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

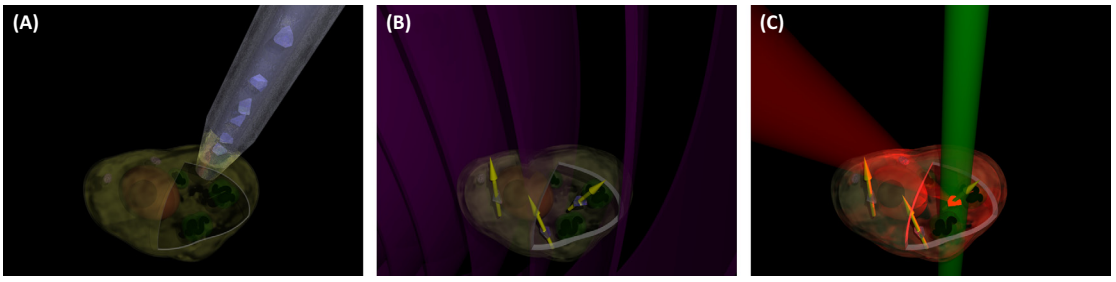

Figure 1. Use of nanodiamonds as ultrasensitive temperature sensors in live cells. **(A)** The cell is injected with several nanodiamonds. **(B)** Electronic spin (yellow arrows) of defect sites in nanodiamonds are excited by microwave radiation. **(C)** Temperature-dependent spin state read-out by analyzing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

Box 2. Imaging methodologies

FRET (Förster resonance energy transfer) – This effect transduces the distance *r* between donor and acceptor fluorophores since the efficiency depends on r^6. It is effective when the analyte causes a displacement between donor and acceptor moieties, or cleaves one moiety altogether. When FRET takes place, an excited fluorescent donor relaxes to its ground state, transferring energy to an acceptor via dipole–dipole coupling. An increase in transfer efficiency results in quenching of donor fluorescence and increase in acceptor fluorescence if the acceptor is fluorescent.

Ratiometric Method – This method is based on the ratio of two independent fluorescence intensities. The analyte interacts with one fluorophore to change its fluorescence yield, while a second fluorophore is insensitive to the presence of the analyte. This technique has the advantage of automatically taking into account variations in excitation intensity, local nanoparticle concentration, etc.

SERS (surface enhanced Raman scattering) – Raman spectroscopy yields the vibrational energies of molecules, which can be used to identify the molecule or its conformation. In the SERS effect the Raman signal of a molecule in the immediate proximity of a metal nanoparticle is enhanced by a factor 10^4–10^{12}, and background fluorescence may also be quenched. Gold or silver substrates are typically used. In the case of a biosensor, the molecule that undergoes enhanced scattering may be the analyte itself, or more commonly, a reporter molecule whose vibrational levels change when it interacts with the analyte.

nanoparticles including ATP [21], cysteine [22], and complex I [23]. We focus on the detection and imaging of the two intensely studied molecular species of most biological importance, reactive oxygen species (ROS) and NADH.

Optical imaging of intracellular ROS

In the intracellular environment, ROS can be products of normal cellular metabolism or derive from exogenous sources. ROS are involved in cell signaling and regulation of various processes (e.g., apoptosis) and their concentration is tightly controlled by antioxidant molecules and enzymes. Pathologies associated with ROS include chronic diseases and cancer. Optical imaging using fluorescent dyes has been used to visualize ROS in cells, but the dyes may not localize to the site of the redox reaction, and nonspecific reactions may occur during their delivery to target tissues or organelles [24]. The dyes are also prone to photobleaching [25] and cytotoxicity [26]. To overcome these issues, nanoparticles have also been considered as probes [27]. It is desirable for the nanoparticle to transduce a signal that is proportional to ROS concentration, and this can be achieved by conjugating the nanoparticle to a ROS-sensitive fluorophore. The nanoparticle can then be exploited to selectively deliver the fluorophore to a target location while the fluorophore provides a signal in proportion to the ROS [27].

For example, a silicate sol–gel nanoprobe, which contains the fluorescent dye 5(6)-carboxyfluorescein, allows detection of ROS concentrations as low as 1 nM [28]. One benefit of this design is that encapsulation of the dye within the inorganic host reduces the leaching of fluorophores, and hence minimizes cell toxicity. Alternatively, chemical changes induced by the ROS can be used to trigger a fluorescent signal. Hyaluronic acid (HA) conjugated with chlorine e6 (Ce6) was used as an indicator of ROS production in stimulated macrophage cells [29]. The presence of ROS causes degradation of the HA [30,31], which releases fluorescent Ce6.

Gold nanoparticles (GNPs) are an alternative scaffold for ROS-sensitive probes, one of which was synthesized by immobilizing fluorescein–HA conjugates on the surface of GNPs [32]. When the fluorophore is on or close to the surface of the GNP, its fluorescence is quenched (Box 1). The presence of ROS releases the fluorophore from the surface of the GNP, allowing the fluorophore to fluoresce (Figure 2).

The situation for very small (<2 nm) gold nanoparticles is subtly different. In this case, the particle and its stabilizing ligand can be thought to form a single hybrid entity, which is luminescent by virtue of its electronic configuration. Reaction with ROS can alter the surface electronic states of the cluster and change its properties. H_2O_2 can be detected, for example, by the effect it has on quenching the fluorescence of the complex formed between 2-nm gold 'nanodots' and 11-mercaptoundecanoic acid [33].

Although most ROS-sensing nanoparticles make use of fluorescence, there are other techniques for the transduction chemical information, even without a fluorophore. Surface enhanced Raman scattering (SERS) (Box 2) is one of the mostly widely known of these methods [1,27]. In this context, gold nanoshells have been used for measuring reductive and oxidative stress in fibroblast cells. The SERS probe molecules were 1,8-diaza-4,5-dithia-1,8-di(2-chloro-[1,4]-naphthoquinone-3-yl)octane and 2-mercapto-benzene-1,4-diol. These molecules have redox-active quinone moieties, which gain or lose H^+ ions with a detectable change in their Raman spectra, thereby allowing the detection of intracellular redox potentials without using fluorescent dyes [34].

The detection of dissolved oxygen is a related issue to the detection of ROS. Some nanoparticle-based schemes for O_2 detection are discussed in recent reviews [35,36]. More recently, polymer core-shell particles with an oxygen-sensitive platinum porphyrin dye (PtTFPP) covalently attached to the hydrophobic core have been examined [37]. These nanoparticles accumulate in cytoplasmic organelles and are fluorescent in the presence of dissolved O_2. More quantitative analyses can be achieved by including a second O_2-insensitive reference fluorophore,which acts as a fluorescence energy transfer (FRET) donor (Box 2) for PtTFPP [38]. Further improvement of this

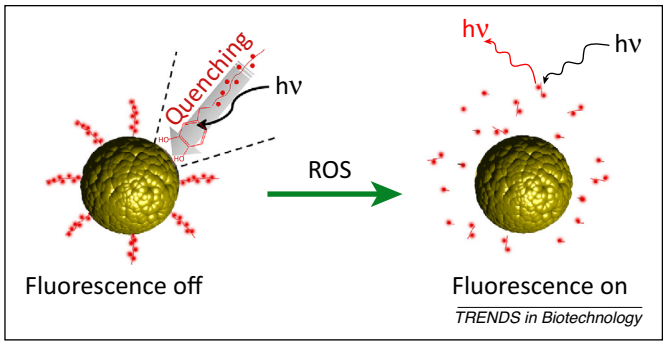

Figure 2. Schematic illustration of a gold nanoparticle (GNP) probe immobilized with fluorescein–hyaluronic acid (HA) conjugates for intracellular reactive oxygen species (ROS) monitoring. When dye is attached to GNP, its fluorescence is quenched, and the presence of ROS cleaves dye from GNP, enabling fluorescence. hν designates a photon.

scheme to obtain sub-cellular resolution would be of great interest.

Optical probes for NADH

NADH is involved in multiple biological processes, and its concentration reflects cellular redox environment, mitochondrial activity, oxidative stress, and metabolic flux [39]. It can be detected by a variety of means including UV-induced autofluorescence (which unfortunately has low sensitivity and induces damage to the cell) and a fluorescent protein that provides a high detection specificity in living cells [40]. One scheme that exploits the strong reduction potential of NADH involves the reduction of Cu^{2+} to Cu^0 by NADH. The Cu^0 precipitates and adsorbs onto the surface of intracellular GNPs to form a shell particle with a gold core [41]. In this study, the intracellular distribution of NADH in HeLa cells was mapped by taking dark field spectra of the nanoparticles with an optical microscope (Figure 3).

Quantum dots (QDs) can be also used as optical labels for NADH probes. For example, CdSe/ZnS QDs functionalized with Nile blue FRET acceptor were used to examine the effects of glucose on the amount of NADH in HeLa [42].

Detection of metal ions

Metal ions are essential co-factors for many enzymes, often required for catalysis of redox reactions. There are multiple pathologies associated with reduced enzyme activity due to faulty metal ion regulation. Many nanoparticle transducers for detecting metal ion concentrations have been investigated. In general, these are based on the modification of a fluorescent signal. The most commonly detected ion is Cu^{2+}, but methods for detecting the presence of Zn^{2+}, Ca^{2+}, K^+, Na^+, Al^{3+}, Fe^{2+}, Mg^{2+}, Pb^{2+}, Cr^{3+}, Hg^{2+}, and $UO_2{}^{2+}$ have also been reported [36,43–46]. Specificity of the response must be verified because fluorescence is also affected by ions such as $H_2PO_4{}^-$, H^+, and $S_2{}^-$ [44,47].

Fluorescent bovine serum albumin (BSA)-coated gold nanoparticles, conjugated to folic acid for improved internalization in HeLa cells, were used as probes for intracellular Cu^{2+} [48]. The fluorescence of the probe was quenched by the presence of Cu^{2+} in the cells. Similar Cu^{2+} fluorescence quenching was seen with BODIPY-functionalized gold nanoparticles in Cu^{2+} treated HeLa cells [49] and fluorescein-functionalized silica nanoparticles [50]. Unfortunately, the data obtained from a single channel measurement is generally not sufficient to quantify Cu^{2+} concentration. A more successful approach has been to take ratiometric measurements of signals from dual emission nanoparticles, such as Rhodamine B-labeled carbon dots ('C dots') [51] or FITC-doped silica nanoparticles linked to RBITC (which have sensitivity down to 10 nM Cu^{2+}) [52]. It is also possible to achieve the same effects with a combination of different nanoparticles, for example, using a red emitting CdSe/ZnS quantum dot (as the reference signal) and a blue emitting carbon dot functionalized with a specific Cu^{2+} receptor (AE-TPEA) [53]. Treatment with Cu^{2+} induced quenching of the C dot fluorescence, but not that of the reference, allowing for a quantitative determination of Cu^{2+} concentration. Zn^{2+} and Ca^{2+} have also been imaged using a ratiometric measurement from nanoparticles [47,54].

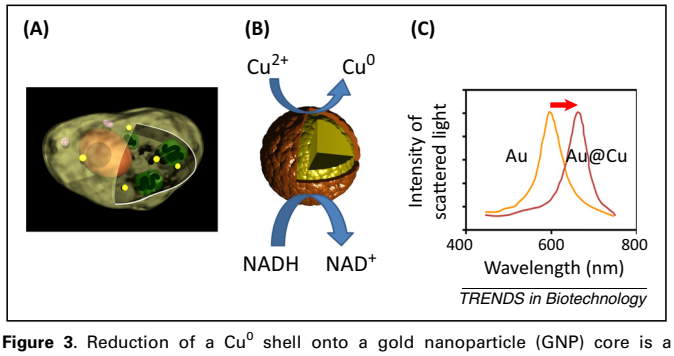

Figure 3. Reduction of a Cu^0 shell onto a gold nanoparticle (GNP) core is a quantitative measure of nicotinamide adenine dinucleotide (NADH). **(A)** Gold nanoparticles are introduced into cell. **(B)** NADH in cell will reduce Cu^{2+} to Cu to form a shell on the Au. **(C)** Formation of the Cu shell causes a marked red shift on the scattering spectrum.

Measurement and mapping of pH

Intracellular pH affects a wide variety of intracellular processes including enzymatic activity, ion channel activity, and cell cycle phase by changing the ionization state of peptides and proteins. Intracellular pH is tightly regulated, and deviations from the optimum range can indicate adverse changes in cell physiology.

Optical transduction of cellular pH has been traditionally achieved using fluorescent or absorptive indicators. Unfortunately, in addition to the general issues that plague the use of all free dyes, pH indicator dyes are also susceptible to interference from the binding of intracellular proteins [55]. Furthermore, fluorescence-based pH indicators generally operate over a very limited pH range, typically approximately one pH unit from the indicator pK_a. Nanoparticle-based pH transducers can overcome many of these limitations by encapsulating the indicator and limiting its interaction with biological molecules or by including a number of different dyes to extend the measurable pH range. Many types of nanoparticles have been developed for optical transduction of pH [56] and to map intracellular (usually endosomal) pH. Although many of the studies using such nanoparticles do not present quantitative information about the mapped pH, an omission that is rarely commented on, there has been recent successful application of nanoparticles to quantitative measurement and mapping of intracellular pH.

For example, GNPs with two fluorophores (pH-insensitive rhodamine and pH-sensitive anthracene) were used to detect endosomal pH in the 4–8 range, with a precision of ±0.3 pH units, which is sufficient to differentiate various locations in the cell [57]. In another study, the pH of endosomes in HeLa cells was monitored with quantum dot (CdS@CdSe) –fluorophore [5(6)-carboxy-X-rhodamine] hybrids. In this case, the DNA linker is sensitive to pH (via conformational changes) and the fluorescent signal is transmitted via FRET [58]. Imaging was straightforward: a single excitation wavelength (460 nm) generated two easily separated fluorescent bands centered on 560 nm and 610 nm. The system produced a precision of ±0.4 unit in the pH range 6.0–7.4.

Mesoporous silica nanoparticles can also be loaded with a pair of pH-sensitive fluorophores such as fluorescein

isothiocyanate (FITC) and Rhodamine B isothiocyanate (RITC). Chen *et al.* [59] used such nanoparticles with negatively or positively charged surfaces to map the pH in HeLa cell endosomes. The pH reported by the negatively charged nanoparticles, which are trapped in the endosomes, was <5. The positively charged nanoparticles, which were able to escape the endosomes and were predominantly located in the cytosol, reported a pH in the range 6.0–6.3.

Although nanoparticles usually enter the cell by the endocytic pathway, accurate measurement of the pH over the lifetime of the vesicle is not straightforward because the evolution of pH can extend over more than two pH units. A focus of recent research has been to increase the measureable pH range. The pH response of silica nanoparticles loaded with FITC and RITC was extended by designing the nanoparticle to exploit the surface–curvature dependence of the fluorophore pK_a [60]. These particles were used to determine the pH in predominantly endocytic regions of HeLa cells over a pH range from 4.5 to 7.5. Large 200-nm polymeric nanoparticles containing Rhodamine 6G (R6G) and 1,3-phenylenediamine have a useful pH range from 3.0 to 8.0, but their use is limited to situations in which the pH monotonically decreases, because protonation of the nanoparticle results in irreversible R6G release [61]. Triple labeling of nanoparticles is another strategy to increase range. For example, acrylamide porous nanoparticles can be loaded with Oregon Green (pK_a 4.1), fluorescein (pK_a 6.0) and Rhodamine B [62].

A different approach entails producing a set of on/off fluorescent nanoparticles, such that each type has a different emission wavelength and sharp (<0.25 pH units) pH transition point [63]. However, implementation of this scheme is more complex because a number of different nanoparticles need to be synthesized.

Two-photon fluorescence, which offers the advantage of reduced optical scattering and reduced cellular auto-fluorescence, has also been used in combination with nanoparticle-based transduction of pH. A ratiometric study exploiting this utilized a probe composed of silver nanoparticle coated with 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) and encapsulated with polyacrylamide, which had a precision of ~0.2 pH units [64]. Another approach that avoids auto-fluorescence involves the use of fluorescence lifetime imaging microscopy (FLIM), in which the image channels represent fluorescence lifetimes rather than the wavelengths and intensities. Cellular auto-fluorescence has a shorter lifetime (2–3 ns) than QDs (five to hundreds of ns); therefore, with time-resolved spectroscopy, the signals can be distinguished easily. Another system utilized CdSe@ZnS nanoparticles capped with mercaptoproprionic acid (which have a pH-dependent fluorescence lifetime) to map changes in pH of endocytic vesicles of cells [65].

SERS has also been used to map the pH of endosomes, utilizing probes such as 4-mercaptobenzoic acid (pMBA) attached to gold nanoaggregates [66] or large (0.5–1 μm) 'nanopeapods' of silica nanotubes containing chains of pMBA-capped gold NPs [67].

Although endosomal pH has attracted substantial interest, it is also possible to use nanoparticles to probe the pH in other parts of a cell. For example, a pHrodo™ dye encapsulated with self-assembled hydrogel nanofibers was used to determine the pH in the cytoplasm, phagosomes, and nucleus [68]. C dots should penetrate even more readily than nanofibers as their very small size facilitates their diffusion into all regions of the cell (Box 3). Dual-labeled (FITC/RITC) C dots, excited at 488 nm, were used to map the pH in both cytoplasmic and nuclear regions of HeLa cells treated with oxidizing reagents [69]. The measurable pH range was 6.0–8.0 with an uncertainty of ~0.2 pH units. Very small (2–7 nm) SiC nanoparticles have been used to map the cytosolic pH in HeLa cells [70].

Box 3. Outstanding questions

- What are the best ways to deliver nanoparticles to parts of the cell such as the cytosol or nucleus for extended quantitative analysis and imaging?
- What options are there for targeting the nanoparticles for specific organelles such as the mitochondria, which are relatively resistant to penetration by any kind of probe?
- To what extent do various nanoparticle transducers perturb the physiological processes within the target region of the cell in any significant manner and how can this be mitigated?

Concluding remarks and future perspectives

Intracellular sensing using nanoparticle-based transduction is a rapidly developing field. Significant progress has been made in mapping intracellular parameters such as temperature, pH, and the concentration of biomolecules such as ROS and NADH. However, the field is still relatively young and other possibilities have yet to be explored in much detail. Intracellular viscosity, for example, can be deduced from the rotation of a non-spherical magnetic particle within the intercellular environment [71]. Could the force required to penetrate an intracellular membrane be determined using a magnetic particle? What other possibilities are there? There is also the important question of whether the measurement perturbs the very system that is being studied. The effect of intracellular nanoparticles on cellular processes is an active but as yet still unexplored field. Many nanoparticles exert quite marked effects, often being cytotoxic or damaging in some way [72], whereas others, such as GNPs larger than 5 nm in size, and nanodiamonds, seem to be practically inert. The processes used to introduce the nanoparticle to the cell also need to be considered. The addition of Cu^{2+} ions in the NADH assay using Au@Cu nanoparticles, for example, might be expected to exert some influence on cellular processes distinct from those due to the copper nanoparticles themselves. Despite these difficulties, the scientific insights to be gained from improved methods of quantitatively transducing information from the intracellular environment into an output we can interpret are potentially immense. Nanoparticle-based techniques are already a useful member of the toolkit that is available, and many exciting new developments in this field may be anticipated.

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