Applications of nanotechnology to biotechnology

Commentary

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The ability to systematically modify the properties of nanostructures by controlling their structure and their surface properties at a nanoscale level makes them extremely attractive candidates for use in biological contexts, from fundamental scientific studies to commercially viable technologies.

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Introduction

The expanding availability of a variety of nanostructures with highly controlled properties in the nanometer size range has sparked widespread interest in their use in biotechnological systems. Size does matter — the fact that nanoparticles are similar in size range to many common biomolecules makes them appear to be natural companions in hybrid systems. More importantly, however, are the new and unique properties that nanostructures bring to biotechnological applications. By controlling structure precisely at nanoscale dimensions, one can control and tailor properties of nanostructures, such as semiconductor nanocrystals and metal nanoshells, in a very accurate manner. In addition, one can make modifications to nanostructures to better suit their integration with biological systems; for example, modifying their surface layer for enhanced aqueous solubility, biocompatibility, or biorecognition. With selected biomolecules bound to nanostructure surfaces, new 'hybrid' nanostructures can be obtained for applications such as biosensing and imaging, or nanostructures can be embedded in other biocompatible materials to modify material properties or impart new functionality.

The idea of merging biological and nonbiological systems at the nanoscale level is not a new one. The broad field of bioconjugate chemistry is based on combining the functionalities of biomolecules and non-biologically derived molecular species for specialized use in applications ranging from markers for research in cell and molecular biology to biosensing, bioimaging and masking of immunogenic moieties to targeted drug delivery [1]. Many current applications of nanostructures in biotechnology are a natural evolution of this approach. In fact, several of the 'breakthrough' applications recently demonstrated using nanostructure–biomolecular hybrids are in fact traditional applications originally addressed by standard molecular bioconjugate techniques that have been revisited with these newly designed nanostructure hybrids.

So one might argue, why replace conventional molecular tags, such as fluorescent chromophores, with nanostructures? Typically, nanostructures possess properties far superior to the molecular species they replace — higher quantum efficiencies, greater scattering or absorbance cross sections, optical activity over more biocompatible wavelengths, and significantly increased chemical or photochemical stability. The systematic control of nanostructure properties obtained by controlled variations in particle size and dimension is in direct contrast to molecular tags, whose properties vary nonsystematically between molecular species. This systematic variation of properties via structure variation not only improves traditional applications, but also leads to new, unique applications well beyond the scope of conventional molecular bioconjugates. A prime example is the optical properties of semiconductor nanocrystals and metal nanoshells, which are new and robust fluorophores, absorbers and scatterers in the near infrared, a region of the electromagnetic spectrum where tissue is essentially transparent. The availability of these new nanostructures will greatly facilitate new *in situ* probes and sensor methods.

In this article, we introduce several successful examples of nanostructures that have been integrated with biomolecular species and applied to relevant problems in biotechnology. The use of bioconjugate semiconductor nanocrystals, or 'quantum dots', as fluorescent biological labels will be discussed. A new and powerful assay based on the optical properties of bioconjugate gold nanoparticles, a new innovation on a traditional bioconjugate technology, will also be described. The biotechnologically friendly properties of gold nanoshells are summarized, and a novel photothermally triggered drug delivery system based on a new nanoshell–polymer composite will also be discussed.

Bioconjugate quantum dots as fluorescent biological labels

Semiconductor nanocrystals are highly light absorbing, luminescent nanoparticles whose absorbance onset and emission maximum shift to higher energy with decreasing particle size, due to quantum confinement effects [2]. These nanocrystals are in the size range of 2–8 nm in diameter. Unlike molecular fluorophores, which typically have very narrow excitation spectra, semiconductor quantum dots absorb light over a very broad spectral range. This makes it possible to optically excite a broad spectrum of quantum dot colors using a single excitation laser wavelength, which enables one to simultaneously probe several markers. Although the luminescence properties of semiconductor nanocrystals have historically been sensitive to their local environment and nanocrystal surface

preparation, recent core-shell geometries where the nanocrystal is encased in a shell of a wider band gap semiconductor have resulted in increased fluorescence quantum efficiencies (> 50%) and greatly improved photochemical stability. In the visible region, CdSe–CdS core-shell nanocrystals have been shown to span the visible region from 550 nm (green) to 630 nm (red). Other materials systems, such as InP and InAs, provide quantum dot fluorophores in the near infrared region of the optical spectrum, a region of high physiological transmissivity. Although neither II-VI nor III-V semiconductor nanocrystals are water soluble, let alone biocompatible, surface functionalization with molecular species such as mercaptoacetic acid or the growth of a thin silica layer on the nanoparticle surface facilitate aqueous solubility [3]. Both the silica layer and the covalent attachment of proteins to the mercaptoacetic acid coating permit the nanoparticles to be biocompatible. Specific binding to cell surfaces, insertion into cells, and binding to cell nuclei have all been demonstrated following conjugation of the nanoparticle with the appropriate targeting protein [2].

A gold nanoparticle bioconjugate-based colorimetric assay

The use of gold colloid in biological applications began in 1971, when Faulk and Taylor invented the immunogold staining procedure. Since that time, the labeling of targeting molecules, especially proteins, with gold nanoparticles has revolutionized the visualization of cellular or tissue components by electron microscopy [4]. The optical and electron beam contrast qualities of gold colloid have provided excellent detection qualities for such techniques as immunoblotting, flow cytometry, and hybridization assays. Conjugation protocols exist for the labeling of a broad range of biomolecules with gold colloid, such as protein A, avidin, streptavidin, glucose oxidase, horseradish peroxidase, and IgG [5].

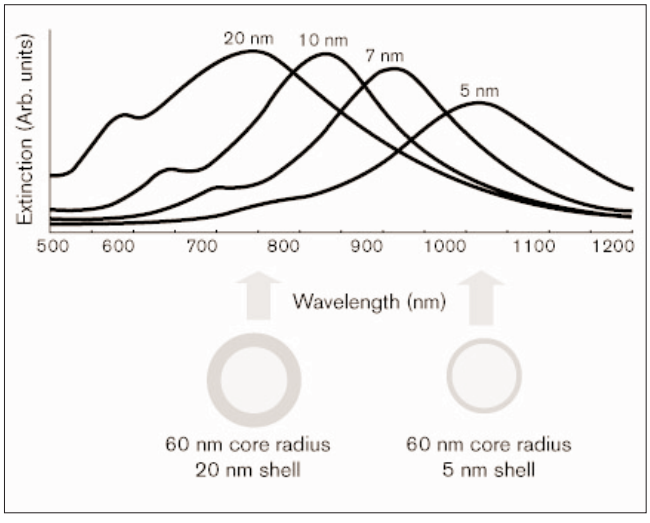
Gold nanoparticle conjugation was recently applied to polynucleotide detection in a manner that exploited the change in optical properties resulting from plasmon–plasmon interactions between locally adjacent gold nanoparticles [6]. The characteristic red of gold colloid has long been known to change to a bluish-purple color upon colloid aggregation. In the case of polynucleotide detection, mercaptoalkyloligonucleotide-modified gold nanoparticle probes were prepared. When a single-stranded target oligonucleotide was introduced into solution, a polymer network was formed consisting of the target oligonucleotide and the conjugated nanoparticles. This condensed network brought the nanoparticles into close enough vicinity to induce a dramatic red-to-blue macroscopic color change. Because of the extremely strong optical absorption of gold colloid, this colorimetric method can be used to detect ~10 fmol of an oligonucleotide, which is 50 times more sensitive than sandwich hybridization detection methods based on fluorescence detection.

A gold nanoshell–polymer composite photothermally triggered drug delivery system

Gold nanoshells are new composite nanoparticles that combine infrared optical activity with the uniquely biocompatible properties of gold colloid. Metal nanoshells are concentric sphere nanoparticles consisting of a dielectric (typically gold sulfide or silica) core and a metal (gold) shell [7]. By varying the relative thickness of the core and shell layers, the plasmon-derived optical resonance of gold can be dramatically shifted in wavelength from the visible region into the infrared over a wavelength range that spans the region of highest physiological transmissivity (see Figure 1) [8]. By varying the absolute size of the gold nanoshell, it can be made to either selectively absorb (for particle diameters <~75 nm) or scatter incident light. Because the gold shell layer is deposited using the same chemical methods used to grow gold colloid, the surface properties of gold nanoshells are virtually identical to those of gold colloid. The same conjugation protocols used to bind a wide variety of biomolecules to gold colloid are therefore readily transferable to gold nanoshell conjugation. Successful gold nanoshell conjugation with enzymes and antibodies has been demonstrated. Gold nanoshells also take advantage of the inherent biocompatibility of gold, not requiring further surface functionalization or protective layer growth.

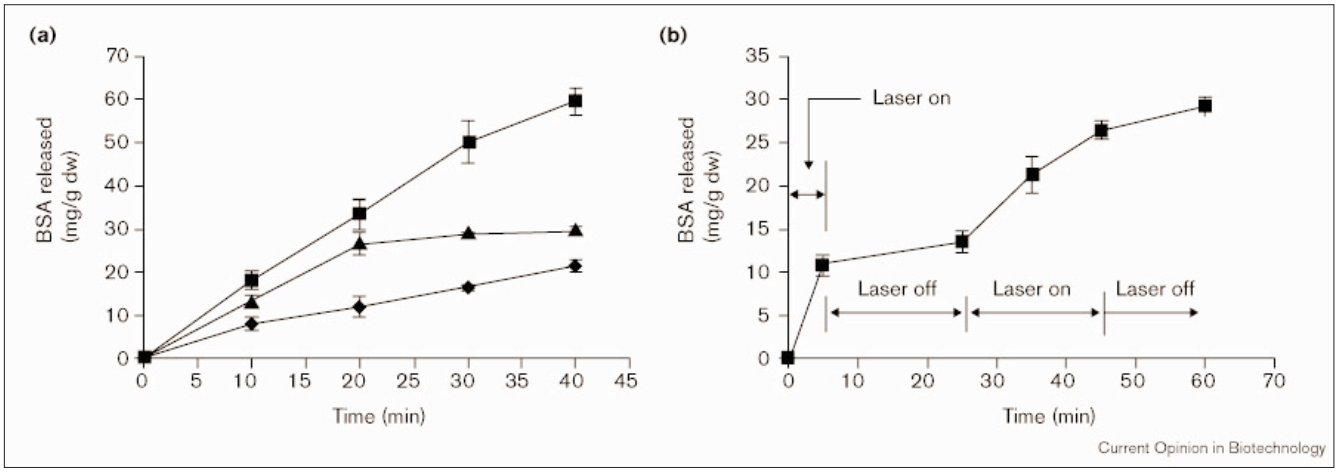
When optically absorbing gold nanoshells are embedded in a matrix, illuminating them at their resonance wavelength causes the nanoshells to transfer heat to their local environment. This photothermal effect can be used to optically 'remote control' drug release in a nanoshell–polymer composite drug delivery material [9]. Copolymers of *N*-isopropylacrylamide (NIPAAm) and acrylamide (AAm)

Figure 1



Optical resonances of gold-shell–silica-core nanoshells, as a function of their core : shell ratio, for particles of 120 nm core diameter. Respective spectra correspond to the nanoparticles depicted beneath. Reproduced from [8] with permission.

Figure 2



(a) Release of bovine serum albumin (BSA) from non-irradiated (diamond), irradiated NIPAAm–co-AAm hydrogels (triangle), and irradiated nanoshell-composite hydrogels (square). Irradiation was at 1064 nm. **(b)** Release of BSA from nanoshell-composite hydrogels in response to sequential irradiation at 1064 nm.

exhibit a critical solution temperature (LCST) that is slightly above body temperature. When the temperature of the copolymer exceeds the LCST, the material collapses causing a burst release of any soluble material held within the polymeric matrix. Gold nanoshells that had been designed to strongly absorb near infrared light have been incorporated into poly(NIPAAm–co-AAm) hydrogels; light at these wavelengths (800–1200 nm), which can be transmitted through tissue with relatively little attenuation, is absorbed by the nanoparticles and converted to heat, thus causing the copolymer to collapse as its temperature exceeds its LCST. Significantly enhanced drug release has been achieved in response to irradiation by Nd:YAG laser light at 1064 nm. The triggered release of methylene blue and proteins of varying molecular weight has also been demonstrated (Figure 2). The nanoshell–composite hydrogels can also release multiple bursts of protein in response to repeated near IR irradiation.

Conclusion

Nanotechnology in the form of nanoparticles whose properties can be precisely tailored by chemical methods is rapidly becoming an important new tool in the arsenal of the biotechnologist. Nanostructures will no doubt lead to new and improved assays and sensing methods, new optically controlled functional materials, new highly specific color-coded probes of cellular function, and new optically based therapeutic methods.

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