Review

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Optical readout of the intracellular environment using nanoparticle transducers

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There is rapid growth in the use of multi-functional nanoparticles as transducers to probe the intracellular environment. New designs of nanoparticles can provide quantitative information at sub-cellular resolution on parameters such as pH, temperature and concentration of nicotinamide adenine dinucleotide (NADH) or selected metal ions. This new work builds on the existing practice of using nanoparticles and fluorescent dyes to provide enhanced microscopic images of cells, but goes beyond it by adding new functionalities and analytical capabilities. In this review, we discuss the recent literature on the development of such nanoparticles for simultaneous biosensing and imaging. We explore and examine the different measurements that will be possible, and analyze the likely accuracy and resolution that could be achieved.

Biosensing using nanoparticles

Nanoparticles with multi-functional properties can now be designed to transduce (see Glossary) biochemical signals at subcellular spatial resolution. Nanoparticles offer several advantages over fluorescent dyes when imaging and probing the intracellular environment. Nanoparticles do not bleach as readily as fluorescent dye probes, and can be readily internalized into cells [1,2]. Many nanoparticles appear to have low cytotoxicity [3,4], and some can capture incident light and amplify the local electromagnetic field [5]. Nanoparticles also have a massive surface-to-volume ratio, thus allowing a very small number of particles to have a surprisingly large effect. An early application of nanoparticles was to produce surface enhanced Raman scattering (SERS). Under suitable conditions it becomes possible to detect an analyte at zeptomolar levels [6]. The application of SERS within live cells has also been investigated for some years and information on this topic is widely available [7,8]. However, there are a number of other, lesser known, ways in which a nanoparticle can be used to transduce information from biochemically-relevant parameters, such as intracellular temperature, pH, or the concentration of metabolites or metal ions into outputs we can detect. These are the focus of the present review.

Transduction of intracellular temperature

The temperature difference between the intra- and extracellular environment, or within the cell, can be a sensitive indicator of the integrity of various cellular processes [9] and how they might be changed by drugs or disease [10,11]. Under static conditions, objects the size of a typical cell will rapidly reach thermal equilibrium in an aqueous environment. However, exo- or endothermic processes within cells (such as metabolism or division) can perturb the intracellular temperature slightly. Intracellular temperature can be measured by inserting devices into a cell, such as a very small thermocouple into the cell, which can give a precision of $<0.1^{\circ}C$ [10], or a microscopic mechanical resonator, which can give a sensitivity of $\sim 0.002^{\circ}C$, equivalent to the temperature difference generated by the 5-pJ heat output of a single brown fat cell [12]. Unfortunately, a cell will almost certainly be significantly perturbed or damaged by such techniques, and insertion of micro-scale devices into cells may be prohibitively difficult. In contrast, photoacoustic thermometry can provide a precision of approximately $0.2^{\circ}C$ [13], which is similar to the 0.1–$0.8^{\circ}C$ precision obtained from various fluorescent dyes [9,14]. Temperature-sensitive enzymes comprise another possible class of probes, and these have a precision

Glossary

Biosensor: is a device for detection and quantification of small biologically-relevant molecules. It interacts with the biochemical and this stimulus is transduced to, for example, an optical signal.
Endocytosis: is a process by which a cell absorbs large molecules or nanoparticles by engulfing them.
Endosomes: are intracellular vesicles into which the foreign matter absorbed by endocytosis are initially placed.
Hyaluronic acid (HA:) is a carbohydrate polymer that is common in tissues and the extracellular matrix.
Nicotinamide adenine dinucleotide (NADH): is a metabolic coenzyme that plays an important role in cell activity. Forms a redox couple with its oxidized form NAD^+.
Reactive oxygen species (ROS): are reactive molecules or free radicals such as H_2O_2, O_2^- or $^{\bullet}OH$. These play an important role in living cells. They are generated naturally by cellular metabolic processes and have a potent effect on signaling, DNA, proteins, lipids, redox homeostasis, metabolism, and aging [27,73,74].
Transduction: is the process of converting a signal from one form (e.g., chemical, magnetic, temperature) to another (e.g., electrical, optical).

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Box 1. Quantum dots, gold nanoparticles, and fluorophores

A quantum dot is an inorganic nanoparticle, typically less than 10 nm in size. The confinement of the electrons produces changes in the electronic structure which, importantly, leads to modifications to the optical properties, such as fluorescence enhancement and tunability. Examples include CdSe@ZnS (the @ symbol indicates a CdSe core surrounded by a ZnS shell). These are used as fluorescent probes and are characterized by narrow emission lines, the wavelength of which can be controlled by the nanoparticle size. They are also brighter than organic dyes and do not photobleach; however, they do have disadvantages such as potential cytotoxicity and fluorescence blinking.

Carbon dots (C dots) are based on graphene or graphene oxide, and are a recently developed platform showing great potential for biosensing applications. The material is inexpensive and nontoxic, unlike many conventional semiconductors. The surface is easily functionalized and can also be doped to change the chemistry and fluorescent properties of the dots.

Gold nanoparticles (GNPs), also known as colloidal gold, also display unusual size-dependent optical properties. Free electrons in the nanoparticles oscillate under the influence of an electromagnetic field and resonate at certain frequencies known as the LSPR (localized surface plasmon resonance), which are determined by the size, shape, and degree of aggregation of the nanoparticles. These resonances cause the electric field in the vicinity of the particle to be greatly enhanced, which can be exploited in a number of ways. It can increase the scattering and absorption of light, it can increase the Raman signal of a nearby molecule (see SERS, Box 2) and can even increase the weak intrinsic fluorescence of gold itself. An excited fluorophore (QD or dye molecule) within 20 or 30 nm of a GNP can interact with its free electrons. Depending on the distance from the GNP, the fluorescence may be enhanced or quenched in a process known as nanoparticle surface energy transfer (NSET).

of approximately 0.7°C [15]. Unfortunately, the latter two transduction modalities are also sensitive to the pH and ionic strength of the intracellular environment, both of which are variable. However, there is a new emerging class of nanoparticle probes that are designed to combine the transduction of intracellular temperature with imaging of the nanoparticle to generate spatial information.

The expansion of the crystal lattice of a quantum dot (QD) (Box 1) due to an increase in temperature will cause a red shift in its emission peak, and this has been used to characterize inhomogeneous temperature distributions within live cells. For example, commercial QDs were used to investigate how administration of Ca^{2+} affected the intracellular temperature of NIH/3T3 cells [16]. A precision of approximately 0.3°C seems to have been obtained. However, blinking of QDs could be a general problem, because it might disrupt a sequence of measurements [17].

Measurement of intracellular temperatures by probing the electronic spin state of defects within nanodiamonds was reported recently [18], with a precision of better than 0.05°C (which could theoretically improve to nearly 0.001°C). The electronic spins within the nitrogen-vacancies are first manipulated using microwave radiation and then probed using electron spin resonance (ESR) spectroscopy (Figure 1). Changes in temperature influence the elastic strain around the N–V centers of the nanodiamond, which causes the spin signal to vary accordingly.

A temperature-sensitive dye can still be safely used to monitor intracellular temperature provided that it can be chemically isolated from the intracellular environment. For example, europium thenoyltrifluoroacetonate can be encapsulated within a poly(methyl methacrylate) (PMMA) nanoparticle [17]. Delivery of the hybrid construct in this case was facilitated by coating it with the cationic polymer poly(allylamine hydrochloride) (PAH).

Another example of a hybrid scheme may be found in conjugates of QD and dyes [19]. In this case, a temperature sensitive-dye (cyanine) is conjugated to a rod-shaped QD of CdS. The change in temperature is not inferred directly from the dye or from the QD, but rather from fluorescence resonance energy transfer (FRET) (Box 2) occurring between the dye and the QD. Estimates of temperature are made by determining the ratio of emission intensities at specific wavelengths. A precision of at least 0.2°C was demonstrated. In another example of using a fluorophore somewhat indirectly, fluorescent molecules were inserted into a nanoparticle of temperature-responsive poly-N-*n*-propylacrylamide (pNNPAM) [20]. The latter has the property that it is more permeable to its aqueous environment at lower temperatures, becoming increasingly less so as the temperature rises above 25°C. Ingress of aqueous ions at low temperatures quenches the fluorophore, but as the temperature rises, the polymer contracts, expelling water and resulting in a systematic increase of fluorescence. The precision is approximately 0.5°C.

Metabolites

Similar to the case of temperature, changes in intracellular metabolite concentrations and dynamics can reflect changes in gene expression as well as the cellular response to the environment, and it would be useful to monitor metabolites visually with high special resolution. A number of different metabolites have been investigated with

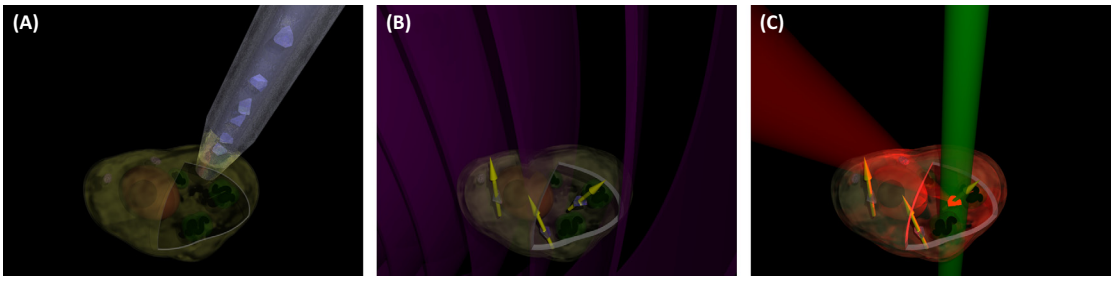

Figure 1. Use of nanodiamonds as ultrasensitive temperature sensors in live cells. **(A)** The cell is injected with several nanodiamonds. **(B)** Electronic spin (yellow arrows) of defect sites in nanodiamonds are excited by microwave radiation. **(C)** Temperature-dependent spin state read-out by analyzing fluorescent emission (red) of selected nanodiamond using probe laser (green).