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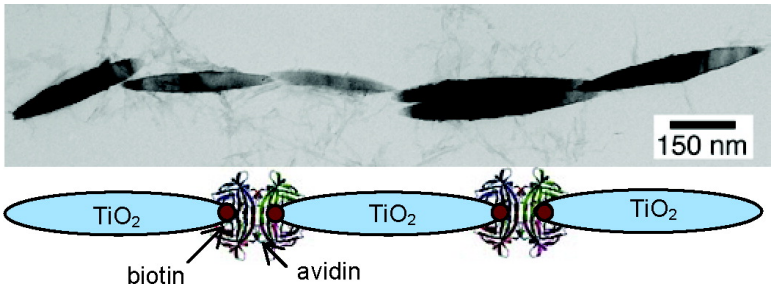

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Assembly and Charge Transfer in Hybrid TiO_2 Architectures Using Biotin−Avidin as a Connector

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Coupling size-dependent optical and electrochemical properties of inorganic nanoparticles with the functionality of biomolecules enables site-selective chemistries that take advantage of integrated properties of hybrids.[1] The functionalization of gold nanoparticles[2] and quantum dots[3] with biomolecules has enabled molecular recognition of oligonucleotides and optical communication between quantum dots and chemisorbed proteins.[4−6] The wiring of redox enzymes with gold nanoparticles has facilitated electrical communication between the biocatalyst and electrode.[7] We have recently developed hybrid nanocomposites which electronically link photoactive TiO_2 nanoparticles to DNA that can be used as a fingerprint of oligonucleotide hybridization.[8] The advantage of using TiO_2 nanocomposites lays in their efficient separation of photogenerated charges that enables redox chemical reactions with attached biomolecules. New challenges in the fabrication of functionalized protein−TiO_2 hybrids arise from coupling light-induced site-specific redox chemistry with conformation of protein. These hybrid architectures and ensuing chemistries can either utilize or alter protein functionality.

In titanium dioxide particulate nanocrystallites, the Ti atoms are hexacoordinated (octahedral) in the bulk, whereas they are pentacoordinated (square pyramidal) at the surface. Enediol ligands such as dopamine (DA) have a large affinity for these undercoordinated surface sites, restoring the Ti atoms to the octahedral coordination and forming irreversible ligand-to-particle charge-transfer complexes.[9] In these hybrid structures localized orbitals of surface attached ligands are electronically coupled with the delocalized electron levels from the conduction band of a TiO_2 semiconductor. As a consequence of this conformation, absorption of light by the TiO_2−dopamine (TiO_2/DA) system yields to the excitation of electrons from the chelating ligands directly into the conduction band of TiO_2 nanocrystallites, without transitioning through the enediol excited state. The photogenerated charge pairs separate over an extended distance (>20 Å), holes localize on the dopamine ligand, and electrons localize in TiO_2 nanoparticles. Our recent progress in the synthesis of TiO_2 nanocrystallites (particles, rods, tubes, sheets) and understanding of their surface structures[10] enable control of the surface sites in differently shaped nanocrystals. This control can be exploited for assembly of complex TiO_2 architectures composed of both axially symmetric and anisotropic crystalline nanomaterials. Taking advantage of the enediol ligands selectivity for Ti surface states, we conjugated biotin to TiO_2 nanocrystallites of different shapes (4.5 nm particles and ∼400 nm elongated rods) using dopamine as a bridging linker. Strong binding in the avidin−biotin system (association constant of 10^{15} M^{-1}) was exploited for the assembly of protein−TiO_2 hybrids and for studies of charge-transfer processes between metal oxide and avidin.

The conjugation of biotin and its analogue, LC-biotin, onto TiO_2 nanocrystallites was performed by the condensation reaction of amino groups on dopamine and *N*-hydroxy-succinimide on biotin derivatives. In the first step, the succinimidyl group on the end of the valeric acid chain of biotin was replaced with dopamine through its terminal amino group. Dopamine end-labeled biotin binds to the surface of TiO_2 nanoparticles through the bidentate complex of dopamine OH groups with undercoordinated Ti surface atoms. The formation of the bidentate complex of dopamine-end-labeled biotin with TiO_2 was demonstrated by the increase in absorption. The conjugation of biotin to TiO_2 was demonstrated using 4-hydroxy-azobenzene-2-carboxylic acid labeled avidin (HABA assay), and fluorescein isothiocyanate (FITC) labeled avidin (Supplement 1 in the Supporting Information).

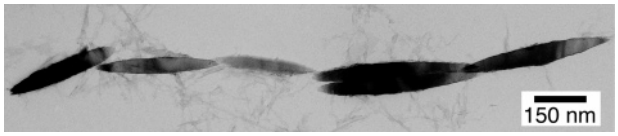

Figure 1. Transmission electron micrograph of the tip-to-tip assembled TiO_2 nanorods using biotin−avidin as the connector.

The assembling of TiO_2−protein hybrid architectures was performed using a procedure similar to the one previously described for gold nanorods.[11] One part of biotinylated TiO_2 nanorod solution, phosphate buffer pH 7, was mixed with the excess of avidin and incubated overnight. The unbound avidin was washed out by repeated centrifugation, decanting, and washing with water. The solution of concentrated TiO_2/DA−biotin−avidin was mixed with the solution of TiO_2/DA−biotin and incubated for a few hours. The resulting binding of avidin with biotin produces almost exclusively tip-to-tip assembly of TiO_2 rods. The number of attached rods depends on the ratio of concentrations (Supplement 2). For low ratio, doublets and, in some cases, triplets were observed. Increasing the concentration of added TiO_2/DA−biotin−avidin hybrids resulted in the formation of more complex structures, such as wirelike assembly, Figure 1. The tip-to-tip assembly of nanorods can be explained by the presence of specific surface sites at the tips of the nanorods. Analogous to the small TiO_2 particles (<20 nm), the high curvature of the surface at the tips of the nanorods gives rise to undercoordinated Ti atoms, promoting the binding of dopamine exclusively to these sites. The structure of these surface defect sites at the tips is such that it dominates the binding and, thus, chemical properties of TiO_2 nanorods.

The light-induced charge separation in avidin−TiO_2 hybrids is an initial step in manipulating conformation of avidin that can result in the changes of protein redox and binding properties and is, thus, exploited for nanomachines. The charge separation and distance-dependent charge transfer in the avidin−TiO_2 hybrids was studied with low temperature electron paramagnetic resonance (EPR) spectroscopy. EPR spectroscopy provides an unambiguous identification of the species involved in the charge separation processes by revealing changes in local symmetry and hyperfine couplings along the pathway of charge carriers. Using EPR we have previously identified $(Ti^{3+})_{latt}$ and dopamine$^+$ (with spin density on the pendant $CH_2-CH_2-NH_2$ side chain) as radical species formed upon

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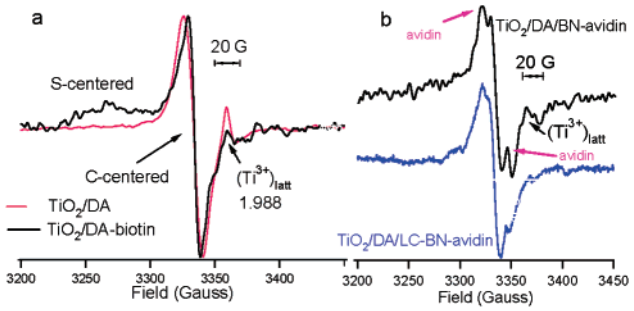
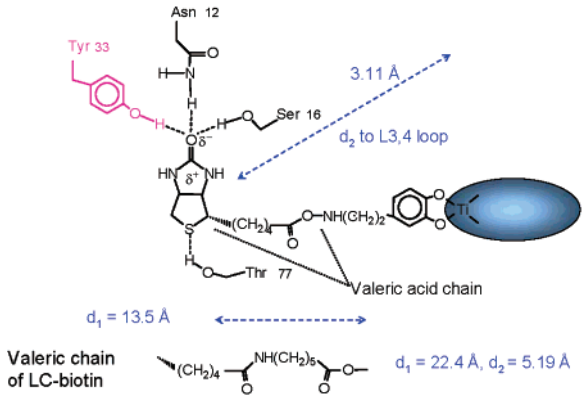


Figure 2. Normalized X-band EPR spectra obtained at 4.6 K after illumination (Xe 300 W lamp) of (a) TiO_2/DA (red line) and TiO_2/DA−biotin (black line) and (b) TiO_2/DA−biotin−avidin hybrids: black line corresponds to biotin, and blue line, to LC-biotin. BN abbreviates for biotin. Frequency 9.0 GHz.

Scheme 1. Schematic Representation of Binding of Avidin to TiO_2 Nanocrystallites via Biotin[a]



[a] The values for distances are Taken from ref 15. The scheme contains the valeric acid chain of LC-biotin, together with corresponding distances.

photoexcitation of the TiO_2/DA complex.[8,9] With biotin conjugated to the pendant side chain of dopamine, the photogenerated electrons and holes from TiO_2/DA separate further, holes localizing at biotin and electrons localizing at TiO_2, Figure 2a. The strong signal of the carbon-centered radical narrows from $\Delta H_{pp} = 16$ G (for DA) to a hyperfine coupling with a constant of 9.8 G (for DA-biotin). This feature is characteristic of charge localized probably at the thiophene ring of biotin.[12] It was suggested that one of the more feasible sites for oxidation of biotin is at the C_2 position of the thiophene which may result in ring scission in basic solution (pK = 9.9 for thiophene ring opening). On the other hand, the protonation of the C_2 intermediary radical in the presence of H^+ or $H_2PO_4^-$ ions occurs at the sulfur atom, with the formation of $=^+$SH.[13] The value for g-tensor of 2.045 agrees with the value for the sulfur cation radicals frequently observed upon radiolysis of organic cyclic monosulfides.[14] Thus, we assign this very weak signal to sulfur cation radicals produced under the steady-state conditions of our experiments at neutral pH in the presence of phosphate buffer.

The formation of TiO_2/DA−biotin−avidin hybrids arises from high affinity of avidin−biotin binding that involves multiple hydrogen bonds, van der Waals interactions between biotin and avidin, and the ordering of surface polypeptide loops that bury the biotin in the protein interior. The hydrogen-bonding network between the binding-site residues of avidin involves predominately the ureido oxygen of the biotin and, in a lesser extent, thiophene sulfur, Scheme 1.[15] When these hybrids were photoexcited, a new EPR spectral feature was observed indicating transfer of photogenerated holes from TiO_2 to avidin, Figure 2b. The line shape of EPR signal is characteristic of tyrosine radical having its spin density in the aromatic ring with 16 G hyperfine coupling to the β-methylene protons.[16,17] This suggests that oxidation of avidin occurs most probably at Tyr 33, because it forms a critical hydrogen bond with the biotin.[18] Tyrosine and tryptophan are two amino acids in avidin prone to oxidation,[19] tyrosine having a more negative redox potential being easier to oxidize. Exchanging biotin with LC-biotin resulted in decreased oxidation of avidin, confirming the site-specific charge-transfer reaction, Figure 2b and Supplement 3. In a complex between LC-biotin and avidin, the distance between dopamine and thiophene ring increases from 13.5 Å (biotin) to 22.4 Å (LC-biotin), and the L3,4 loop is disordered,[15] which all contribute to the larger distance between avidin and TiO_2.

The EPR spectra demonstrated that light-induced site-specific charge transfer from TiO_2 to avidin changes the redox state of protein. The research of the light-induced changes in avidin's functionality in binding biotin, as well as charge transfer across wirelike and other complex architectures, is underway.

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Supporting Information Available: The details on the synthesis and characterization of biotinylated TiO_2, assembly of nanorods, and EPR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5796.
(2) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757.
(3) Gerion, D.; Chen, F.; Kannan, B.; Fu, A.; Park, W. J.; Chen, D. J.; Majumdar, A.; Alivisatos, A. P. *Anal. Chem.* **2003**, *75*, 4766.
(4) Memedova, N. N.; Kotov, N. A.; Rogach, A. L.; Studer, J. *Nano Lett.* **2001**, *1*, 281.
(5) Willnard, D. M.; Carillo, L. L.; Jung, J.; Van Orden, A. *Nano Lett.* **2001**, *1*, 469.
(6) Medintz, I. L.; Clapp, A. R.; Mattoussi, H.; Goldman, E. R.; Fisher, B.; Nauro, J. M. *Nat. Mater.* **2003**, *2*, 630.
(7) Xiao, Y.; Patolsky, F.; Katz, E.; Hainfeld, J. F.; Willner, I. *Science* **2003**, *299*, 1877.
(8) Rajh, T.; Saponjic, Z.; Liu, J.; Dimitrijevic, N. M.; Scherer, N. F.; Vega-Arroyo, M.; Zapol, P.; Curtiss, L. A.; Thurnauer, M. C. *Nano Lett.* **2004**, *4*, 1017.
(9) Rajh, T.; Chen, L. X.; Lukas, K.; Liu, T.; Thurnauer, M. C.; Tiede, D. M. *J. Phys. Chem. B* **2002**, *106*, 10543.
(10) Saponjic, Z.; Dimitrijevic, N.; Tiede, D.; Goshe, A.; Zuo, X.; Chen, L.; Barnard, A.; Zapol, P.; Curtiss, L.; Rajh, T. *Adv. Mater.*, in press.
(11) Caswell, K. K.; Wilson, J. N.; Bunz, U. H. F.; Murphy, C. J. *J. Am. Chem. Soc.* **2003**, *125*, 13914.
(12) Rao, D. N. R.; Symons, M. C. R. *J. Chem. Soc., Perkin Trans. 2* **1983**, 135.
(13) Moorthy, P. N.; Hayon, E. *J. Org. Chem.* **1977**, *42*, 879.
(14) Bonazzola, L.; Michaut, J. P.; Roncin, J. *J. Chem. Phys.* **1985**, *83*, 2727.
(15) Pazy, Y.; Kulik, T.; Bayer, E. A.; Wilchek, M.; Livnah, O. *J. Biol. Chem.* **2002**, *277*, 30892.
(16) Barry, B. A.; El-Deeb, M. K.; Sandusky, P. O.; Babcock, G. T. *J. Biol. Chem.* **1990**, *265*, 20139.
(17) (a) Faller, P.; Rutherford, A. W.; Debus, R. J. *Biochemistry* **2002**, *41*, 12914. (b) Faller, P.; Goussias, C.; Rutherford, A. W.; Un, S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 8732.
(18) Livnah, O.; Bayer, E. A.; Wilchek, M.; Sussman, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5076.
(19) (a) Havran, L.; Billova, S.; Palecek, E. *Electroanalysis* **2004**, *16*, 1139. (b) Masarik, M.; Kizek, R.; Kramer, K. J.; Billova, S.; Brazdova, M.; Vacek, J.; Bailey, M.; Jelen, F.; Howard, J. A. *Anal. Chem.* **2003**, *75*, 2663.

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