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focus

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Nanoparticle- and nanorod-biomaterial hybrid systems for sensor, circuitry and motor applications*

I. Willner**
Institute of Chemistry, The Hebrew University of Jerusalem, Israel

Resum

Els sistemes híbrids biomolècula-nanopartícula (NP) serveixen com a sistemes funcionals per a la construcció de sensors, nanocircuits i instruments.

S'explica el desenvolupament de sensors, basats en NP funcionalitzats, mitjançant l'exemple del contacte elèctric de la glucosa oxidasa amb Au NP (1.4 nm) modificats amb el cofactor NAD i amb l'exemple de la detecció òptica de l'activitat de la telomerasa en cèl·lules de càncer utilitzant NP de CdSe-ZnS del tipus *core-shell*.

Es descriu l'ús d'enzims DNA per a la detecció amplificada de l'activitat de DNA/telomerasa. Una estructura hemina/G-quàdruplex serveix com a marcador biocatalític per a la detecció quimioluminescent o colorimètrica de l'activitat de DNA/telomerasa.

La utilització de NP per a nanocircuits i instruments es presenta mitjançant la polimerització de la G-actina funcionalitzada amb Au NP i el posterior creixement dels filaments per a la generació contínua dels nanofilaments de Au. La polimerització seqüencial de la G-actina funcionalitzada amb Au NP produeix nanofilaments amb funcions de motor. Els filaments immobilitzats sobre una interfície de miosina es comencen a moure mentre s'afegeix l'ATP com a fons d'energia. Aquests nanoobjectes es mouen sobre la superfície a una velocitat que correspon a 250 nm × sec^{-1}.

Un altre aspecte de nanobiotecnologia inclou l'acoblament de les biomolècules amb nano- i microelements per a donar sistemes funcionals híbrids. S'estudia aquest aspecte seguint la ruptura de les seqüències específiques del dúplex d'ADN funcionalitzat amb partícules magnètiques sobre les bigues voladisses mitjançant l'endonucleasa Mse I en presència d'un camp magnètic extern.

Paraules clau: Nanopartícules, quantum dots, nanofilaments, nanobiotecnologia, monocapa biosensora, elèctrode, enzim, DNA, DNAzim, partícules magnètiques, endonucleasa, quimioluminescència, *beacon*.

Abstract

Biomolecule-nanoparticle (NP) hybrid systems provide functional assemblies for the construction of sensors, nanocircuitry and devices.

The development of sensors based on functionalized NPs will be exemplified with the electrical contacting of glucose oxidase with Au NPs (1.4 nm) functionalized with the FAD cofactor, and with the optical detection of telomerase activity in cancer cells using CdSe-ZnS core-shell NPs.

The use of DNAzymes for the amplified detection of DNA/telomerase activity is described. A hemin/G-quadruplex structure provides a biocatalytic label for the chemiluminescent or colorimetric detection of DNA/telomerase activity.

The use of biomolecule-NP hybrid systems for nanocircuitry and devices is addressed by the polymerization of Au NPs-functionalized G-actin followed by the catalytic enlargement of the filaments to generate continuous, conductive Au-nanowires. Sequential polymerization of Au-NP-modified G-actin and G-actin yields patterned nanowires with motor functions. The immobilization of the filaments on a myosin interface results in the motility of the filaments upon addition of the ATP fuel. The nano-objects move on the surface at a speed corresponding to 250 nm·sec^{-1}. A further facet of nanobiotechnology involves the coupling of biomolecules with nano/micro elements to functional hybrid systems. This aspect is discussed by following the sequence-specific scission of a duplex DNA functionalized with magnetic particles on a cantilever by the endonuclease Mse I in the presence of an external magnetic field.

Keywords: nanoparticles, quantum dots, nanowires, nanobiotechnology, biosensing monolayer, electrode, enzyme, DNA, DNAzyme, magnetic particles, endonuclease, chemiluminescence, beacon.

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**Author for correspondence: Itamar Willner. Institute of Chemistry, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel. Tel. 972-2-6585272. Fax: 972-2-6527715. Email: willnea@vms.huji.ac.il

Biomaterials, such as enzymes, antibodies or nucleic acids and metallic or semiconductor nanoparticles (NPs) exhibit similar dimensions. The combination of these two kinds of materials yields hybrid systems of new functionalities [1-3]. The present article discusses and presents some functions of biomolecule-NPs hybrid systems, and will highlight the utility of these systems for biosensing, nanocircuitry and the formation of nanodevices.

Redox enzymes lack direct electrical contact with electrodes. This originates from the spatial separation of the redox-center from the electrode by the insulating protein assembly. Gold NPs modified with an N-hydroxysuccinimide tethering unit was used [4] as electrical contacting unit of the enzyme glucose oxidase, GOx, Figure 1. The cofactor N^6-(2-aminoethyl)-flavin adenine dinucleotide (1) was covalently linked to a Au-NP, and apo-GOx was reconstituted on the cofactor-functionalized NP. The resulting Au-NP/enzyme hybrid was then linked to an Au-electrode using different dithiols (2)-(4) as bridges. Figure 2(A) shows the cyclic voltammograms generated by the enzyme-modified electrode, in the presence of variable concentrations of glucose. The electrocatalytic currents increase as the concentration of glucose are elevated, and the appropriate calibration curve was extracted, Figure 2(B).

The electrocatalytic currents imply that the Au-NPs electrically contact the enzyme with the electrode and activate the biocatalyst towards the oxidation of glucose. From the saturation current that is observed, I_{max} = 460 μA·cm^{-2}, and knowing the anzyme surface coverage, 1 × 10^{-12} mole·cm^{-2}, the electron transfer turnover rate was calculated to be 5000 s^{-1}. This is an unprecedented effective electrical contacting, far higher than the turnover rate of the enzyme with its native electron acceptor, oxygen (ca. 700 s^{-1}). This effective electrical contacting has important consequences on the properties of the enzyme-electrode as a biosensor, since the amperometric output of the system turns to be insensitive to oxygen or other interfering substances, such as ascorbic acid or uric acid. This concept was further developed to electrically contact GOx with electrode supports by other nano-assemblies such as carbon nanotubes [5] or supramolecular rotaxane systems [6].

For example, carbon nanotubes (CNTs) were assembled on Au-electrodes, and the amino-FAD cofactor, (1), was covalent-

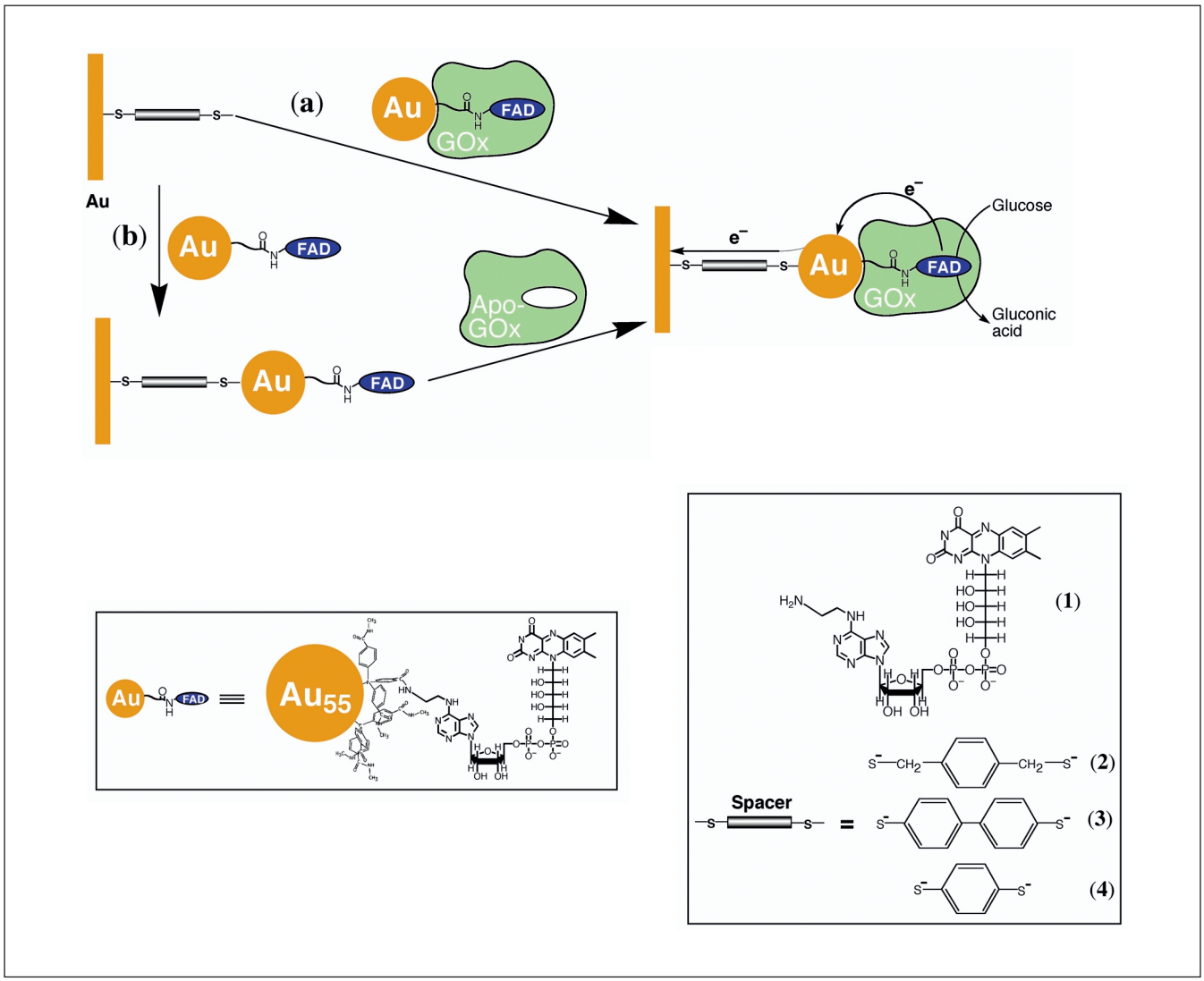

Figure 1. Electrical contacting of GOx by the assembly of GOx reconstituted on a Au-NP associated with a Au-electrode for the bioelectrocatalyzed oxidation of glucose.

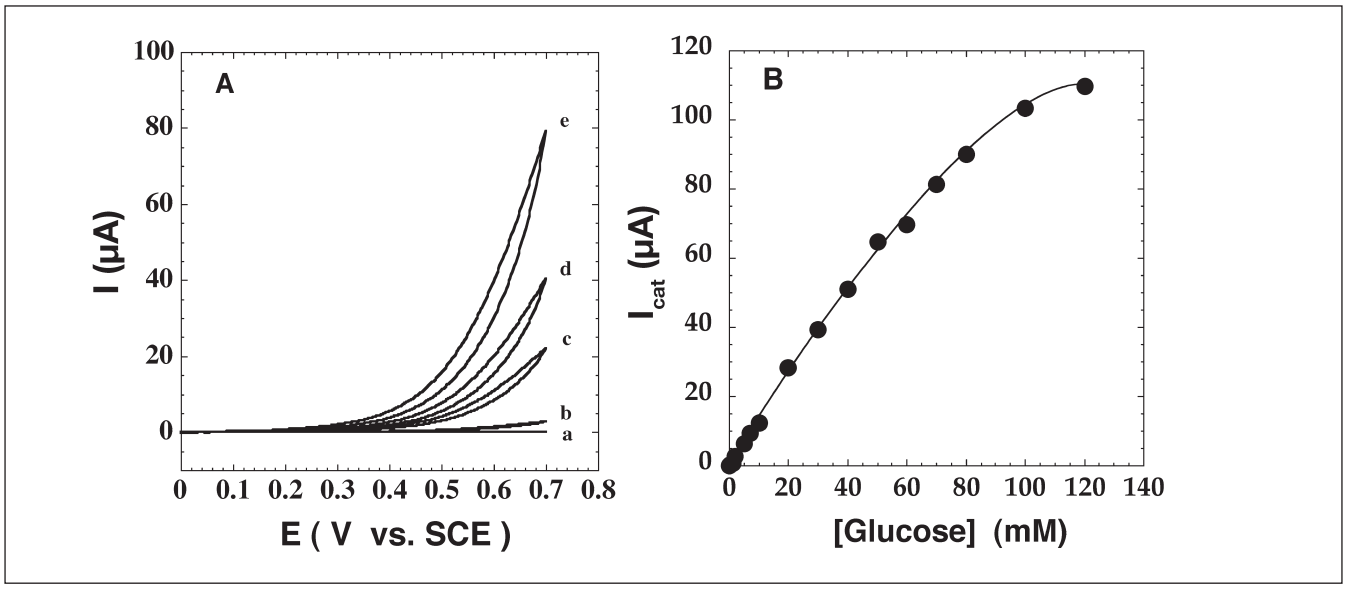

Figure 2. (A) Cyclic voltammograms corresponding to the bioelectrocatalyzed oxidation of different concentrations of glucose by the GOx-Au-NP-modified electrode. Glucose concentrations correspond to: (a) 0 mM, (b) 1 mM, (c) 10 mM, (d) 20 mM, (e) 50 mM. Data recorded in 0.1 M phosphate buffer, pH = 7, 35°C, scan rate 5 $mV \cdot s^{-1}$. (B) Calibration curve corresponding to the amperometric responses of the electrode at E = 0.6 V vs. SCE at variable concentrations of glucose.

ly linked to the ends of the carbon nanotubes, Figure 3. Apo-GOx was then reconstituted on the cofactor-functionalized CNTs [7]. Systems consisting of CNTs of controlled lengths were employed in this study. Figure 4(A) shows the cyclic voltammograms of the enzyme-modified electrode consisting of CNTs with a length of 25-30 nm, in the presence of variable concentrations of glucose. The observed electrocatalytic anodic currents indicate that the CNTs electrically contact GOx with the electrode support. From the saturated anodic current we estimate an electron transfer turnover rate of ca. 4500 s^{-1} for the system, implying an effective electrical contact. Figure 4(B) shows the effect of the CNTs length on the resulting currents. Evidently, as the linking CNTs are longer, the bioelelectrocatalytic anodic currents are lower, a result, that at a first

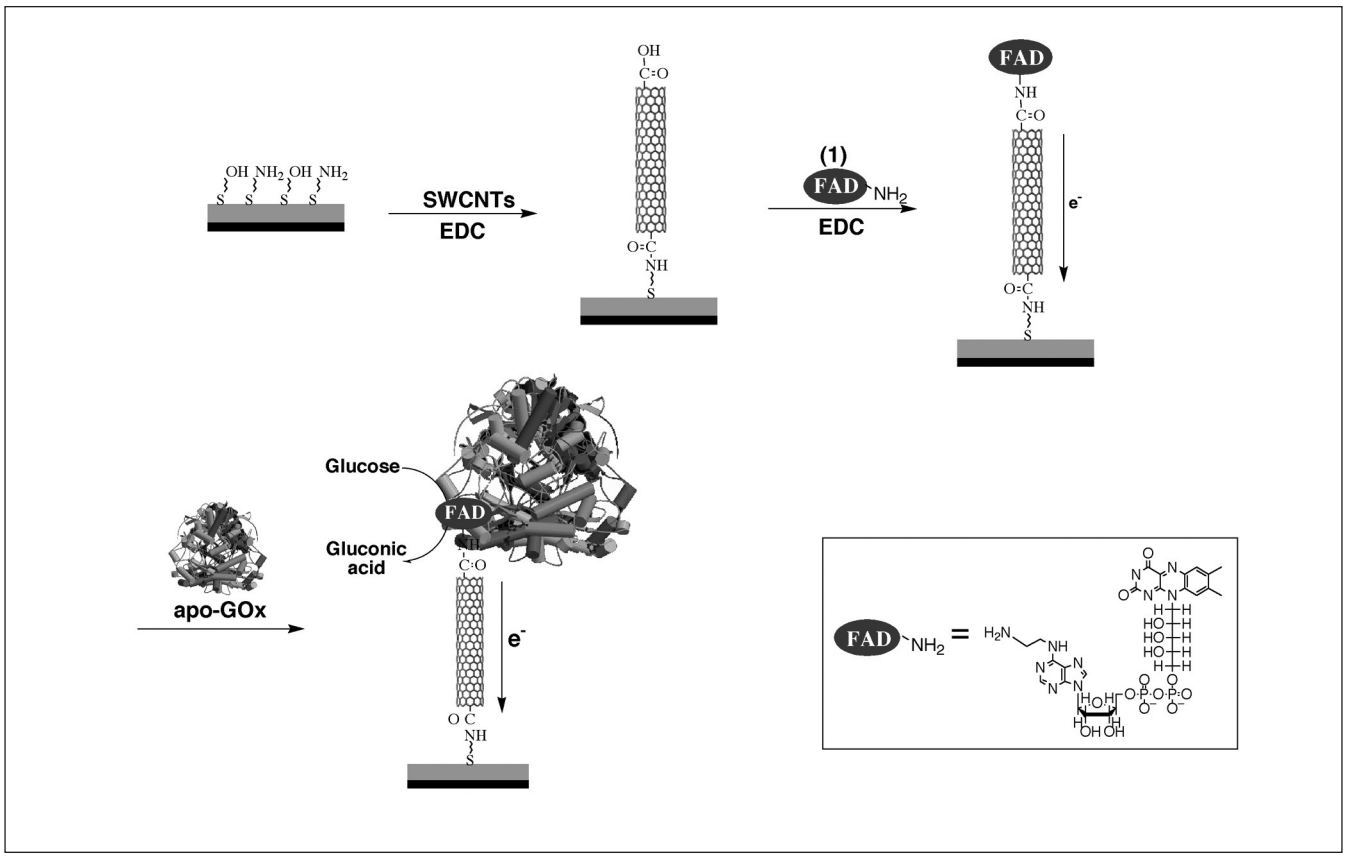

Figure 3. Assembly of the glucose oxidase-reconstituted CNT bioelectrocatalytic electrode.

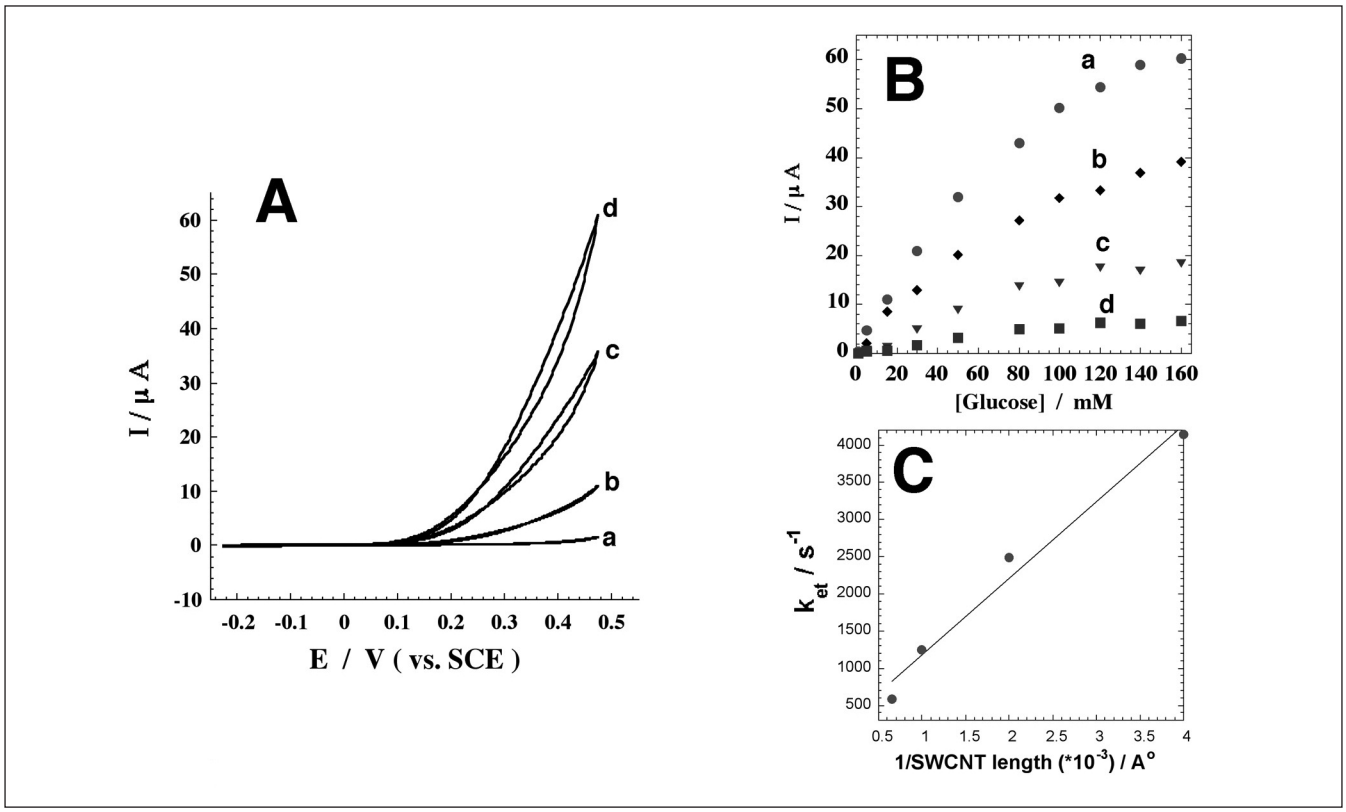

Figure 4. (A) Cyclic voltammograms corresponding to the bioelectrocatalyzed oxidation of different concentrations of glucose by the GOx/CNT (25 nm) electrode. Glucose concentrations correspond to: (a) 0 mM; (b) 20 mM; (c) 60 mM; (d) 160 mM. (B) Amperometric responses corresponding to the bioelectrocatalyzed oxidation of variable concentrations of glucose using GOx/CNT electrodes consisting of CNTs of different lengths: (a) 25nm; (b) 50 nm; (c) 100 nm; (d) 150 nm. (C) Analysis of the turnover rates of the GOx/CNT electrodes as a function of 1/L (L = length of CNT).

glance, contradicts the ballistic electrical conductivity properties of CNTs. The observed results were rationalized by the following explanations: (i) The results demonstrate that CNTs electrically contact the redox enzyme across long distances, albeit the electrical contact (transport rate of electrons) is retarded as the CNTs are longer. (ii). The length dependence of the electrical contacting efficiency of the enzyme was attributed to defect sites present in the CNTs. The CNTs were prepared by the oxidative scission of long CNTs followed by their chromatographic separation and functionalization and fractionation into their respective sizes. This synthetic procedure did not only introduce carboxylic acid residues at the end of the CNTs (functionalities that are essential for the assembly of the bioelectrocatalytic construct), but introduced the carboxylic acid units and other oxidized residues at the sidewalls of the CNTs. These oxidation sites perturb the conjugation of the graphite sidewalls and act as defects. The contact of the transported charge with a defect site requires its back scattering and the need to find an alternative fully conjugated path. As the probability for defect sites increases with the length of the CNTs, the back scattering effect and the retardation in the charge transport should be enhanced with the lengths of the CNTs connector units. Indeed, this phenomenon was theoretically treated, and a linear relation between the charge transport rates and 1/L (L = length of the CNTs) was predicted. Figure 4(C) shows the analysis of the experimental turnover rates extracted from Figure 4(B) for the different lengths of the CNTs according to the theoretical paradigm. One may see that the linearity of the plot supports the mechanism for electron transport and electrical contacting by the CNTs.

The unique optical properties of semiconductor NPs were used for the optical detection of DNA or to follow telomerase activity in cancer cells [8]. The telomers are nucleic acid segments tethered to the chromosomes. They protect the chromosomal DNA from environmental erosion, and are shortened upon cell proliferation. At a certain degree of shortening, the telomers trigger the termination of the cell's life cycle. In certain cells there is an accumulation of the telomerase enzyme. Telomerase is a ribonucleoprotein that constantly synthesizes the telomer repeat units [9,10]. As a result, the cells are transformed to immortal cancer or malignant cells [11]. Indeed, elevated amounts of telomerase were detected in over 95% of different cancer cells and the enzyme is considered as a versatile marker for cancer, cells [12]. Figure 5 outlines the principle of applying CdSe-ZnS core-shell NPs for the optical detection of telomerase activity. A thiolated nucleic acid, (5), recognized by telomerase was linked to the CdSe-ZnS particles. The modified particles were then interacted with the HeLa cancer cell extract, which included telomerase, in the presence of the dNTP mixture which included Texas Red-modified dUTP, (6). Telomerase induced the telomerization process of the primer (5), in analogy to the biological process occurring in the cancer cell. This process introduces, however, the Texas-Red dye as label into the telomer units. The dye is selected in such a way that upon photoexcitation of the semiconductor quantum dot, fluorescence resonance energy transfer (FRET) to the dye occurs,

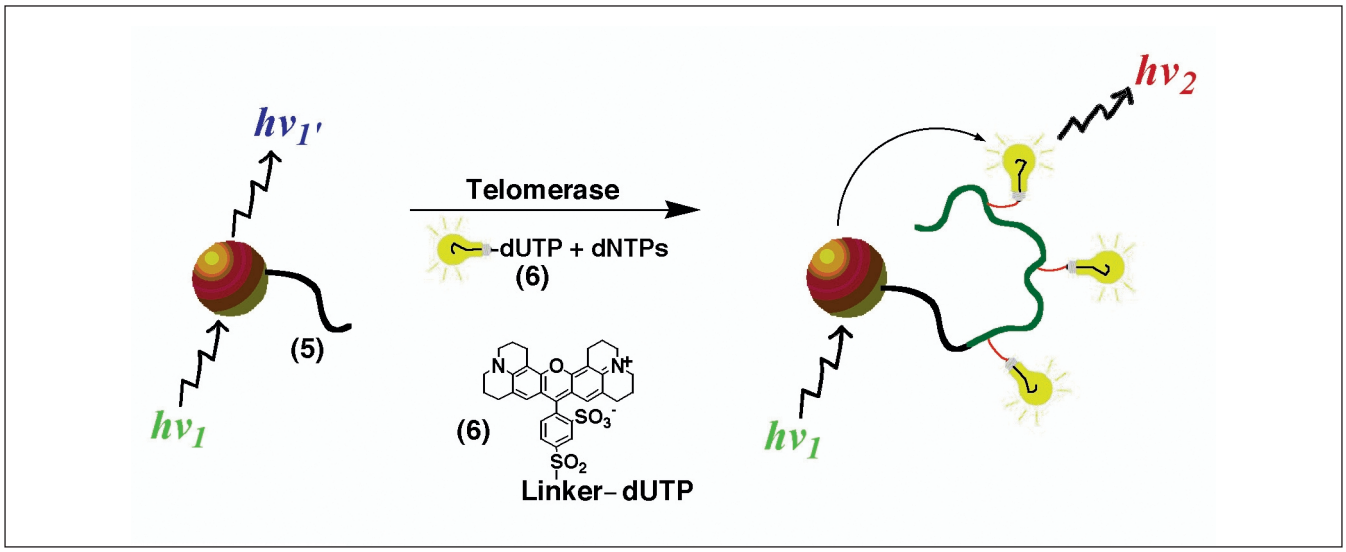

Figure 5. Optical detection of telomerase activity by the use of semiconductor quantum dots.

resulting in the activation of the dye fluorescence. Since the FRET process is dependent on the intimate contact between the donor and energy acceptor (NP and dye, respectively), only the dye units incorporated into the telomers will be photonically stimulated by the NPs. Figure 6(A) shows the fluorescence features of the system as telomerization proceeds. The fluorescence intensity of the CdSe-ZnS NPs at λ = 560 nm decreases as telomerization proceeds, and simultaneously the fluorescence of Texas Red at λ = 610 nm is intensified, implying that as the telomerization is prolonged, higher amounts of the dye are incorporated in the telomer units. A powerful confirmation that telomerization occurred in the CdSe/ZnS NPs is the AFM image shown in Figure 6(B). Two telomer chains, ca. 300 nm each, are linked to the central NP, suggesting that ca. 1000 bases were incorporated into the primer (5) as a result of the telomerization process.

One of the major challenges in developing DNA sensors relates to the sensitivity of the resulting detection system. One means to enhance the sensitivity process is the development of an amplification path. Usually, the amplification of a biosensing process is achieved by the conjugation of a catalytic label to the biorecognition complex. The catalyst, bound to a single recognition site, transforms numerous molecules of the substrate to the product within a unit of time, and thus the electronic or optical detection of the products amplifies the single recognition events, yet their disadvantage is the encountered non-specific binding of the proteins to the sensing interface. We introduced a catalytic label based on catalytic nucleic acids, and DNAzymes, for the analysis of DNA, and specifically, of telomerase activity. The advantages of using catalytic nucleic acids as labels for analyzing DNA rests on the fact that electrostatic repulsions between the DNA and the catalyst prevent non-specific adsorption phenomena, yet the binding of the catalyst to the DNA recognition complex is a result of specific hybridization complementarity. We describe here two general methods to amplify the detection of telomerase activity by DNAzyme units. DNAzymes can be prepared for a variety of reactions using the SELEX protocol [13,14]. One of the reported DNAzymes is a G-quadruplex generating nucleic acid,(7), which was formed to intercalate hemin (8). The resulting hemin-quadruplex complex exhibited peroxidase-like activi-

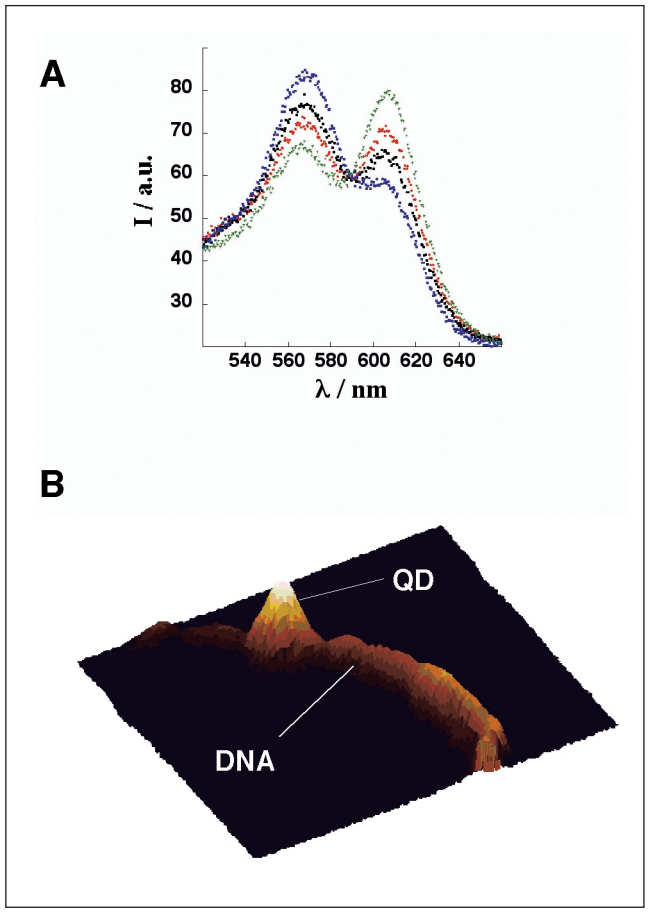

Figure 6. (A)Fluorescence spectra of the (5)-functionalized CdSe-ZnS quantum dots upon telomerization of (5) in the presence of HeLa cells extract (10,000 cells) in the presence of dNTP and (6) for different time intervals: before addition of telomerase; and after 10, 30 and 60 minutes; in the presence of telomerase before the addition of dNTPs and Texas Red-dUTP. (B) AFM image of the CdSe/ZnS-telomer conjugate.

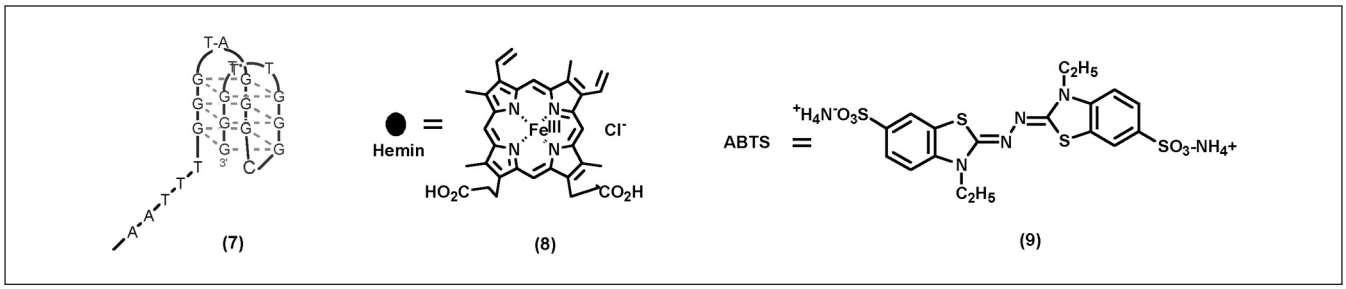

Figure 7. A hemin/G-quadruplex that catalyzes the oxidation of ABTS or stimulates the generation of chemiluminescence.

ties, and it was found to catalyze the oxidation of ABTS, (9), to the colored dye $ABTS^{\bullet+}$ in the presence of H_2O_2 [15]. We found that the hemin/G-quadruplex catalyzes the generation of chemiluminescence in the presence of H_2O_2/luminol [16], Figure 7. The application of the DNAzyme for the amplification of DNA or the analysis of telomerase activities is described here in two configurations: In one system [17], Figure 8(A), a primer (5), recognized by telomerase, is assembled on a Au-electrode. Telomerization of the primer in the presence of the dNTPs mixture and the Hela cancer cells

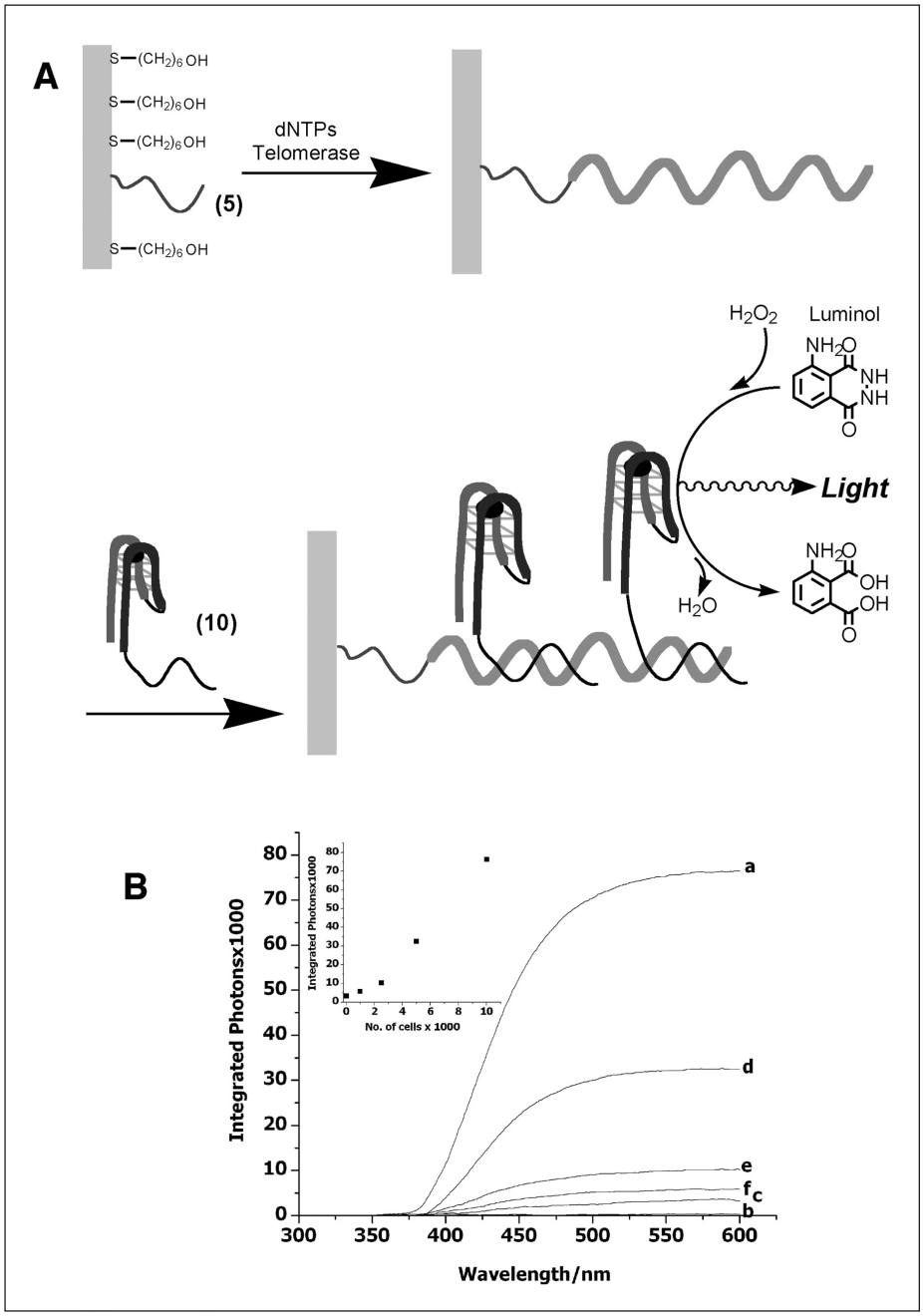

Figure 8. (A) Chemiluminescence detection of telomers using the hemin-G-quadruplex tethered to the nucleic acid complementary to the telomer repeat units as amplifying catalyst. (B) Integrated light intensities observed upon analyzing: (a) 10,000 HeLa cells; (b) 10,000 heat-treated HeLa cells; (c) 10,000 cells in the absence of the DNAzyme; (d) to (f) Analysis of 5,000, 2,500, 1,000 HeLa cells, respectively. The DNAzyme, 2.5 μM, is applied in all systems unless otherwise stated. Inset: Calibration curve corresponding to the chemiluminescence detection of HeLa cells.

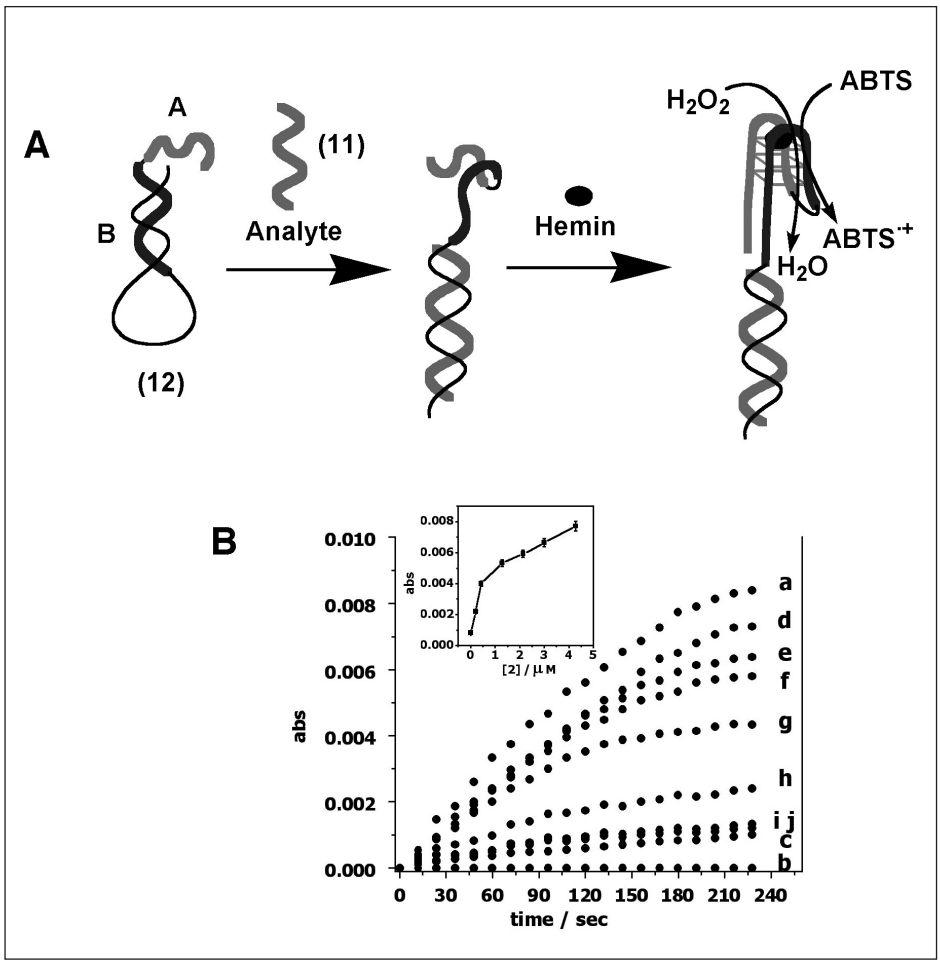

Figure 9. (A) Analysis of telomerase activity by a functional DNAzyme beacon system. (B) Absorbance changes as a result of the oxidation of ABTS upon analyzing the DNA (11) with the catalytic beacon (12): (a) 4.3 μM of (11); (b) in the absence of (11); (c) in the presence of (11) and hemin, but without (12); (d) to (h) in the presence of 3.0, 2.15, 1.30, 0.40 and 0.20 M of (11); (i) and (j) analyzing single base mismatches of (11), 4.3 μM.

extract results in the telomers, consisting of constant repeat units. Hybridization of the nucleic acid (10), that includes a sequence complementary to the telomer repeat units, that is tethered to the hemin/G-quadruplex DNAzyme units, yields a catalytically active interface that stimulates the generation of chemiluminescence in the presence of H_2O_2/luminol. Figure 8(B) shows the integrated light intensity emitted by the system upon analyzing different concentrations of Hela cells, and the respective calibration curve. The system is capable of analyzing the Hela cancer cells with a sensitivity limit corresponding to 500-1000 cells. The second DNAzyme-based configuration for the amplified analysis of DNA is depicted in Figure 9(A). A catalytic beacon for the amplified detection of DNA, (11), was tailored, where the single strand loop of the hairpin structure of the beacon, (12), is complementary to the analyte DNA, (11). The stem double stranded pact includes a segment B that is tethered to segment A, where the composite segments A + B, provide the sequence to self assemble into the DNAzyme in the presence of hemin [18] Upon the hybridization of (12) with (11), the beacon is opened, and the DNAzyme assembles into the bioreactive unit that catalyzes the oxidation of ABTS to $ABTS^{\bullet+}$ by means of H_2O_2. Figure 9(B) shows the spectroscopic analysis of different concentrations of the DNA (11) by means of the pseudo-DNAzyme (12).

The use of biomolecule-nanoparticle hybrid systems has invaluable importance for the future development of nanostructures, and specifically, functional nanostructures [1-3]. The miniaturization of objects by optical lithography reaches its theoretical limits. It is well accepted that an alternative "bottom-up" fabrication of objects may be a feasible approach to overcome these size limitations for the synthesis of objects. The use of biomolecules as templates to generate nanostructures is a promising and viable method to construct nanostructures. There are several reasons why biomolecules (proteins or DNA) attract our imagination to fabricate nano-objects: (i) Mother Nature provides ingenious functional protein structures. Furthermore, genetic engineering of proteins yield man-made biomolecules of predesigned structures and functions. Also, polypeptides of pre-designed composition may be synthesized, and these may self-assemble into nano-tubes or nano-fibers that may act as templates for the synthesis of nano-objects. Similarly, DNA of predesigned base sequences and ingenious 2D or 3D shapes can be synthesized. These nucleic acids provide unique environments with base-addressability for the assembly of materials. (ii) Mother Nature provides a battery of enzymes that hydrolyze proteins or DNA, couple nucleic acids (ligase), or replicate DNA strands (polymerase). These enzymes are biocatalytic nano-tools for the manipulation of the biomolecules and the effective generation of the desired templates. (iii) Functional units present in the protein/DNA may be used as reaction sites or catalytic centers for the chemical growth of the nano-objects. For example, the binding of cations to peptide bonds

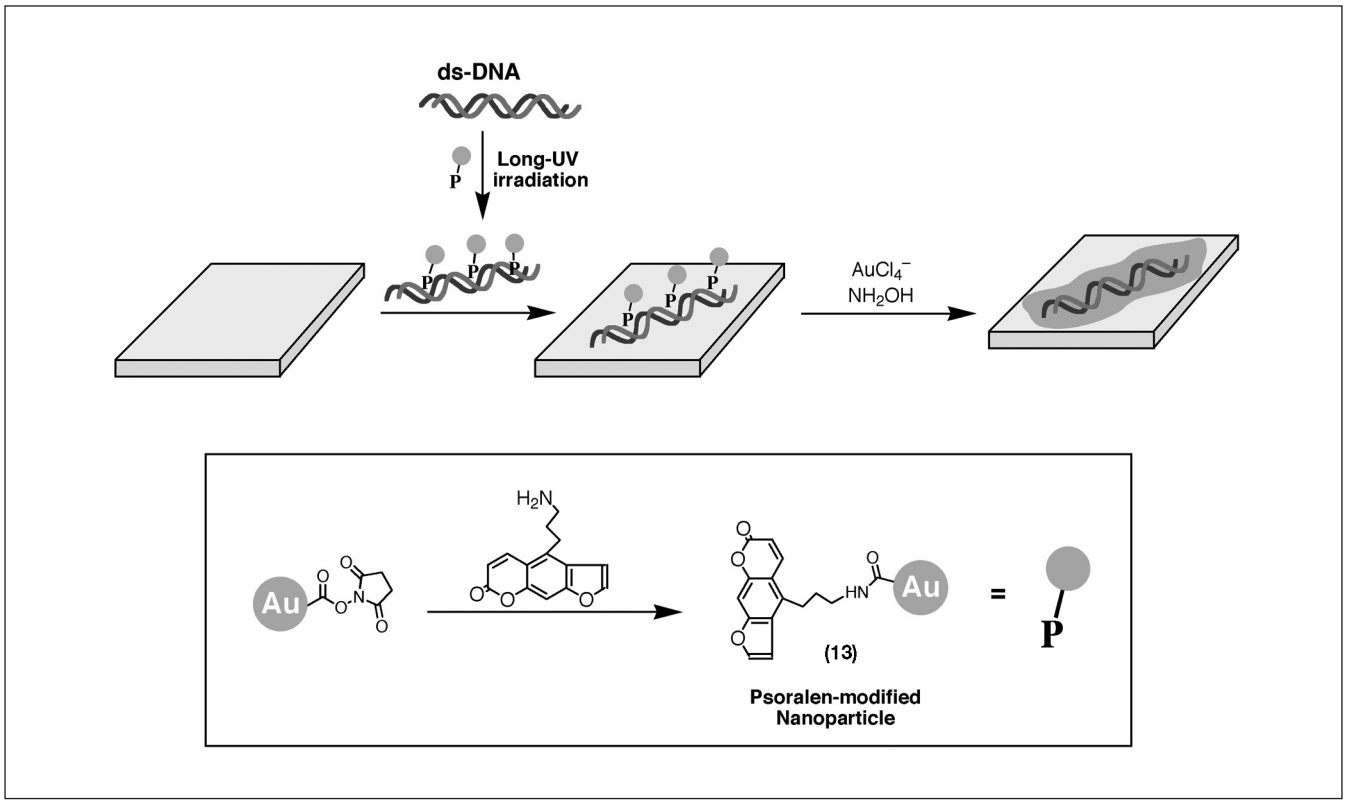

Figure 10. Assembly of a Au NP wire on a DNA template.

of proteins or phosphate sites of DNA, or the intercalation of chemical components in DNA base-pairs represent a means to concentrate chemical reactants on the biomolecular templates. (iv) The formation of protein-DNA complexes, such as RecA/DNA or protein-aptamer complexes, allows the formation of composite biomolecular assemblies with addressable domains. (v) DNAzymes constructed into the DNA templates may be used as active catalysts for the synthesis of the nanoobjects.

Figure 10 depicts a method to fabricate Au-NP nanowires. The Au-NPs (1.4 nm) are functionalized with the psoralen-intercalator to yield the hybrid intercalator-functionalized particles, (13). The modified Au NPs were intercalated into polyA/poly T double-stranded DNA and fixed to the DNA template by the photochemical crosslinking of the intercalator to the thymine units [19]. Figure 11 shows the AFM image of the resulting NP nanowire. Wires exhibiting a length of 600-700 nm and height corresponding to 4 nm are formed.

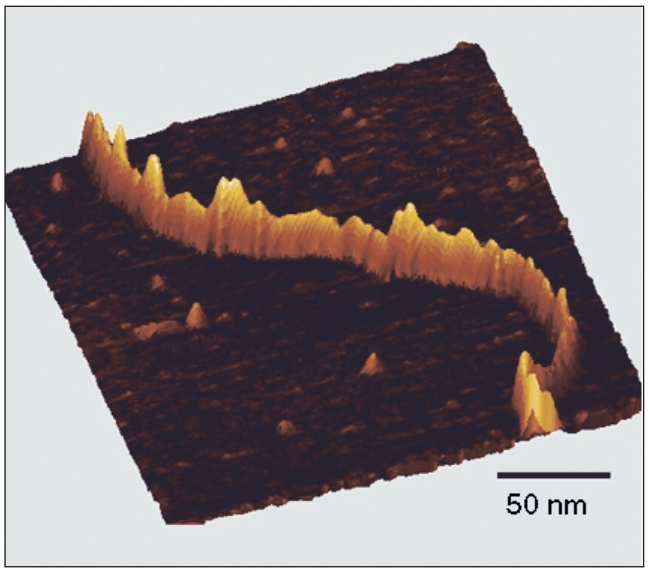

Figure 11. AFM image of psoralen-functionalized Au-NPs intercalated into polyA/poly T and photochemically crosslinked.

The use of proteins as templates for the synthesis of nanowires and functional nanodevices will be addressed by the application of the motor-protein actin as the template for generation of nanowires and a nanotransporter device [20]. The G-actin monomer was modified with the maleimide-functionalized 1.4 nm Au NPs. This reaction yields a hybrid consisting of a single Au NP attached to a G-actin unit (linked by a cysteine bridge). The Au NP-functionalized G-actin was polymerized in the presence of ATP and crosslinked with phalloidin. The resulting Au NP-modified filaments, Figure 12(A), were then enlarged with $AuCl_4^-/NH_2OH$ to yield continuous Au wires that were 1 μm to 2μm long with a width and height of 80-110 nm. Figure 13(A) shows the AFM image of the resulting metallic nanowire. Figure 13(B) shows the SEM image of the gold wire positioned between two microelectrodes. The metal nanowire reveals a resistance of 300 ± 40Ω and exhibits conductivity features of bulk gold.

Figure 12(B) shows the scheme for the generation of a patterned actin-Au-nanowire-actin hybrid system. In the first step, the Au NP-functionalized G-actin units were polymerized in the presence of ATP/phalloidin. The resulting filaments were then reacted with unsubstituted G-actin to yield actin segments on the two ends of the Au-NP-actin wire. The Au NPs were then enlarged by the catalytic deposition of gold on the NPs using

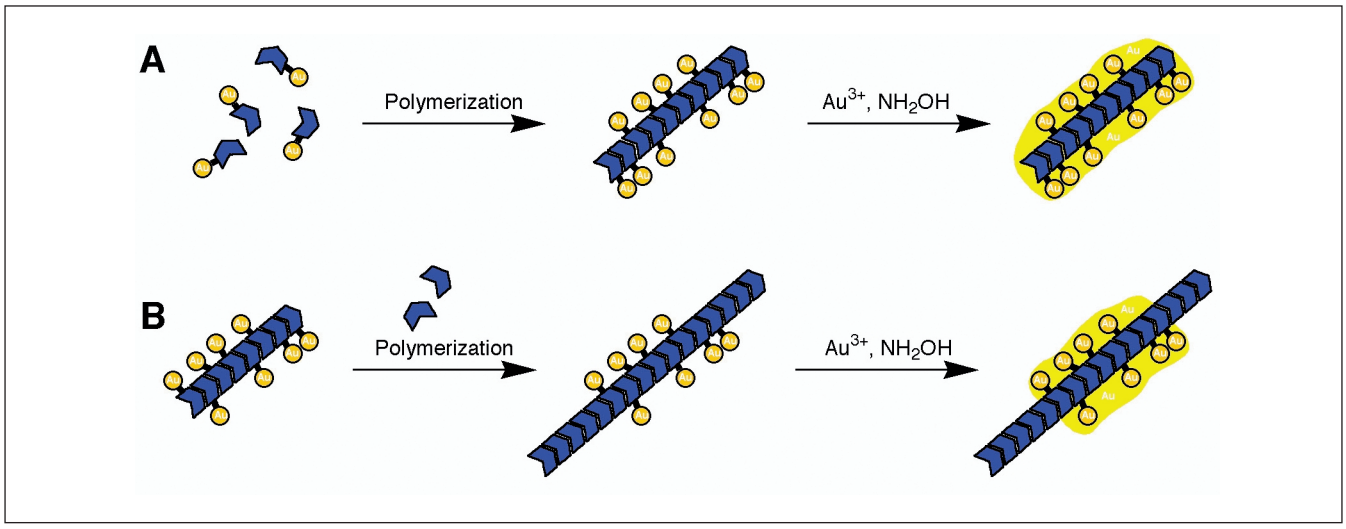

Figure 12. (A) The fabrication of a Au nanowire on an actin template. (B) The synthesis of an actin-Au-nanowire-actin hybrid system.

the $AuCl_4^-/NH_2OH$ system. Figure 14 shows the image of the resulting nanostructure that consists of two F-actin motor proteins separated by a gold wire. This nano-object was deposited on a myosin layer deposited on a glass support. Upon the addition of ATP the motility of the nanostructures was observed, and the wires moved on the surface at a speed corresponding to 250 ± 50 nm·sec^{-1}.

A further facet of future nanobiotechnology involves the coupling of biomolecules, specifically DNA, with nano/micro objects such as cantilevers, and the use of their hybrid structures for biosensing or nanoscale machinery. For example, the electrostatic repulsive interactions between double-stranded DNA units generated by hybridization of the nucleic acids at cantilever surfaces, resulted in a surface stress, and the mechanical deflection of the cantilever [21]. We have extended the concept [22] by demonstrating the amplified detection of biorecognition complexes using biomolecule-functionalized magnetic particles as tags and an external magnetic field, Fi-

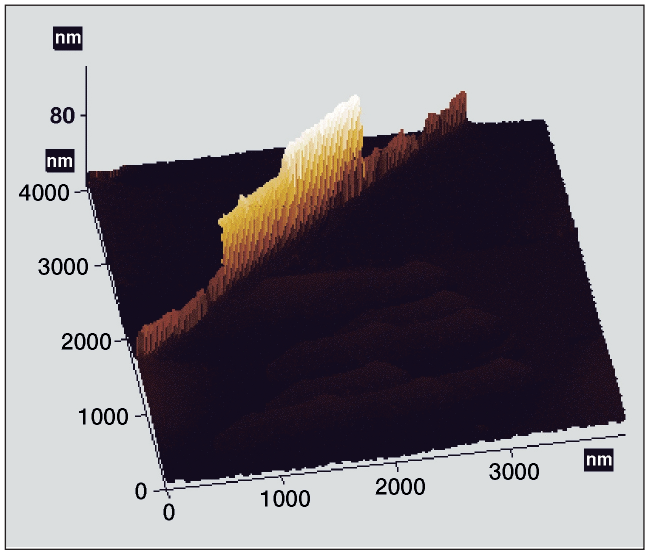

Figure 14. AFM image of the actin-Au nanowire-actin nanomotor.

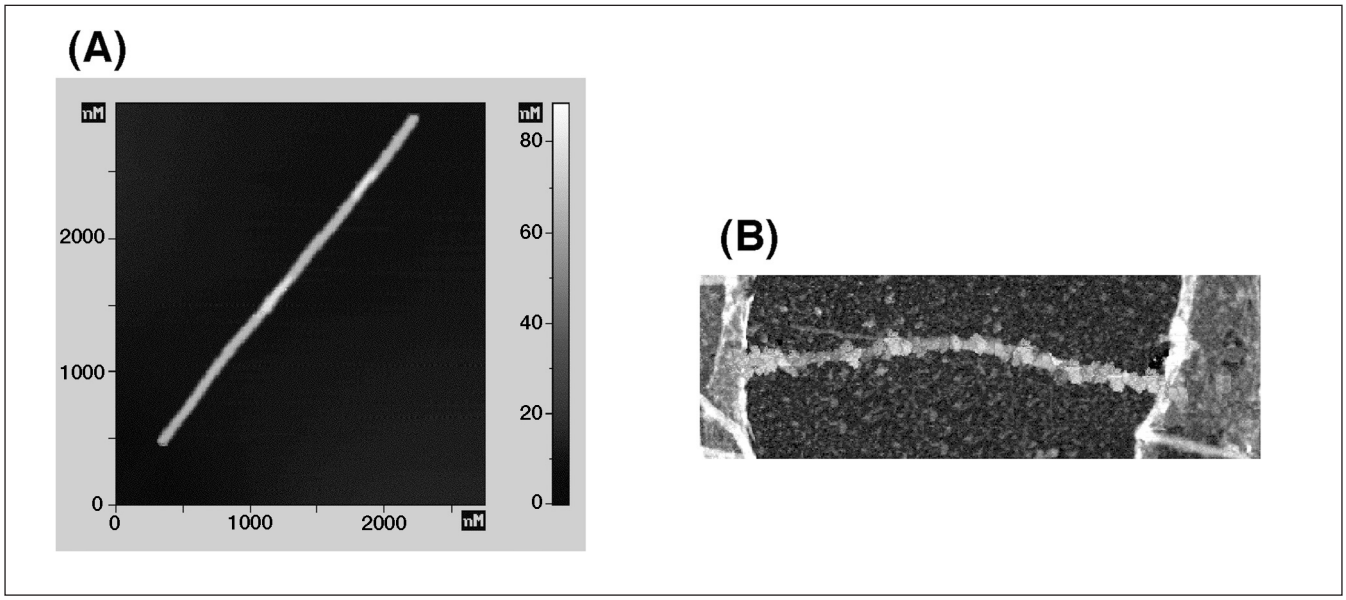

Figure 13. (A) AFM image of Au-nanowire on the actin template. (B) SEM image of the Au-nanowire on the actin template that bridges and contacts two Au microelectrodes.

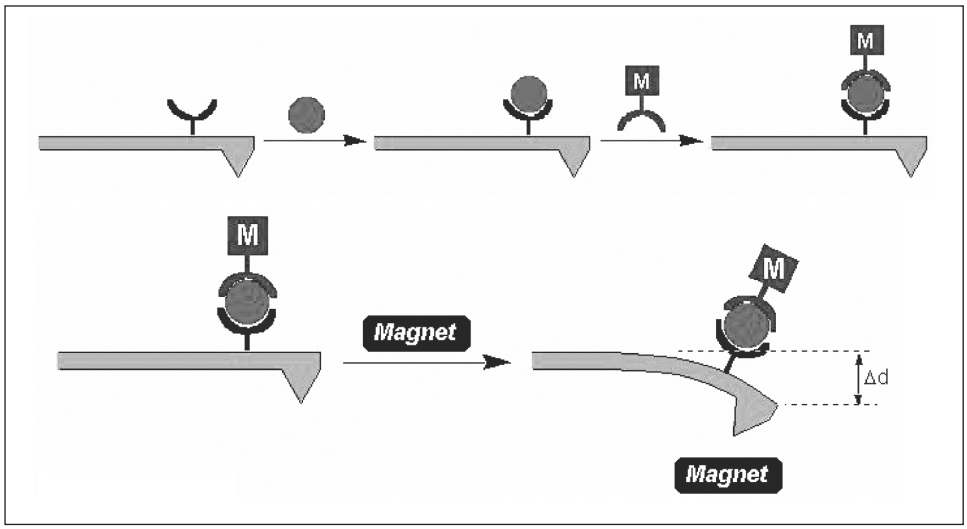

Figure 15. Amplified analysis of biorecognition events on a cantilever using functional magnetic particles and an external magnet.

gure 15. The biorecognition complex is generated on the cantilever support, and subsequently, the biomloecule-magnetic particle tag is coupled to the complex, and the cantilever is deflected under the application of the external magnetic field. This concept was recently extended to sense the sequence-specific scission of nucleic acids by endonucleases [23]. This is exemplified here with the specific scission of a double-stranded DNA by the endonuclease Mse I. The thiolated nucleic acid (14) was assembled on an Au-coated cantilever, and the (15)-functionalized magnetic particles were hybridized on the surface, see Figure 16 (A). The resulting double-stranded DNA includes the specific sequence for scission by Mse I (marked in Figure

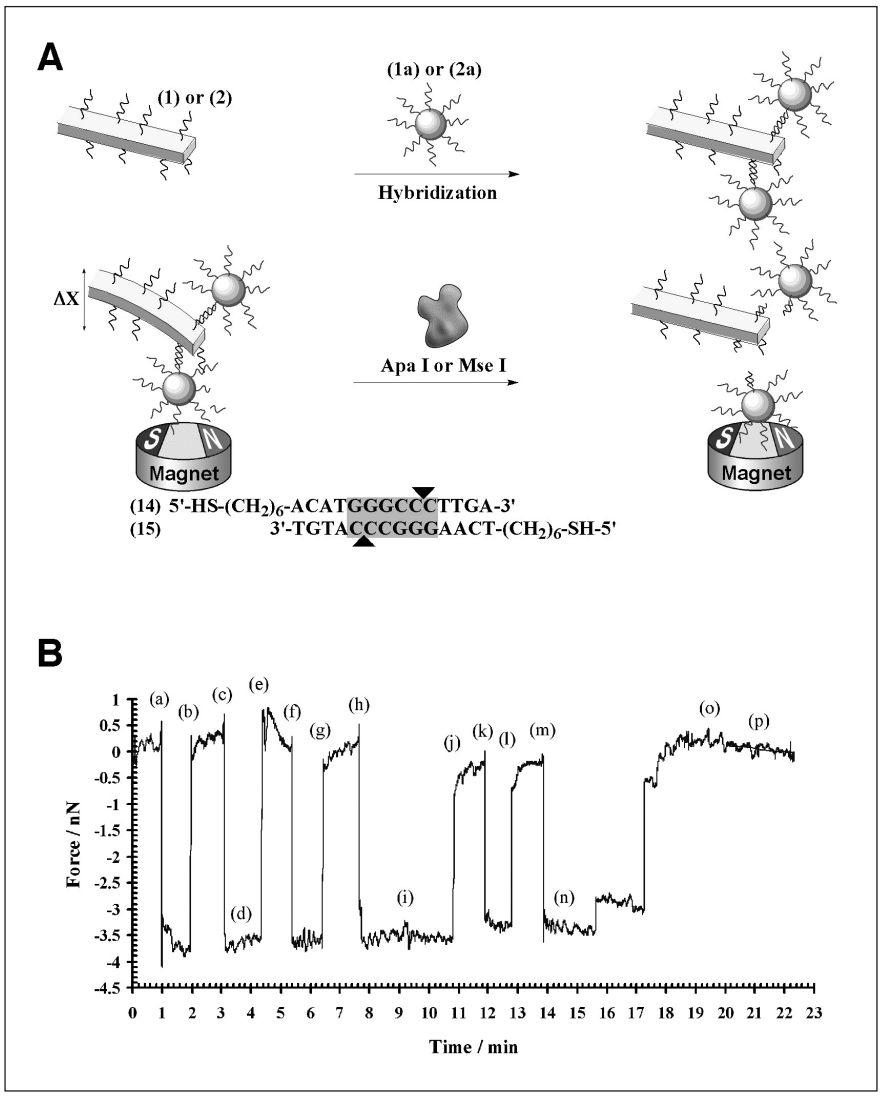

Figure 16. (A) Magneto-mechanical analysis of the scission of magnetic particle-functionalized duplex DNAs on a cantilever under an external magnetic field. (B) Magneto-mechanical analysis of the scission of the magnetic particle-functionalized duplex (14)/(15) on a cantilever by the endonuclease Mse I. Points: (a) to (h) "ON" and "OFF" attraction and retraction of the functionalized cantilever by applying and removing the external magnet. (i) Injection of the foreign endonuclease Apa I. (j) to (m) Further mechanical deflection and retraction of the cantilever by the external magnet. (n) Injection of Mse I while applying the external magnet and deflecting the cantilever. (o) and (p) Applying and removing the external magnet after scission of the duplex DNA by Mse I.

16 (A)). Thus, the cleavage of the double-stranded DNA is anticipated to release the magnetic particles, and the deflected cantilever would retract to its rest position. Figure 16 (B) shows the time-dependent analysis of the scission of the duplex (14)/(15) by Mse I by the amplified magnetic field-induced mechanical deflection/retraction of the cantilever by means of an external magnet. The positioning of the magnet below the cantilever, and the removal of the external magnet from the cantilever, repeatedly deflect and retract the cantilever, reversibly. The use of the endonuclease Apa I that does not recognize the sequence of the (14)/(15) duplex, does not affect the mechanical deflection/retraction of the lever in the presence/absence of the external magnet. The addition of Mse I under conditions where the cantilever is deflected by means of the external magnet, results in the retraction of the cantilever to its rest position, even under the applied magnetic field. The further removal/application of the external magnet on the system has no effect on the position of the cantilever. These results indicate that the scission of the duplex (14)/(15) resulted in the removal of the magnetic particles, a process that could be followed by the magnetic field-induced mechanical deflection of the cantilever. Similar sequence-specific cleavage processes of other duplex structures, using other nucleases were observed [23], implying that the process is specific.

This assay has summarized some of our research activities in the area of nanobioelectronics, nanobiosensing and nanobiotechnology. We have addressed different facets of possible applications of biomolecule-hybrid systems. The development of biosensors based on nanoparticle systems is a viable technology. Based on the progress in the field we can, however, anticipate that exciting functional systems and devices will originate from these studies.

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About the author

Itamar Willner obtained his PhD in 1978 and then from 1986 has been a professor at the Institute of Chemistry of the Hebrew University of Jerusalem, Israel. He holds several awards including:Max Planck Research Award for International Cooperation - 1998, Israel Chemical Society Award - 2001, Israel Prize in Chemistry - 2002, The Klachky Family Prize for the Advancement of the Frontiers of Science - 2003. He is a member of the Israel Academy of Sciences and Humanities and the European Academy of Sciences and Arts. He has published over 400 papers and 20 patents in the fields of bioelectronics, molecular electronics and molecular machinery, nanoparticle superstructures on surfaces, molecular optoelectronics and optobioelectronics, DNA-based electronic and photonic systems and photoinduced electron transfer and artificial photosynthesis.