Review

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Gold-nanoparticle-based biosensors for detection of enzyme activity

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Optimal enzyme activity is essential for maintenance of physiological homeostasis. A variety of both non-genetic and genetic disruptions can excessively activate or silence intrinsic enzyme activities, with pathological outcomes. Many pharmacological agents are activators and inhibitors of enzymes. It is essential, therefore, in the development of drugs as enzyme activators and inhibitors, that enzyme activities be accurately measured under physiological and pathological conditions. Different biochemical assays have been developed for this purpose, some of which are based on nanostructured materials. This review focuses on gold nanoparticle (GNP)-based structures for the sensing and measurement of enzyme activities in biological specimens. Here we provide an overview and critical analysis of such assays, identify their advantages and limitations, and discuss interesting features of GNPs to be exploited for future applications in pharmacology.

Gold nanoparticles in biosensing

Gold nanoparticles (GNPs) have been used in the arts and medicine for centuries. They provided color for medieval cathedral windows and until the 18th century were believed to have life-prolonging and rejuvenating benefits if taken internally as *aurum potabilis*. Modern scientific evaluation of colloidal gold began with Michael Faraday's work in the 1850s, which recognized that the color observed was due to the minute size of the gold. The technological advances of the 20th century made it possible to determine their exact morphological, physical, and chemical properties. In 1971, Faulk and Taylor were the first to use GNPs as immunocytochemical markers in transmission electron microscopy [1]. GNPs can be attached to many traditional biological probes such as antibodies, lectins, superantigens, glycans, nucleic acids, and receptors. Particles of different sizes are easily distinguishable in electron micrographs, allowing simultaneous multiple-labeling experiments. They can also be used for labeling thin cryosections for scanning electron microscopy, and samples for light microscopy. The unique properties of GNPs, such as their high absorption coefficient (see Glossary), scattering flux, luminescence, and conductivity, as well as their ability to enhance electromagnetic fields, quench (or enhance) fluorescence, and catalyze

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Glossary

Absorption coefficient: the absorption coefficient determines how far light of a particular wavelength can penetrate into a material before it is absorbed.
Antibody: an antibody (Ab), also known as an immunoglobulin (Ig), is a large Y-shaped protein produced by B cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. Each tip of the Y of an antibody has a structure that is specific for one particular structure on its target, allowing these two structures to bind together with precision. As a consequence, an antibody can tag or neutralize its target directly (e.g., by blocking a part of a microbe that is essential for its survival and invasive character).
Avidin–biotin interaction: avidin is a protein that can bind up to four molecules of biotin simultaneously with a high degree of affinity and specificity. The strong affinity between avidin and biotin is widely used in biomedical research and industry.
Dipole moment: measure of net molecular polarity that can be defined as the product of the charge magnitude (q) and the distance between the charges (d).
Förster resonance energy transfer (FRET): energy transfer between two chromophores, a donor and an acceptor, via a nonradiative mechanism without absorption or emission of photons.
Nanocage: particles of nanometer scale shaped in the form of a cage.
Quantum dots: nanometer-sized semiconductor particles composed of various compounds of elements such as cadmium, zinc, tellurium, selenium, and sulfur. Quantum dots react to electricity or light by emitting their own light across the visible wavelength range from 470 to 730 nm. Quantum dots provide greater sensitivity and up to 1000 times brighter live-cell imaging compared to conventional organic fluorophores. Quantum dots also have the advantage that they do not photobleach on exposure to light. In addition, quantum dots of different sizes can be excited by a single-wavelength light source, allowing simultaneous detection and multicolor imaging.
Quantum yield: the quantum yield of a fluorophore determines how much energy input is needed to produce a certain intensity of emitted light. It is defined as the ratio of the number of photons emitted to the number of photons absorbed.
Rayleigh scattering: elastic scattering of light or other electromagnetic radiation by particles much smaller than the wavelength of the light; named after the British physicist Lord Rayleigh.
Scattering flux: number of scattered particles per unit area per unit time.
Surface plasmon absorption peak: the wavelength at which maximum light absorption occurs due to the resonance conditions: matching between the natural frequency of surface electrons oscillating (surface plasmon) and the frequency of light photons.
Surface plasmon resonance (SPR): collective oscillation of electrons in solid or liquid stimulated by incident light. The resonance condition is established when the frequency of light photons matches the natural frequency of surface electrons oscillating against the restoring force of positive nuclei. SPR in nanometer-sized structures is called localized surface plasmon resonance.
Tat peptide: a domain of Tat protein that enters cells by crossing the cell membrane; it is therefore known as a cell-penetrating peptide. Tat protein is produced by a lentivirus (such as HIV) within infected cells. It greatly increases the rate of viral transcription and replication and is also secreted extracellularly, where it plays a role in increasing viral replication in newly infected cells and in enhancing the susceptibility of T cells to infection.
Telomerase: enzyme that adds DNA sequence repeat units at the 3′ end of the DNA so that chromosomes are not shortened during cell division. Telomerase is particularly active in cancer cells.
Two-photon luminescence (TPL): emission of light after the simultaneous absorption of two photons of identical or different frequencies. The energy of the emitted photon is equal to the sum of the energies of the two photons.

reactions, provide numerous ways to exploit these particles for sensing purposes. Today, GNPs are used as catalysts and as imaging and therapeutic agents [2]. In addition, because of their remarkable versatility in biological and medical applications [3], GNPs have been proposed as signal transducers for biosensors [3].

Biosensors are analytical devices comprising two elements in spatial proximity: a biological recognition element, which is able to interact with the target, and a transducer, which converts the recognition event into a measurable signal. The myriad shapes and surfaces of GNPs available today, their relatively easy conjugation to molecules of interest, and their low toxicity are favorable features for biological applications. In biosensors, GNPs are the transducers, producing a measurable signal on biological recognition of the primary event occurring in close proximity, most often on their surfaces. GNP-based tests for various biomarkers are already commercially available (e.g., Nanosphere, Merck, and BBInternational), offering highly sensitive and specific detection of proteins and nucleic acids associated with infections, and with heart, kidney, and genetic diseases.

The life and death of complex organisms depend on the activity of enzymes. Enzymes are indispensable, for example, for regulation of signal transduction, for determining cell morphology function and differentiation, for adaptation to stressful stimuli, and for maintenance of cell homeostasis. The synthesis and activity of enzymes in living organisms strongly depend on the circumstances. For example, caspase 1, a key enzyme in inflammation and programmed cell death, exists mostly in its inactive precursor form in normal healthy cells. Cell exposure to whole pathogens or crystalline structures, such as asbestos, leads to the assembly of NLRP3 inflammasomes and subsequent activation of caspase 1. Active caspase 1 processes the cytokines IL-1β and IL-18 and induces pyroptotic cell death. Measurements of enzyme activities and the post-translational modifications they are subject to are important in the exploration of physiological and pathological processes (e.g., inflammation and cancer) and are crucial to enzyme research, enzyme engineering, and drug discovery. In traditional enzyme assays, catalytic activity is usually detected using labeled substrates or indirect sensor systems that produce a detectable spectroscopic signal on reaction. Although these approaches, and commercially available assays in general (e.g., GenScript), are adequate for many applications, they are limited in sensitivity and are restricted to *in vitro* use.

Advances in nanotechnology have now made it possible to measure minute changes in enzyme activity with high accuracy and precision. Nevertheless, few current nanoparticle-based assays are suitable for assessing enzyme-catalyzed transformations *in vivo*. The focus of this review is on the use of GNP-based assays for sensing enzyme activity in biological analytes (i.e., detection of enzyme-specific chemical reactions in biological environments). This detection depends on the transfer, removal, or addition of functional groups, the cleavage or joining of bonds, or the oxidation and reduction catalyzed by enzymes. Assays designed to quantify the amount of enzyme as a protein (rather than its catalytic activity) in a sample fall outside the scope of this review, but have been discussed elsewhere [2–4].

GNP-based assays for enzyme activity detection

Despite the many interesting physicochemical properties of GNPs, the majority of GNP-based enzyme assays exploit one of the following two GNP characteristics: (i) the surface plasmon absorption of GNPs; and (ii) their ability to quench fluorescence. Because of these properties, GNPs have been used in colorimetric-based and Förster resonance energy transfer (FRET)-based enzyme assays. In addition to colorimetric and FRET-based assays, other detection systems have been described for GNP-based enzyme activity determination, most notably electrochemical and light-scattering measurements.

Colorimetric assays

The surface plasmon absorption peak for GNPs is very sensitive to the environment, shifting towards longer wavelengths (red shift) and broadening significantly on GNP aggregation. In other words, the absorption peak for the dispersion changes towards longer wavelengths when the particles aggregate. This color change is easily detectable (Figure 1A). Conversely, the color shift can also occur in the reverse direction (Figure 1B); such a change may occur if the aggregated particles are linked to cleavable molecules. Cleavage of these molecules can be initiated by a change in the biological environment or an exogenous stimulus. These stimuli then cause the GNP aggregates to fragment into individual particles, leading to a blue shift in the absorption spectrum of the dispersion. The extent of aggregation/separation is proportional to the absorption peak shift, so the signal is quantifiable and provides a direct measure of the aggregation- or separation-inducing agent (e.g., an active enzyme) (Figure 1A,B). Biochemical assays based on color changes resulting from enhanced enzyme expression or changes in enzyme activity are popular in research and clinical laboratories because they are simple and do not depend on sophisticated instrumentation. However, these colorimetric assays can suffer from numerous problems owing to the presence of interfering molecules in biological samples that can absorb visible light in the same spectral region as the dispersed and/or aggregated particles. When this happens, it is difficult to distinguish the absorption peaks for GNPs and contributing biomolecules.

Colloidal gold is stabilized sterically or electrostatically by surface molecules. Aggregation or separation of GNPs due to enzyme activity can occur in several ways (Figure 1). An enzyme could compromise the stabilizing forces either by cleaving the surface molecules or by changing their charges, thus inducing aggregation of the particles. Linking the GNPs to antibodies recognizing the enzyme-modified surface molecule or using avidin–biotin interactions will also result in aggregation [5]. Conversely, interconnected (and therefore aggregated) GNPs can be separated when the link is enzymatically severed (Figure 1B).

Colorimetric assays are by far the most frequently used technique in GNP-based enzyme assays. The relatively simple measurement, inexpensive instrumentation, and robustness and potential for high-throughput assay make