Nanobiotechnology: Protein−Nanomaterial Interactions

ARTICLE *in* BIOTECHNOLOGY PROGRESS · MARCH 2007

Impact Factor: 2.15 · DOI: 10.1021/bp060388n · Source: PubMed

TOPICAL PAPER

Nanobiotechnology: Protein−Nanomaterial Interactions

Ravi S. Kane*,† and Abraham D. Stroock*,‡

Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York 12180, and Department of Chemical and Biomolecular Engineering, Cornell University, Ithaca, New York 14853

We review recent research that involves the interaction of nanomaterials such as nanoparticles, nanowires, and carbon nanotubes with proteins. We begin with a focus on the fundamentals of the structure and function of proteins on nanomaterials. We then review work in three areas that exploit these interactions: (1) sensing, (2) assembly of nanomaterials by proteins and proteins by nanomaterials, and (3) interactions with cells. We conclude with the identification of challenges and opportunities for the future.

Introduction

In recent times, there has been an increasing interest in interfacing biological molecules with nanomaterials and in understanding, controlling, and applying biomolecule−nanomaterial interactions for sensing, delivery, and the assembly of nanocomposites. This research is one of the pillars of the emerging field of nanobiotechnology. This manuscript focuses on recent progress in understanding and applying *protein*-nanomaterial interactions.

Protein Structure and Function on Nanomaterials

Interfacing proteins with nanomaterials is useful for applications ranging from sensing to cellular delivery. For instance, the recognition properties of proteins may find use both in sensing and in directing the assembly of nanomaterials into controlled architectures. The retention of protein structure and activity on nanoscale supports is critical for these applications. It is, however, also of fundamental interest to understand how nanomaterial properties such as size and surface chemistry influence the structure, activity, and stability of conjugated proteins.

Vertegel et al. (*1*) characterized the secondary structure and activity of chicken egg lysozyme adsorbed onto silica nanoparticles of various diameters. They reported a change in protein structure upon adsorption, with a greater loss in α-helix content on particles with larger diameters. The loss of activity of the adsorbed protein correlated well with the decrease in α-helix content. Lundqvist et al. (*2*) studied the adsorption of human carbonic anhydrase variants onto silica nanoparticles of varying sizes and also observed a larger perturbation of protein secondary structure on particles with larger diameters. These studies suggested that smaller nanoparticles, perhaps as a result of higher surface curvature, promoted the retention of native-like protein structure and function when compared to larger particles. Other studies suggest that the dependence of protein structure retention on nanomaterial curvature is protein-dependent. Roach et al. (*3*) studied the effects of the diameter of silica nanoparticles on the secondary structure of two proteins differing in size and shape, bovine serum albumin and fibrinogen. While bovine serum albumin was increasingly less ordered on larger nanoparticles, consistent with the studies described above, fibrinogen was denatured to a *greater* extent on smaller nanoparticles. Karajanagi et al. (*4*) observed a similar protein-dependence in the retention of secondary structure and activity upon adsorption onto single-walled carbon nanotubes (SWNTs). Chymotrypsin exhibited a nearly complete loss of activity and a significant perturbation in secondary structure, whereas soybean peroxidase (SBP) retained more of its native structure and activity upon adsorption onto SWNTs.

The curvature of a nanoscale support can influence not just protein structure and activity but also protein stability. Asuri et al. (*5, 6*) recently reported the ability of SWNTs to stabilize proteins under harsh conditions (e.g., at elevated temperatures or in solutions containing organic solvents) to a greater extent than conventional flat supports. For instance, the half-life of SBP adsorbed onto SWNTs at 95 °C or in nearly neat methanol was approximately 2-fold longer than that of SBP adsorbed onto flat supports under similar conditions (*6*). Their results suggest that lateral interactions between adjacent adsorbed proteins contribute to protein deactivation in harsh environments and that these unfavorable lateral protein−protein interactions are suppressed on highly curved supports such as SWNTs relative to flat surfaces (*6*). These results also suggest that the enhancements in protein stability should not be unique to SWNTs; similar enhancements in SBP stability were observed on other curved supports including silica and gold nanoparticles and fullerene aggregates.

The surface chemistry of a nanoparticle also provides control over the structure and function of adsorbed proteins. Hong et al. (*7*) studied the recognition of chymotrypsin by CdSe nanoparticles functionalized with different thioalkyl and thioalkylated oligo(ethylene glycol) ligands. Three different levels

* To whom correspondence should addressed: (R.S.K.) Ph: 518-276-2536. E-mail: kaner@rpi.edu. (A.D.S.) Ph: 607-255-4276. Fax: 607-255-9166. E-mail: ads10@cornell.edu.

† Rensselaer Polytechnic Institute.

‡ Cornell University.

10.1021/bp060388n CCC: $37.00
Published on Web 03/03/2007

of interaction of chymotrypsin with the nanoparticles were demonstrated: (i) enzyme inhibition with denaturation by nanoparticles functionalized with mercaptoundecanoic acid, (ii) enzyme inhibition with retention of structure by nanoparticles presenting a tetra(ethylene glycol)-carboxylic acid functionality, and (iii) no interaction with nanoparticles presenting a tetra-(ethylene glycol) group without the carboxylic acid "recognition element". Bayraktar et al. (*8*) demonstrated the ability to disrupt protein−protein interactions using surface-functionalized gold nanoparticles; they designed nanoparticles that bound selectively to cytochrome *c* (Cyt c) or cytochrome *c* peroxidase (CCP), thereby inhibiting enzyme turnover. Surface-functionalized nanoparticles with gold cores (2 nm) were prepared using thiolates with oligo(ethylene glycol) groups terminated in carboxylate (Au-TCOOH) and trimethylamine (Au-TTMA) functionalities. Au-TTMA bound selectively to CCP, which presents a negative patch on its surface, whereas Au-TCOOH bound selectively to Cyt c, whose surface is rich in basic residues. You et al. (*9*) fabricated amino-acid-functionalized gold nanoparticles that modulate the catalytic activity of α-chymotrypsin. They proposed that the amino acid monolayer on the nanoparticle controls both the capture of substrate by the active site and the release of product through electrostatic interactions, thereby influencing substrate specificities and catalytic constants. Amino-acid-functionalized nanoparticles also influence the binding and denaturation of α-chymotrypsin (*10*). A correlation was observed between the hydrophobicity index of amino acid side chains and the binding affinity and denaturation rates: the hydrophobic side chains had little effect on chymotrypsin structure, whereas the hydrophilic side chains destabilized the protein. Roach et al. (*3*) found that bovine serum albumin and fibrinogen were more denatured on hydrophobic surfaces than on hydrophilic ones. It is likely that changes in the secondary structure of adsorbed proteins depend on both the identity of the adsorbed protein and the mode of presentation of chemical functionalities.

Sensing

Applications of nanomaterials in sensing and imaging (*11*−*15*) have attracted considerable interest in recent times, and approaches to sensing based on quantum dots and other nanoparticles have been reviewed previously (*14*, *15*). Numerous assays based on conjugates of nanomaterials with antibodies or antigens have also been developed. We focus here on approaches to sensing that make use of the ability of *proteins* to modulate nanomaterial properties.

Cui et al. (*16*) demonstrated the sensitive real-time sensing of biological and chemical species based on changes in the conductance of boron-doped silicon nanowires (SiNWs). The conductance of these p-doped SiNWs was found to increase upon the adsorption of a negatively charged protein (streptavidin) and decrease following the adsorption of a positively charged protein (an antibody). Biotin-functionalized SiNWs were used to detect streptavidin down to at least a picomolar concentration range. Furthermore, antigen-functionalized SiNWs showed reversible antibody binding and concentration-dependent detection in real time. More recently, Lieber and co-workers have used nanowire-based sensors for the label-free detection of small-molecule−protein interactions (*17*) and for the ultrasensitive detection of DNA and DNA sequence variations (*18*); they have also demonstrated the spatially resolved sensitive detection, stimulation, and inhibition of neuronal signals using high-density nanowire transistor arrays (*19*).

The modulation of the properties of carbon nanotubes by proteins has also been used for the detection of both small molecules and proteins. Chen et al. (*20*) demonstrated the ability to detect clinically important biomolecules, antibodies associated with autoimmune diseases, using SWNTs. The binding of streptavidin and monoclonal antibodies resulted in a significant change in the conductance of the SWNT devices, thereby enabling detection at low concentrations. Extensive characterization revealed that electronic effects occurring at the metal-nanotube contacts due to protein adsorption made a more significant contribution to the electronic biosensing signal than adsorption along the exposed length of the nanotube (*21*).

The enzymatic activity of adsorbed proteins may also enable the manipulation of nanomaterial properties. Besteman et al. (*22*) attached glucose oxidase to SWNTs and reported an increase in conductance upon the addition of glucose, the substrate for the enzyme. Strano and co-workers have used SWNT-glucose oxidase conjugates to develop optical sensors for glucose (*23*). Glucose oxidase catalyzed the conversion of glucose to gluconolactone with hydrogen peroxide as the coproduct; partial reduction of ferricyanide moieties adsorbed on the SWNTs by hydrogen peroxide resulted in an increase in fluorescence intensity, thereby enabling the fluorescence of SWNTs to be coupled reversibly to the glucose concentration.

Assemblies of Proteins and Nanomaterials

In exploiting protein−nanomaterial interactions to form superstructure, investigators have both used proteins to organize nanomaterials and nanomaterials to organize proteins; we will discuss examples of each strategy. The strength and selectivity of protein−protein interactions make proteins excellent candidates to serve as linkers between nanoparticles and nanowires to form ordered structures. In particular, a number of teams have used antigen−antibody interactions (*24*−*26*) and biotin−avidin interactions (*27*−*30*) to direct assembly. Assemblies of conductive and semiconductive superstructures have been formed by these strategies: Searson's group used biotin−avidin to direct the end-to-end assembly of Au/Pt/Au multisegment nanowires (*30*); Kotov and collaborators used antigen−antibody interactions to assemble junctions of pre-assembled wires of semiconducting CdTe (*31*). Kotov and collaborators were able to measure local electrical transport but not global conductivity and hypothesized that the insulating gap formed by the protein linkages inhibited electron transfer between wires. Investigators have used bacterial S-layer proteins to direct the deposition (*32*) and growth (*33*) of nanoparticles into two-dimensional crystals on flat substrates. Groups have used proteins preassembled into microtubules to direct the assembly of nanoparticles (*34*, *35*). Hancock and Williams demonstrated that microtubules functionalized with magnetic nanoparticles could be oriented over macroscopic dimensions with an external field. Belcher pioneered the use of preassembled viral coat proteins to direct the assembly of nanomaterials. In recent work, Belcher and co-workers genetically modified M13 viruses to display specific binding peptides (streptavidin-binding peptide and hexahistidine peptide) (*36*) and tuned surface charge (*37*).

Recent efforts have used nanomaterials to organize proteins (*38*, *39*) and virus particles (*40*). Dragnea and collaborators have made virus-like particles by assembling viral coat proteins around gold cores (∼12 nm diameter) functionalized with acid-terminated tetra(ethylene glycol) thiols; transmission electron micrographs demonstrate significant organization of the assembled proteins. They hypothesize that creation of a negative particle that mimics the native nucleic acid core is important for the assembly process. Levy's group demonstrated significant ordering of intact virus particles around 200-nm-scale polymeric

cores (*40*); their goal was to influence transfection efficiency (cf. next section).

Cellular Interaction of Nanomaterials

The potential for specific interactions of nanomaterials with living cells leads to opportunities to develop new tools with which to communicate with and study cells. We will focus our attention on studies that explore the potential for therapeutic applications of nanoparticles. There is also significant interest in the toxicology of nanomaterials; we direct the reader to recent reviews of this topic (*41*−*44*).

The therapeutic as well as the toxicological activity of nanomaterials depends on the specific interactions with the cell surface and mechanisms of transmission into the cytosol and nucleus. A number of groups have explored the use of specific biological species on the surface of nanomaterials to control attachment and internalization (*40*, *45*−*48*). In order to promote delivery to the nucleus, de la Fuente et al. (*45*) attached Tat protein-derived peptide sequences to gold nanoparticles; they demonstrated Tat peptide-dependent transmission of the nanoparticles to the nuclei of human fibroblast cells in TEM images. Levy and co-workers demonstrated that the presentation of adenovirus vectors on the surface of a biodegradable nanoparticle allowed for significant rates of transfection of mammalian cells to be achieved even in the absence of specific cell surface receptors (*40*); the authors do not propose a mechanism for this effect. Several groups have investigated the impact of surface functionalization on the interaction of CNT with cells (*46*−*48*). Pantarotto's group demonstrated that carbon nanotubes functionalized with a peptide can translocate across the cell membrane of human and murine fibroblasts (*46*); they suggested that the mechanism of nanotube uptake was not by endocytosis as the internalization was not affected by temperature or endocytosis inhibitors. Dai and co-workers functionalized carbon nanotubes with proteins and showed that they could enter both adherent and nonadherent human cancer cells. In these studies, the nanotubes were seen to bring fluorescein-labeled streptavidin into cells. In distinction from Pantarotto, these authors suggest that uptake is due to adsorption-mediated endocytosis (*49*); continued investigation of the translocation process of CNTs is clearly needed. Zettl and Bertozzi have functionalized CNT with glycoproteins to mimic the chemistry of cell surfaces (*47*). These coated tubes were found to be non-cytotoxic and to bind to cell surfaces via specific interactions; uncoated tubes were cytotoxic.

For therapeutic applications of nanomaterials as vehicles for drug delivery, pharmacokinetic studies have been performed. Two recent studies point to the importance of bio-inert surface coatings to define desirable distribution and residence time: in vitro, Pishko demonstrated that poly(ethylene glycol)-coated nanoparticles (200 nm diameter) were significantly less likely to be taken up by macrophages than uncoated particles (*50*); in vivo, Amiji showed that encapsulation of a chemotherapeutic agent within a poly(ethylene glycol)-coated nanoparticle led to significantly larger accumulation of drug in the tumor relative to solution injection (*50*). In a number of studies, nanomaterials have been tested as direct mediators of therapeutic cytotoxicity for the treatment of cancer. For example, Hirsch and co-workers showed that nanoshells that absorb strongly in the near-infrared (NIR) can be used to destroy solid tumors in vivo by delivery of heat locally via adsorption of extracorporeally applied NIR radiation (*52*). In another example, Drezek and co-workers (*12*, *13*) employed nanoshells to simultaneously image and destroy carcinoma cells in the breast; the particles were delivered selectively to the tumor by functionalization with antibodies to epitopes that are overexpressed by the tumor cell (*13*). In an analogous fashion, functionalized SWNTs have been exploited to target cancer cells selectively for thermal destruction based on irradiation of the nanotubes with NIR light after internalization into cells (*53*). In a distinct application, nanotube conjugates have been tested as vectors for vaccination. Pantarotto et al. covalently attached an antigenic epitope from the foot-and-mouth disease virus to SWNTs; the resulting conjugates were immunogenic in vivo (*46*).

Conclusions

As these examples illustrate, the multiplicity of interactions between proteins and nanomaterials form the basis for advances in materials processing as well as for numerous applications in nanobiotechnology ranging from biosensing to delivery. Nonetheless, important challenges remain, such as scaling up the manufacture of nanomaterials and protein−nanomaterial conjugates, development of processing methods based on protein−nanomaterial assembly that allow for the growth of long range order and registration (e.g., to lithographically defined electrodes), creation of standard building blocks and linkages to allow for the rapid development of new assemblies, and a complete assessment of the potential toxicological effects of nanomaterials. The development of a broad mechanistic understanding of protein−nanomaterial interactions and of the mode of action of these bioconjugates is important to allow future design efforts to proceed in a more systematic manner. Potential applications of nanomaterial−protein conjugates are limited only by one's imagination and range from novel sensing techniques to therapeutics, drug delivery vehicles, and smart nanocomposites.

References and Notes

(1) Vertegel, A. A.; Siegel, R. W.; Dordick, J. S. Silica nanoparticle size influences the structure and enzymatic activity of adsorbed lysozyme. *Langmuir* **2004**, *20* (16), 6800−6807.

(2) Lundqvist, M.; Sethson, I.; Jonsson, B. H. Protein adsorption onto silica nanoparticles: Conformational changes depend on the particles' curvature and the protein stability. *Langmuir* **2004**, *20* (24), 10639−10647.

(3) Roach, P.; Farrar, D.; Perry, C. C. Surface tailoring for controlled protein adsorption: Effect of topography at the nanometer scale and chemistry. *J. Am. Chem. Soc.* **2006**, *128* (12), 3939−3945.

(4) Karajanagi, S. S.; Vertegel, A. A.; Kane, R. S.; Dordick, J. S. Structure and function of enzymes adsorbed onto single-walled carbon nanotubes. *Langmuir* **2004**, *20* (26), 11594−11599.

(5) Asuri, P.; Karajanagi, S. S.; Dordick, J. S.; Kane, R. S. Directed assembly of carbon nanotubes at liquid-liquid interfaces: Nanoscale, conveyors for interfacial biocatalysis. *J. Am. Chem. Soc.* **2006**, *128* (4), 1046−1047.

(6) Asuri, P.; Karajanagi, S. S.; Yang, H. C.; Yim, T. J.; Kane, R. S.; Dordick, J. S. Increasing protein stability through control of the nanoscale environment. *Langmuir* **2006**, *22*, 5833.

(7) Hong, R.; Fischer, N. O.; Verma, A.; Goodman, C. M.; Emrick, T.; Rotello, V. M. Control of protein structure and function through surface recognition by tailored nanoparticle scaffolds. *J. Am. Chem. Soc.* **2004**, *126* (3), 739−743.

(8) Bayraktar, H.; Ghosh, P. S.; Rotello, V. M.; Knapp, M. J. Disruption of protein-protein interactions using nanoparticles: Inhibition of cytochrome c peroxidase. *Chem. Commun.* **2006** (13), 1390−2.

(9) You, C. C.; Agasti, S. S.; De, M.; Knapp, M. J.; Rotello, V. M. Modulation of the catalytic behavior of α-chymotrypsin at monolayer-protected nanoparticle surfaces. *J. Am. Chem. Soc.* **2006**, *128* (45), 14612−14618.

(10) You, C. C.; De, M.; Han, G.; Rotello, V. M. Tunable inhibition and denaturation of α-chymotrypsin with amino acid-functionalized gold nanoparticles. *J. Am. Chem. Soc.* **2005**, *127* (37), 12873−12881.

(11) Lewis, J. D.; Destito, G.; Zijlstra, A.; Gonzalez, M. J.; Quigley, J. P.; Manchester, M.; Stuhlmann, H. Viral nanoparticles as tools for intravital vascular imaging. *Nat. Med.* **2006**, *12* (3), 354−360.
(12) Loo, C.; Hirsch, L.; Lee, M. H.; Chang, E.; West, J.; Halas, N.; Drezek, R. Gold nanoshell bioconjugates for molecular imaging in living cells. *Opt. Lett.* **2005**, *30* (9), 1012−1014.
(13) Loo, C.; Lowery, A.; Halas, N.; West, J.; Drezek, R. Immunotargeted nanoshells for integrated cancer imaging and therapy. *Nano Lett* **2005**, *5* (4), 709−711.
(14) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. Quantum dot bioconjugates for imaging, labelling and sensing. *Nat. Mater.* **2005**, *4* (6), 435−446.
(15) Penn, S. G.; He, L.; Natan, M. J. Nanoparticles for bioanalysis. *Curr. Opin. Chem. Biol.* **2003**, *7* (5), 609−615.
(16) Cui, Y.; Wei, Q. Q.; Park, H. K.; Lieber, C. M. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. *Science* **2001**, *293* (5533), 1289−1292.
(17) Wang, W. U.; Chen, C.; Lin, K. H.; Fang, Y.; Lieber, C. M. Label-free detection of small-molecule-protein interactions by using nanowire nanosensors. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (9), 3208−3212.
(18) Hahm, J.; Lieber, C. M. Direct ultrasensitive electrical detection of dna and DNA sequence variants using nanowire nanosensors. *Nano Lett.* **2004**, *4* (1), 51−54.
(19) Patolsky, F.; Timko, B. P.; Yu, G.; Fang, Y.; Greytak, A. B.; Zheng, G.; Lieber, C. M. Detection, stimulation, and inhibition of neuronal signals with high-density nanowire transistor arrays. *Science* **2006**, *313* (5790), 1100−1104.
(20) Chen, R. J.; Bangsaruntip, S.; Drouvalakis, K. A.; Kam, N. W. S.; Shim, M.; Li, Y. M.; Kim, W.; Utz, P. J.; Dai, H. J. Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (9), 4984−4989.
(21) Chen, R. J.; Choi, H. C.; Bangsaruntip, S.; Yenilmez, E.; Tang, X. W.; Wang, Q.; Chang, Y. L.; Dai, H. J. An investigation of the mechanisms of electronic sensing of protein adsorption on carbon nanotube devices. *J. Am. Chem. Soc.* ***2004***, 126 (5), 1563−1568.
(22) Besteman, K.; Lee, J. O.; Wiertz, F. G. M.; Heering, H. A.; Dekker, C. Enzyme-coated carbon nanotubes as single-molecule biosensors. *Nano Lett.* **2003**, *3* (6), 727−730.
(23) Barone, P. W.; Baik, S.; Heller, D. A.; Strano, M. S. Near-infrared optical sensors based on single-walled carbon nanotubes. *Nat. Mater.* **2005**, *4* (1), 86−92.
(24) Shenton, W.; Davis, S. A.; Mann, S. Directed self-assembly of nanoparticles into macroscopic materials using antibody-antigen recognition. *Adv. Mater.* **1999**, 11 (6), 449−452.
(25) Yang, J.; Mayer, M.; Kriebel, J. K.; Garstecki, P.; Whitesides, G. M. Self-assembled aggregates of IgGs as templates for the growth of clusters of gold nanoparticles. *Angew. Chem., Int. Ed.* **2004**, *43* (12), 1555−1558.
(26) Hegde, S.; Kapoor, S.; Joshi, S.; Mukherjee, T. Self-assembly of Ag nanoparticle-biotin composites into long fiberlike microstructures. *J. Colloid Interface Sci.* **2006**, *297* (2), 637−643.
(27) Connolly, S.; Fitzmaurice, D. Programmed assembly of gold nanocrystals in aqueous solution. *Adv. Mater.* **1999**, *11* (14), 1202−1205.
(28) Park, S. J.; Lazarides, A. A.; Mirkin, C. A.; Letsinger, R. L. Directed assembly of periodic materials from protein and oligonucleotide-modified nanoparticle building blocks. *Angew. Chem., Int. Ed.* **2001**, *40* (15), 2909−2912.
(29) Smorodin, T.; Beierlein, U.; Kotthaus, J. P. Contacting gold nanoparticles with carbon nanotubes by self-assembly. *Nanotechnology* **2005**, *16* (8), 1123−1125.
(30) Salem, A. K.; Chen, M.; Hayden, J.; Leong, K. W.; Searson, P. C. Directed assembly of multisegment Au/Pt/Au nanowires. *Nano Lett.* **2004**, *4* (6), 1163−1165.
(31) Wang, Y.; Tang, Z. Y.; Tan, S. S.; Kotov, N. A. Biological assembly of nanocircuit prototypes from protein-modified CdTe nanowires. *Nano Lett.* **2005**, *5* (2), 243−248.
(32) Mark, S. S.; Bergkvist, M.; Yang, X.; Angert, E. R.; Batt, C. A. Self-assembly of dendrimer-encapsulated nanoparticle arrays using 2-D microbial S-layer protein biotemplates. *Biomacromolecules* **2006**, *7* (6), 1884−1897.
(33) Allred, D. B.; Sarikaya, M.; Baneyx, F.; Schwartz, D. T. Electrochemical nanofabrication using crystalline protein masks. *Nano Lett.* **2005**, *5* (4), 609−613.
(34) Platt, M.; Muthukrishnan, G.; Hancock, W. O.; Williams, M. E. Millimeter scale alignment of magnetic nanoparticle functionalized microtubules in magnetic fields. *J. Am. Chem. Soc.* **2005**, *127* (45), 15686−15687.
(35) Behrens, S.; Habicht, W.; Wagner, K.; Unger, E. Assembly of nanoparticle ring structures based on protein templates. *Adv. Mater.* **2006**, *18* (3), 284−289.
(36) Nam, K. T.; Peelle, B. R.; Lee, S. W.; Belcher, A. M. Genetically driven assembly of nanorings based on the M13 virus. *Nano Lett.* **2004**, *4* (1), 23−27.
(37) Yoo, P. J.; Nam, K. T.; Qi, J.; Lee, S. K.; Park, J.; Belcher, A. M.; Hammond, P. T. Spontaneous assembly of viruses on multilayered polymer surfaces. *Nat. Mater.* **2006**, *5* (3), 234−240.
(38) Chen, C.; Daniel, M. C.; Quinkert, Z. T.; De, M.; Stein, B.; Bowman, V. D.; Chipman, P. R.; Rotello, V. M.; Kao, C. C.; Dragnea, B. Nanoparticle-templated assembly of viral protein cages. *Nano Lett.* **2006**, *6* (4), 611−615.
(39) Chen, C.; Kwak, E. S.; Stein, B.; Kao, C. C.; Dragnea, B. Packaging of gold particles in viral capsids. *J. Nanosci. Nanotechnol.* **2005**, *5* (12), 2029−2033.
(40) Chorny, M.; Fishbein, I.; Alferiev, I. S.; Nyanguile, E.; Gaster, R.; Levy, R. J. Adenoviral gene vector tethering to nanoparticle surfaces results in receptor-independent cell entry and increased transgene expression. *Mol. Ther.* **2006**, *14* (3), 382−391.
(41) Garnett, M. C.; Kallinteri, P. Nanomedicines and nanotoxicology: some physiological principles. *Occup. Med.* **2006**, *56* (5), 307−311.
(42) Gwinn, M. R.; Vallyathan, V. Nanoparticles: Health effects−pros and cons. *Environ. Health Persp.* **2006**, *114* (12), 1818−1825.
(43) Hurt, R. H.; Monthioux, M.; Kane, A. Toxicology of carbon nanomaterials: Status, trends, and perspectives on the special issue. *Carbon* **2006**, *44* (6), 1028−1033.
(44) Lam, C. W.; James, J. T.; McCluskey, R.; Arepalli, S.; Hunter, R. L. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit. Rev. Toxicol.* **2006**, *36* (3), 189−217.
(45) de la Fuente, J. M.; Berry, C. C. Tat peptide as an efficient molecule to translocate gold nanoparticles into the cell nucleus. *Bioconjugate Chem.* **2005**, *16* (5), 1176−1180.
(46) Pantarotto, D.; Briand, J. P.; Prato, M.; Bianco, A. Translocation of bioactive peptides across cell membranes by carbon nanotubes. *Chem. Commun.* **2004** (1), 16−17.
(47) Chen, X.; Tam, U. C.; Czlapinski, J. L.; Lee, G. S.; Rabuka, D.; Zettl, A.; Bertozzi, C. R. Interfacing carbon nanotubes with living cells. *J. Am. Chem. Soc.* **2006**, *128* (19), 6292−6293.
(48) Kam, N. W. S.; Liu, Z. A.; Dai, H. J. Carbon nanotubes as intracellular transporters for proteins and DNA: An investigation of the uptake mechanism and pathway. *Angew. Chem., Int. Ed.* **2006**, *45* (4), 577−581.
(49) Kam, N. W. S.; Jessop, T. C.; Wender, P. A.; Dai, H. J. Nanotube molecular transporters: Internalization of carbon nanotube-protein conjugates into mammalian cells. *J. Am. Chem. Soc.* **2004**, *126* (22), 6850−6851.
(50) Zahr, A. S.; Davis, C. A.; Pishko, M. V. Macrophage uptake of core-shell nanoparticles surface modified with poly(ethylene glycol). *Langmuir* **2006**, *22* (19), 8178−8185.
(51) Shenoy, D. B.; Amiji, M. A., Poly(ethylene oxide)-modified poly-(epsilon-caprolactone) nanoparticles for targeted delivery of tamoxifen in breast cancer. *Int. J. Pharm.* **2005**, *293* (1−2), 261−270.
(52) Hirsch, L. R.; Jackson, J. B.; Lee, A.; Halas, N. J.; West, J. A whole blood immunoassay using gold nanoshells. *Anal. Chem.* **2003**, *75* (10), 2377−2381.
(53) Kam, N. W. S.; O'Connell, M.; Wisdom, J. A.; Dai, H. J. Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (33), 11600−11605.

Received December 20, 2006. Accepted February 8, 2007.

BP060388N