Magneto-Switchable Electrocatalytic and Bioelectrocatalytic Transformations

Eugenii Katz, Laila Sheeney-Haj-Ichia, and Itamar Willner*[a]

Abstract: Magnetic switching of redox reactions and bioelectrocatalytic transformations is accomplished in the presence of relay-functionalized magnetite particles (Fe_3O_4). The electrochemistry of a naphthoquinone (**1**), pyrroloquinoline quinone (**2**; PQQ), microperoxidase-11 (**3**), a ferrocene derivative (**4**) and a bipyridinium derivative (**5**), functionalized magnetic particles, is switched "ON" and "OFF" by an external magnet upon the attraction of the magnetic particles to an electrode or their retraction from the electrode, respectively. The magneto-switchable activation and deactivation of the electrochemical oxidation of the ferrocene-functionalized magnetic particles and the electrochemical reduction of the bipyridinium-functionalized magnetic particles are used for the triggering of mediated bioelectrocatalytic oxidation of glucose, in the presence of glucose oxidase (GOx), and bioelectrocatalytic reduction of nitrate (NO_3^-), in the presence of nitrate reductase (NR), respectively. Magnetic particles functionalized with a PQQ–NAD^+ dyad are used for the magnetic switching of the bioelectrocatalytic oxidation of lactate in the presence of lactate dehydrogenase (LDH). The coupling of these particles with a ferrocene-monolayer-functionalized electrode allows the dual and selective sensing of lactate and glucose in the presence of LDH and GOx, respectively, by using an external magnet to switch the detection mode.

Keywords: biosensors · cyclic voltammetry · electrocatalysis · magnetic particles · magnetic properties

Introduction

The development of signal-controlled redox functionalities in molecular,[1] macromolecular,[2] and biomolecular[3] systems attracts substantial research efforts directed to the design of information storage and processing systems, the development of new electronic materials, and the tailoring of molecular machinery systems. Photochemically controlled redox functions of molecular and supramolecular systems in solution were reported by the use of photoisomerizable components. Photoisomerization of molecular structures was reported to yield redox-activated states,[4] and light-induced translocations of redox units in supramolecular catenane or rotaxane assemblies, by the photoisomerization of a light-sensitive molecular unit or photoinduced electron transfer, were accomplished.[5, 6] Similarly, pH-induced translocation of redox functionalities between distinct states in rotaxanes[7] represents a chemically controlled molecular mechanical function. The integration of photoisomerizable, redox-activated, molecular functionalities with electrodes in the form of monolayers was suggested as a general means for the electrochemical (amperometric) transduction of photonic information recorded by the photoactive interface.[8] Also, the photochemical translocation of a tethered redox-active cyclodextrin in a rotaxane system associated with an electrode was suggested as a light-driven "molecular machine" that electrochemically transduces its operational configuration.[9] Photoswitchable bioelectrocatalytic assemblies were suggested as a general method to develop optobioelectronic systems.[10] Tethering of photoisomerizable groups to redox enzymes,[11] reconstitution of apo-flavoenzymes with a photoisomerizable FAD-cofactor,[12] the use of photoisomerizable electron mediators,[13] and the organization of photoisomerizable monolayers as "command interfaces" that control the electrical contact of redox-proteins with the electrode[14] were reported as functional enzyme-based bioelectronic systems for the amperometric transduction of photonic signals.

Recently, we reported on the development of magneto-switchable bioelectrocatalytic systems in which external magnetic fields switch the bioelectrocatalytic functions of redox enzymes "ON" and "OFF".[15] Here we wish to report the comprehensive studies on magneto-controlled redox systems, magneto-switchable electrocatalysis, and bioelectrocatalysis. We discuss the magneto-switchable bioelectrocatalysis of flavoenzymes and of NAD^+-dependent enzymes, and demonstrate the use of the magneto-triggered biocatalytic processes for the specific sensing of two different substrates in a mixture.

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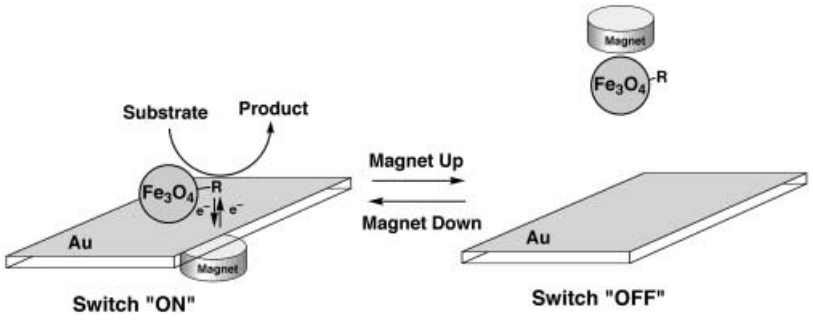
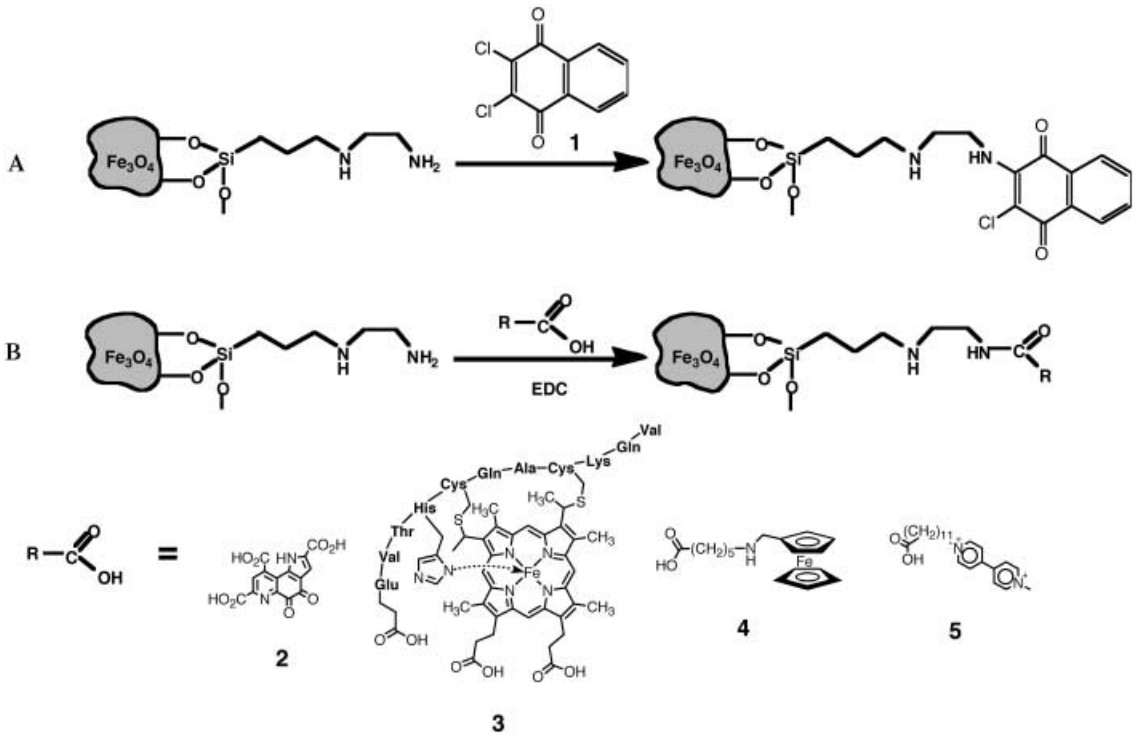
Results and Discussion

The magnetic switching of redox functions and electrocatalytic transformations at an electrode surface are depicted in Scheme 1. The redox-active component (**R**) is covalently linked to the magnetic particles. Positioning of the external magnet below the electrode attracts the functionalized particles to the electrode, thus enabling the redox transformation to occur. Positioning of the magnet above the electrochemical cell retracts the magnetic particles from the electrode surface, leading to the blocking of the electrical contact between the functionalized particles and the electrode support. By the reversible positioning of the external magnet below the electrode and above the electrochemical cell, the redox responses of the system are cycled between "ON" and "OFF" states. Provided the redox component linked to the magnetic particle acts as electrocatalyst for the reduction (or oxidation) of a substrate in solution, the respective electrocatalytic transformations might be switched "ON" and "OFF" by the appropriate positioning of the external magnet with respect to the electrode support.

The first aim of the study was to demonstrate the covalent mode of a redox modifier bound to magnetic particles. Since redox potentials of covalently bound and physically adsorbed molecules are often similar,[16, 17] it is not easy to distinguish these two binding modes, unless they have very different stability. However, even stability of the species immobilized by covalent binding or by adsorption might be sometimes comparable.[18] It is known that nucleophilic addition of amino groups to quinones[19] yields amino–quinone derivatives with redox potentials significantly negatively shifted due to the electron-donating effect of the amino group. This chemical process was used for the immobilization of 1,4-naphthoquinones on electrode surfaces functionalized with amino groups,[20] and resulted in the formation of covalently bound aminonaphthoquinone molecules on electrode surfaces. Accordingly, this approach was used to modify the magnetic particles. The magnetic particles were functionalized with [3-(2-aminoethyl)aminopropyl]-trimethoxysilane. The resulting particles were treated with 2,3-dichloro-1,4-naphthoquinone (**1**) to yield 2-amino-3-chloro-1,4-naphthoquinone-functionalized magnetic particles. The resulting particles were washed with ethanol to remove physi-



Scheme 1. Magneto-switched electron transfer and electrocatalyzed transformation in the presence of redox-active units linked to magnetic particles.



Scheme 2. Synthesis of relay-functionalized magnetic particles by the covalent linkage of redox-active units to magnetic particles functionalized with [3-(2-aminoethyl)aminopropyl]siloxane film: A) Linkage of 2,3-dichloro-1,4-naphthoquinone to the functionalized particles. B) Carbodiimide coupling of electron-relay carboxylic derivatives to the amino groups of the siloxane layer.

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cally adsorbed unreacted 2,3-dichloro-1,4-naphthoquinone, Scheme 2A.

It has been shown before that only the terminal primary amino group of the silane reacts with the quinone.[20a] Figure 1A, curve a, shows the differential pulse voltammogram of the aminonaphthoquinone-functionalized magnetic particles, $E^o = -0.40$ V (pH = 8.0), that are attracted to the electrode by means of the external magnet. Figure 1A, curve b, shows the differential pulse voltammogram of the aminonaphthoquinone-functionalized magnetic particle in the presence of **1** that has not been washed out and is physically adsorbed on the particles. The redox wave of **1** appears at $E^o = -0.19$ V and that of the aminonaphthoquinone-functionalized particles is negatively shifted by 210 mV, $E^o = -0.40$ V. This potential shift is typical for aminoquinone derivatives,[20] and it is consistent with the fact that the electron-withdrawing chloride substituent is replaced by the electron-donating amine functionality.[19] The potential difference between the physically adsorbed precursor and the covalently bound modifier provides a proof of covalent coupling of the modifier to the aminosiloxane thin film associated with the surface of the magnetic particles.

Figure 1B shows the differential pulse voltammograms of the aminonaphthoquinone-functionalized magnetic particles upon positioning the external magnet below and above the electrode, curves a and b, respectively. Clearly, when the functionalized particles are attracted to the electrode, the aminonaphthoquinone units form electrical contacts with the electrode surface, giving rise to the amperometric response. Retraction of the magnetic particles from the electrodes blocks the redox response of the functionalized particles. The small residual peak in the differential pulse voltammogram (curve b) originates from incomplete removal of the functionalized magnetic particles from the electrode. The inset in Figure 1B shows reversible increase and decrease of the peak current of the differential pulse voltammograms upon translocation of the magnetic particles to and from the electrode, respectively, by means of the external magnet. It should be noted that this experiment was performed under argon, and, thus, only the electrochemical response of the aminonaphthoquinone is reversibly activated and inhibited by the external magnet.

The magneto-switched electrochemical process of the aminoquinone immobilized on the magnetic particles can be further used to activate secondary redox reactions. It is known that the aminonaphthoquinone electrocatalyzes the reduction of dioxygen (O_2) to yield H_2O_2.[21] Figure 1C shows the magneto-switched electrocatalyzed reduction of O_2 by the aminonaphthoquinone-modified particles. Magnetic attraction of the particles to the electrode surface results in an electrocatalytic cathodic current from the quinone-mediated reduction of oxygen, whereas the magnetic retraction of the functionalized magnetic particles blocks the electrocatalyzed reduction of O_2 (Figure 1C, curves a and b, respectively). The residual cathodic current observed at the electrode when the aminonaphthoquinone-functionalized magnetic particles are removed from the electrode (curve b) corresponds to the noncatalytic reduction of O_2 at the electrode. It should be noted that in the experiments described, the Au electrode surface was functionalized with a mercaptohexanol monolayer in order to retard the noncatalytic reduction of O_2. The inset in Figure 1C, shows the reversible activation and deactivation of the electrocatalytic reduction of O_2 upon attraction and removal of the aminonaphthoquinone-func-

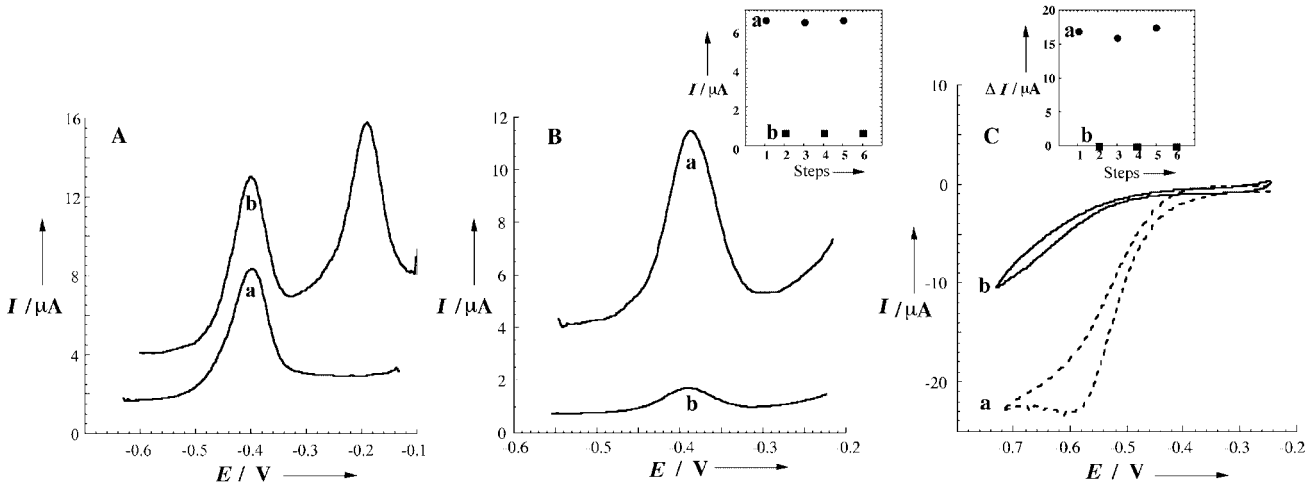

Figure 1. A) Differential pulse voltammograms of an Au-electrode with the magnetically attracted magnetic particles (10 mg). Curve a: Covalently functionalized with aminonaphthoquinone. Curve b: Covalently functionalized with aminonaphthoquinone in the presence of physically adsorbed 2,3-dichloro-1,4-naphthoquinone. B) Differential pulse voltammograms corresponding to the aminonaphthoquinone-functionalized particles. Curve a: Magnetically attracted to the electrode surface. Curve b: Magnetically retracted from the electrode surface. Inset: Reversible changes of the peak current of the differential pulse voltammograms upon magnetic switching "ON" (points a) and "OFF" (points b) of the redox response of the aminonaphthoquinone-functionalized particles. C) Cyclic voltammograms corresponding to the electrocatalyzed reduction of O_2 in the presence of the aminonaphthoquinone-functionalized particles and a Au electrode modified with a mercaptohexanol monolayer. Curve a: Switched "ON" by attraction of the magnetic particles to the electrode. Curve b: Switched "OFF" by retraction of the magnetic particles from the electrode. Inset: Reversible changes of the electrocatalytic current (measured at $E = -0.6$ V) upon attraction of the magnetic particles to the electrode (points a) and retraction of the magnetic particles from the electrode (points b). The data were recorded in 0.1 M phosphate buffer, pH = 8.0. The differential pulse voltammograms were measured at an unmodified Au electrode under Ar with a potential scan rate of 20 mV s^{-1}, pulse height 5 mV. The cyclic voltammograms were measured at a mercaptohexanol monolayer-modified Au electrode in the background electrolyte solution equilibrated with air at a potential scan rate of 10 mV s^{-1}.

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tionalized magnetic particles to and from the electrode surface induced by the translocation of the external magnet below the electrode and above the cell, respectively.

A series of carboxylic-acid-functionalized redox-active compounds were covalently coupled to the amino-functionalized magnetic particles (Scheme 2B). Pyrroloquinoline quinone (**2**, PQQ), was covalently linked to the particles. Figure 2A shows the cyclic voltammograms of the PQQ-modified magnetic particles, upon their attraction to the electrode and retraction from the conductive support with the external magnetic field (curves a and b, respectively). The cyclic voltammogram recorded when the PQQ-functionalized magnetic particles rest on the electrode surface (curve a), shows a quasi-reversible electrochemical process for PQQ,[22] $E^{o} = -0.16$ V (pH = 8.0), with a peak-to-peak separation of $\Delta E_p = 45$ mV (potential scan rate 100 mV s^{-1}). The observed value is smaller than the theoretically expected value of $\Delta E_p = 60$ mV for the diffusionally controlled cyclic voltammogram,[23] but still larger than $\Delta E_p = 0$ mV predicted by the Laviron theory for a surface-confined redox species.[24] This originates from intermolecular interactions of the redox species on the electrode and roughness irregularities on the conductive support. Indeed the increase in the observed peak-to-peak separation value has been explained by the second Laviron's theoretical approximation,[25] and was experimentally confirmed for PQQ-monolayer-modified electrodes.[22] A further reason for the increase in the ΔE_p is the slow kinetics of the electrochemical process.[24] When a slow potential scan rate is applied ($v < 20$ mV s^{-1}) the peak-to-peak separation is independent of the potential scan rate and equal to 20 mV, Figure 2B. At higher scan rates ($v > 100$ mV s^{-1}) ΔE_p is proportional to the logarithm of the potential scan rate according to the Laviron theory.[24] The PQQ formal potential (E^{o}) remains constant at different potential scan rates (v), because the anodic and cathodic peaks are shifted symmetrically: $\delta E_{pa}/\delta(\log v) \approx \delta E_{pc}/\delta(\log v) \approx 35$ mV. Thus, it can be assumed that the anodic and cathodic transfer coefficients are similar: $\alpha_a \approx \alpha_c \approx 0.5$. The peak current values of the cyclic voltammograms increase linearly upon the increase of the potential scan rate (Figure 2B, inset); this provides further support to the surface-confined electrochemical process involving PQQ. The electron-transfer constant of $k_{et} = 2$ s^{-1} was calculated from the Laviron theory for the electrochemical process of the immobilized PQQ.[24] Figure 2A, curve b, shows a cyclic voltammogram of the electrode when the PQQ-functionalized magnetic particles are lifted up by the external magnet. A very small redox wave of the residual particles is observed. The PQQ-modified magnetic particles can be reversibly attracted to or retracted from the electrode surface by positioning the external magnet below or above the electrochemical cell, respectively, thus providing the reversible magnetic switch for the PQQ-redox process (Figure 2A, inset).

PQQ acts as an electrocatalyst for the oxidation of 1,4-dihydronicotinamide adenine dinucleotide, NADH,[26] and the enhanced electrocatalytic activity was demonstrated in the presence of added Ca^{2+} as co-catalyst.[27] Figure 2C shows a voltammogram of the magneto-switchable electrocatalyzed oxidation of NADH by the PQQ-modified magnetic particles. The electrical contact of the magnetically attracted PQQ-functionalized particles results in the electrocatalyzed oxidation of NADH; this is reflected by the electrocatalytic anodic current (Figure 2C, curve a). Blocking the electrical communication between the PQQ-modified particles and the electrode fully inhibits the electrocatalyzed oxidation of NADH (Figure 2C, curve b). The electrocatalytic current was reversibly switched "ON" and "OFF" by positioning the external magnet below and above the electrochemical cell, respectively (Figure 2C, inset).

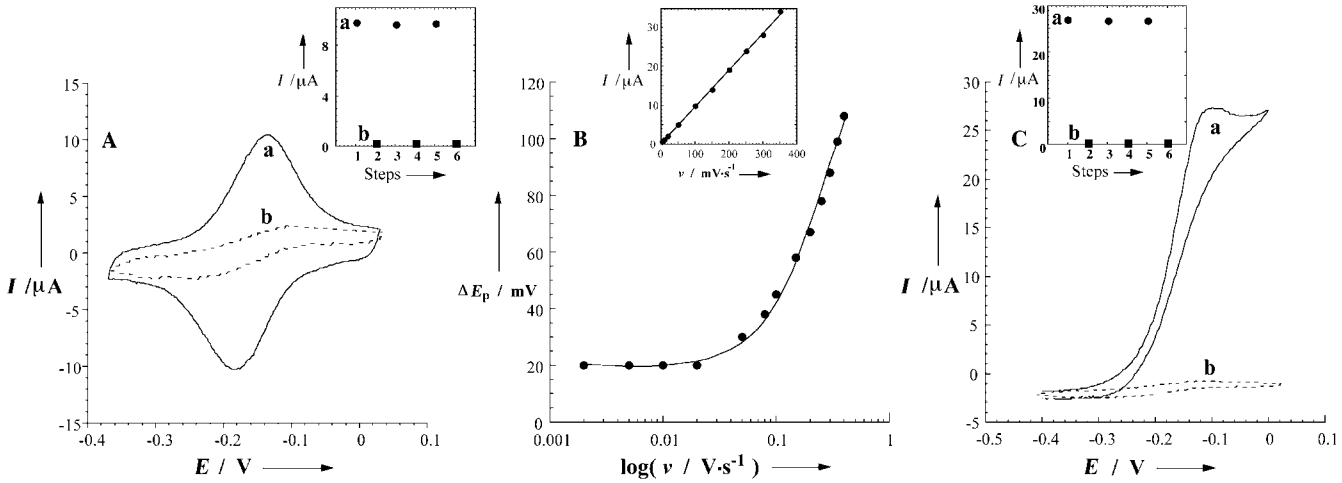

Figure 2. A) Cyclic voltammograms at an Au-electrode in the presence of PQQ-functionalized magnetic particles (10 mg). Curve a: The particles are magnetically attracted to the electrode surface. Curve b: The particles are magnetically retracted from the electrode. Inset: Reversible changes of the peak current of the cyclic voltammograms upon the magnetic switching ("ON": points a; "OFF": points b) of the electrochemical process of the PQQ associated with the magnetic particles. B) Dependence of the peak-to-peak separation of the cyclic voltammograms of PQQ-functionalized magnetic particles attracted to the Au electrode on the potential scan rate. Inset: Dependence of the peak current on the potential scan rate. C) Cyclic voltammograms at an Au-electrode in the presence of the PQQ-functionalized magnetic particles (10 mg) and NADH (50 mM). Curve a: When the magnetic particles are attracted to the electrode. Curve b: When the magnetic particles are retracted from the electrode. Inset: Reversible changes of the electrocatalytic current (measured at $E =$ 0.05 V) upon attraction of the magnetic particles to the electrode (points a) and retraction of the magnetic particles from the electrode (points b). The data were obtained in Tris buffer (0.1M, pH = 8.0) in the presence of $CaCl_2$ (10 mM) under argon. Potential scan rates are A) 100 mV s^{-1}; C) 2 mV s^{-1}.

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Similarly, the heme-containing undecapeptide, microperoxidase-11 (**3**, MP-11) was covalently linked to magnetic particles (Scheme 2B). Figure 3A, curve a, shows the cyclic voltammogram of the electrode with the MP-11-functionalized magnetic particles attracted to the electrode surface. The redox wave of MP-11, $E^{o} = -0.40$ V, is similar to that observed for MP-11 assembled as a monolayer on an Au electrode.[28] The cyclic voltammogram of the system recorded upon lifting the magnetic particles by positioning the external magnet above the electrochemical cell does not show any redox activity of MP-11 (Figure 3A, curve b). The redox activity of the MP-11-functionalized particles is reversibly switched between "ON" and "OFF" states by the appropriate positioning of the external magnet (Figure 3A, inset). The heme site of MP-11 electrocatalyzes the reduction of H_2O_2 to H_2O.[28] The reduction of H_2O_2 proceeds, however, at a substantially more positive potential than that of MP-11, owing to the formation of a [heme-Fe^{IV}=O]$^{\bullet+}$ intermediate complex upon interaction of the heme with H_2O_2.[29] The magneto-switched MP-11-electrocatalyzed reduction of H_2O_2 is nicely demonstrated in Figure 3B. The magnetic attraction of the MP-11-functionalized magnetic particles to the electrode stimulates the catalytic reduction of H_2O_2; this is reflected by a high electrocatalytic cathodic current (curve a), whereas magnetic retraction of the particles prohibits the electrocatalyzed reduction of H_2O_2. The inset in Figure 3B shows the reversible changes of the electrocatalytic cathodic current developed in the system upon the magneto-controlled switching of the electrocatalytic process.

The electrical activation of redox enzymes is a key process in bioelectronics, since it enables the use of the electrochemically activated biocatalysts as the active materials for biosensors, biofuel cells, and biomaterial-based electronic devices.[30] Redox enzymes usually lack direct electrical communication with electrodes, owing to the structural insulation of their redox sites by the protein matrixes. Different methods by which redox enzymes can form electrical contacts with electrodes have been developed; these include the application of diffusional electron mediators,[31] covalent tethering of redox-relays to the proteins,[32] and the reconstitution of proteins with synthetic relay-functionalized cofactors.[33] Thus, the covalent attachment of electron relays that activate redox enzymes to magnetic particles could lead to the development of magneto-switchable bioelectrocatalytic systems.

Accordingly, the amine-functionalized magnetic particles were modified with *N*-(ferrocenylmethyl)aminohexanoic acid (**4**), Scheme 2B. The ferrocene unit, upon oxidation to the ferrocenylium cation, acts as an electron mediator for many biocatalyzed oxidative transformations.[31, 34] Figure 4A shows the differential pulse voltammograms that demonstrate the magneto-switchable properties of the ferrocene-modified magnetic particles. Magnetic attraction of the particles to the electrode allows the oxidation of ferrocene (curve a), while the magnetic removal of the particles from the conductive supports blocks the oxidation of the ferrocene units (curve b). Addition of glucose oxidase and glucose to the system leads to the magneto-switchable bioelectrocatalyzed oxidation of glucose, Figure 4B. Attraction of the magnetic

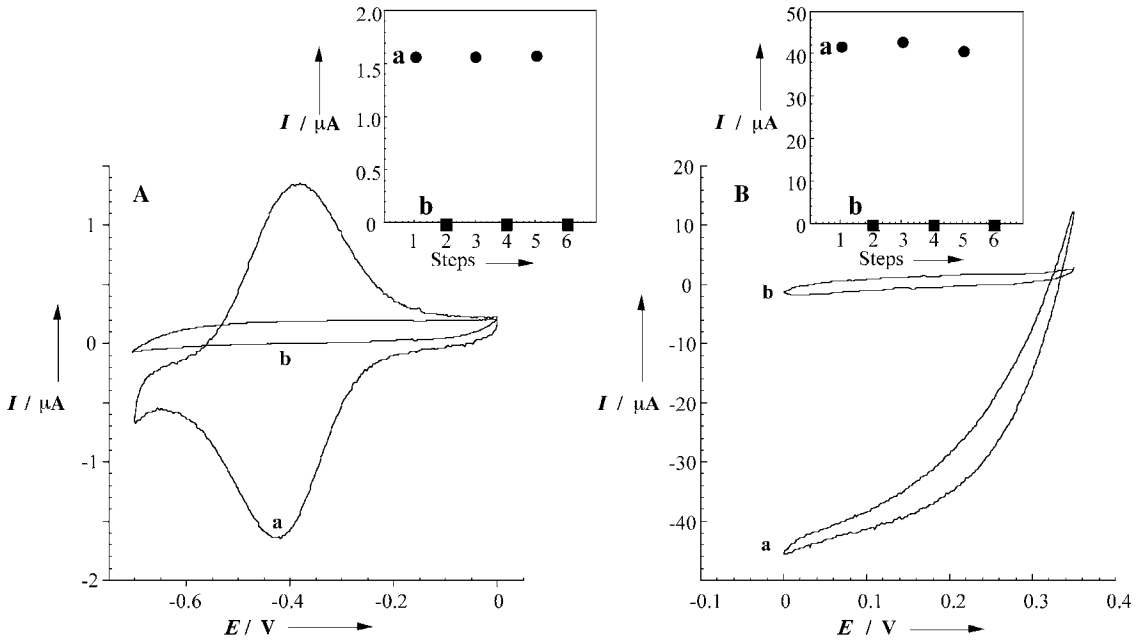

Figure 3. A) Cyclic voltammograms of an Au-electrode recorded in the presence of MP-11-functionalized magnetic particles (10 mg). Curve a: The magnetic particles are attracted to the electrode surface. Curve b: The magnetic particles are retracted from the electrode. Inset: Reversible changes of the peak current of the cyclic voltammograms upon the magnetic switching ("ON": points a; "OFF": points b) of the electrochemical process of MP-11 associated with the magnetic particles. B) Cyclic voltammograms at an Au-electrode in the presence of MP-11-functionalized magnetic particles (10 mg) and H_2O_2 (10 mM). Curve a: When the redox functions of the MP-11-modified particles are switched "ON" by attraction of the magnetic particles to the electrode. Curve b): When the redox functions of the MP-11-modified particles are switched "OFF" by retraction of the magnetic particles from the electrode. Inset: Reversible changes of the electrocatalytic current (measured at $E = 0.0$ V) upon attraction of the magnetic particles to the electrode (points a) and retraction of the magnetic particles from the electrode (points b). The data were recorded in phosphate buffer (0.1M, pH = 7.0) under argon. Potential scan rates are: A) 100 mV s^{-1}; B) 10 mV s^{-1}.

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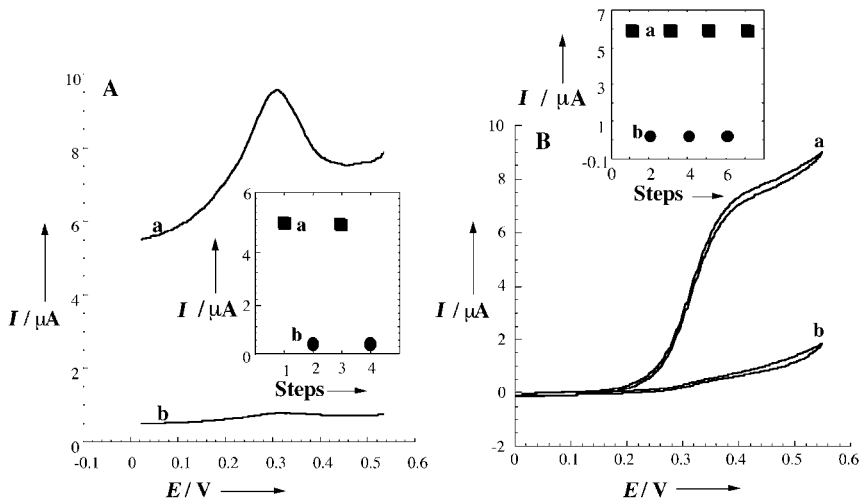

Figure 4. A) Differential pulse voltammograms of an Au-electrode in the presence of the ferrocene-functionalized magnetic particles (10 mg). Curve a: The particles are magnetically attracted to the electrode surface. Curve b: The particles are magnetically retracted from the electrode. Inset: Reversible changes of the peak current of the differential pulse voltammograms upon magnetic activation (points a) and deactivation (points b) of the electrochemical process of the ferrocene units. B) Cyclic voltammograms at an Au-electrode in the presence of the ferrocene-functionalized magnetic particles (10 mg), glucose (10 mM), and GOx (1 mg mL^{-1}). Curve a: The redox functions of the particles are switched "ON" by their attraction to the electrode. Curve b: The redox functions of the particles are switched "OFF" by their retraction from the electrode. Inset: Reversible changes of the electrocatalytic current (measured at $E = 0.45$ V) upon attraction of the magnetic particles to the electrode (points a) and retraction (points b) of the magnetic particles from the electrode. The data were recorded in phosphate buffer (0.1M, pH = 7.0) under argon. The differential pulse voltammograms were measured at a potential scan rate of 20 mV s^{-1}, pulse height 5 mV. The cyclic voltammograms were measured at a potential scan rate of 5 mV s^{-1}.

particles to the electrode affects the oxidation of the ferrocene units to ferrocenyl cation, which mediates the oxidation of the active site of glucose oxidase (GOx); this in turn catalyzes the oxidation of glucose to gluconic acid. The overall bioelectrocatalyzed oxidation of glucose is reflected by an electrocatalytic anodic current at the redox potential of the ferrocene units (Figure 4B, curve a). Retraction of the ferrocene-modified magnetic particles from the electrode by positioning the magnet above the electrochemical cell prohibits the electrochemical oxidation of the ferrocene sites and the subsequent biocatalyzed oxidation of glucose (Figure 4B, curve b). By the cyclic positioning of the external magnet below and above the electrochemical cell, the bioelectrocatalyzed oxidation of glucose is reversibly switched between "ON" and "OFF" states, respectively (Figure 4B, inset).

Similarly, a magneto-switchable bioelectrocatalytic reductive transformation was demonstrated by the application of bipyridinium-functionalized magnetic particles. *N*-Methyl-*N*′-(dodecanoic acid)-4,4′-bipyridinium (**5**) was coupled to the amine-functionalized magnetic particles, Scheme 2B. Reduction of bipyridinium salts to the respective bipyridinium radical cations yields an active electron carrier for the activation of various enzymes such as nitrate reductase,[35] nitrite reductase,[36] glutathione reductase,[37] formate dehydrogenase,[38] hydrogenase,[39] and others[31] towards the reduction of nitrate, nitrite, glutathione, CO_2, H^+, etc., respectively. We demonstrate here the magneto-switchable bioelectrocatalyzed reduction of nitrate (NO_3^-) to nitrite (NO_2^-) in the presence of nitrate reductase (NR). Figure 5A shows the differential pulse voltammograms of the bipyridinium-functionalized magnetic particles upon attraction (curve a) and retraction (curve b) of the particles to and from the electrode surface, respectively, by the external magnet. Clearly, the bipyridinium units are reduced at the electrode support, while their retraction from the electrode blocks the electrical contact

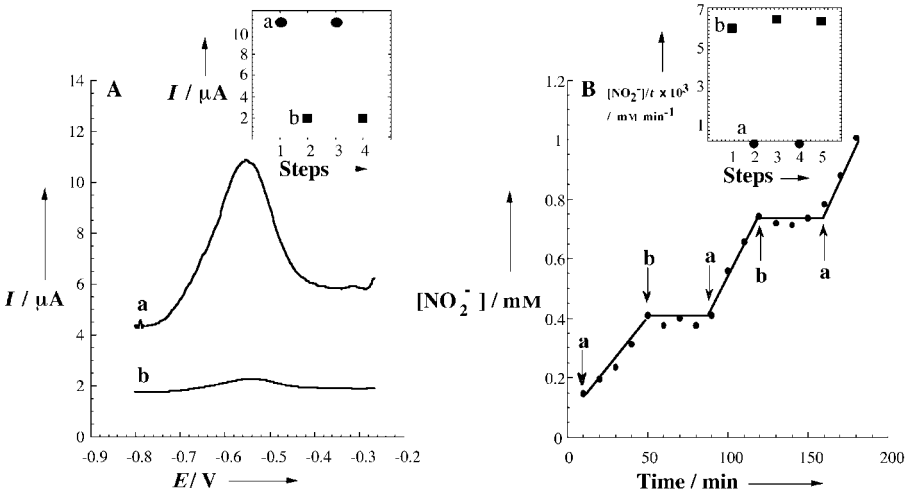

Figure 5. A) Differential pulse voltammograms at an Au-electrode in the presence of the **5**-functionalized magnetic particles (10 mg). Curve a: The particles are magnetically attracted to the electrode surface. Curve b: The particles are magnetically retracted from the electrode surface. Inset: Reversible changes of the peak current of the differential pulse voltammograms upon the magnetic switching ("ON": points a; "OFF": points b) of the electrochemical process of the bipyridinium units associated with the magnetic particles. B) Formation of nitrite ions (NO_2^-) upon the bioelectrocatalytic reduction of nitrate (0.1M KNO_3) in the presence of nitrate reductase (5 units per mL) and **5**-functionalized magnetic particles (10 mg). A potential corresponding to $E = -0.7$ V is applied on the electrode: At points a the particles are magnetically attracted to the electrode surface. At points b the particles are magnetically retracted from the electrode. Inset: Reversible changes of the rate of the bioelectrocatalytic formation of nitrite upon the magnetic switching ("ON": points a; "OFF": points b) of the the NO_3^- reduction. The data were recorded in phosphate buffer (0.1M, pH = 7.0) under argon. Potential scan rate 20 mV s^{-1}; pulse height 5 mV.

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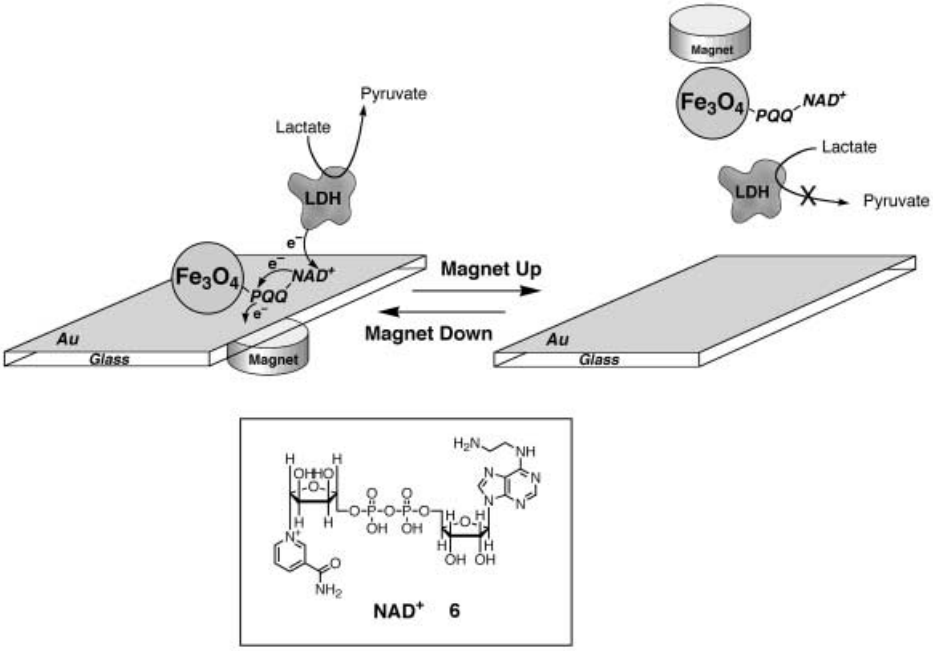
between the electrode and the bipyridinium sites. The magneto-controlled switching of the redox activity of the bipyridinium sites can be cycled reversibly (Figure 5A, inset).

Figure 5B shows the magneto-controlled formation of nitrite (NO_2^-) upon the bioelectrocatalyzed reduction of nitrate (NO_3^-) in the presence of nitrate reductase (NR). At point a the magnet is positioned below the electrode that is biased at a potential of $E = -0.7$ V; this results in the reduction of the bipyridinium units linked to the magnetic particles. The reduced relay units mediate the NR-biocatalyzed reduction of NO_3^- with a rate corresponding to approximately 6.5×10^{-3} mM min^{-1}. At point b the magnet is translocated to the upper position; this results in the blocking of the reduction of NO_3^- in the system, which is reflected by a constant concentration of NO_2^- in the system. At the next point a the magnet is repositioned below the electrode, resulting in the reactivation of the bioelectrocatalyzed reduction of NO_3^- with the rate of approximately 6.5×10^{-3} mM min^{-1}. The inset in Figure 5B shows the reversible switching "ON" and "OFF" of the bioelectrocatalytic process upon cyclic translocation of the external magnet.

The magneto-switching of bioelectrocatalytic transformations has been broadened to NAD$^+$-dependent redox enzymes. The family of NAD(P)$^+$-dependent enzymes represents a broad class of redox biocatalysts.[31] The electrical activation of NAD(P)$^+$-dependent enzymes requires the electrochemical regeneration of NAD(P)$^+$ by the oxidation of NAD(P)H. Different catalysts, such as quinones; phenazine, phenoxazine, and phenothiazine derivatives; and other redox mediators were applied for the electrochemical regeneration of NAD(P)$^+$.[40] PQQ was reported as an effective catalyst for the electrochemical oxidation of NAD(P)H to NAD(P)$^+$, and enhanced electrocatalytic functions were observed in the presence of Ca^{2+} as co-catalyst.[26, 27] Recently, we applied the electrocatalytic properties of PQQ to assemble an integrated enzyme electrode for the bioelectrocatalyzed oxidation of lactate.[41] In this study, an affinity complex[42] between lactate dehydrogenase and a PQQ-NAD$^+$ monolayer, associated with an electrode surface, was crosslinked with glutaric dialdehyde to yield an integrated electrode; vectorial electron transfer in this system leads to the biocatalyzed oxidation of lactate to pyruvate by the electrocatalyzed regeneration of the NAD$^+$ cofactor.

We applied this concept to develop a magneto-switchable system for the bioelectrocatalyzed oxidation of lactate to pyruvate in the presence of the NAD$^+$-dependent enzyme lactate dehydrogenase (LDH), Scheme 3. Amino-NAD$^+$ (**6**) was covalently linked to the PQQ-functionalized magnetic particles. In the presence of the lactate and LDH in solution, oxidation of lactate generates a reduced $PQQH_2$–NADH film on the magnetic particles. The conversion of lactate to pyruvate is, however, blocked when the functional layer associated with the magnetic particles is reduced. Since the molar amount of the functional film on the particles is minute, the conversion yield of lactate to pyruvate is negligible. Attraction of the magnetic particles to the electrode enables the electrochemical regeneration of NAD$^+$ by the electrochemical oxidation of $PQQH_2$ to PQQ and further oxidation of NADH to NAD$^+$. This enables the continuous cyclic oxidation of lactate to pyruvate. Retraction of the magnetic particles from the electrode blocks the electrochemical regeneration of NAD$^+$ and the bioelectrocatalyzed oxidation of lactate is switched off. Figure 6 shows the magneto-switchable bioelectrocatalyzed oxidation of lactate to pyruvate. Positioning of the magnet below the electrode (points a) activates the oxidation of lactate with a rate that corresponds to approximately 0.13 mM min^{-1}. Retraction of the magnetic particles from the electrode by the positioning of the external magnet above the cell (points b) blocks the biocatalyzed oxidation of lactate.

An important question that should be addressed in our discussion relates to the potential practical utility of such systems, beyond the demonstration of the concept of magneto-switchable bioelectrocatalysis. We examined the possibility to use magneto-switchable bioelectrocatalysis for the development of bifunctional electrocatalytic sensors, in which the selective electrochemical sensing of one substrate out of two target analytes is possible. The electrochemical sensing of two different substrates in a mixture, in the presence of two different redox-enzymes, is a general problem in bioelectronics. For example, the specific analysis of a substrate in a



Scheme 3. Magneto-switched bioelectrocatalytic oxidation of lactate in the presence of lactate dehydrogenase (LDH) and magnetic particles functionalized with PQQ-NAD$^+$.

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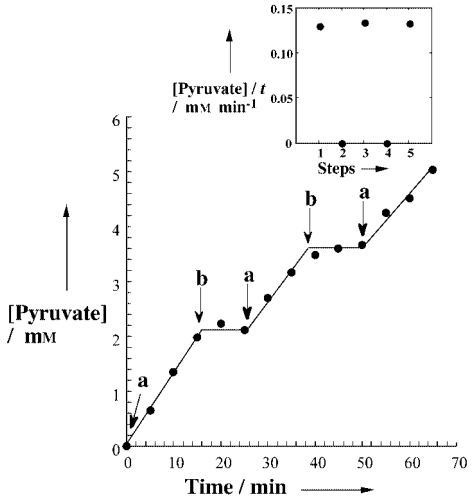
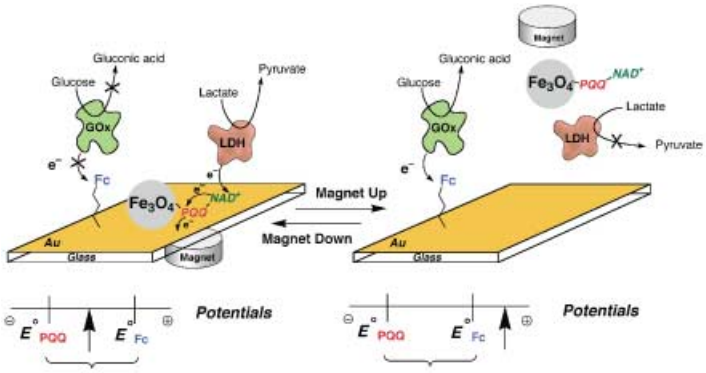


Figure 6. Formation of pyruvate upon the bioelectrocatalytic oxidation of lactate (0.1 M) in the presence of lactate dehydrogenase (2 mg mL^{-1}) and PQQ–NAD$^+$-functionalized magnetic particles with a potential of $E = 0.05$ V applied on the electrode. Curve a: The magnetic particles are attracted to the electrode surface. Curve b: The magnetic particles are retracted from the electrode surface. Inset: Reversible changes of the rate of the bioelectrocatalytic formation of pyruvate upon the magnetic switching ("ON": points a; "OFF": points b) of the biocatalytic process. The data were recorded in Tris-HCl buffer (0.1 M, pH 7.0) and $CaCl_2$ (10 mM) under argon.



Scheme 4. Magneto-controlled dual biosensing of glucose and lactate in the presence of glucose oxidase (GOx), lactate dehydrogenase (LDH), magnetic particles functionalized with PQQ-NAD$^+$, and an Au-electrode modified with a monolayer of ferrocene units.

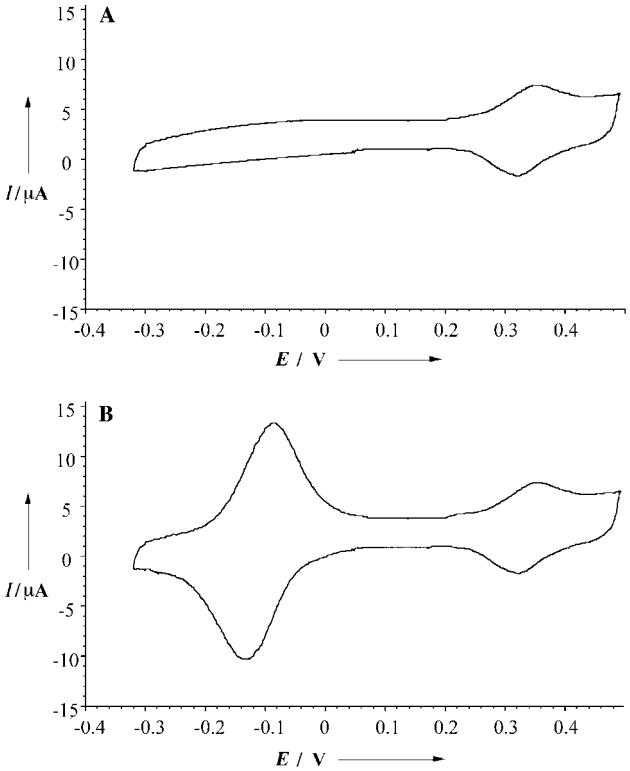


Figure 7. A) Cyclic voltammogram of the ferrocene-monolayer-functionalized Au electrode when the NAD$^+$-PQQ-functionalized magnetic particles are retracted from the electrode by the external magnet. B) Cyclic voltammogram of the ferrocene-monolayer-functionalized Au electrode in the presence of the NAD$^+$-PQQ-functionalized magnetic particles attracted to the electrode by the magnet. The data were recorded under argon in the presence of the magnetic particles (20 mg) in Tris-HCl buffer (0.1 M, pH 7.0), $CaCl_2$ (10 mM). Potential scan rate, 100 mV s^{-1}.

mixture of two substrates that are oxidized at close oxidation potentials is difficult, since the amperometric response is the sum of two simultaneously operating bioelectrocatalyzed transformations. Previous studies[43] have suggested the use of photoswitchable redox enzymes to resolve the problem. That is, by the use of two enzymes, whereby one of the biocatalysts is photo-switched to an "OFF" state and one of the substrates is selectively analyzed by the non-photoactive enzyme. The subsequent photoactivation of the second enzyme to the "ON" function leads to the cumulative amperometric response resulting from the bioelectrocatalyzed oxidation of the two substrates. The subtraction of the amperometric response, which results from the single-enzyme operation, from the latter amperometric response yields the amperometric signal that corresponds to the bioelectrocatalyzed oxidation of the second substrate. The magnetic attraction or retraction of magnetic particles that activate or deactivate a bioelectrocatalyzed transformation at the electrode, respectively, enables the compartmentalization of two redox transformations. This is exemplified with the specific analysis of glucose or lactate in the presence of the two enzymes glucose oxidase (GOx), and lactate hydrogenase (LDH), respectively, Scheme 4. An Au electrode is modified with a ferrocene-functionalized monolayer. This redox-active monolayer acts as a mediator for the activation of GOx towards the bioelectrocatalyzed oxidation of glucose. The magnetic particles are modified with a PQQ–NAD$^+$ monolayer, which acts as the catalyst–cofactor component for the regeneration of the NAD$^+$-cofactor and the activation of the bioelectrocatalyzed oxidation of lactate by LDH (vide supra). Figure 7A shows the cyclic voltammogram of the ferrocene-monolayer-functionalized electrode when the magneticparticles are retracted from the electrode by positioning of the external magnet above the cell. The quasi-reversible redox wave of the ferrocene units is observed at $E^o = 0.32$ V, the potential at which the bioelectrocatalyzed oxidation of glucose occurs. Figure 7B shows the cyclic voltammogram of the system when the modified magnetic particles are attracted to the ferrocene-monolayer-functionalized electrode by means of the external magnet. Two quasi-reversible redox

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waves are observed at $E^{o} = -0.13$ V and $E^{o} = 0.32$ V; these potentials correspond to the PQQ units of the PQQ–NAD^{+}-functionalized magnetic particles and the ferrocene units associated with the electrode, respectively. Thus, by the retraction of the magnetic particles from the electrodes, the selective bioelectrocatalyzed oxidation of glucose may be stimulated by the application of a potential of $E > 0.32$ V which is capable of oxidizing the ferrocene monolayer. Attraction of the magnetic particles to the electrode by means of the external magnet, and the application of a potential that is less positive than the redox potential of the ferrocene monolayer, but sufficiently positive to oxidize the PQQ units, $-0.13 < E < 0.32$ V (see Scheme 4), results in the selective oxidation of lactate.

Figure 8A, curve b, shows the selective bioelectrocatalyzed oxidation of glucose by the ferrocene-monolayer-functionalized electrode in the presence of GOx and LDH and the two substrates, glucose and lactate, in solution when the modified magnetic particles are retracted from the electrode by means of the external magnet positioned above the electrochemical cell. An electrocatalytic anodic current is observed at the potential characteristic of the ferrocene monolayer; this indicates that the bioelectrocatalyzed oxidation of glucose proceeds effectively. The inset in Figure 8A shows the amperometric responses of the system in the presence of variable concentrations of glucose (curve a) and lactate (curve b), with the applied potential on the electrode corresponding to $E = 0.5$ V. Clearly, the system reveals selectivity towards the oxidation of glucose when the external magnet is positioned above the cell and the magnetic particles are retracted from the electrode. Figure 8B shows the cyclic voltammograms of the system that includes the ferrocene-functionalized electrode, GOx, LDH, and the substrates glucose and lactate, when the PQQ–NAD^{+}-modified particles are attracted to the electrode support and the potential applied on the electrode is limited to the range -0.36 V to $+0.15$ V. In this potential range, the ferrocene units are electrochemically inactive, but the PQQ components reveal redox activity. An electrocatalytic anodic current is observed at $E > -0.13$ V, the redox potential of the PQQ units, implying that the PQQ-mediated bioelectrocatalyzed oxidation of lactate proceeds in the system. The inset in Figure 8B shows the amperometric responses of the system upon the interaction of the ferrocene-functionalized electrode with the magnetically-associated particles in the presence of variable concentrations of lactate (curve a) and glucose (curve b), with a potential applied on the electrode of $E = 0.05$ V. Clearly, the system responds to the addition of lactate, but is insensitive to the addition of glucose. This is consistent with the fact that at this potential the ferrocene units are redox inactive and, consequently, the mediated bioelectrocatalyzed oxidation of glucose is blocked. On the other hand, at this applied potential, the bioelectrocatalyzed oxidation of lactate by the NAD^{+}-dependent enzyme LDH is effectively stimulated. Biocatalyzed oxidation of lactate to pyruvate yields the reduced NADH cofactor, which is oxidized by the PQQ units to NAD^{+}, on the functionalized particles. The electrochemical oxidation of the generated $PQQH_2$ drives the continuous electrochemical regeneration of the NAD^{+}-cofactor and the coupled biocatalyzed oxidation of lactate to pyruvate. Thus, the system enables the reversible and cyclic selective analysis of lactate or glucose by means of an external magnetic field.

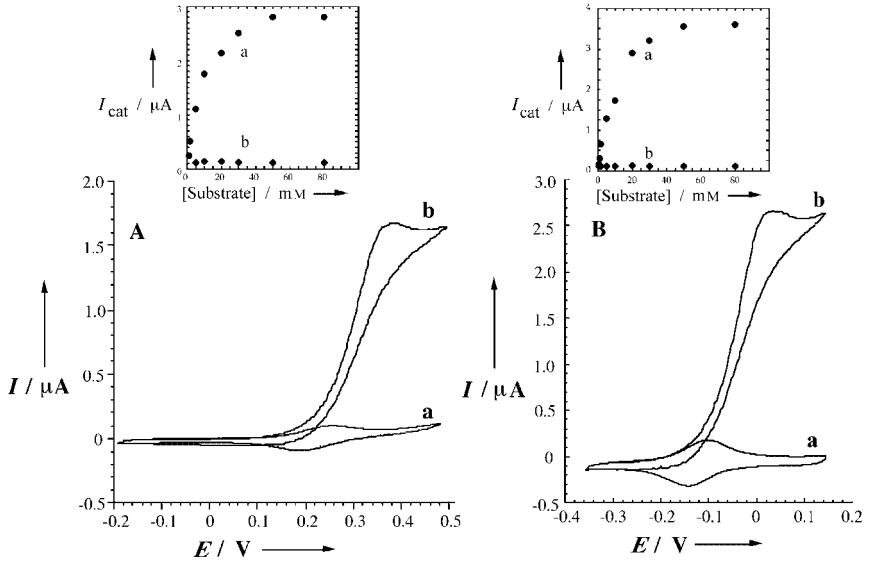

Figure 8. A) Cyclic voltammograms of the ferrocene-monolayer-functionalized Au-electrode when the NAD^{+}-PQQ-functionalized magnetic particles are retracted from the electrode by the external magnet. Curve a: In the presence of GOx (1 mg mL^{-1}) and LDH (2 mg mL^{-1}). Curve b: In the presence of GOx (1 mg mL^{-1}), LDH (2 mg mL^{-1}), glucose (50 mM), and lactate (20 mM). Inset: Calibration plot of the amperometric response of the system with the magnet in the "up" position and the applied potential $E = 0.50$ V. Curve a: At different concentrations of glucose. Curve b: At different concentrations of lactate. B) Cyclic voltammograms of the ferrocene-monolayer-functionalized Au electrode in the presence of the NAD^{+}-PQQ-functionalized magnetic particles attracted to the electrode by the magnet. Curve a: In the presence of GOx (1 mg mL^{-1}) and LDH (2 mg mL^{-1}). Curve b: In the presence of GOx (1 mg mL^{-1}), LDH (2 mg mL^{-1}), glucose (50 mM), lactate (20 mM). Inset: Calibration plot of the amperometric responses of the system with the magnet in the "down" position and the applied potential $E = 0.05$ V. Curve a: At different concentrations of lactate. Curve b: At different concentrations of glucose. The data were recorded under argon in the presence of the magnetic particles (20 mg) in Tris-HCl buffer (0.1 M, pH 7.0), $CaCl_2$ (10 mM). Potential scan rate, 5 mV s^{-1}.

Conclusion

The report has discussed the development of the new concepts of magneto-switchable electrocatalysis and bioelectrocatalysis. Mechanical translocation of chemical components associated with magnetic particles, by means of an external magnetic field, is a common practice in the separation, concentration and transport of chemical components.[44] The approach developed by us couples an interfacial electron transfer to a magneto-con-

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trolled translocation of magnetic particles functionalized with redox-active components. The ability to trigger the redox functionalities of the electroactive components associated with the magnetic particles establishes a novel switching system, whereby magnetic signals recorded by the system are electronically transduced by the electrochemical response of the assembly.

The present study has addressed the magneto-switchable functions of enzymes by the application of electron relay-modified or relay-cofactor-functionalized magnetic particles. We have demonstrated that magneto-switchable bioelectrocatalysis can be applied to develop multisensor devices for the selective electrochemical analysis of different substrates. Although it is difficult to define the broad perspectives of magneto-switchable electrocatalysis and bioelectrocatalysis at this stage, one may envisage some potential future applications of such systems in designing magnetic valves that control the electrochemical generation or uptake of chemical ingredients in reactors, or magnetic valves that trigger the switchable electrochemical generation and transport of therapeutic compounds. Biological and redox-active species attached to magnetic particles can also find broad applications in immunosensors and DNA sensors.[45]

Experimental Section

Chemicals: *N*-Methyl-*N*′-(dodecanoic acid)-4,4′-bipyridinium (**5**),[46] *N*-(ferrocenylmethyl)- aminohexanoic acid (**4**)[14b] and N^6-(2-aminoethyl)-β-nicotinamide adenine dinucleotide (amino-NAD$^+$; **6**)[47] were synthesized and purified as described before. Glucose oxidase (GOx, E.C. 1.1.3.4 from *Aspergillus niger*), nitrate reductase (NR, E.C. 1.6.6.2 from *Aspergillus* species), lactate dehydrogenase (LDH, E.C. 1.1.1.27 from rabbit muscle, type II), microperoxidase-11, pyrroloquinoline quinone (PQQ), 4-(2-hydroxyethyl)piperazine- 1-ethanesulfonic acid sodium salt (HEPES), tris-(hydroxymethyl)aminomethane hydrochloride (Tris), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), and all other chemicals were purchased from Aldrich or Sigma and used as supplied. Ultrapure water from a "Seralpur Pro 90 CN" source was used in all experiments.

Magnetic particles—preparation and modification: Magnetic particles (Fe_3O_4, saturated magnetization ca. 65 emu g^{-1}), with an approximate average diameter of 1 μm, were prepared according to the published procedure[48] without including the surfactant into the reaction medium. The magnetic particles were silanized with [3-(2-aminoethyl)aminopropyl]trimethoxysilane (5% v/v) in dry toluene for 2 h under reflux. *N*-(Ferrocenylmethyl)aminohexanoic acid (**4**; 1×10^{-3} M), *N*-methyl-*N*′-(dodecanoic acid)-4,4′-bipyridinium (**5**; 1×10^{-3} M), pyrroloquinoline quinone (PQQ, **2**; 1×10^{-3} M), or microperoxidase-11, (**3**; 1×10^{-4} M) were covalently linked to the amino-functionalized magnetic particles (100 mg) suspended in HEPES buffer (5 mL, 0.1 M, pH 7.2) in the presence of EDC (5×10^{-3} M) as a coupling reagent for 5 hours. The resulting redox-functionalized magnetic particles were washed with water, separated with a centrifuge, and dried in a dessicator. The PQQ-functionalized magnetic particles (50 mg) were further reacted with amino-NAD$^+$ (**6**; 1×10^{-4} M) in HEPES buffer (5 mL, 0.1 M, pH 7.2) in the presence of EDC (5×10^{-3} M) for 5 hours, washed with water, separated with a centrifuge, and dried. 2,3-Dichloro-1,4-naphthoquinone (**1**; 200 mg suspension in 100 mL ethanol) was treated with the amino-functionalized magnetic particles (100 mg) upon reflux overnight. The resulting aminonaphthoquinone-modified magnetic particles were washed with ethanol, then with water and dried in a dessicator.

Electrochemical measurements: Cyclic voltammetry, differential pulse voltammetry, and constant potential electrolysis were performed by using an electrochemical analyzer (EG&G, VersaStat) linked to a computer (EG&G software 270/250). The Au-coated (50 nm gold layer) glass plate (Analytical-μSystem, Germany) was used as a working electrode (0.3 cm^2 area exposed to the solution). An electrochemical cell (1 mL), made up of an Au-plate working electrode in a horizontal position at the bottom of the cell, a glassy carbon auxiliary electrode, and a saturated calomel electrode (SCE), was used for the electrochemical measurements. The potentials are reported versus SCE. A 5 mm diameter magnet (NdFeB/Zn-coated magnet with a remanent magnetization of 10.8 kG) was used to move the modified magnetic particles. A special holder allows the placement of the external magnet below the bottom of the cell, allowing the magnetic particles to be attracted to the working electrode, or above the cell, allowing the retraction of the magnetic particles from the working electrode.

Most of the experiments were performed with a bare Au working electrode. Two kinds of Au-modified working electrodes were employed in the specifically mentioned experiments. A mercaptohexanol-functionalized surface was prepared by treatment of an Au electrode with mercaptohexanol (1×10^{-3} M) in ethanol for 2 hours, followed by rinsing the surface with ethanol. A ferrocene-monolayer-functionalized Au-electrode was obtained in the following way. An Au electrode was soaked in a solution of cystamine in water (0.02 M) for 2 hours and was then washed with water. The resulting electrode was treated with **4** (1×10^{-3} M) in HEPES buffer (0.1 M, pH 7.2) in the presence of EDC (5×10^{-3} M) for 2 hours, and then washed with water.

Analysis of products of the biocatalytic reactions: The bioelectrocatalytic reduction of NO_3^- ions (1 mM KNO_3 in 0.1 M phosphate buffer, pH = 7.0) was performed by the application of a potential of $E=-0.7$ V on the working electrode in the presence of nitrate reductase (5 units per mL) and **5**-functionalized magnetic particles, which were magnetically attracted to the electrode surface, under argon. Nitrite formed upon the bioelectrocatalytic reduction of nitrate was determined by a spectrometric method based on the diazotation of sulphanylamide and coupling with *N*-1-naphthylethylenediamine dihydrochloride.[49] Samples (25 μL) of the cell-solution were taken at certain time intervals during the electrolysis and placed in a cuvette. Then 1 mL of each of the reagents was added, and the absorption at $\lambda=540$ nm was measured after 10 minutes. Using an appropriate calibration curve, the amounts of NO_2^- at certain time intervals during the electrolysis were determined.

The bioelectrocatalyzed oxidation of lactate (1 mM lactic acid in 0.1 M Tris-HCl buffer, pH 7.0, $CaCl_2$, 10 mM) was performed by the application of a potential corresponding to $E=0.05$ V on the working electrode in the presence of LDH, 2 mg mL^{-1}, and NAD$^+$-PQQ-functionalized magnetic particles attracted by the external magnet to the electrode surface, under argon. Samples (25 μL) of the cell solution were taken at several time intervals during electrolysis, and pyruvate formed upon the bioelectrocatalytic oxidation of lactate was analyzed by HPLC (Shodex KC-811 anionic exchange column, eluent 0.1% H_3PO_4, UV-detector $\lambda=210$ nm).

Acknowledgement

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