanoparticles.[112a,f, 246] By the appropriate design of complementary affinity interactions between biomaterial-capped metal particles (for example, Au particles) three-dimensional superlattices were assembled.[246k] The organization of similar superlattices on transducer elements is anticipated to yield novel sensing matrices.[247] The unique photonic and electronic features of nanoparticles[248] suggest that the organization of biomaterial/nanoparticle composites would yield novel optobioelectronic systems, such as new photo-cells for the effective light-to-electrical energy conversion or optoelectronic sensors. Double-stranded oligonucleotide assemblies with sub-monolayer coverage were used as a mold for the deposition of metal nanowires.[249] This opens up novel routes for the generation of nanoscale metal networks with nanometric spatial resolution.

Finally, the spatial patterning of surfaces with chemical functionalities, and the ability to modify surfaces with biomaterials with micro- or nanoscale structures, has made tremendous progress in recent years. This opens the way to construct parallel multisensor systems, biosensing libraries, and bioelectronic arrays for parallel or consecutive operation. It is, however, mandatory to develop further chemical methods to specifically address the target domains on the patterned transducer element. The photochemical activation of nano-domains,[213b] or electrochemically driven synthesis at microelectrodes,[92d, 97g, 144a,c, 250] could provide future challenging goals. The development of the various scanning microscopy techniques, such as atomic force microscopy (AFM) or scanning tunneling microscopy (STM) has enabled the microscopic imaging of biomaterial patterns.[251] The ability to follow complementary affinity force interactions of biomaterials by AFM permits not only the basic understanding of the molecular forces characterizing the macroscopic bioelectronic systems,[252] but also allows the patterning of microstructures using these forces.[98g, 113a,b, 253] The possibility of illuminating nano-domains using a near-field-scanning optical microscope (NSOM) opens the way to activate layered photoswitchable biomaterials associated with electrodes for dense optical recording and electronic transduction.[254] Clearly, the architecture of biomaterial layers on transducer elements is envisaged to offer exciting perspectives at the frontiers of chemistry, biology, physics, medicine, and material science.

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[1] a) W. Göpel, P. Heiduschka, *Biosens. Bioelectron.* **1994**, *9*, III – XIII; b) W. Göpel, *Biosens. Bioelectron.* **1995**, *10*, 35 – 59.
[2] a) W. Göpel, P. Heiduschka, *Biosens. Bioelectron.* **1995**, *10*, 853 – 883; b) M. Mrksich, G. M. Whitesides, *Ann. Rev. Biophys. Biomol. Struct.* **1996**, 55 – 78; c) H.-L. Schmidt, W. Schuhmann, *Biosens. Bioelectron.* **1996**, *11*, 127 – 135.
[3] C.-J. Zhong, M. D. Porter, *Anal. Chem.* **1995**, *67*, 709A – 715A.
[4] a) C. Bourdillon, C. Demaille, J. Gueris, J. Moiroux, J.-M. Savéant, *J. Am. Chem. Soc.* **1993**, *115*, 12264 – 12269; b) C. Bourdillon, C. Demaille, J. Moiroux, J.-M. Savéant, *J. Am. Chem. Soc.* **1994**, *116*, 10328 – 10329; c) C. Bourdillon, C. Demaille, J. Moiroux, J.-M. Savéant, *Acc. Chem. Res.* **1996**, *29*, 529 – 535.
[5] M. Yang, M. E. McGovern, M. Thompson, *Anal. Chim. Acta* **1997**, *346*, 259 – 275.
[6] A. Bardea, E. Katz, A. F. Bückmann, I. Willner, *J. Am. Chem. Soc.* **1997**, *119*, 9114 – 9119.
[7] a) P. Pantano, T. H. Morton, W. G. Kuhr, *J. Am. Chem. Soc.* **1991**, *113*, 1832 – 1833; b) T. Hoshi, H. Takeshita, J. Anzai, T. Osa, *Anal. Sci.* **1995**, *11*, 311 – 312; c) X. Du, J. Anzai, T. Osa, R. Motohashi, *Electroanalysis* **1996**, *8*, 813 – 816; d) Q. Chen, Y. Kobayashi, H. Takeshita, T. Hoshi, J. Anzai, *Electroanalysis* **1998**, *10*, 94 – 97; e) J. Anzai, H. Takeshita, Y. Kobayashi, T. Osa, T. Hoshi, *Anal. Chem.* **1998**, *70*, 811 – 817.
[8] I. Willner, S. Rubin, Y. Cohen, *J. Am. Chem. Soc.* **1993**, *115*, 4937 – 4938.
[9] a) R. S. Sethi, *Biosens. Bioelectron.* **1994**, *9*, 243 – 264; b) P. N. Bartlett, P. Tebbutt, R. C. Whitaker, *Prog. React. Kinet.* **1991**, *16*, 55 – 155.
[10] a) R. M. Ianniello, T. J. Lindsay, A. M. Yacynych, *Anal. Chem.* **1982**, *54*, 1980 – 1984; b) A. L. Ghindilis, I. N. Kurochkin, *Biosens. Bioelectron.* **1994**, *9*, 353 – 357.
[11] a) H. S. Bresler, M. J. Lenkevich, J. F. Murdock, Jr., A. L. Newman, R. O. Robbin in *Biosensor Design and Application* (ACS Symp. Ser. **1992**, *511*, 89 – 104); b) V. M. Mirsky, C. Krause, K. D. Heckmann, *Thin Solid Films* **1996**, *284*, 939 – 941; c) C. Krause, V. M. Mirsky, K. D. Heckmann, *Langmuir* **1996**, *12*, 6059 – 6064.
[12] a) D. Athey, M. Ball, C. J. McNeil, R. D. Armstrong, *Electroanalysis* **1995**, *7*, 270 – 273; b) T. M. Nahir, E. F. Bowden, *J. Electroanal. Chem.* **1996**, *410*, 9 – 15.
[13] a) M. Lion-Dagan, I. Ben-Dov, I. Willner, *Colloids Surf. B* **1997**, *8*, 251 – 260; b) I. Ben-Dov, I. Willner, E. Zisman, *Anal. Chem.* **1997**, *69*, 3506 – 3512; c) A. Bardea, A. Dagan, I. Ben-Dov, B. Amit, I. Willner, *Chem. Commun.* **1998**, 839 – 840.
[14] a) F. Sevilla III, T. Kullick, T. Scheper, *Biosens. Bioelectron.* **1994**, *9*, 275 – 281; b) A. N. Reshetilov, M. V. Donova, D. V. Dovbnya, A. M. Boronin, T. D. Leathers, R. V. Greene, *Biosens. Bioelectron.* **1996**, *11*, 401 – 408; c) N. Kazanskaya, A. Kukhtin, M. Manenkova, A. N. Reshetilov, L. Yarysheva, O. Arzhakova, A. Volynskii, N. Bakeyev, *Biosens. Bioelectron.* **1996**, *11*, 253 – 261.
[15] a) S. M. Amador, J. M. Pachence, R. Fischetti, J. P. McCauley, Jr., A. B. Smith, J. K. Blasic, *Langmuir* **1993**, *9*, 812 – 817; b) D. Hobara, K. Niki, C. Zhou, G. Chumanov, T. M. Cotton, *Colloids Surf. A* **1994**, *93*, 241 – 250; c) B. Liedberg, C. Nylander, I. Lundström, *Biosens. Bioelectron.* **1995**, *10*, I – IX; d) C. E. Jordan, R. M. Corn, *Anal. Chem.* **1997**, *69*, 1449 – 1456.
[16] a) E. Katz, V. Heleg-Shabtai, B. Willner, I. Willner, A. F. Bückmann, *Bioelectrochem. Bioenerg.* **1997**, *42*, 95 – 104; b) I. Willner, E. Katz, B. Willner, R. Blonder, V. Heleg-Shabtai, A. F. Bückmann, *Biosens. Bioelectron.* **1997**, *12*, 337 – 356; c) U. J. Krull, M. S. Heimlich, K. M. R. Kallury, P. A. E. Piunno, J. D. Brennan, R. S. Brown, D. P. Nikolelis, *Can. J. Chem.* **1995**, *73*, 1239 – 1250.
[17] a) J. M. Laval, J. Moiroux, C. Bourdillon, *Biotechnol. Bioeng.* **1991**, *38*, 788 – 796; b) S. Kuwabata, R. Tsuda, H. Yoneyama, *J. Am. Chem. Soc.* **1994**, *116*, 5437 – 5443; c) R. Yuan, S. Kuwabata, H. Yoneyama, *Chem. Lett.* **1996**, 137 – 138.
[18] a) M. Lion-Dagan, E. Katz, I. Willner, *J. Am. Chem. Soc.* **1994**, *116*, 7913 – 7914; b) I. Willner, M. Lion-Dagan, S. Marx-Tibbon, E. Katz, *J. Am. Chem. Soc.* **1995**, *117*, 6581 – 6592; c) I. Willner, M. Lion-Dagan, E. Katz, *Chem. Commun.* **1996**, 623 – 624; d) I. Willner, R. Blonder, E. Katz, A. Stocker, A. F. Bückmann, *J. Am. Chem. Soc.* **1996**, *118*, 5310 – 5311.
[19] a) E. Katz, A. Y. Shkuropatov, O. I. Vagabova, V. A. Shuvalov, *Biochim. Biophys. Acta* **1989**, *976*, 121 – 128; b) E. Katz, A. Y. Shkuropatov, V. A. Shuvalov, *Bioelectrochem. Bioenerg.* **1990**, *23*, 239 – 247; c) A. A. Solov'ev, E. Katz, V. A. Shuvalov, Y. E. Erokhin, *Bioelectrochem. Bioenerg.* **1991**, *26*, 29 – 41.
[20] I. Willner, *Acc. Chem. Res.* **1997**, *30*, 347 – 356.
[21] a) R. Blonder, E. Katz, Y. Cohen, N. Itzhak, A. Riklin, I. Willner, *Anal. Chem.* **1996**, *68*, 3151 – 3157; b) A. L. Ghindilis, P. Atanasov, M. Wilkins, E. Wilkins, *Biosens. Bioelectron.* **1998**, *13*, 113 – 131; c) T. Ihara, M. Nakayama, M. Murata, K. Nakano, M. Maeda, *Chem. Commun.* **1997**, 1609 – 1610.
[22] A. L. Ghindilis, A. Makower, F. W. Scheller, *Anal. Methods Instrum.* **1995**, *2*, 129 – 132.
[23] J.-M. Kauffman, G. G. Guilbault in *Biosensor Principles and Applications* (Eds.: L. J. Blum, P. R. Coulet), Marcel Dekker, New York, **1991**, chap. 4, pp. 63 – 82.
[24] a) C. Bourdillon, J. P. Bourgeois, D. Thomas, *Biotechnol. Bioeng.* **1979**, *21*, 1877 – 1879; b) C. Bourdillon, J. P. Bourgeois, D. Thomas, *J. Am. Chem. Soc.* **1980**, *102*, 4231 – 4235; c) H. J. Wieck, C. Shea, A. M. Yacynych, *Anal. Chim. Acta* **1982**, *142*, 277 – 279; d) G. Urban, G. Jobst, F. Kohl, A. Jachimowicz, F. Olcaytug, O. Tilado, P. Goiser, G. Nauer, F. Pittner, T. Schalkhammer, E. Mann-Buxbaum, *Biosens. Bioelectron.* **1991**, *6*, 555 – 562; e) J. P. Lowry, K. McAteer, S. E. Atrash, R. D. O'Neill, *J. Chem. Soc. Chem. Commun.* **1994**, 2483 – 2484.
[25] A. Heller, *J. Phys. Chem.* **1992**, *96*, 3579 – 3587.
[26] a) R. A. Marcus, N. Sutin, *Biochim. Biophys. Acta* **1985**, *811*, 265 – 322; b) *Electron Transfer in Inorganic, Organic and Biological Systems* (Adv. Chem. Ser. **1991**, *228*).
[27] A. Curulli, I. Carelli, O. Trischitta, G. Palleschi, *Biosens. Bioelectron.* **1997**, *12*, 1043 – 1055.
[28] F. W. Scheller, F. Schubert, B. Neumann, D. Pfeiffer, R. Hintsche, I. Dransfeld, U. Wollenberger, R. Renneberg, A. Warsinke, G. Johansson, M. Skoog, X. Yang, V. Bogdanovskaya, A. Bückmann, S. Y. Zaitsev, *Biosens. Bioelectron.* **1991**, *6*, 245 – 253.
[29] a) S. A. Wring, J. P. Hart, *Analyst* **1992**, *117*, 1215 – 1229; b) J. M. Dicks, W. J. Aston, G. Davis, A. P. F. Turner, *Anal. Chim. Acta* **1986**, *182*, 103 – 112; c) I. Rosen-Margalit, J. Rishpon, *Biosens. Bioelectron.* **1993**, *8*, 315 – 323; d) L. Gorton, H. I. Karan, P. D. Hall, T. Inagaki, Y. Okamoto, T. A. Skotheim, *Anal. Chim. Acta* **1990**, *228*, 23 – 30.
[30] a) D. Avnir, S. Braun, O. Lev, M. Ottolenghi, *Chem. Mater.* **1994**, *6*, 1605 – 1614; b) V. Glezer, O. Lev, *J. Am. Chem. Soc.* **1993**, *115*, 2533 – 2534.
[31] a) B. A. Gregg, A. Heller, *J. Phys. Chem.* **1991**, *95*, 5970 – 5975; b) E. J. Calvo, R. Etchenigue, C. Danilowicz, L. Diaz, *Anal. Chem.* **1996**, *68*, 4186 – 4193; c) S. Cosnier, *Electroanalysis* **1997**, *9*, 894 – 902; d) I. Willner, E. Katz, N. Lapidot, P. Bäuerle, *Bioelectrochem. Bioenerg.* **1992**, *29*, 29 – 45; e) S.-Y. Liu, C.-F. Li, D.-D. Zhang, Y. Zhang, Z.-H. Mo, Q. Cai, A.-R. Zhu, *J. Electroanal. Chem.* **1994**, *364*, 31 – 36; f) P. D. Hale, T. Inagaki, H. I. Karan, Y. Okamoto, T. A. Skotheim, *J. Am. Chem. Soc.* **1989**, *111*, 3482 – 3484; g) E. J. Calvo, C. Danilowicz, L. Diaz, *J. Electroanal. Chem.* **1994**, *369*, 279 – 282; h) G. Arai, M. Masuda, I. Yasumori, *Bull. Chem. Soc. Jpn.* **1994**, *67*, 2962 – 2966.
[32] a) S. B. Adeloju, G. G. Wallace, *Analyst* **1996**, *121*, 699 – 703; b) S. A. Emr, A. M. Yacynych, *Electroanalysis* **1995**, *7*, 913 – 923; c) C. G. J. Koopal, R. J. M. Nolte, *Bioelectrochem. Bioenerg.* **1994**, *33*, 45 – 53.
[33] J. D. Swalen, D. L. Allara, J. D. Andrade, E. A. Chandross, S. Caroff, J. Israelshvili, T. J. McCarthy, R. Murray, R. F. Pease, J. F. Rabolt, K. J. Wynne, H. Yu, *Langmuir* **1987**, *3*, 932 – 950.

[34] a) D. J. C. Yates, *J. Phys. Chem.* **1961**, *65*, 746 – 753; b) E. W. Thornton, P. G. Harrison, *J. Chem. Soc. Faraday Trans. 1* **1975**, 461 – 472; c) H. O. Finklea, R. Vithanage, *J. Phys. Chem.* **1982**, *86*, 3621 – 3626.

[35] a) C. Widrig, M. Majda, *Anal. Chem.* **1987**, *59*, 754 – 760; b) A. Proctor, J. F. Castner, L. B. Wingard, Jr., D. M. Hercules, *Anal. Chem.* **1985**, *57*, 1644 – 1649.

[36] a) H. Angerstein-Kozlowska, B. E. Conway, W. B. A. Sharp, *J. Electroanal. Chem.* **1973**, *43*, 9 – 36; b) L. D. Burke, M. B. C. Roche, *J. Electroanal. Chem.* **1984**, *164*, 315 – 334; c) J. Facci, R. W. Murray, *J. Electroanal. Chem.* **1980**, *112*, 221 – 229.

[37] J. R. Lenhard, R. W. Murray, *J. Electroanal. Chem.* **1977**, *78*, 195 – 201.

[38] a) M. R. Tarasevich, *Electrochemistry of Carbon Materials*, Nauka, Moscow, **1984**; b) V. J. Razumas, J. J. Jasaitis, J. J. Kulys, *Bioelectrochem. Bioenerg.* **1984**, *12*, 297 – 322; c) B. R. Puri in *Chemistry and Physics of Carbon*, *Vol. 6* (Ed.: P. L. Walker, Jr.), Marcel Dekker, New York, **1970**.

[39] a) B. F. Watkins, J. R. Behling, E. Kariv, L. L. Miller, *J. Am. Chem. Soc.* **1975**, *97*, 3549 – 3550; b) B. E. Firth, L. L. Miller, M. Mitani, T. Rogers, J. Lennox, R. W. Murray, *J. Am. Chem. Soc.* **1976**, *98*, 8271 – 8272; c) C. P. Jester, R. D. Rocklin, R. W. Murray, *J. Electrochem. Soc.* **1980**, *127*, 1979 – 1985; d) K. J. Stutts, R. M. Wrighton, *Anal. Chem.* **1983**, *55*, 1576 – 1579.

[40] a) R. Larsson, L. Y. Johansson, L. Jonsson, *J. Appl. Electrochem.* **1981**, *11*, 489 – 492; b) H. Gomathi, G. P. Rao, *J. Electroanal. Chem.* **1985**, *190*, 85 – 94.

[41] a) J. A. Osborn, R. M. Ianniello, H. J. Wieck, T. F. Decker, S. L. Gordon, A. M. Yacynych, *Biotechnol. Bioeng.* **1982**, *24*, 1653 – 1669; b) I. Rosen, J. Rishpon, *J. Electroanal. Chem.* **1989**, *258*, 27 – 39.

[42] a) J. F. Evans, T. Kuwana, *Anal. Chem.* **1979**, *51*, 358 – 365; b) N. Oyama, A. P. Brown, F. C. Anson, *J. Electroanal. Chem.* **1978**, *87*, 435 – 441; c) N. Oyama, F. C. Anson, *J. Electroanal. Chem.* **1978**, *88*, 289 – 297.

[43] K. A. Daube, D. J. Harrison, T. E. Mallouk, A. J. Ricco, S. Chao, M. S. Wrighton, W. A. Hendrickson, A. J. Drube, *J. Photochem.* **1985**, *29*, 71 – 88.

[44] a) A. W. C. Lin, P. Yeh, A. M. Yacynych, T. Kuwana, *J. Electroanal. Chem.* **1977**, *84*, 411 – 419; b) A. M. Yacynych, T. Kuwana, *Anal. Chem.* **1978**, *50*, 640 – 645; c) R. M. Ianniello, H. J. Wieck, A. M. Yacynych, *Anal. Chem.* **1983**, *55*, 2067 – 2070.

[45] a) R. M. Ianniello, A. M. Yacynych, *Anal. Chem.* **1981**, *53*, 2090 – 2095; b) R. M. Ianniello, T. J. Lindsay, A. M. Yacynych, *Anal. Chem.* **1982**, *54*, 1098 – 1101; c) G. Jonsson, L. Gorton, *Biosensors* **1985**, *1*, 355 – 368.

[46] R. M. Ianniello, A. M. Yacynych, *Anal. Chim. Acta* **1981**, *131*, 123 – 132.

[47] V. J. Razumas, J. J. Jasaitis, J. J. Kulys, *Bioelectrochem. Bioenerg.* **1983**, *10*, 427 – 439.

[48] J. A. Osborn, A. M. Yacynych, D. C. Roberts, *Anal. Chim. Acta* **1986**, *183*, 287 – 292.

[49] R. F. Antrim, A. M. Yacynych, H. J. Wieck, G. W. Lutter, *Analyst* **1988**, *113*, 341 – 344.

[50] R. M. Ianniello, A. M. Yacynych, *Anal. Chim. Acta* **1983**, *146*, 249 – 253.

[51] R. A. Kamin, G. S. Wilson, *Anal. Chem.* **1980**, *52*, 1198 – 1205.

[52] C. A. Koval, F. C. Anson, *Anal. Chem.* **1978**, *50*, 223 – 229.

[53] R. D. Rocklin, R. W. Murray, *J. Electroanal. Chem.* **1979**, *100*, 271 – 282.

[54] M. Umana, D. R. Rolison, R. Nowak, P. Daum, R. W. Murray, *Surface Sci.* **1980**, *101*, 295 – 309.

[55] M. H. Smit, A. E. G. Cass, *Anal. Chem.* **1990**, *62*, 2429 – 2436.

[56] N. Oyama, K. B. Yap, F. C. Anson, *J. Electroanal. Chem.* **1979**, *100*, 233 – 246.

[57] C. Bourdillon, V. Thomas, D. Thomas, *Enzyme Microbiol. Technol.* **1982**, *4*, 175 – 180.

[58] a) W. Schuhmann, R. Lammert, B. Uhe, H.-L. Schmidt, *Sens. Actuators B* **1990**, *1*, 537 – 541; b) W. Schuhmann, H. Wohlschlager, R. Lammert, H.-L. Schmidt, U. Loffler, H.-D. Wiemhofer, W. Göpel, *Sens. Actuators B* **1990**, *1*, 571 – 575.

[59] P. Bianco, J. Haladjian, C. Bourdillon, *J. Electroanal. Chem.* **1990**, *283*, 151 – 163.

[60] J. M. Laval, C. Bourdillon, *J. Electroanal. Chem.* **1983**, *152*, 125 – 141.

[61] a) R. F. Lane, A. T. Hubbard, *J. Phys. Chem.* **1973**, *77*, 1401 – 1410; b) R. F. Lane, A. T. Hubbard, *J. Phys. Chem.* **1973**, *77*, 1411 – 1421; c) M. Sharp, M. Petersson, K. Edstrom, *J. Electroanal. Chem.* **1979**, *95*, 123 – 130; d) J. T. Hupp, M. J. Weaver, *J. Electrochem. Soc.* **1984**, *131*, 619 – 622.

[62] a) S. Mazur, T. Matusinovic, K. Cammann, *J. Am. Chem. Soc.* **1977**, *99*, 3888 – 3890; b) R. Nowak, F. A. Schultz, M. Umana, H. D. Abruña, R. W. Murray, *J. Electroanal. Chem.* **1978**, *94*, 219 – 225; c) G. Jonsson, L. Gorton, L. Pettersson, *Electroanalysis* **1989**, *1*, 49 – 55; d) E. Katz, *J. Electroanal. Chem.* **1993**, *361*, 109 – 144.

[63] a) A. P. Brown, F. C. Anson, *J. Electroanal. Chem.* **1977**, *83*, 203 – 206; b) H. Jaegfeldt, A. Torstensson, L. Gorton, G. Johansson, *Anal. Chem.* **1981**, *53*, 1979 – 1982.

[64] E. Katz, *J. Electroanal. Chem.* **1994**, *365*, 157 – 164.

[65] a) I. Haller, *J. Am. Chem. Soc.* **1978**, *100*, 8050 – 8055; b) E. Katz, A. Y. Shkuropatov, B. D. Sviridov, V. A. Shuvalov, O. I. Vagabova, *Zhurn. Fiz. Khim.* **1986**, *60*, 1312 – 1314.

[66] H. O. Finklea, H. Abruña, R. W. Murray, *Adv. Chem. Ser.* **1980**, *184*, 253 – 268.

[67] P. R. Moses, R. W. Murray, *J. Electroanal. Chem.* **1977**, *77*, 393 – 399.

[68] C. Zou, M. S. Wrighton, *J. Am. Chem. Soc.* **1990**, *112*, 7578 – 7584.

[69] C. M. Elliott, R. W. Murray, *Anal. Chem.* **1976**, *48*, 1247 – 1254.

[70] a) W. J. Albery, A. J. Hillman, *Annu. Rep. Prog. Chem. Sect. C* **1981**, *78*, 377 – 437; b) R. W. Murray in *Electroanalytical Chemistry*, Vol. 13 (Ed.: A. J. Bard), Marcel Dekker, New York, **1984**, pp. 191 – 368; c) R. W. Murray, *Acc. Chem. Res.* **1980**, *13*, 135 – 141; d) M. S. Wrighton, *Science* **1986**, *231*, 32 – 37; e) H. D. Abruña, *Coord. Chem. Rev.* **1988**, *86*, 135 – 189.

[71] S. Yoshida, H. Kanno, T. Watanabe, *Anal. Sci.* **1995**, *11*, 251 – 256.

[72] S. K. Bhatia, M. J. Cooney, L. C. Shriver-Lake, T. L. Fare, F. S. Ligler, *Sens. Actuators B* **1991**, *3*, 311 – 317.

[73] C. D. Bain, E. B. Troughton, Y.-T. Tao, J. Eval, G. M. Whitesides, R. G. Nuzzo, *J. Am. Chem. Soc.* **1989**, *111*, 321 – 335.

[74] R. G. Nuzzo, F. A. Fusco, D. L. Allara, *J. Am. Chem. Soc.* **1987**, *109*, 2358 – 2368.

[75] a) E. B. Troughton, C. D. Bain, G. M. Whitesides, R. G. Nuzzo, D. L. Allara, M. D. Porter, *Langmuir* **1988**, *4*, 365 – 385; b) E. Katz, N. Itzhak, I. Willner, *J. Electroanal. Chem.* **1992**, *336*, 357 – 362; c) M. W. J. Beulen, B.-H. Huisman, P. A. van der Heijden, C. F. J. M. van Veggel, M. G. Simons, E. M. E. F. Biemond, P. J. de Lange, D. N. Reinhoudt, *Langmuir* **1996**, *12*, 6170 – 6172.

[76] A. J. Arduengo, J. R. Moran, J. Rodriguez-Parada, M. D. Ward, *J. Am. Chem. Soc.* **1990**, *112*, 6153 – 6154.

[77] J. Y. Gui, D. A. Stern, F. Lu, A. T. Hubbard, *J. Electroanal. Chem.* **1991**, *305*, 37 – 55.

[78] D. Datta, H. A. O. Hill, H. Nakayama, *J. Electroanal. Chem.* **1991**, 297, 309 – 314.

[79] a) H. A. Finklea in *Electroanalytical Chemistry*, *Vol. 19* (Eds.: A. J. Bard, I. Rubinstein), Marcel Dekker, New York, **1996**, pp. 109 – 335; b) E. Delamarche, B. Michel, H. A. Biebuyck, C. Gerber, *Adv. Mater.* **1996**, *8*, 719 – 729; c) A. Ulman, *Chem. Rev.* **1996**, *96*, 1533 – 1554.

[80] a) C. D. Bain, J. Evall, G. M. Whitesides, *J. Am. Chem. Soc.* **1989**, *111*, 7155 – 7164; b) R. G. Nuzzo, B. R. Zegarski, L. H. Dubois, *J. Am. Chem. Soc.* **1987**, *109*, 733 – 740; c) R. G. Nuzzo, L. H. Dubois, D. L. Allara, *J. Am. Chem. Soc.* **1990**, *112*, 558 – 569.

[81] a) J. J. Hickman, C. Zou, D. Ofer, P. D. Harvey, M. S. Wrighton, *J. Am. Chem. Soc.* **1989**, *111*, 7271 – 7272; b) J. J. Hickman, P. E. Laibinis, D. I. Auerbach, C. Zou, T. J. Gardner, G. M. Whitesides, M. S. Wrighton, *Langmuir* **1992**, *8*, 357 – 359; c) M. P. Soriaga, E. Binamira-Soriaga, A. T. Hubbard, J. B. Benziger, K.-W. P. Pang, *Inorg. Chem.* **1985**, *24*, 65 – 73; d) J. L. Stickney, M. P. Soriaga, A. T. Hubbard, S. E. Anderson, *J. Electroanal. Chem.* **1981**, *125*, 73 – 88; e) E. Katz, A. A. Solov'ev, *J. Electroanal. Chem.* **1990**, *291*, 171 – 186.

[82] a) M. M. Walczak, C. Chung, S. M. Stole, C. A. Widrig, M. D. Porter, *J. Am. Chem. Soc.* **1991**, *113*, 2370 – 2378; b) I. Taniguchi, M. Iseki, H. Yamaguchi, K. Yasukouchi, *J. Electroanal. Chem.* **1984**, *175*, 341 – 348; c) L. S. Pinheiro, M. L. A. Temperini, *J. Electroanal. Chem.* **1990**, *295*, 169 – 181; d) P. Fenter, P. Eisenberger, J. Li, N. Camillone, S. Bernasek, G. Scoles, T. A. Ramanarayanan, K. S. Liang, *Langmuir* **1991**, *7*, 2013 – 2016; e) P. E. Laibinis, G. M. Whitesides, *J. Am. Chem. Soc.* **1992**, *114*, 1990 – 1995; f) P. E. Laibinis, G. M. Whitesides, D. L.

Allara, Y.-T. Tao, A. N. Parikn, R. G. Nuzzo, *J. Am. Chem. Soc.* **1991**, *113*, 7152 – 7167; g) P. E. Laibinis, C. D. Bain, G. M. Whitesides, *J. Phys. Chem.* **1991**, *95*, 7017 – 7021.

[83] M. E. Bothwell, M. P. Soriaga, *J. Electroanal. Chem.* **1990**, *295*, 123 – 129.

[84] B. Reynders, M. Stratmann, *Fresenius J. Anal. Chem.* **1991**, *341*, 406 – 407.

[85] a) T. T.-T. Li, M. J. Weaver, *J. Am. Chem. Soc.* **1984**, *106*, 6107 – 6108; b) T. T.-T. Li, M. J. Weaver, *J. Electroanal. Chem.* **1985**, *188*, 121 – 129; c) M. J. Weaver, T. T.-T. Li, *J. Phys. Chem.* **1986**, *90*, 3823 – 3829.

[86] E. Katz, V. V. Borovkov, R. P. Evstigneeva, *J. Electroanal. Chem.* **1992**, *326*, 197 – 212.

[87] C. W. Sheen, J.-X. Shi, J. Martensson, A. N. Parikh, D. L. Allara, *J. Am. Chem. Soc.* **1992**, *111*, 1514 – 1515.

[88] Y. Gu, Z. Lin, R. A. Butera, V. S. Smentkowski, D. H. Waldeck, *Langmuir* **1995**, *11*, 1849 – 1851.

[89] a) A. R. Bishop, R. G. Nuzzo, *Curr. Opin. Coll. Interf. Sci.* **1996**, *1*, 127 – 136; b) D. L. Allara, *Biosens. Bioelectron.* **1995**, *10*, 771 – 783; c) D. Mandler, I. Turyan, *Electroanalysis* **1996**, *8*, 207 – 213.

[90] a) N. Mohri, M. Inoue, Y. Arai, K. Yoshikawa, *Langmuir* **1995**, *11*, 1612 – 1616; b) J. B. Schlenoff, M. Li, H. Ly, *J. Am. Chem. Soc.* **1995**, *117*, 12528 – 12536; c) L. Sun, R. C. Thomas, R. M. Crooks, *J. Am. Chem. Soc.* **1991**, *113*, 8550 – 8552; d) R. C. Thomas, L. Sun, R. M. Crooks, *Langmuir* **1991**, *7*, 620 – 622; e) Z. Zhang, T. L. Beck, J. T. Young, F. J. Boerio, *Langmuir* **1996**, *12*, 1227 – 1234.

[91] a) T. L. Freeman, S. D. Evans, A. Ulman, *Langmuir* **1995**, *11*, 4411 – 4417; b) S. V. Atre, B. Liedberg, D. L. Allara, *Langmuir* **1995**, *11*, 3882 – 3893; c) C. G. Worley, R. W. Linton, E. T. Samulski, *Langmuir* **1995**, *11*, 3805 – 3810; d) K. de Weldige, M. Rohwerder, E. Vago, H. Viefhaus, M. Stratmann, *Fresenius J. Anal. Chem.* **1995**, *353*, 329 – 332; e) D. G. Castner, K. Hinds, D. W. Grainger, *Langmuir* **1996**, *12*, 5083 – 5086.

[92] a) M. G. Samant, K. Kunimatsu, R. Viswanathan, H. Seki, G. Pacchioni, P. S. Bargus, M. R. Philpott, *Langmuir* **1991**, *7*, 1261 – 1268; b) S. D. Evans, E. Urankar, A. Ulman, N. Ferris, *J. Am. Chem. Soc.* **1991**, *113*, 4121 – 4131; c) H. C. Yang, D. L. Dermody, C. Xu, A. J. Ricco, R. M. Crooks, *Langmuir* **1996**, *12*, 726 – 735; d) Y. Sato, S. Ye, T. Haba, K. Uosaki, *Langmuir* **1996**, *12*, 2726 – 2736; e) B. L. Frey, R. M. Corn, *Anal. Chem.* **1996**, *68*, 3187 – 3193; f) R. V. Duevel, R. M. Corn, *Anal. Chem.* **1992**, *64*, 337 – 342; g) I. T. Bae, M. Sandifer, Y. W. Lee, D. A. Tryk, C. N. Sukenik, D. A. Scherson, *Anal. Chem.* **1995**, *67*, 4508 – 4513.

[93] a) R. A. Drawhorn, N. L. Abbott, *J. Phys. Chem.* **1995**, *99*, 16511 – 16515; b) S. E. Creager, J. Clarke, *Langmuir* **1994**, *10*, 3675 – 3683; c) P. E. Laibinis, R. G. Nuzzo, G. M. Whitesides, *J. Phys. Chem.* **1992**, *96*, 5097 – 5105; d) P. E. Laibinis, M. A. Fox, J. P. Folkers, G. M. Whitesides, *Langmuir* **1991**, *7*, 3167 – 3173; e) S. D. Evans, R. Sharma. A. Ulman, *Langmuir* **1991**, *7*, 156 – 161; f) C. D. Bain, G. M. Whitesides, *J. Am. Chem. Soc.* **1988**, *110*, 5897 – 5898; g) C. D. Bain, G. M. Whitesides, *Angew. Chem.* **1989**, *101*, 522 – 528; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 506 – 512; h) C. D. Bain, G. M. Whitesides, *J. Am. Chem. Soc.* **1988**, *110*, 6560 – 6561; i) C. D. Bain, G. M. Whitesides, *J. Am. Chem. Soc.* **1988**, *110*, 3665 – 3666; j) C. D. Bain, G. M. Whitesides, *Science* **1988**, *240*, 62 – 63.

[94] a) B. Leidberg, P. Tengvall, *Langmuir* **1995**, *11*, 3821 – 3827; b) J. P. Folkers, P. E. Laibinis, G. M. Whitesides, *Langmuir* **1992**, *8*, 1330 – 1341; c) M. D. Porter, T. B. Bright, D. L. Allara, C. E. D. Chidsey, *J. Am. Chem. Soc.* **1987**, *109*, 3559 – 3568

[95] a) R. F. Debono, G. D. Loucks, D. D. Manna, U. J. Krull, *Can. J. Chem.* **1996**, *74*, 677 – 688; b) K. A. Peterlinz, R. Georgiadis, *Langmuir* **1996**, *12*, 4731 – 4740; c) T. T. Ehler, N. Malmberg, K. Carron, B. P. Sullivan, L. J. Noe, *J. Phys. Chem. B* **1997**, *101*, 3174 – 3180; d) G. B. Sigal, M. Mrksich, G. M. Whitesides, *Langmuir* **1997**, *13*, 2749 – 2755; e) T. T. Ehler, N. Malmberg, L. J. Noe, *J. Phys. Chem. B* **1997**, *101*, 1268 – 1272.

[96] a) W. Pan, C. J. Durning, N. J. Turro, *Langmuir* **1996**, *12*, 4469 – 4473; b) Y. J. Lee, C. Jeon, W. Paik, K. Kim, *Langmuir* **1996**, *12*, 5830 – 5837.

[97] a) J. A. M. Sondag-Huethorst, L. G. J. Fokkink, *Langmuir* **1995**, *11*, 2237 – 2241; b) K. Sinniah, J. Cheng, S. Terrettaz, J. E. Reutt-Robey, C. J. Miller, *J. Phys. Chem.* **1995**, *99*, 14500 – 14505; c) W. R. Everett, I. Fritsch-Faules, *Anal. Chim. Acta* **1995**, *307*, 253 – 268; d) W. R. Fawcett, M. Fedurco, Z. Kovácová, *Langmuir* **1994**, *10*, 2403 – 2408; e) J. A. M. Sondag-Huethorst, L. G. J. Fokkink, *J. Electroanal. Chem.* **1994**, *367*, 49 – 57; f) A. Badia, R. Back, B. Lennox, *Angew. Chem.* **1994**, *106*, 2429 – 2431; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2332 – 2335; g) D.-F. Yang, C. P. Wilde, M. Morin, *Langmuir* **1996**, *12*, 6570 – 6577; h) C. E. D. Chidsey, D. N. Loiacono, *Langmuir* **1990**, *6*, 682 – 691; i) E. Katz, I. Willner, *Langmuir* **1997**, *13*, 3364 – 3373.

[98] a) W. Li, J. A. Virtanen,. R. M. Penner, *J. Phys. Chem.* **1994**, *98*, 11751 – 11755; b) S. J. Stranick, A. N. Parikh, Y.-T. Tao, D. L. Allara, P. S. Weiss, *J. Phys. Chem.* **1994**, *98*, 7636 – 7646; c) Y.-T. Kim, R. L. McCarley, A. J. Bard, *Langmuir* **1993**, *9*, 1941 – 1944; d) L. Sun, R. M. Crooks, *Langmuir* **1993**, *9*, 1951 – 1954; e) R. L. McCarley, D. J. Dunaway, R. J. Willicut, *Langmuir* **1993**, *9*, 2775 – 2777; f) Y.-T. Kim, A. J. Bard, *Langmuir* **1992**, *8*, 1096 – 1102; g) I. Touzov, C. B. Gorman, *J. Phys. Chem. B* **1997**, *101*, 5263 – 5276; g) R. Heinz, J. P. Rabe, *Langmuir* **1995**, *11*, 506 – 511; i) R. J. Willicut, R. L. McCarley, *Anal. Chim. Acta* **1995**, *307*, 269 – 276.

[99] a) C. A. Alves, E. L. Smith, M. D. Porter, *J. Am. Chem. Soc.* **1992**, *114*, 1222 – 1227; b) J. T. Woodward, D. K. Schwartz, *J. Am. Chem. Soc.* **1996**, *118*, 7861 – 7862; c) M. Jaschke, H. Schönherr, H. Wolf, H.-J. Butt, E. Bamberg, M. K. Besocke, H. Ringsdorf, *J. Phys. Chem.* **1996**, *100*, 2290 – 2301; d) J.-B. D. Green, M. T. McDermott, M. D. Porter, *J. Phys. Chem.* **1996**, *100*, 13342 – 13345; e) K. Tamada, M. Hara, H. Sasabe, W. Knoll, *Langmuir* **1997**, *13*, 1558 – 1566; f) T. Han, J. M. Williams, T. P. Beebe, Jr., *Anal. Chim. Acta* **1995**, *307*, 365 – 376; g) W. B. Caldwell, D. J. Campbell, K. Chen, B. R. Herr, C. A. Mirkin, A. Malik, M. K. Durbin, P. Dutta, K. G. Huang, *J. Am. Chem. Soc.* **1995**, *117*, 6071 – 6082; h) C. A. McDermott, M. T. McDermott, J.-B. Green, M. D. Porter, *J. Phys. Chem.* **1995**, *99*, 13257 – 13267.

[100] a) L. Strong, G. M. Whitesides, *Langmuir* **1988**, *4*, 546 – 558; b) B. Hagenhoff, A. Benninghoven, J. Spinke, M. Liley, W. Knoll. *Langmuir* **1993**, *9*, 1622 – 1624; c) J. Huang, J. C. Hemminger, *J. Am. Chem. Soc.* **1993**, *115*, 3342 – 3343; d) J. Li, J. Huang, R. T. McIver, Jr., J. C. Hemminger, *J. Am. Chem. Soc.* **1992**, *114*, 2428 – 2432; e) M. G. Samant, C. A. Brown, J. G. Gordon II, *Langmuir* **1991**, *7*, 437 – 439; f) N. Camillone III, T. Y. B. Leung, P. Schwartz, P. Eisenberger, G. Scoles, *Langmuir* **1996**, *12*, 2737 – 2746; g) S. Mouradian, C. M. Nelson, L. M. Smith, *J. Am. Chem. Soc.* **1996**, *118*, 8639 – 8645; h) T. D. McCarley, R. L. McCarley, *Anal. Chem.* **1997**, *69*, 130 – 136; i) S. D. Evans, A. Ulman, *Chem. Phys. Lett.* **1990**, *170*, 462 – 466; j) A.-S. Duwez, S. D. Paolo, J. Ghijsen, J. Riga, M. Deleuze, J. Delhalle, *J. Phys. Chem. B* **1997**, *101*, 884 – 890.

[101] H. Ron, I. Rubinstein, *Langmuir* **1994**, *10*, 4566 – 4573.

[102] a) S. E. Creager, G. K. Rowe, *J. Electroanal. Chem.* **1994**, *370*, 203 – 211; b) T. Ohtsuka, Y. Sato, K. Uosaki, *Langmuir* **1994**, *10*, 3658 – 3662; c) G. K. Rowe, S. E. Creager, *Langmuir* **1994**, *10*, 1186 – 1192; d) L. Tender, M. T. Carter, R. W. Murray, *Anal. Chem.* **1994**, *66*, 3173 – 3181; e) K. Weber, S. E. Creager, *Anal. Chem.* **1994**, *66*, 3164 – 3172; f) K. Shimazu, I. Yagi, Y. Sato, K. Uosaki, *Langmuir* **1992**, *8*, 1385 – 1387; g) D. D. Popenoe, R. S. Deinhammer, M. D. Porter, *Langmuir* **1992**, *8*, 2521 – 2530; h) J. Redepenning, J. M. Flood, *Langmuir* **1996**, *12*, 508 – 512; i) J. N. Richardson, S. R. Peck, L. S. Curtin, L. M. Tender, R. H. Terrill, M. T. Carter, R. W. Murray, G. K. Rowe, S. E. Creager, *J. Phys. Chem.* **1995**, *99*, 766 – 772; j) W. R. Everett, T. L. Welch, L. Reed, I. Fritsch-Faules, *Anal. Chem.* **1995**, *67*, 292 – 298; k) S. R. Peck, L. S. Curtin, L. M. Tender, M. T. Carter, R. H. Terrill, R. W. Murray, J. P. Collman, W. A. Little, H. M. Duan, C. Dong, A. M. Hermann, *J. Am. Chem. Soc.* **1995**, *117*, 1121 – 1126; l) M. T. Carter, G. K. Rowe, J. N. Richardson, L. M. Tender, R. H. Terrill, R. W. Murray, *J. Am. Chem. Soc.* **1995**, *117*, 2896 – 2899; m) L. A. Hockett, S. E. Creager, *Langmuir* **1995**, *11*, 2318 – 2321; n) J. F. Smalley, S. W. Feldberg, C. E. D. Chidsey, M. R. Linford, M. D. Newton, Y.-P. Liu, *J. Phys. Chem.* **1995**, *99*, 13141 – 13149; o) D. M. Collard, M. A. Fox, *Langmuir* **1991**, *7*, 1192 – 1197; p) C. E. D. Chidsey, C. R. Bertozzi, T. M. Putvinski, A. M. Mujsce, *J. Am. Chem. Soc.* **1990**, *112*, 4301 – 4306; q) H. C. DeLong, J. J. Donohue, D. A. Buttry, *Langmuir* **1991**, *7*, 2196 – 2202; r) S. E. Creager, G. K. Rose, *Anal. Chim. Acta* **1991**, *246*, 233 – 239; s) K. Uosaki, Y. Sato, H. Kita, *Langmuir* **1991**, *7*, 1510 – 1514.

[103] a) E. Katz, N. Itzhak, I. Willner, *Langmuir* **1993**, *9*, 1392 – 1396; b) X. Tang, T. W. Schneider, D. A. Buttry, *Langmuir* **1994**, *10*, 2235 – 2240; c) X. Tang, T. W. Schneider, J. W. Walker, D. A. Buttry, *Langmuir*

1996, *12*, 5921 – 5933; d) S. Yamada, H. Kohrogi, T. Matsuo, *Chem. Lett.* **1995**, 639 – 640; e) K. A. B. Lee, *Langmuir* **1990**, *6*, 709 – 712; f) H. C. DeLong, D. A. Buttry, *Langmuir* **1990**, *6*, 1319 – 1322.

[104] a) E. Katz, H.-L. Schmidt, *J. Electroanal. Chem.* **1993**, *360*, 337 – 342; b) E. Katz, D. D. Schlereth, H.-L. Schmidt, *J. Electroanal. Chem.* **1994**, *367*, 59 – 70; c) E. Katz, H.-L. Schmidt, *J. Electroanal. Chem.* **1994**, *368*, 87 – 94; d) L. Zhang, T. Lu, G. W. Gokel, A. E. Kaifer, *Langmuir* **1993**, *9*, 786 – 791; e) J. J.Hickman, D. Ofer, P. E. Laibinis, G. M. Whitesides, M. S. Wrighton, *Science* **1991**, *252*, 688 – 691; f) J. Lukkari, K. Kleemola, M. Meretoja, J. Kankare, *Chem. Commun.* **1997**, 1099 – 1100.

[105] a) J. E. Hutchison, T. A. Postlethwaite, R. W. Murray, *Langmuir* **1993**, *9*, 3277 – 3283; b) T. Akiyama, H. Imahori, Y. Sakata, *Chem. Lett.* **1994**, 1447 – 1450; c) J. Zak, H. Yuan, M. Ho, L. K. Woo, M. D. Porter, *Langmuir* **1993**, *9*, 2772 – 2774; d) T. R. E. Simpson, M. J. Cook, M. C. Petty, S. C. Thorpe, D. A. Russell, *Analyst* **1996**, *121*, 1501 – 1505; e) T. A. Postlethwaite, J. E. Hutchison, K. W. Hathcock, R. W. Murray, *Langmuir* **1995**, *11*, 4109 – 4116; f) T. R. E. Simpson, D. J. Revell, M. J. Cook, D. A. Russell, *Langmuir* **1997**, *13*, 460 – 464; g) K. Uosaki, T. Kondo, X.-Q. Zhang, M. Yanagida, *J. Am. Chem. Soc.* **1997**, *119*, 8367 – 8368; h) S. Yamada, Y. Koide, T. Matsuo, *J. Electroanal. Chem.* **1997**, *426*, 23 – 26.

[106] a) H.-Y. Chen, D.-M. Zhou, J.-J. Xu, H.-Q. Fang, *J. Electroanal. Chem.* **1997**, *422*, 21 – 25; b) D. D. Schlereth, E. Katz, H.-L. Schmidt, *Electroanalysis* **1995**, *7*, 46 – 54; c) D. D. Schlereth, E. Katz, H.-L. Schmidt, *Electroanalysis* **1994**, *6*, 725 – 734; d) C. Ruan, F. Yang, C. Lei, J. Deng, *Anal. Chem.* **1998**, *70*, 1721 – 1725.

[107] a) J. Redepenning, H. M. Tunison, H. O. Finklea, *Langmuir* **1993**, *9*, 1404 – 1407; b) H. O. Finklea, D. D. Hanshew, *J. Am. Chem. Soc.* **1992**, *114*, 3173 – 3181; c) M. Maskus, H. D. Abruña, *Langmuir* **1996**, *12*, 4455 – 4462; d) H. O. Finklea, L. Liu, M. S. Ravenscroft, S. Punturi, *J. Phys. Chem.* **1996**, *100*, 18852 – 18858.

[108] a) P. He, J. Ye, Y. Fang, I. Suzuki, T. Osa, *Electroanalysis* **1997**, *9*, 68 – 73; b) H. Yamamoto, Y. Maeda, H. Kitano, *J. Phys. Chem. B* **1997**, *101*, 6855 – 6860; c) M. Lahav, K. T. Ranjit, E. Katz, I. Willner, *Chem. Commun.* **1997**, 259 – 260; d) M. Lahav, K. T. Ranjit, E. Katz, I. Willner, *Isr. J. Chem.* **1997**, *37*, 185 – 195.

[109] H. Schönherr, G. J. Vancso, B.-H. Huisman, F. C. J. M. van Veggel, D. N. Reinhoudt, *Langmuir* **1997**, *13*, 1567 – 1570.

[110] a) T. Lu, L. Zhang, G. W. Gokel, A. E. Kaifer, *J. Am. Chem. Soc.* **1993**, *115*, 2542 – 2543; b) K. T. Ranjit, S. Marx-Tibbon, I. Ben-Dov, I. Willner, *Angew. Chem.* **1997**, *109*, 90 – 93; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 147 – 150; c) K. T. Ranjit, S. Marx-Tibbon, I. Ben-Dov, B. Willner, I. Willner, *Isr. J. Chem.* **1997**, *36*, 407 – 419.

[111] a) W. B. Caldwell, K. Chen, C. A. Mirkin, S. J. Babinec, *Langmuir* **1993**, *9*, 1945 – 1947; b) X. Shi, W. B. Caldwell, K. Chen, C. A. Mirkin, *J. Am. Chem. Soc.* **1994**, *116*, 11598 – 11599; c) T. Akiyama, H. Imahori, A. Ajawakom, Y. Sakata, *Chem. Lett.* **1996**, 907 – 908; d) H. Tokuhisa, R. M. Crooks, *Langmuir* **1997**, *13*, 5608 – 5612.

[112] a) F. C. Meldrum, J. Flath, W. Knoll, *Langmuir* **1997**, *13*, 2033 – 2049; b) M. Miyake, H. Matsumoto, M. Nishizawa, T. Sakata, H. Mori, S. Kuwabata, H. Yoneyama, *Langmuir* **1997**, *13*, 742 – 746; c) H. Fan, G. P. López, *Langmuir* **1997**, *13*, 119 – 121; d) A. Badia, L. Cuccia, L. Demers, F. Morin, R. B. Lennox, *J. Am. Chem. Soc.* **1997**, *119*, 2682 – 2692; e) C. Demaille, M. Brust, M. Tsionsky, A. J. Bard, *Anal. Chem.* **1997**, *69*, 2323 – 2328; f) A. Doron, E. Katz, I. Willner, *Langmuir* **1995**, *11*, 1313 – 1317; g) S. Ogawa, K. Hu, F.-R. Fan, A. J. Bard, *J. Phys. Chem. B* **1997**, *101*, 5701 – 5711.

[113] a) G. Bar, S. Rubin, A. N. Parikh, B. I. Swanson, T. A. Zawodzinski Jr., M.-H. Whangbo, *Langmuir* **1997**, *13*, 373 – 377; b) S. Xu, G. Liu, *Langmuir* **1997**, *13*, 127 – 129; c) S. Akari, D. Horn, H. Keller, W. Schrepp, *Adv. Mater.* **1995**, *7*, 549 – 551; d) J. L. Wilbur, H. A. Biebuyck, J. C. MacDonald, G. M. Whitesides, *Langmuir* **1995**, *11*, 825 – 831.

[114] a) M. Nishizawa, T. Sunagawa, H. Yoneyama, *J. Electroanal. Chem.* **1997**, *436*, 213 – 218; b) L. Yan, C. Mazzolin, A. Terfort, G. M. Whitesides, *Langmuir* **1997**, *13*, 6704 – 6712; c) S. Imabayashi, D. Hobara, T. Kakiuchi, W. Knoll, *Langmuir* **1997**, *13*, 4502 – 4504.

[115] a) C.-X. Zhao, J. Zhang, Z.-F. Liu, *Chem. Lett.* **1997**, 473 – 474; b) R. Wang, T. Iyoda, D. A. Tryk, K. Hashimoto, A. Fujishima, *Langmuir* **1997**, *13*, 4644 – 4651; c) E. Katz, M. Lion-Dagan, I. Willner, *J. Electroanal. Chem.* **1995**, *382*, 25 – 31; d) E. Katz, I. Willner, *Electroanalysis* **1995**, *7*, 417 – 419; e) M. Lion-Dagan, E. Katz, I. Willner, *J. Chem. Soc. Chem. Commun.* **1994**, 2741 – 2742; f) S. Marx-Tibbon, I. Ben-Dov, I. Willner, *J. Am. Chem. Soc.* **1996**, *118*, 4717 – 4718; g) A. Doron, E. Katz, M. Portnoy, I. Willner, *Angew. Chem.* **1996**, *108*, 1626 – 1628; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1535 – 1537; h) A. Doron, M. Portnoy, M. Lion-Dagan, E. Katz, I. Willner, *J. Am. Chem. Soc.* **1996**, *118*, 8937 – 8944; i) E. Katz, B. Willner, I. Willner, *Biosens. Bioelectron.* **1997**, *12*, 703 – 719; j) A. Doron, E. Katz, G. Tao, I. Willner, *Langmuir* **1997**, *13*, 1783 – 1790; k) K. Morigaki, Z.-F. Liu, K. Hashimoto, A. Fujishima, *J. Phys. Chem.* **1995**, *99*, 14771 – 14777; l) I. Willner, A. Doron, E. Katz, *J. Phys. Org. Chem.* **1998**, *11*, 546 – 560.

[116] a) I. Willner, E. Katz, A. Riklin, R. Kasher, *J. Am. Chem. Soc.* **1992**, *114*, 10965 – 10966; b) I. Willner, N. Lapidot, A. Riklin, R. Kasher, E. Zahavy, E. Katz, *J. Am. Chem. Soc.* **1994**, *116*, 1428 – 1441; c) E. Katz, A. Riklin, I. Willner, *J. Electroanal. Chem.* **1993**, *354*, 129 – 144.

[117] I. Willner, E. Katz, B. Willner, *Electroanalysis* **1997**, *9*, 965 – 977.

[118] I. Willner, A. Riklin, B. Shoham, D. Rivenzon, E. Katz, *Adv. Mater.* **1993**, *5*, 912 – 915.

[119] a) Y. Kajiya, T. Okamoto, H. Yoneyama, *Chem. Lett.* **1993**, 2107 – 2110; b) A. Riklin, I. Willner, *Anal. Chem.* **1995**, *67*, 4118 – 4126; c) S. E. Creager, K. G. Olsen, *Anal. Chim. Acta* **1995**, *307*, 277 – 289; d) S. Kuwabata, T. Okamoto, Y. Kajiya, H. Yoneyama, *Anal. Chem.* **1995**, *67*, 1684 – 1690; e) M. A. McRipley, R. A. Linsenmeier, *J. Electroanal. Chem.* **1996**, *414*, 235 – 246.

[120] W. Jin, F. Bier, U. Wollenberger, F. Scheller, *Biosens. Bioelectron.* **1995**, *10*, 823 – 829.

[121] B. Shoham, Y. Migron, A. Riklin, I. Willner, B. Tartakovsky, *Biosens. Bioelectron.* **1995**, *10*, 341 – 352.

[122] I. Willner, A. Riklin, *Anal. Chem.* **1994**, *66*, 1535 – 1539.

[123] T. Sawaguchi, T. Matsue, I. Uchida, *Bioelectrochem. Bioenerg.* **1992**, *29*, 127 – 133.

[124] a) B. A. Kuznetsov, N. A. Byzova, G. P. Shumakovich, *J. Electroanal. Chem.* **1994**, *371*, 85 – 92; b) W. H. Koppenol, C. A. J. Vroonland, R. Brams, *Biochem. Biophys. Acta* **1978**, *503*, 499 – 508.

[125] a) T. Lötzbeyer, W. Schuhmann, E. Katz, J. Falter, H.-L. Schmidt, *J. Electroanal. Chem.* **1994**, *377*, 291 – 294; b) A. N. J. Moore, E. Katz, I. Willner, *J. Electroanal. Chem.* **1996**, *417*, 189 – 192; c) A. Narvaez, E. Dominguez, I. Katakis, E. Katz, K. T. Ranjit, I. Ben-Dov, I. Willner, *J. Electroanal. Chem.* **1997**, *430*, 217 – 233; d) L. Jiang, A. Glidle, C. J. McNeil, J. M. Cooper, *Biosens. Bioelectron.* **1997**, *12*, 1143 – 1155.

[126] a) G. J. Leggett, C. J. Roberts, P. M. Williams, M. C. Davies, D. E. Jackson, S. J. B. Tendler, *Langmuir* **1993**, *9*, 2356 – 2362; b) G. J. Leggett, M. C. Davis, D. E. Jackson, C. J. Roberts, S. J. B. Tendler, P. M. Williams, *J. Phys. Chem.* **1993**, *97*, 8852 – 8854; c) M.-C. Parker, M. C. Davies, S. J. B. Tendler, *J. Phys. Chem.* **1995**, *99*, 16155 – 16161.

[127] a) F. R. Shu, G. S. Wilson, *Anal. Chem.* **1976**, *48*, 1679 – 1686; b) M. Lion-Dagan, S. Marx-Tibbon, E. Katz, I. Willner, *Angew. Chem.* **1995**, *107*, 1730 – 1733; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1604 – 1606; c) R. A. Williams, H. W. Blanch, *Biosens. Bioelectron.* **1994**, *9*, 159 – 167.

[128] E. Mann-Buxbaum, F. Pittner, T. Schalkhammer, A. Jachimowicz, G. Jobst, F. Olcaytug, G. Urban, *Sens. Actuators B* **1990**, *1*, 518 – 522.

[129] T. Miyasaka, K. Koyama, T. Watanabe, *Chem. Lett.* **1990**, 627 – 630.

[130] a) H.-G. Hong, P. W. Bohn, S. G. Sligar, *Anal. Chem.* **1993**, *65*, 1635 – 1638; b) H.-G. Hong, M. Jiang, S. G. Sligar, P. W. Bohn, *Langmuir* **1994**, *10*, 153 – 158; c) M. A. Firestone, M. L. Shank, S. G. Sligar, P. W. Bohn, *J. Am. Chem. Soc.* **1996**, *118*, 9033 – 9041.

[131] E. Katz, V. Heleg-Shabtai, I. Willner, H. K. Rau, W. Haehnel, *Angew. Chem.* **1998**, *112*, 3443 – 3447; *Angew. Chem. Int. Ed.* **1998**, *37*, 3253 – 3256.

[132] P. S. Stayton, J. M. Olinger, S. T. Wollman, E. M. Thurman, P. W. Bohn, S. G. Sligar in *Synthetic Microstructures in Biological Research* (Eds.: J. M. Schnur, M. Peckerar), Plenum, New York, **1992**, pp. 163 – 170.

[133] K. Narasimhan, L. B. Wingard, Jr., *Anal. Chem.* **1986**, *58*, 2984 – 2987.

[134] K. T. Kinnear, H. G. Monbouquette, *Langmuir* **1993**, *9*, 2255 – 2257.

[135] G. Puu, I. Gustafson, P.-A. Ohlsson, G. Olofsson, A. Sellström in *Progress in Membrane Technology* (Eds.: J. C. Gomes-Fernandez, D. Chapman, L. Racker), Birkhäuser, Basel, **1991**, pp. 279 – 290.

[136] M. Kühner, R. Tampe, E. Sackmann, *Biophys. J.* **1994**, *67*, 217 – 226.

[137] R. Naumann, A. Jonczyk, R. Kopp, J. Esch, H. Ringsdorf, W. Knoll, P. Gräber, *Angew. Chem.* **1995**, *107*, 2168 – 2171; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2056 – 2058.

[138] K. L. Prime, G. M. Whitesides, *J. Am. Chem. Soc.* **1993**, *115*, 10714 – 10721.

[139] M. J. Tarlov, E. F. Bowden, *J. Am. Chem. Soc.* **1991**, *113*, 1847 – 1849.

[140] U. R. Achtnich, L. X. Tiefenauer, R. Y. Andres, *Biosens. Bioelectron.* **1992**, *7*, 279 – 290.

[141] B. L. Frey, C. E. Jordan, S. Kornguth, R. M. Corn, *Anal. Chem.* **1995**, *67*, 4452 – 4457.

[142] a) W. Knoll, G. Pirwitz, K. Tamada, A. Offenhäusser, M. Hara, *J. Electroanal. Chem.* **1997**, *438*, 199 – 205; b) N. Dontha, W. B. Nowall, W. G. Kuhr, *Anal. Chem.* **1997**, *69*, 2619 – 2625.

[143] a) R. Köneke, C. Menzel, R. Ulber, K. Schügerl, T. Scheper, M. Saleemuddin, *Biosens. Bioelectron.* **1996**, *11*, 1229 – 1236; b) P. L. Edmiston, S. S. Saavedra, *J. Am. Chem. Soc.* **1998**, *120*, 1665 – 1671.

[144] J. Madoz, B. A. Kuznetzov, F. J. Medrano, J. L. Garcia, V. M. Fernandez, *J. Am. Chem. Soc.* **1997**, *119*, 1043 – 1051.

[145] I. Willner, V. Heleg-Shabtai, R. Blonder, E. Katz, G. Tao, A. F. Bückmann, A. Heller, *J. Am. Chem. Soc.* **1996**, *118*, 10321 – 10322.

[146] E. Katz, D. D. Schlereth, H.-L. Schmidt, A. J. J. Olsthoorn, *J. Electroanal. Chem.* **1994**, *368*, 165 – 171.

[147] L.-H. Guo, G. McLendon, H. Razafitrimo, Y. Gao, *J. Mater. Chem.* **1996**, *6*, 369 – 374.

[148] a) D. D. Schlereth, *J. Electroanal. Chem.* **1997**, *425*, 77 – 85; b) D. D. Schlereth, R. P. H. Kooyman, *J. Electroanal. Chem.* **1997**, *431*, 285 – 295; c) L. Bertilsson, H.-J. Butt, G. Nellest, D. D. Schlereth, *Biosens. Bioelectron.* **1997**, *12*, 839 – 852; d) D. D. Schlereth, R. P. H. Kooyman, *J. Electroanal. Chem.* **1998**, *444*, 231 – 240.

[149] a) H. A. O. Hill, N. J. Walton, I. J. Higgins, *FEBS Lett.* **1981**, *126*, 282 – 284; b) A. E. G. Cass, G. Davis, H. A. O. Hill, D. J. Nancarrow, *Biochim. Biophys. Acta* **1985**, *828*, 51 – 57; c) F. A. Armstrong, H. A. O. Hill, N. J. Walton, *Q. Rev. Biophys.* **1986**, *18*, 261 – 322; d) W. Jin, U. Wollenberger, F. F. Bier, A. Makower, F. W. Scheller, *Bioelectrochem. Bioenerg.* **1996**, *39*, 221 – 225.

[150] M. Brunori, *Biosens. Bioelectron.* **1994**, *9*, 633 – 636.

[151] a) P. A. Adams in *Peroxidases in Chemistry and Biology*, *Vol. 2* (Eds.: J. Everse, K. E. Everse), CRC, Boston, **1991**, chap. 7, pp. 171 – 200; b) G. Ranghino, G. Antonini, P. Fantucci, *Isr. J. Chem.* **1994**, *34*, 239 – 244.

[152] F. Patolsky, E. Katz, V. Heleg-Shabtai, I. Willner, *Chem. Eur. J.*, **1998**, *4*, 1068 – 1073.

[153] a) V. Heleg-Shabtai, E. Katz, I. Willner, *J. Am. Chem. Soc.* **1997**, *119*, 8121 – 8122; b) V. Heleg-Shabtai, E. Katz, S. Levi, I. Willner, *J. Chem. Soc. Perkin Trans. 2* **1997**, 2645 – 2651.

[154] I. Willner, E. Katz, V. Heleg-Shabtai, W. Haehnel, H. K. Rau, *J. Am. Chem. Soc.* **1999**, *121* 6455 – 6468.

[155] A. L. Ghindilis, P. Atanasov, E. Wilkins, *Electroanalysis* **1997**, *9*, 661 – 674.

[156] A. I. Yaropolov, V. Malovik, S. D. Varfolomeev, I. V. Berezin, *Dokl. Akad. Nauk SSSR* **1979**, *249*, 1399 – 1401.

[157] a) F. A. Armstrong, A. M. Lannon, *J. Am. Chem. Soc.* **1987**, *109*, 7211 – 7212; b) F. A. Armstrong, A. M. Bond, F. N. Büchi, A. Hamnett, H. A. O. Hill, A. M. Lannon, O. C. Lettington, C. G. Zoski, *Analyst* **1993**, *118*, 973 – 978; c) M. S. Mondal, H. A. Fuller, F. A. Armstrong, *J. Am. Chem. Soc.* **1996**, *118*, 263 – 264.

[158] E. Csöregi, G. Johnsson-Pettersson, L. Gorton, *J. Biotechnol.* **1993**, *30*, 315 – 320.

[159] T. Ruzgas, L. Gorton, J. Emneus, E. Csöregi, G. Marko-Varga, *Anal. Proc.* **1995**, *32*, 207 – 208.

[160] I. V. Berezin, V. A. Bogdanovskaya, S. D. Varfolomeev, M. R. Tarasevich, A. I. Yaropolov, *Dokl. Akad. Nauk SSSR* **1978**, *240*, 615 – 618.

[161] a) "Bioelectrochemistry": M. R. Tarasevich in *Comprehensive Treatise of Electrochemistry*, *Vol. 10* (Eds.: S. Srinivasan, Y. A. Chizmadzehv, J. O M. Bockris, B. E. Conway, E. Yeager), Plenum, New York, **1985**, pp. 275 – 301; b) T. Ruzgas, L. Gorton, J. Emneus, G. Marko-Varga, *J. Electroanal. Chem.* **1995**, *391*, 41 – 49.

[162] L.-H. Guo, H. A. O. Hill, G. A. Lawrance, G. S. Sanghera, D. J. Hopper, *J. Electroanal. Chem.* **1989**, *266*, 379 – 396.

[163] L.-H. Guo, H. A. O. Hill, D. J. Hopper, G. A. Lawrance, G. S. S. Anghera, *J. Biol. Chem.* **1990**, *265*, 1958 – 1963.

[164] A. L. Burrows, H. A. O. Hill, T. A. Leese, W. S. McIntire, H. Nakayama, G. S. Sanghera, *Eur. J. Biochem.* **1991**, *199*, 73 – 78.

[165] T. Ikeda, F. Matsushita, M. Senda, *Biosens. Bioelectron.* **1991**, *6*, 299 – 304.

[166] a) J. J. Kulys, R. D. Schmid, *Bioelectrochem. Bioenerg.* **1990**, *24*, 305 – 311; b) U. Wollenberger, V. Bogdanovskaya, S. Bobrin, F. Scheller, M. Tarasevich, *Anal. Lett.* **1990**, *23*, 1795 – 1808; c) L. Gorton, G. Bremle, E. Csöregi, G. Jönsson-Pettersson, B. Persson, *Anal. Chim. Acta* **1991**, *249*, 43 – 54; d) T. Tatsuma, T. Watanabe, T. Watanabe, *J. Electroanal. Chem.* **1993**, *356*, 245 – 253; e) T. Tatsuma, T. Watanabe, T. Watanabe, *Sens. Actuators B* **1993**, *13 – 14*, 752 – 753.

[167] a) T. A. Buttler, K. Johansson, L. Gorton, G. A. Marko-Varga, *Anal. Chem.* **1993**, *65*, 2628 – 2636; b) G. Marko-Varga, K. Johansson, L. Gorton, *J. Chromatogr.* **1994**, *660*, 153 – 167.

[168] V. Kacaniklic, K. Johansson, G. Marko-Varga, L. Gorton, G. Jönsson-Pettersson, E. Czöregi, *Electroanalysis* **1994**, *6*, 381 – 390.

[169] R. M. Paddock, E. F. Bowden, *J. Electroanal. Chem.* **1989**, *260*, 487 – 494.

[170] M. F. Cardosi, *Electroanalysis* **1994**, *6*, 89 – 95.

[171] J. Zhao, R. Henkens, J. Stonehuerner, J. P. O'Daly, A. L. Crumbliss, *J. Electroanal. Chem.* **1992**, *327*, 109 – 119.

[172] a) U. Wollenberger, J. Wang, M. Ozsoz, E. Gonzalez-Romero, F. Scheller, *Bioelectrochem. Bioenerg.* **1991**, *26*, 287 – 296; b) I. C. Popescu, G. Zetterburg, L. Gorton, *Biosens. Bioelectron.* **1995**, *10*, 443 – 461.

[173] a) D. L. Williams, A. P. Doig, Jr., A. Korosi, *Anal. Chem.* **1970**, *42*, 118 – 121; b) P. Janada, J. Weber, *J. Electroanal. Chem.* **1991**, *300*, 119 – 124.

[174] a) A. E. G. Cass, G. Davis, G. D. Francis, H. A. O. Hill, W. J. Aston, I. J. Higgins, E. V. Plotkin, L. D. L. Scott, A. P. F. Turner, *Anal. Chem.* **1984**, *56*, 667 – 671; b) A. E. G. Cass, G. Davis, H. A. O. Hill, I. J. Higgins, E. V. Plotkin, A. P. F. Turner, W. J. Aston in *Charge and Field Effects in Biosystems* (Eds.: M. J. Allen, P. N. R. Usherwood), Abacus, Tunbridge Wells, UK, **1984**, pp. 475 – 484; c) G. Davis, H. A. O. Hill, I. J. Higgins, A. P. F. Turner in *Implantable Sensors for Closed Loop Prosthetic Systems* (Ed.: W. H. Ko), Futura, New York, **1985**, pp. 189 – 195; d) G. Davis, M. J. Green, H. A. O. Hill, *Enzyme Microb. Technol.* **1986**, *8*, 1203 – 1208; e) S. L. Brooks, R. E. Ashby, A. P. F. Turner, M. R. Calder, D. J. Clarke, *Biosensors* **1987/88**, *3*, 45 – 51; f) J. M. Dicks, S. Hattori, I. Karube, A. P. F. Turner, T. Yokozawa, *Ann. Biol. Clin.* **1989**, *47*, 607 – 619.

[175] C. Iwakura, Y. Kajiya, H. Yoneyama, *J. Chem. Soc. Chem. Commun.* **1988**, 1019 – 1020.

[176] N. C. Foulds, C. R. Lowe, *J. Chem. Soc. Faraday Trans. 1* **1986**, *82*, 1259 – 1264.

[177] A. P. F. Turner, S. P. Hendry, M. F. Cardosi, *Biosensors*, *Instrumentation and Processing*, *The World Biotech. Report*, *Vol. 1*, Online Publications, Pinner, **1987**, pp. 125 – 137.

[178] S. P. Hendry, A. P. F. Turner, *Horm. Metab. Res. Suppl. Ser.* **1988**, *20*, 37 – 40.

[179] Y. Kajiya, R. Tsuda, H. Yoneyama, *J. Electroanal. Chem.* **1991**, *301*, 155 – 161.

[180] a) M. R. Tarasevich, *Bioelectrochem. Bioenerg.* **1979**, *6*, 587 – 597; b) H. A. O. Hill, I. J. Higgins, *Philos. Trans. R. Soc. London Ser. A* **1981**, *302*, 267 – 273.

[181] J. J. Kulys, R. A. Vidziuaite, *Anal. Lett.* **1983**, *16*, 197 – 207.

[182] E. J. D'Costa, I. J. Higgins, A. P. F. Turner, *Biosensors* **1986**, *2*, 71 – 87.

[183] W. H. Muller, S. J. Churchouse, P. M. Vadgama, *Anasyst* **1985**, *110*, 925 – 928.

[184] T. Matsue, N. Kasai, M. Narumi, M. Nishizawa, H. Yamada, I. Uchida, *J. Electroanal. Chem.* **1991**, *300*, 111 – 118.

[185] a) S. Marx-Tibbon, E. Katz, I. Willner, *J. Am. Chem. Soc.* **1995**, *117*, 9925 – 9926; b) P. Alzari, N. Anicet, C. Bourdillon, J. Moiroux, J.-M. Savéant, *J. Am. Chem. Soc.* **1996**, *118*, 6788 – 6789; c) S. J. Sadeghi, G. Gilardi, G. Nicolosi, A. E. G. Cass, *Chem. Commun.* **1997**, 517 – 518; d) A. D. Ryabov, Y. N. Firsova, V. N. Gozal, E. S. Ryabova, A. N. Shevelkova, L. T. Troitskaya, T. V. Demeschik, V. I. Sokolov, *Chem. Eur. J.* **1998**, *4*, 806 – 813.

[186] G. Tao, E. Katz, I. Willner, *Chem. Commun.* **1997**, 2073 – 2074.

[187] a) Y. Degani, A. Heller, *J. Phys. Chem.* **1987**, *91*, 1285 – 1289; b) Y. Degani, A. Heller, *J. Am. Chem. Soc.* **1988**, *110*, 2615 – 2620; c) W. Schuhmann, T. J. Ohara, H.-L. Schmidt, A. Heller, *J. Am. Chem. Soc.*

1991, *113*, 1394 – 1397; d) P. N. Bartlett, R. G. Whitaker, M. J. Green, J. Frew, *J. Chem. Soc. Chem. Commun.* **1987**, 1603 – 1604.
[188] A. Badia, R. Carlini, A. Fernandez, F. Battaglini, S. R. Mikkelsen, A. M. English, *J. Am. Chem. Soc.* **1993**, *115*, 7053 – 7060.
[189] W. Schuhmann, *Biosens. Bioelectron.* **1995**, *10*, 181 – 193.
[190] A. Riklin, E. Katz, I. Willner, A. Stoker, A. F. Bückmann, *Nature* **1995**, *376*, 672 – 675.
[191] I. Willner, B. Willner, *React. Polym.* **1994**, *22*, 267 – 279.
[192] I. Willner, R. Kasher, E. Zahavy, N. Lapidot, *J. Am. Chem. Soc.* **1992**, *114*, 10963 – 10965.
[193] a) N. C. Foulds, C. R. Lowe, *Anal. Chem.* **1988**, *60*, 2473 – 2478; b) W. Schuhmann, C. Kranz, J. Huber, H. Wohlsläger, Sy*nth. Met.* **1993**, *61*, 31 – 35; c) S. Cosnier, C. Innocent, Y. Jouanneau, *Anal. Chem.* **1994**, *66*, 3198 – 3201; d) S. Cosnier, L. Allien, L. Coche-Guérente, C. Innocent, P. Labbé, P. Mailley, *Sens. Mater.* **1996**, *8*, 169 – 177.
[194] a) P. D. Hale, L. I. Boguslavsky, T. Inazaki, H. I. Karan, H. S. Lee, T. A. Skotheim, *Anal. Chem.* **1991**, *63*, 677 – 682; b) T. Inagaki, H. S. Lee, P. D. Hale, T. A. Skotheim, Y. Okamoto, *Macromolecules* **1989**, *22*, 4641 – 4643; c) T. Inagaki, H. S. Lee, T. A. Skotheim, Y. Okamoto, *J. Chem. Soc. Chem. Commun.* **1989**, 1181 – 1183; d) P. D. Hale, T. Inagaki, H. S. Lee, H. I. Karan, Y. Okamoto, T. A. Skotheim, *Anal. Chim. Acta* **1990**, *228*, 31 – 37.
[195] a) T. Kaku, H. I. Karan, Y. Okamoto, *Anal. Chem.* **1994**, *66*, 1231 – 1235; b) M. G. Loughram, J. M. Hall, A. P. F. Turner, *Electroanalysis* **1996**, *8*, 870 – 875.
[196] S. Cosnier, K. Le Lous, *Talanta* **1996**, *43*, 331 – 337.
[197] A. Ehrenberg, H. Theorell, *Acta Chem. Scand.* **1955**, *9*, 1193 – 1205.
[198] a) F. A. Armstrong, H. A. O. Hill, N. J. Walton, *Acc. Chem. Res.* **1988**, *21*, 407 – 413; b) J. E. Frew, H. A. O. Hill, *Eur. J. Biochem.* **1988**, *172*, 261 – 269.
[199] a) I. Taniguchi, K. Toyosawa, H. Yamaguchi, K. Yasukouchi, *J. Chem. Soc. Chem. Commun.* **1982**, 1032 – 1033; b) I. Taniguchi, S. Yoshimoto, K. Nishiyama, *Chem. Lett.* **1997**, 353 – 354; c) B. D. Lamp, D. Hobara, M. D. Porter, K. Niki, T. M. Cotton, *Langmuir* **1997**, *13*, 736 – 741; d) H. A. O. Hill, D. J. Page, N. J. Walton, *J. Electroanal. Chem.* **1987**, *217*, 141 – 158; e) Y. Xie, S. Dong, *Bioelectrochem. Bioenerg.* **1992**, *29*, 71 – 79.
[200] P. M. Allen, H. A. O. Hill, N. J. Walton, *J. Electroanal. Chem.* **1984**, *178*, 69 – 86.
[201] a) Z.-X. Huang, M. Feng, Y.-H. Wang, J. Cui, D.-S. Zou, *J. Electroanal. Chem.* **1996**, *416*, 31 – 40; b) R. Santucci, A. Faraoni, L. Campanella, G. Tranchida, M. Brunori, *Biochem. J.* **1991**, *273*, 783 – 786.
[202] S. Bagby, P. D. Barker, K. DiGleria, H. A. O. Hill, V. J. Lowe, *Biochem. Soc. Trans.* **1988**, *16*, 958 – 959.
[203] G. Li, H. Chen, D. Zhu, *Anal. Chim. Acta* **1996**, *319*, 275 – 276.
[204] X. Qu, T. Lu, S. Dong, C. Zhou, T. M. Cotton, *Bioelectrochem. Bioenerg.* **1994**, *34*, 153 – 156.
[205] T. Lu, X. Yu, S. Dong, C. Zhou, S. Ye, T. M. Cotton, *J. Electroanal. Chem.* **1994**, *369*, 79 – 86.
[206] K. R. Brown, A. P. Fox, M. J. Natan, *J. Am. Chem. Soc.* **1996**, *118*, 1154 – 1157.
[207] a) T. Sakurai, F. Nose, *Chem. Lett.* **1995**, 1075 – 1076; b) T. Sakurai, F. Nose, T. Fujiki, S. Suzuki, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2855 – 2862; c) T. Sakurai, *Chem. Lett.* **1996**, 481 – 482; d) T. D. Martin, S. A. Monheit, R. J. Niichel, S. C. Peterson, C. H. Campbell, D. C. Zapien, *J. Electroanal. Chem.* **1997**, *420*, 279 – 290.
[208] K. Nishiyama, H. Ishida, I. Taniguchi, *J. Electroanal. Chem.* **1994**, *373*, 255 – 258.
[209] a) R. Santucci, H. Reinhard, M. Brunori, *J. Am. Chem. Soc.* **1988**, *110*, 8536 – 8537; b) P. A. Mabrouk, *Anal. Chem.* **1996**, *68*, 189 – 191.
[210] a) E. Katz, T. Lötzbeyer, D. D. Schlereth, W. Schuhmann, H.-L. Schmidt, *J. Electroanal. Chem.* **1994**, *373*, 189 – 200; b) M. J. Lobo, A. J. Miranda, P. Tuñón, *Electroanalysis*, **1997**, *9*, 191 – 201; c) I. Katakis, E. Dominguez, *Mikrochim. Acta* **1997**, *126*, 11 – 32; d) E. Lorenzo, F. Pariente, L. Hernàndez, F. Tobalina, M. Darder, Q. Wu, M. Maskus, H. D. Abruña, *Biosens. Bioelectron.* **1998**, *13*, 319 – 332.
[211] a) G. Prentice, *CHEMTECH* **1984**, *14*, 684 – 701; b) "Enzymatic Conversion of Biomass for Fuels Production": G. Tayhas, R. Palmore, G. M. Whitesides, *ACS Symp. Ser.* **1994**, *566*, 271 – 290; c) K. Kordesch, *Ber. Bunsen-Ges. Phys. Chem.* **1990**, *94*, 902 – 907; d) C. Van Dijk, C. Laane, C. Veeger, *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 245 – 249.
[212] a) I. Willner, E. Katz, F. Patolsky, A. F. Bückmann, *J. Chem. Soc. Perkin Trans. 2* **1998**, 1817 – 1822; b) I. Willner, G. Arad, E. Katz, *Bioelectrochem. Bioenerg.* **1998**, *44*, 209 – 214.
[213] a) S. Britland, E. Perez-Arnaud, P. Clark, B. McGinn, P. Connoly, G. Moores, *Biotechnol. Prog.* **1992**, *8*, 155 – 160; b) S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, D. Solas, *Science* **1991**, *251*, 767 – 773.
[214] a) S. K. Bhatia, J. J. Hickman, F. S. Ligler, *J. Am. Chem. Soc.* **1992**, *114*, 4432 – 4433; b) M. N. Wybourne, J. C. Wu, M. Yan, S. X. Cai, J. F. W. Keana, *J. Vac. Sci. Technol. B* **1993**, *11*, 2210 – 2213; c) C. S. Dulcey, J. H. Georger, V. Krauthamer, D. A. Stenger, T. L. Fare, J. M. Calvert, *Science* **1991**, *252*, 551 – 554; d) J. M. Calvert, J. H. Georger, M. C. Peckerar, P. E. Pehrsson, J. M. Schnur, P. E. Schoen, *Thin Solid Films* **1992**, *211*, 359 – 363; e) W. J. Dressick, J. M. Calvert, *Jpn. J. Appl. Phys.* **1993**, *32*, 5829 – 5893.
[215] a) A. Kumar, N. L. Abbott, E. Kim, N. A. Biebuyck, G. M. Whitesides, *Acc. Chem. Res.* **1995**, *28*, 219 – 226; b) R. Singhvi, A. Kumar, G. P. Lopez, G. N. Stephanopoulos, D. I. C. Wang, G. M. Whitesides, D. E. Ingber, *Science* **1994**, *264*, 696 – 698; c) W. J. Dressick, C. S. Dulcey, J. H. Georger, Jr., J. M. Calvert, *Chem. Mater.* **1993**, *5*, 148 – 150; d) J. M. Calvert, *J. Vac. Sci. Technol. B* **1993**, *11*, 2155 – 2163.
[216] a) N. L. Abbott, A. Kumar, G. M. Whitesides, *Chem. Mater.* **1994**, *6*, 596 – 602; b) N. L. Abbott, G. M. Whitesides, *Langmuir* **1994**, *10*, 1493 – 1497; c) N. L. Abbott, G. M. Whitesides, L. M. Racz, J. Szekely, *J. Am. Chem. Soc.* **1994**, *116*, 290 – 294; d) N. L. Abbott, J. P. Folkers, G. M. Whitesides, *Science* **1992**, *257*, 1380 – 1382.
[217] a) J. L. Wilbur, E. Kim, Y. Xia, G. M. Whitesides, *Adv. Mater.* **1995**, *7*, 649 – 652; b) Y. Xia, E. Kim, M. Mrksich, G. M. Whitesides, *Chem. Mater.* **1996**, *8*, 601 – 603; c) E. Kim, A. Kumar, G. M. Whitesides, *J. Electrochem. Soc.* **1995**, *142*, 628 – 633; d) N. L. Jeon, R. G. Nuzzo, Y. Xia, M. Mrksich, G. M. Whitesides, *Langmuir* **1995**, *11*, 3024 – 3026; e) C. B. Gorman, H. A. Biebuyck, G. M. Whitesides, *Chem. Mater.* **1995**, *7*, 252 – 254; f) C. D. James, R. C. Davis, L. Kam, H. G. Craighead, M. Issacson, J. N. Turner, W. Shain, *Langmuir* **1998**, *14*, 741 – 744; g) A. Bernard, E. Delamarche, H. Schmid, B. Michel, H. R. Bosshard, H. Biebuyck, *Langmuir* **1998**, *14*, 2225 – 2229.
[218] A. Kumar, H. A. Biebuyck, N. L. Abbott, G. M. Whitesides, *J. Am. Chem. Soc.* **1992**, *114*, 9188 – 9189.
[219] A. Kumar, G. M. Whitesides, *Science* **1994**, *263*, 60 – 62.
[220] a) A. Kumar, H. A. Biebuyck, G. M. Whitesides, *Langmuir* **1994**, *10*, 1498 – 1511; b) A. Kumar, G. M. Whitesides, *Appl. Phys. Lett.* **1993**, *63*, 2002 – 2004.
[221] P. C. Hidber, P. F. Nealey, W. Helbig, G. M. Whitesides, *Langmuir* **1996**, *12*, 5209 – 5215.
[222] A. C. Pease, D. Solas, E. J. Sullivan, M. T. Cronin, C. P. Homes, S. P. A. Fodor, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5022 – 5026.
[223] a) L. F. Rozsnyai, D. R. Benson, S. P. A. Fodor, P. G. Schultz, *Angew. Chem.* **1992**, *104*, 801 – 803; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 759 – 761; b) S. K. Bhatia, J. L. Teixeira, M. Anderson, L. C. Shriver-Lake, J. M. Calvert, J. H. Georger, J. J. Hickman, C. S. Dulcey, P. E. Schoen, F. S. Ligler, *Anal. Biochem.* **1993**, *208*, 197 – 205.
[224] G. P. López, H. A. Biebuyck, R. Härter, A. Kumar, G. M. Whitesides, *J. Am. Chem. Soc.* **1993**, *115*, 10774 – 10781.
[225] G. Sundarababu, H. Gao, H. Sigrist, *Photochem. Photobiol.* **1995**, *61*, 540 – 544.
[226] T. Matsuda, T. Sugawara, *Langmuir* **1995**, *11*, 2267 – 2271.
[227] M. Yan, S. X. Cai, M. N. Wybourne, J. F. W. Keana, *J. Am. Chem. Soc.* **1993**, *115*, 814 – 816.
[228] a) V. N. R. Pillai, *Synthesis* **1980**, 1 – 26; b) R. W. Binkley in *Synthetic Organic Photochemistry* (Ed.: W. M. Horspool), Plenum, New York, **1984**, pp. 375 – 423.
[229] a) D. J. Pritchard, H. Morgan, J. M. Cooper, *Angew. Chem.* **1995**, *107*, 84; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 91 – 93; b) M. Hengsakul, A. E. G. Cass, *Bioconjugate Chem.* **1996**, *7*, 249 – 254.
[230] a) I. Willner, R. Blonder, A. Dagan, *J. Am. Chem. Soc.* **1994**, *116*, 9365 – 9366; b) I. Willner, B. Willner, *Adv. Mater.* **1995**, *7*, 587 – 589; c) R. Blonder, I. Ben-Dov, A. Dagan, I. Willner, E. Zisman, *Biosens. Bioelectron.* **1997**, *12*, 627 – 644.
[231] I. Willner, R. Blonder, *Thin Solid Films* **1995**, *266*, 254 – 257.

[232] a) C. Pale-Grosdemange, E. S. Simon, K. L. Prime, G. M. Whitesides, *J. Am. Chem. Soc.* **1991**, *113*, 12 – 20; b) K. L. Prime, G. M. Whitesides, *Science* **1991**, *252*, 1164 – 1167; c) G. P. López, M. W. Albers, S. L. Schreiber, R. Carroll, E. Peralta, G. M. Whitesides, *J. Am. Chem. Soc.* **1993**, *115*, 5877 – 5878.

[233] a) G. P. López, H. A. Biebuyck, G. M. Whitesides, *Langmuir* **1993**, *9*, 1513 – 1516; b) G. P. López, H. A. Biebuyck, D. C. Frisbie, G. M. Whitesides, *Science* **1993**, *260*, 647 – 649.

[234] a) I. Willner, S. Rubin, *Angew. Chem.* **1996**, *108*, 419; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 367 – 385; b) I. Willner, B. Willner, *Bioelectrochem. Bioenerg.* **1997**, *42*, 43 – 57; c) I. Willner, S. Rubin, *React. Polym.* **1993**, *21*, 177 – 186.

[235] R. Blonder, E. Katz, I. Willner, V. Wray, A. F. Bückmann, *J. Am. Chem. Soc.* **1997**, *119*, 11 747 – 11 757.

[236] a) P. Skládal, *Electroanalysis* **1997**, *9*, 737 – 745; b) B. Lu, M. R. Smyth, R. O'Kennedy, *Analyst* **1996**, *121*, 29R-32R; c) S.-C. Huang, K. D. Caldwell, J.-N. Lin, H.-K. Wang, J. N. Herron, *Langmuir* **1996**, *12*, 4292 – 4298.

[237] G. Wallraff, J. Labadie, P. Brock, R. DiPietro, T. Nguyen, T. Huynh, W. Hinsberg, G. McGall, *CHEMTECH* **1997**, 22 – 32.

[238] Y. Cohen, S. Levi, S. Rubin, I. Willner, *J. Electroanal. Chem.* **1996**, *417*, 65 – 75.

[239] a) L. X. Tiefenauer, S. Kossek, C. Padeste, P. Thiébaud, *Biosens. Bioelectron.* **1997**, *12*, 213 – 223; b) A. L. Ghindilis, O. V. Skorobogat'ko, V. P. Gavrilova, A. I. Yaropolov, *Biosens. Bioelectron.* **1992**, *7*, 301 – 304; c) E. Katz, I. Willner, *J. Electroanal. Chem.* **1996**, *418*, 67 – 72.

[240] a) A. A. Suleiman, G. G. Guilbault, *Analyst* **1994**, *119*, 2279 – 2282; b) G. G. Guilbault, B. Hock, R. Schmid, *Biosens. Bioelectron.* **1992**, *7*, 411 – 419; c) C. Kösslinger, S. Drost, F. Abert, H. Wolf, S. Koch, P. Woias, *Biosens. Bioelectron.* **1992**, *7*, 397 – 404; d) C. R. Suri, M. Raje, G. C. Mishra, *Biosens. Bioelectron.* **1994**, *9*, 325 – 332; e) J. Horácek, P. Skládal, *Anal. Chim. Acta* **1997**, *347*, 43 – 50; f) C. R. Suri, G. C. Mishra, *Biosens. Bioelectron.* **1996**, *11*, 1199 – 1205; g) H. O. Ghourchian, N. Kamo, *Anal. Chim. Acta* **1995**, *300*, 99 – 105; h) J. L. N. Harteveld, M. S. Nieuwenhuizen, E. R. J. Wils, *Biosens. Bioelectron.* **1997**, *12*, 661 – 667.

[241] a) I. Moser, T. Schalkhammer, F. Pittner, G. Urban, *Biosens. Bioelectron.* **1997**, *12*, 729 – 737; b) J. Wang, X. Cai. G. Rivas, H. Shiraishi, N. Dontha, *Biosens. Bioelectron.* **1997**, *12*, 587 – 599; c) J. Li, G. Cheng, S. Dong, *Electroanalysis* **1997**, *9*, 834 – 837.

[242] a) J. Wang, P. E. Nielsen, M. Jiang, X. Cai, J. R. Fernandes, D. H. Grant, M. Ozsoz, A. Beglieter, M. Mowat, *Anal. Chem.* **1997**, *69*, 5200 – 5202; b) C. Nicolini, V. Erokhin, P. Facci, G. A. Ross, P. Paschkevitsch, *Biosens. Bioelectron.* **1997**, *12*, 613 – 618; c) F. Caruso, E. Rodda, D. N. Furiong, K. Niikura, Y. Okahata, *Anal. Chem.* **1997**, *69*, 2043 – 2049; d) Y. Okahata, M. Kawase, K. Niikura, F. Ohtake, H. Furusawa, Y. Ebara, *Anal. Chem.* **1998**, *70*, 1288 – 1296.

[243] A. Bardea, A. Dagan, I. Ben-Dov, B. Amit, I. Willner, *Chem. Commun.*, **1998**, 839 – 840.

[244] F. Patolsky, B. Filanovsky, E. Katz, I. Willner, *J. Phys. Chem. B* **1998**, *102*, 10359 – 10367.

[245] a) S. Toyama, Y. Ikariyama, *Chem. Lett.* **1997**, 1083 – 1084; b) A. J. Thiel, A. G. Frutos, C. E. Jordan, R. M. Corn, L. M. Smith, *Anal. Chem.* **1997**, *69*, 4948 – 4956; c) C. E. Jordan, A. G. Frutos, A. J. Thiel, R. M. Corn, *Anal. Chem.* **1997**, *69*, 4939 – 4947; E. Kaganer, R. Pogreb, D. Davidov, I. Willner, *Langmuir* **1999**, *15*, 3920 – 3923.

[246] a) S. Rubin, G. Bar, T. N. Taylor, R. W. Cutts, T. A. Zawodzinski, Jr., *J. Vac. Sci. Technol. A* **1996**, *14*, 1870 – 1876; b) T. Sato, D. Brown, B. F. G. Johnson, *Chem. Commun.* **1997**, 1007 – 1008; c) K. C. Grabar, K. J. Allison, B. E. Baker, R. M. Bright, K. R. Brown, R. G. Freeman, A. P. Fox, C. D. Keating, M. D. Musick, M. J. Natan, *Langmuir* **1996**, *12*, 2353 – 2361; d) S. E. Gilbert, O. Cavalleri, K. Kern, *J. Phys. Chem.* **1996**, *100*, 12 123 – 12 130, e) M. Gao, X. Zhang, B. Yang, J. Shen, *J. Chem. Soc. Chem. Commun.* **1994**, 2229 – 2230; f) M. Brust, R. Etchenique, E. J. Calvo, G. J. Gordillo, *Chem. Commun.* **1996**, 1949 – 1950; g) D. Bethell, M. Brust, D. J. Schiffrin, C. Kiely, *J. Electroanal. Chem.* **1996**, *409*, 137 – 143; h) S. Yabuki, F. Mizutani, *Electroanalysis* **1997**, *9*, 23 – 25; i) S. Peschel, G. Schmid, *Angew. Chem.* **1995**, *107*, 1568 – 1569; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1442 – 1443; j) J. H. Fendler, F. C. Meldrum, *Adv. Mater.* **1995**, *7*, 607 – 632; k) R. Blonder, L. Sheeney, I. Willner, *Chem. Commun.* **1998**, 1393 – 1394; l) R. G. Freeman, K. C. Grabar, K. J. Allison, R. M. Bright, J. A. Davis, A. P. Guthrie, M. B. Hommer, M. A. Jackson, P. C. Smith, D. G. Walter, M. J. Natan, *Science* **1995**, *267*, 1629 – 1632; m) K. C. Grabar, P. C. Smith, M. D. Musick, J. A. Davis, D. G. Walter, M. A. Jackson, A. P. Guthrie, M. J. Natan, *J. Am. Chem. Soc.* **1996**, *118*, 1148 – 1153; n) K. C. Grabar, R. G. Freeman, M. B. Hommer, M. J. Natan, *Anal. Chem.* **1995**, *67*, 735 – 743; o) M. D. Musick, C. D. Keating, M. H. Keefe, M. J. Natan, *Chem. Mater.* **1997**, *9*, 1499 – 1501; p) S. J. Green, J. J. Stokes, M. J. Hostetler, J. Pietron, R. W. Murray, *J. Phys. Chem. B* **1997**, *101*, 2663 – 2668.

[247] a) C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607 – 609; b) A. P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Willson, C. J. Loweth, M. P. Bruchez, Jr., P. G. Schultz, *Nature* **1996**, *382*, 609 – 611; c) R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, *Science* **1997**, *277*, 1078 – 1081; d) J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, *120*, 1959 – 1964.

[248] A. P. Alivisatos, *Science* **1996**, *271*, 933 – 937.

[249] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, *Nature* **1998**, *391*, 775 – 778.

[250] a) J. J. Calvente, Z. Kovácová, M. D. Sachez, R. Andreu, W. R. Fawcett, *Langmuir* **1996**, *12*, 5696 – 5803; b) M. M. Walczak, D. D. Popenoe, R. S. Deihammer, B. D. Lamp, C. Chung, M. D. Porter, *Langmuir* **1991**, *7*, 2687 – 2693; c) D.-F. Yang, M. Morin, *J. Electroanal. Chem.* **1998**, *441*, 173 – 181; d) C.-J. Zhong, J. Zak, M. D. Porter, *J. Electroanal. Chem.* **1997**, *421*, 9 – 13.

[251] a) S. Kossek, C. Padeste, L. X. Tiefenauer, H. Siegenthaler, *Biosens. Bioelectron.* **1998**, *13*, 31 – 43; b) E. P. Friis, J. E. G. Andersen, L. L. Madsen, P. Møller, J. Ulstrup, *J. Electroanal. Chem.* **1997**, *431*, 35 – 38. c) M. Quinto, A. Ciancio, P. G. Zmabonin, *J. Electroanal. Chem.* **1998**, *448*, 51 – 59.

[252] W. R. Bowen, N. Hilal, R. W. Lovitt, C. J. Wright, *J. Colloid Interf. Sci.* **1998**, *197*, 348 – 352.

[253] a) P. Avouris, *Acc. Chem. Res.* **1995**, *28*, 95 – 102; b) M. Jaschke, H.-J. Butt, *Langmuir* **1995**, *11*, 1061 – 1064; c) J. Wang, J. R. Kenseth, V. W. Jones, J.-B. D. Green, M. T. McDermott, M. D. Porter, *J. Am. Chem. Soc.* **1997**, *119*, 12 796 – 12 799.

[254] P. K. Wei, J. H. Hsu, B. R. Hsieh, W. S. Fann, *Adv. Mater.* **1996**, *8*, 573 – 576.