Controlling the direction of photocurrents by means of CdS nanoparticles and cytochrome *c*-mediated biocatalytic cascades†

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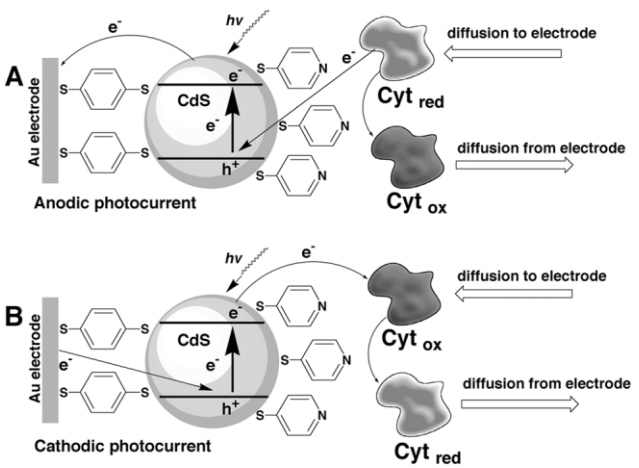
Cathodic or anodic photocurrents are generated by a monolayer of CdS nanoparticles in the presence of the oxidized or reduced states of cytochrome *c*, respectively, and the photocurrents are amplified by enzyme-generated biocatalytic cascades mediated by cytochrome *c*.

The unique photophysical properties of semiconductor nanoparticles (NPs) have recently been applied to follow biorecognition or biocatalytic processes.[1] The fluorescence properties of semiconductor NPs were used to follow the formation of immunocomplexes[2] and membrane–cell interactions.[3] Fluorescence resonance energy transfer (FRET) from semiconductor NPs to appropriate dyes was employed to detect DNA[4,5] or telomerase activity in cancer cells[5] and to assay DNA cleavage.[6] Also, the photoinduced electron–hole generation in semiconductor NPs was employed to develop photoelectrochemical systems consisting of semiconductor NP–biomolecule hybrid systems. Photocurrents were generated by layered CdS NP–DNA assemblies associated with electrodes.[7] By the incorporation of a redox-active intercalator in a CdS NP–double-stranded DNA the photocurrent direction (cathodic or anodic) could be switched by applying the appropriate potential to the electrode.[8] The conjugation of semiconductor NPs and enzymes is scarce. As far as we are aware, only the activation of the photocurrent by CdS NPs using the hydrolytic biocatalyst acetylcholine esterase was demonstrated,[9] while the application of hybrid systems composed of redox proteins/enzymes and semiconductor NPs for photoelectrochemical applications is not established. Here we wish to report on the photocurrent generation by coupling reduced or oxidized cytochrome *c* (Cyt *c*) to CdS NPs linked to an electrode, and on the amplified generation of photocurrents using Cyt *c*-mediated biocatalytic cascades. We demonstrate that the photocurrent direction (cathodic or anodic) is controlled by the redox state of Cyt *c*.

Cytochrome *c* is a redox protein that exists in the oxidized and reduced states and can operate as an electron acceptor and an electron donor, respectively, in photochemical systems.[10] Photoelectrodes based on monolayer-immobilized bacterial photosynthetic reaction centers, that use the reduced state of Cyt *c* as an electron donor, were reported.[11] Cyt *c* lacks direct electrical contact with electrode surfaces. Pyridine units assembled on Au electrodes as monolayers act, however, as promoters that reversibly bind Cyt *c*, resulting in the structural alignment of the hemoprotein on the electrode support, and its electrical contacting.[12] The interaction of Cyt *c* with pyridine-functionalized monolayers is weak,[13] and the currents associated with the reversible electrochemical reduction/oxidation process are diffusion-controlled.[12] We studied photocurrents generated by the monolayer of the pyridine-functionalized CdS NPs in the presence of oxidized or reduced Cyt *c* (see the ESI†). CdS NPs (*ca.* 5.5 nm) were prepared in water using polyphosphate as a capping agent.[14] The CdS NPs were assembled as a monolayer on a Au electrode using 1,4-benzene dithiolate as linker, Scheme 1. Microgravimetric quartz crystal microbalance (QCM) measurements indicate that the coverage of the dithiol linker corresponds to *ca.* 1.4×10^{-10} mol cm^{-2}, and the surface coverage of the CdS NP monolayer corresponds to *ca.* 1.4×10^{12} NPs cm^{-2}. The resulting CdS NP monolayer was then modified with 4-mercaptopyridine, surface coverage 1.4×10^{14} molecules cm^{-2}, which translates to *ca.* 100 pyridine units per single CdS NP.

Irradiation of the CdS-modified electrode (0.3 cm^2, with no potential applied on the electrode, under Ar) in the presence of reduced Cyt *c*, 5×10^{-5} M, results in the photocurrent action spectrum depicted in Fig. 1(A) , curve (a). Control experiments reveal that no photocurrent is generated in the absence of reduced Cyt *c* or the CdS NPs, or in the absence of the mercaptopyridine-capping layer associated with the CdS NPs. The photocurrent action spectrum follows the spectral features of the CdS NPs,



Scheme 1 Photoelectrochemical systems composed of a monolayer of CdS NPs and soluble Cyt *c*. (A) Anodic photocurrent generation in the presence of reduced Cyt *c*. (B) Cathodic photocurrent generation in the presence of oxidized Cyt *c*.

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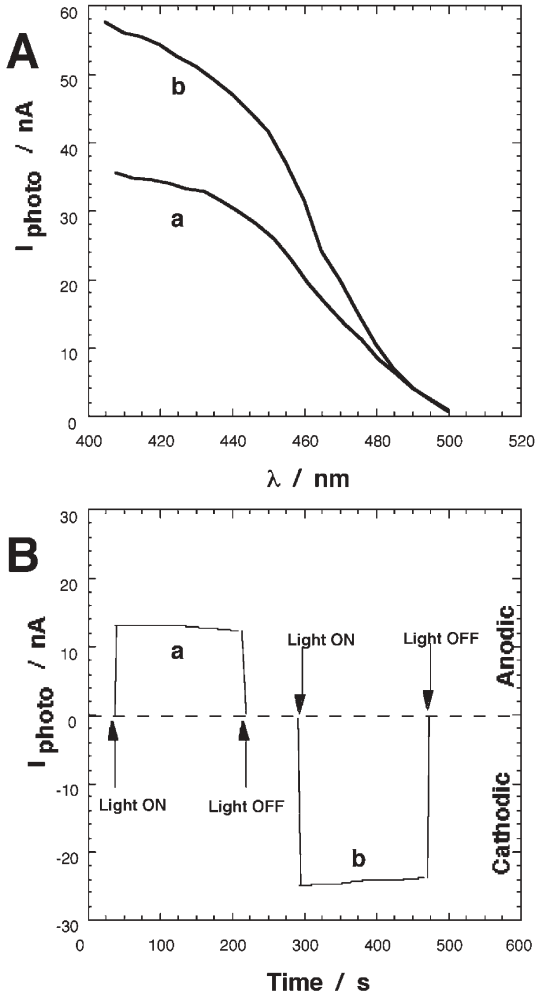
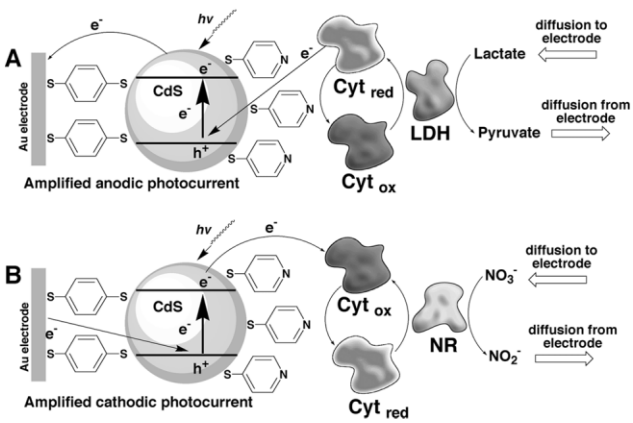


Fig. 1 (A) Action spectra of the photocurrents generated by a monolayer of CdS NPs, under no potential applied on the electrode, in the presence of: (a) reduced Cyt *c*, (b) oxidized Cyt *c*. (B) Photocurrents of different directions generated by the system, upon application of 0 V on the electrode, in the presence of: (a) reduced Cyt *c*, (b) oxidized Cyt *c*. The measurements were performed in 0.1 M phosphate buffer, pH 7.0, under Ar, with the Cyt *c* concentrations of 5×10^{-5} M.

implying that the photocurrent originates from the photoexcitation of the semiconductor NPs.[7] Similarly, the irradiation of the CdS NP-modified electrode in the presence of the oxidized Cyt *c*, 5×10^{-5} M, results in the generation of a photocurrent too. The photocurrent action spectrum observed in the presence of oxidized Cyt *c* is shown in Fig. 1(A), curve (b). The photocurrent intensity in the presence of oxidized Cyt *c* is *ca.* 2-fold higher than in the presence of reduced Cyt *c*. Also for this system, control experiments reveal that no photocurrents are generated upon irradiation of systems in the absence of the CdS NPs, or the oxidized Cyt *c*, or upon the elimination of the mercaptopyridine capping monolayer on the CdS NPs. It should be noted that the photocurrents shown in Fig. 1(A), curves (a) and (b), were recorded using a lock-in amplifier, and thus the direction of the photocurrent cannot be directly derived. Connecting the photoactivated electrode to a potentiostat, and monitoring the resulting photocurrent at $\lambda = 420$ nm upon application of 0 V (*vs.* SCE) on the photoelectrode, Fig. 1(B), reveals that, in the presence of the oxidized Cyt *c*, a cathodic current is generated, whereas in the presence of reduced Cyt *c* an anodic current is formed (0 V is close to the middle point potential of Cyt *c*).

The quantum yield of the photocurrent generation (taking into account the light flux, its reduction by the semitransparent electrode and the absorbance of the CdS monolayer) was estimated to be *ca.* 4.2% and *ca.* 2.2% for the cathodic and anodic photocurrents, respectively. That is, the anodic photocurrent is generated by the transfer of conduction-band electrons to the electrode and the concomitant transfer of electrons from reduced Cyt *c* to valence-band holes. The diffusional exchange of the photochemically generated oxidized Cyt *c* with the solubilized reduced Cyt *c*, acting as an electron donor, leads to the steady-state photocurrent, Scheme 1(A). Similarly, the cathodic photocurrent is generated by the transfer of conduction-band electrons to oxidized Cyt *c*, with the concomitant supply of electrons to valence-band holes by the electrode, Scheme 1(B). The diffusion exchange of the photogenerated reduced Cyt *c* with solubilized oxidized Cyt *c*, which operates as an electron acceptor, results in the steady-state cathodic photocurrent. The observed difference in the values of the anodic and cathodic photocurrents correlates with the faster electron transfer process to the oxidized Cyt *c* comparing the oxidation of reduced Cyt *c* measured by chronoamperometry.[15] This phenomenon was attributed to the different reorganization energies of the protein structures upon the electrochemical reduction and oxidation processes.[15] The effect of Cyt *c* concentration (in the reduced or oxidized states) was examined and, as the concentration of Cyt *c* increases, the cathodic or anodic photocurrents are almost linearly enhanced.

The Cyt *c*-stimulated generation of anodic or cathodic photocurrents was coupled to secondary Cyt *c*-mediated biocatalytic cascades that amplify the photoelectrochemical processes, Scheme 2. The reduced Cyt *c*–CdS NPs photoelectrochemical system was coupled to the biocatalytic system composed of lactate dehydrogenase (LDH) and lactate, Scheme 2(A). Fig. 2, curve (a) shows the photocurrents generated by the system in the presence of different concentrations of lactate (measured at $\lambda = 420$ nm; the potential of 0 V *vs.* SCE was applied to the photoelectrode). The photocurrent is enhanced as the lactate concentration increases,



Scheme 2 Photoelectrochemical systems composed of a monolayer of CdS NPs, soluble Cyt *c* and the respective biocatalytic systems: (A) amplified anodic photocurrent generation in the presence of reduced Cyt *c*, LDH and lactate; (B) amplified cathodic photocurrent generation in the presence of oxidized Cyt *c*, NR and nitrate.

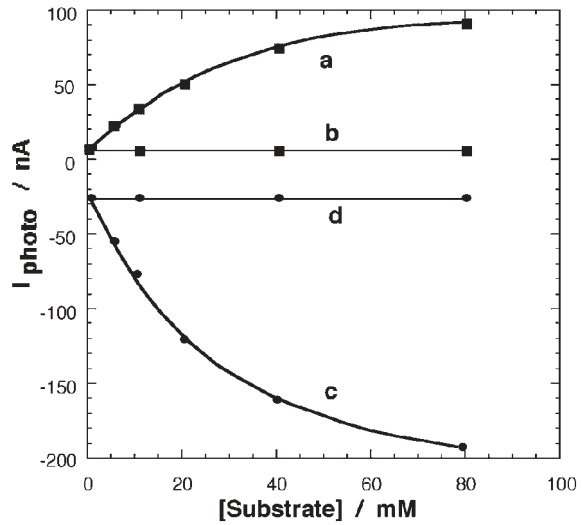


Fig. 2 Photocurrents generated by a monolayer of CdS in the presence of: (a) reduced Cyt *c*, LDH, 1 mg mL^{-1}, and variable concentration of lactate; (b) reduced Cyt *c* and variable concentration of lactate (in the absence of LDH); (c) oxidized Cyt *c*, NR, 1 mg mL^{-1}, and variable concentration of nitrate; (d) oxidized Cyt *c* and variable concentration of nitrate (in the absence of NR). The data were obtained in 0.1 M phosphate buffer, pH 7.0, under Ar, upon application of 0 V on the electrode.

and it levels off to a saturation value at a lactate concentration of *ca*. 70 mM. The saturated photocurrent value in the system is *ca*. 9-fold higher than the value observed with reduced Cyt *c* only, implying that the photocurrent was, indeed, amplified. Control experiments indicate that no enhanced photocurrent is observed in the system in the absence of LDH, Fig. 2, curve (b). Similarly, the oxidized Cyt *c*/CdS-NPs system was coupled to the biocatalytic system consisting of nitrate reductase (NR) and nitrate (NO_3^-), Scheme 2(B). Fig. 2, curve (c), depicts the photocurrents (at λ = 420 nm; the potential of 0 V *vs*. SCE was applied to the photoelectrode) generated in the presence of different concentrations of NO_3^-. The saturated cathodic photocurrent is *ca*. 8-fold higher than in the presence of oxidized Cyt *c* only. Also, a non-amplified photocurrent is observed in the absence of NR, Fig. 2, curve (d), indicating that the biocatalyst is essential to amplify the photocurrent. The amplification of the anodic or cathodic photocurrents in the presence of LDH/lactate or NR/NO_3^-, respectively, is attributed to the biocatalytic regeneration of reduced Cyt *c* (with the LDH/lactate system) or of oxidized Cyt *c* (with the NR/NO_3^- system) at the CdS NP interfaces, respectively. These biocatalytic transformations eliminate the need for diffusional exchange of the active cofactors at the CdS NP surfaces, and preserve their high concentrations at the photoactive supports. It should be noted that the photocurrents reported for all of the systems are reproducible with an accuracy of $\pm 5\%$.

To conclude, the present study has demonstrated the control of the direction of the photocurrents generated by CdS NPs by means of the Cyt *c* added in different oxidation states. By the activation of secondary Cyt *c*-mediated biocatalytic processes the anodic or cathodic photocurrents were amplified.

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