# Optical nanoparticle sensors for quantitative intracellular imaging

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Real-time measurements of biological/chemical/physical processes, with no interferences, are an ultimate goal for *in vivo* intracellular studies. To construct intracellular biosensors that meet such a goal, nanoparticle (NP) platforms seem to be most promising, because of their small size and excellent engineerability. This review describes the development of NP-based opical sensors and their intracellular applications. The sensor designs are classified into two types, based on the sensor structures regarding analyte receptor and signal transducer. Type 1 sensors, with a single component for both receptor and transducer, work by mechanisms similar to those of 'molecular probes'. Type 2 sensors, with a separate component for receptor and transducer, work by different mechanisms that require the presence of specific NPs. A synergistic increase in optical signal or selectivity has been reported for these second type of NP sensors. With ongoing rapid advances in nanotechnology and instrumentation, these NP systems will soon be capable of sensing at the single-molecule level, at the point of interest within the living cell, and capable of simultaneously detecting multiple analytes and physical parameters.

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Intracellular imaging of the biochemistry and biophysics of live cells has been of prime interest for decades. Numerous approaches have been proposed to achieve real-time, noninvasive analysis of chemical and physical properties at unperturbed cellular physiological status. Significant progress in the visualization of biological processes has been achieved by the dramatic advances in imaging processing technologies with high-performance computer systems as well as by the continuous development of new analyte-specific fluorescent molecular probes. These molecular probes, however, have several drawbacks that limit the indicator dyes available for reliable intracellular measurements. The indicator molecules have to be in a cell-permeable form, which often requires properly derivatized indicator molecules. The measurement is often skewed by intracellular sequestration to specific organelles inside the cell, or by nonspecific binding to proteins and other cell components. The cytotoxicity of the available dyes is sometimes a problem, as the mere presence of

these dye molecules may chemically perturb the cell. Furthermore, the dye is usually not 'ratiometric', i.e., has only a single spectral peak, which then requires technologically more demanding techniques, such as picosecond lifetime resolution or phase-sensitive detection. We note that just loading into the cell a separate reference dye, for ratiometric measurements, is not a solution, because of the aforementioned sequestration and nonspecific binding.

In an attempt to solve these problems, while maintaining minimal physical interference, a new type of sensor has been developed, utilizing nanoparticles (NPs) as platforms for immobilizing the sensor chemistry. The NP sensor is physically noninvasive owing to its small size. An NP sensor of 20–600 nm in diameter takes up only 1 ppm to 1 ppb of a mammalian cell's volume.[1] There are also standard methods for delivery of NPs into cells, such as via a gene gun, pico-injection, liposome incorporation, or endocytosis. This prevents unnecessary modification (derivatization) of the indicator dyes. The inert protective matrix of the NPs eliminates interferences such as protein binding and/or membrane/organelle sequestration.[2] The NP matrix also obviates the toxicity problem by protecting the cellular contents from the indicator dyes and vice versa. The cell

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viability after NP sensor delivery is about 99%, relative to control cells,[3] indicating negligible physical and chemical perturbation to the cell. Moreover, a ratiomentric sensor can be easily constructed by co-loading of the indicator and reference components within the NP matrix. We note that the NP sensors can also be attached with specific molecular targeting moieties, enabling the measurements of analytes at specific cells or organelles of the cells.[4]

The NP has a high surface-to-volume ratio that allows high accessibility of analytes to the indicator dyes/receptors as well as targeting factors towards specific cells or components of cells. Each NP can be loaded with a high amount of components (single or multiple) within the NP matrix as well as on the surface. High loaded amounts of dyes in close proximity to each other either within the restricted NP volume or on the NP surface allow multiple interactions with the sensing components, resulting in signal amplification.[5] It is noteworthy that similar amplification effects have been reported for targeting efficiency by NPs with multiple targeting moieties on the surface.[6]

Since the first NP sensor called PEBBLE (Photonic Explorer for Biomedical use with Biologically Localized Embedding) was reported by Kopelman and colleagues,[7,8] a number of possibilities have been proposed for immobilizing the sensor chemistry within various kinds of NP matrixes, so as to construct NP sensors for specific intracellular applications. It is also noted that optical detection has remains the most widely used method for sensing and imaging of biological systems.

This review focuses on the uses of synthetic NPs of 1–1000 nm in diameter for the design of optical sensors and their applications to intracellular measurements. It covers only untethered, that is free, NP sensors which are suitable for *in situ* measurements in three dimensions; it does not cover mechanically fixed sensors like fiber-tip or film on glass slide, even when they utilize NPs.

## OPTICAL NP SENSOR DESIGN

The basic structure of a sensor requires two components: an analyte recognizer that binds the target analyte, and a transducer that signals binding.

### Optical Transduction Modality

Fluorescence is a highly sensitive, specific means for monitoring cell activity, and a number of fluorescent reporters can be analyzed simultaneously. Fluorescence has been and will be a major transduction modality but has limiting factors such as photobleaching and interference due to autofluorescence from cellular components.

Surface-enhanced Raman scattering (SERS) is a recently evolving optical modality for intracellular NP sensors and is complementary to fluorescence.[9,10] It was found that the SERS effect is provided by a very small number of molecules located at special sites in the gap between two nearly touching gold nanocrystals.[11] Because of the high specificity of a Raman spectrum, minute amounts of chemicals inside living cells might be identified by their unique fingerprint spectra.[12] SERS requires the so-called 'SERS-active substrates' such as nanometer-sized silver or gold structures, which target molecules that get attached to them. Surface plasmon resonance (SPR) is another metal NP–based optical transducer that draws much interest in biological detection, including immunoassay.[13] SERS and SPR are free from photobleaching and self-quenching of the marker molecule. However, their sensitivity/reproducibility still needs validation.

## NP Matrix

NP matrices should exhibit excellent chemical stability and biocompatibility. A variety of NP matrices have been utilized for the design of optical nanosensors, as listed in Table 1.

Polymeric NPs of various matrices and sizes with surface-located reactive functional groups can be prepared by various synthetic methods.[53,54] The sensor components can be loaded into the NP matrix by various methods, including encapsulation, covalent linkage, physical adsorption, etc. The matrix for polymeric NP-based sensors is selected by the accessibility of an analyte to a recognition element and the loading efficiency, within NP matrix, of indicator/receptor and signal transducer.

Liposomes or micelles present limited utility for biological sensing within the membrane-rich cellular environments, as they tend to mix with the native cell membranes, degrading the sensor structure. However, polymer-capped stabilized liposomes or micelles have been utilized for designing sensors for intracellular measurements.[40,41]

Semiconductor quantum dots (QDs) are brighter and more stable against photobleaching than organic fluorophores, allowing real-time and continuous monitoring.[55] A study shows that the fluorescence emission of QDs remains bright and stable inside cells for at least 14 days.[56] The biosensing applications of QDs are usually based on fluorescence resonance energy transfer (FRET).[55,57,58]

**TABLE 1** | Matrices for Optical NP Sensors

| NP type | Optical transduction modality | Matrix | References |
|---|---|---|---|
| Polymeric NP | Fluorescence | Poly(acrylamide) | 3,8,14–20 |
| | | Poly(decylmethacrylate) | 21–24 |
| | | Poly(ethylene glycol) | 25 |
| | | Poly(methacrylate) | 26 |
| | | Poly(n-butylacrylate) | 27 |
| | | Polystyrene | 28–30 |
| | | Dendrimer | 31 |
| | | Latex | 32 |
| | | Organically modified silica | 33,34 |
| | | Silica | 35–39 |
| Polymerized liposome | Fluorescence | 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) liposome with polymethacrylate shell | 40 |
| Plymerized micelle | | Silane-capped (polymerized) mixed micelle | 41 |
| Quantum dot | Fluorescence | CdS | 42,43 |
| | | ZnS-coated CdSe | 44–46 |
| Metal | SERS, fluorescence | Gold | 47–49 |
| | SERS | Silver | 50 |
| Metal/polymer hybrid | SERS, SPR | Gold nanoshell over silica | 51,52 |

NP, nanoparticles; SERS, surface-enhanced Raman scattering; SPR, surface plasmon resonance

In a metallic or metal-coated polymer NP, incident light can couple to the plasmon excitation of the metal.[59] This leads to enhanced optical detection schemes utilizing SERS and plasmon resonance. These metal (gold, silver, or gold-coated silver) NPs have been utilized to detect a wide range of biological molecules through binding events involving interactions with surface-coated specific molecules that offer distinct SERS[48,50,51,60] and SPR.[52] Gold NPs have also been utilized to construct an optical biosensor for DNAs.[47]

## Sensor Classification

The NP optical sensors that have been developed so far for intracellular measurements can be classified into two types (see Figure 1): (1) Type 1 sensor where the incorporated single component, usually fluorescent molecular probe, serves as an analyte recognizer as well as an optical signal transducer; (2) Type 2 sensor where the analyte recognizer and optical transducer are distinct. Type 2 sensors enable a synergistic signal and selectivity enhancement as well as sensitivity control that cannot be achieved with free molecular probes.

In a Type 1 sensor, fluorescent or Raman-active dyes are either encapsulated in or covalently linked to polymeric or metallic NPs. Upon binding with the analyte, the spectral change (fluorescence quenching/enhancement, fluorescence lifetime change or fluorescence peak shift, SERS) of the indicator dyes occurs. The sensitivity and selectivity of the sensor mostly depend on the incorporated indicator dye but are also affected by the NP matrix.

In a Type 2 sensor, nonfluorescent selective analyte-recognition elements or receptors (enzymes, antibodies, ligands, or aptamers) are either encapsulated in or covalently linked to the polymeric or metallic NPs. Binding of a specific analyte to the receptors produces an effect on the optical reporters that consist of co-loaded fluorescent dyes or the NP themselves (as for QDs or metallic NPs).

Both Type 1 and 2 sensors have been developed to detect a variety of intracellular analytes, as exemplified in the following sections.

# NP SENSORS FOR ION SENSING

## Type 1 Sensors
### Fluorescent Sensors

Type 1 fluorescent sensors, also called direct ion measurement PEBBLEs, have been developed for sensing $H^+$, $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Cu^{+/2+}$, and
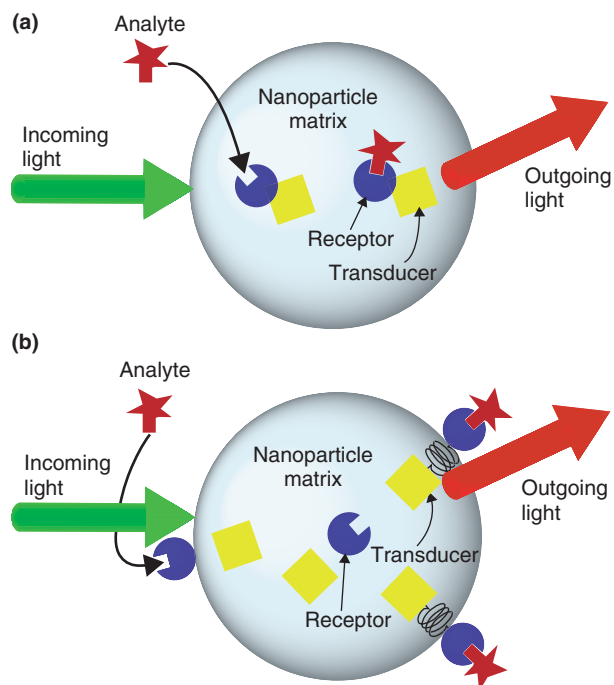
**(a)**



**(b)**



**FIGURE 1** | Schematic presentation of two kinds of nanoparticles (NP) sensors: (a) Type 1 where a single component serves as receptor and transducer; (b) Type 2 where receptor and transducer are separated but they communicate in order to produce optical signal change upon binding.

$Fe^{3+}$.[3,8,14–18,61,62] The design includes a fluorescent indicator and a reference dye entrapped in or covalently linked to an NP. The polyacrylamide NP has been used exclusively for this type of ion sensor because of its neutral and hydrophilic nature, which allows ions to readily permeate the NP matrix and interact with the indicator dye. The indicator dyes are mostly fluorescent molecular probes, but analyte-sensitive biological molecules, such as a red fluorescent protein, have also been used.[17] Type 1 ion PEBBLEs have been applied successfully for intracellular measurements of pH, $Mg^{2+}$, and $Ca^{2+}$. As an example, ratiometric calcium nano-PEBBLEs, containing the 'Calcium Green- 1′ ('Molecular

Probes') dye as sensing component and the sulforhodamine dye as reference, have been used to measure calcium release from mitochondria upon introduction of toxins.[63] Figure 2[64] shows a confocal microscope image of C6 glioma cells containing these PEBBLEs, after their selective delivery by liposomes (to the cytosol only). The sulforhodamine fluorescence is red (reference peak) in the image, while that of Calcium Green is yellow/green. The ratio of the Calcium Green/sulforhodamine intensity gives a good indication of cellular (cytosolar) calcium levels, regardless of dye or PEBBLE concentration, or fluctuations in light source intensity. The toxin, *m*-dinitrobenzene (DNB), was introduced on the left side of the sample (microscope slide) and allowed to diffuse to the right. The effect of DNB is a severe disruption of the mitochondrial function, followed by uncontrolled release of calcium (onset of a mitochondrial permeability transition). This caused calcium PEBBLEs inside the cytosol of different cells to 'light up' from left to right as a function of time. As a result, high resolution in both the spatial and temporal domains was obtained.

Another interesting intracellular application of Type 1 PEBBLE sensors was made with the $Mg^{2+}$ PEBBLEs, to study the chemical changes induced inside human macrophage cells by invading salmonella bacteria.[65] The $Mg^{2+}$ measurements by the PEBBLE sensors showed conclusively that $Mg^{2+}$ is not an important contributor in the control of pathogens by macrophages, in contradiction to previous reports.[66]

A different kind of direct ion NP sensor was designed using additional layers of polyelectrolytes on the surface of NPs for immobilizing indicator dyes.[67] In this work, the potassium ion indicator, potassium-binding benzofuran isophthalate, was immobilized within poly(styrene sulfonate)/poly(allylamine hydrochloride) films assembled on the surface of fluorescent europium NPs. The fluorescence from the (commercial) core nanoparicle serves as reference for a ratiometric measurement. The indicator retains its sensitivity to potassium ions after immobilization within

**FIGURE 2** | Confocal microscope image (time snapshot) of three human C6 glioma cells that contain Calcium Green/sulfarhodamine Photonic Explorer for Biomedical use with Biologically Localized Embeddings (PEBBLEs) (with *m*—dinitrobenzene (DNB) toxin dffusing from left to right). (Reprinted, with permission, from Ref. 64. Copyright 2003 Taylor & Francis Group).
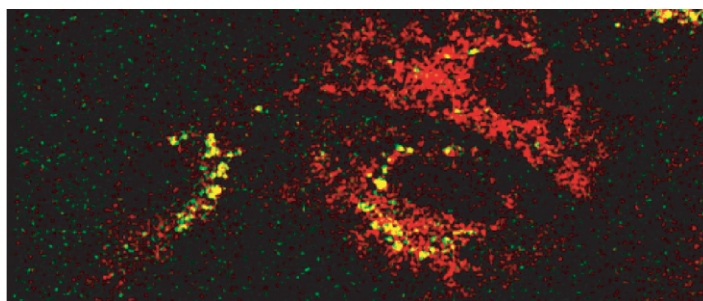
the films and exhibits sensitivity toward increases in potassium concentration over a broad range.

### SERS Sensors

An SERS pH sensor was developed with silver NPs (50–80 nm in diameter) functionalized with *para*-mercaptobenzoic acid (4-MBA).[50] The SERS spectrum from the functionalized silver NPs shows a characteristic response to the pH 6–8 of the surrounding solution. There was a large variability in the measured pH, as the SERS spectrum was observed only from aggregated particle clusters. These sensors were delivered into living Chinese Hamster Ovary (CHO) cells by passive uptake. The NP sensors retained their robust signal and sensitivity to pH within a cell. The spectrum indicates that the pH surrounding the NP is below 6, which is consistent with the particles being located inside a lysosome (pH 5).

A similar SERS pH sensor was designed on the basis of a gold nanoshell/silica core NP coated with a layer of *para*-MBA.[52] The nanosensor was capable of measuring pH in its local vicinity continuously over the range of 5.80–7.60 pH units.

## Type 2 Sensors

Type 2 NP sensors for cobalt, copper, hydrogen, nickel, potassium, silver, sodium, zinc, and chloride ions have been developed.

NP sensors called ion-correlation PEBBLEs have been developed for $Na^+$, $K^+$, and $Cl^-$ ions.[21–23] The sensor is made of poly(decylmethacrylate) (PDMA) NPs embedded with three components: a nonfluorescent ionophore that binds selectively to the ion of interest, a fluorescent hydrogen ion–selective dye that plays the role of a reporter, and a lipophilic additive that maintains ionic strength. The operation of the entire system is based on having a thermodynamic equilibrium that controls ion exchange (for sensing cations) or ion co-extraction (for sensing anions), i.e. an equilibrium-based correlation between different ion species. The degree of protonation measured from the fluorescence change of the hydrogen ion–selective dye is related to the concentration of the analyte ion by the theory developed for optical absorption–based ion-correlation sensors.[68,69] The hydrophobic PDMA matrix is selected to ensure a local chemical equilibrium among embedded components within NPs in the aqueous phase. The composition of the matrix, i.e. the cross-linker-to-monomer ratio, was found to affect the dynamic response range. Intracellular measurements of $K^+$ and $Cl^-$ ions were made by this type of PEBBLE sensors. The PDMA $K^+$ PEBBLE sensors, as an example, were introduced into rat C6 glioma cells using

a BioRad (Hercules, CA) Biolistic PDS-1000/He gene gun system.[21] The confocal images confirmed that the sensors were localized in the cytoplasm of the cells. The response of the PEBBLE sensors inside the cells to the addition of kainic acid, a $K^+$-channel-opening agonist, indicated an increase in $K^+$ concentration, the expected trend. Another $K^+$ NP sensor based on the same mechanism was designed with a different matrix, i.e. a poly(*n*-butyl acrylate) (PnBA) nano sphere of less than 200 nm in diameter.[27] This study shows that the composition of the three sensor components (ionophore, hydrogen-sensitive dye, and lipophilic additive) affects the characteristics of the sensors, such as its dynamic range, selectivity, and response time. It should be noted that the selectivity of these two Type 2 $K^+$ NP sensors[21,27] is higher than that of Type 1 $K^+$ NP sensor[67] by a factor of 1000–10,000.

Several FRET-based Type 2 ion NP sensors have been designed.

A Type 2 pH nanosensor was developed by coating a pH-insensitive fluorescent polystyrene bead (200 nm in diameter) with a layer of polyaniline (PANI) of only a few nanometers thick.[28] Plain PANI films display no fluorescence in the visible and near-IR range, but they do display characteristic pH-dependent absorption spectra that are due to protonation and deprotonation, respectively, of the emeraldine form of the PANI. Because of the fluorescence spectra of the beads being overlapped with the absorption spectra of PANI, the fluorescence intensity changes in accordance with the changes in pH.

Silica NPs have been utilized for two different designs of Type 2 fluorescence sensors for copper ions. In one design, the surface of silica NPs was covalently linked with a picolinamide subunit (selective $Cu^{2+}$ ligand) and fluorescent dansylamide.[36–38] The grafting of the ligand and the dye subunits to the NP's surface not only ensures the intercomponent communication in the sensor but also induces cooperative processes in the binding of the substrate. The sensitivity of the sensors was tuned by changing the ligand-to-dye ratio. In another silica-based design, silica NPs were prepared from a monomer containing chemosensor-like unit (similar to a molecular probe) made by coupling polyamine chains (receptor) and dansyl units (fluorophore).[5] These sensors may be classified as Type 1.5 sensors. The sensors were selective for copper, cobalt, and nickel ions and showed a greatly improved sensitivity from the occurrence of multicomponent cooperative photophysical processes.

Another Type 2 copper ion sensor was developed utilizing latex NPs. The hydrophobic fluorophore (BODIPY) is entrapped within the particle core, and the copper-chelating receptor (cyclam) is covalently

linked to the polymer backbone. The fluorescence of the dye is quenched upon binding $Cu^{2+}$ to cyclam because of FRET between the dye and copper cyclam complexes. The response of the sensors is fast, with 90% quenching within 1 s.[32,70]

QDs have also been used for designing ion nanosensors. The ligands coated on the surface of QDs were found to have a profound effect on the luminescence response of QDs to physiologically important metal cations. L-Cysteine- and thioglycerol-capped CdS QDs were used to detect zinc and copper ions in physiological buffer samples, respectively. The detection limits were 0.8 µM for zinc (II) and 0.1 µM for copper (II) ions.[42] Pentapeptide Gly-His-Leu-Leu-Cys-coated CdS QDs (2.4 ± 1.5 nm by transmission electron microscopy (TEM)) were designed to detect $Cu^{2+}$ and $Ag^+$ selectively, with high sensitivity, below 0.5 µ м.[43]

It is noted that the copper ion nanosensor has been the most studied among Type 2 NP sensors. Table 2 compares Type 1 and 2 NP sensors for copper ion that have been developed so far. It should be noted that, none of them has been applied for intracellular studies because the dynamic ranges of the developed sensors are above the normal unbound copper ion level, which is only femtomolar.[71,72] These sensors may be applied for cells under stressed conditions that could increase the free copper ion concentration to micromolar levels.[73] In order to study the copper ion homeostasis under normal conditions, a sensor with higher sensitivity needs to be developed.

## NP SENSORS FOR SMALL MOLECULES

### Dissolved Oxygen Sensor

All the NP sensors that have been developed for detecting dissolved oxygen belong to Type 1. The first NP sensor for dissolved oxygen was developed using hydrophilic silica NPs paired with ruthenium indicator dyes, and reference dyes.[35] The sensor was used successfully for the reliable oxygen imaging done inside live cells. NP sensors with enhanced sensitivity and dynamic range were developed using the more sensitive platinum-based oxygen-sensitive dyes and reference dyes, embedded in a hydrophobic matrix, organically modified silica (ormosil),[33] or PDMA.[24] The hydrophobic matrix is usually better suited for oxygen sensing than the hydrophilic one because of its higher oxygen solubility. The embedded platinum (II) octaethylporphine ketone, an oxygen-sensitive dye, has infrared (IR) fluorescence and makes the sensors work in human plasma samples,[24] unaffected by light scattering and autofluorescence. These PEBBLE nanosensors exhibit a perfectly linear Stern–Volmer calibration curve over the entire range of dissolved oxygen concentration, an ideal but previously unachieved goal for any fluorescent oxygen sensors. The sensitivity was very high with $Q_{DO}$ of 97–97.5% is the quenching response to dissolved oxygen, defined by

$$Q_{DO} = (I_{N_2} - I_{O_2})/I_{N_2} \times 100 \qquad (1)$$

where $I_{N_2}$ is the fluorescence intensity of the indicator dye or the indicator/reference intensity ratio in fully deoxygenated water, and $I_{O_2}$ is that in fully oxygenated water.

These oxygen nanosensors were also successfully applied for real-time imaging of oxygen inside live cells, monitoring metabolic changes inside live C6 Glioma cells.[33]

Oxygen sensors with additional layers of polyelectrolytes on the NP surface have been developed. The polyelectrolyte layers are used either to control the dye loading or to systematically assemble

**TABLE 2** | Copper Ion[a] NP Sensors

| Sensor type | Matrix | NP size (nm) | Recognition component | Signal producer | Optical signal | Detectable range | References |
|---|---|---|---|---|---|---|---|
| Type 1 | Poly (acrylamide) | 85 | DsRed | DsRed | Fluorescence | 200–5000 nM | 17 |
| Type 2 | Silica | 18–75 | Picolinamide | Dansylamide | Fluorescence | 4.7–200µM | 36–38 |
| | Silica | 30 | Polyamine | Dansyl unit | Fluorescence | 50–1000µM | 5 |
| | Latex | 16 | Cyclam | BODIPY derivative | Fluorescence | 1 nM–5µM | 32,70 |
| | CdS QD | 3.5 | Thioglycerol | QD | Fluorescence | 0.1–1600µM | 42 |
| | CdS QD | 2.4 | Peptide (Gly-His-Leu-Leu-Cys | QD | Fluorescence | 100 nM–2µM | 43 |

[a]Type 1 NP sensor based on DsRed is designed to sense both $Cu^+$ and $Cu^{2+}$, while all Type 2 NP sensors are made for $Cu^{2+}$ only.
NP, nanoparticles; QD, quantum dots

the sensors on the cell membranes. In one realization, commercial fluorospheres (100 nm) are coated with a multilayer of polyelectrolytes via layer-by-layer self-assembly, and then a ruthenium-based oxygen-sensitive fluorophore, (tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II), is post-loaded within the deposited polyelectrolyte multilayers.[74] The fluorescent NPs act as physical scaffolds and provide a reference peak for a ratiometric measurement. The sensitivity was medium level, with $Q_{DO}$ of 60%. The sensors were successfully delivered to the interior of human dermal fibroblasts via endocytosis with no apparent loss in cell viability. In another design, the same ruthenium-based dye is entrapped in commercial polystyrene beads of 100 nm in diameter, and poly(ethyleneimine) (PEI) is covalently linked to the NP surface via glutaraldehyde chemistry.[29] These nanosensors were assembled on individual *Saccharomyces cerevisiae* cells via electrostatic interactions between the positively charged PEI and negatively charged cell surfaces. This work demonstrates a proof of concept for self-assembly of nanosensors onto individual cell surfaces in a controlled manner for noninvasive examination of the oxygen concentration in the proximity of individual yeast cells.

Oxygen nanosensors were also developed on the basis of a nanometer-sized, polymerized phospholipid vesicle (liposome).[40] The liposomes of 150 nm diameter were prepared from 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) or DOPC doped with small (< 1%) mole percentages of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanol amine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE). These vesicles were then stabilized via a cross-linking polymerization of hydrophobic methacrylate monomers, partitioned into the hydrophobic interior of the DOPC bilayer. For oxygen detection, a ruthenium-based dye was encapsulated into the aqueous interior of the polymerized liposome. NBD-PE was used as a reference dye for ratiometric measurements. The Stern–Vomer plot provides a straight line over the entire dissolved oxygen range and the $Q_{DO}$ is 76%.

The oxygen NP sensors described above have all utilized fluorescence intensity for measurements. Lifetime measurement based oxygen sensors were also constructed by encapsulating Pt(II)-tetrapentafluorophenyl-porphyrin (PtPFPP) in polystyrene beads of 0.3–1µm in diameter.[30] The sensors were injected into plant cells using glass microcapillaries, and an optical multifrequency phase-modulation technique was used to discriminate the sensor signal from the strong autofluorescence of the plant tissue. The same sensors were injected into the salivary glands of the blowfly to quantify the changes in oxygen content within individual gland tubules during hormone-induced secretory activity.[75]

The NP sensors for dissolved oxygen are summarized in Table 3.

## NP Sensors for Reactive Oxygen Species

NP sensors have been developed for two molecular reactive oxygen species (ROS) (singlet oxygen and hydrogen peroxide) and one radical ROS (hydroxyl radical). These sensors were designed to show irreversible responses towards ROS, due to high reactivities and short lifetimes of the ROS.

### Singlet Oxygen Sensor

Ratiometric NP sensors for singlet oxygen have been developed using ormosil NPs.[34] These sensors incorporate the singlet oxygen–sensitive 9,10-dimethyl anthracene as an indicator dye and a singlet oxygen–insensitive dye, octaethylporphine, as a reference dye for ratiometric fluorescence-based analysis. The encapsulation of these dyes into the hydrophobic ormosil matrix results in a higher specificity toward singlet oxygen, as the matrix blocks the entry of short-lived polar ROS, such as OH and superoxide radicals. These nanoprobes have been used to monitor the singlet oxygen produced by 'dynamic nanoplatforms' that were developed for photodynamic therapy.[76]

### OH Radical Sensors

The hydroxyl radical is the most reactive ROS, presenting two problems for the construction of sensors: (1) inability to penetrate significantly into any matrix without being destroyed; (2) ability to oxidize (and photobleach) most potential reference dyes. A sensor was designed to get around these problems by attaching the hydroxyl indicator dye coumarin-3-carboxylic acid (CCA) onto the NP surface, while encapsulating the reference dye deep inside it.[20] The detection of this probe was based on the irreversible hydroxylation of a nonfluorescent form of CCA, resulting in a fluorescent product (7-hydroxycoumarin-3-carboxylic acid). This nanoprobe demonstrates a proof of principle of a ratiometric hydroxyl radical probe, with good sensitivity and reversibility.

### Hydrogen Peroxide Sensor

A poly(ethylene glycol) (PEG) hydrogel nanosphere (250–350 nm) with the encapsulated enzyme horseradish peroxide (HRP) was prepared and utilized as a sensor for hydrogen peroxide, based on the Amplex Red assay.[25] In the presence of HRP, Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine)

**TABLE 3 | Dissolved Oxygen NP Sensors**

| Sensor type | Matrix | NP size | Oxygen indicator | Optical signal | $Q_{DO}$ (%) | References |
|---|---|---|---|---|---|---|
| Type 1 | Silica | 20–300 nm | Ru(II)- tris(4,7-diphenyl-1,10-phenanthroline) dichloride | Fluorescence intensity | 80 | 35 |
| | Ormosil | 120 nm | Pt(II) octaethylporphine ketone | Fluorescence intensity | 97 | 33 |
| | Ormosil | 120 nm | Pt(II) octaethylporphine | Fluorescence intensity | 97 | 33 |
| | Poly(decyl methacrylate) | 150–250 nm | Pt(II) octaethylporphine ketone | Fluorescence intensity | 97.5 | 24 |
| | Commercial fluorophore with multilayer of polyelec-trolytes | 100 nm | Ru(II)- tris(4,7-diphenyl-1,10-phenanthroline) dichloride | Fluorescence intensity | 60 | 74 |
| | Polystyrene with covalently linked polyethylen-imine | 100 nm | Ru(II)- tris(4,7-diphenyl-1,10-phenanthroline) dichloride | Fluorescence intensity | Not available | 29 |
| | Polymerized liposome | 150 nm | Ru(II)- tris(4,7-diphenyl-1,10-phenanthroline) dichloride | Fluorescence intensity | 76 | 40 |
| | Polystyrene | 300 nm–1μm | Pt(II)-tetra-pentafluorophenyl-porphyrin | Fluorescence lifetime | Not available | 30,75 |

reacts with $H_2O_2$, in a 1:1 stoichiometry, to produce the red fluorescent oxidation product, resorufin. The response of the HRP-loaded PEG NPs changed as a function of $H_2O_2$ concentration in the presence of externally introduced Amplex Red, indicating that the enzyme activity of HRP was still maintained within the NPs. The HRP-loaded NPs were introduced via phagocytosis inside macrophages and were found to respond to exogenous $H_2O_2$ (100μm) as well as endogenous peroxide induced by lipopolysaccharide (1 μg/mL).

## Glucose Sensor

A poly(acrylamide) NP-based fluorescent glucose sensor was developed by incorporating glucose oxidase (GOx), an oxygen-sensitive ruthenium-based dye, and a reference dye.[19] This is a Type 2 sensor in which the enzymatic oxidation of glucose to gluconic acid results in the local depletion of oxygen, which is measured by the oxygen-sensitive dye. It should be noted that the traditional 'naked' molecular probes cannot be used to achieve this kind of synergistic task. The dynamic range was found to be ∼ 0.3–8 mM, with a linear range between 0.3 and 5 mM.

## Maltose Sensor

Three different designs of QD-based maltose sensors have been reported with maltose-binding proteins (MBPs) as maltose receptors. Two of them utilize the β-cyclodextrin-acceptor dye conjugates that are capable of binding within the saccharide-binding pocket of MBP and thus compete effectively with maltose, the MBP's preferred substrate.[44] In one configuration, a β-cyclodextrin–QSY9 conjugate is bound to an MBP located on the QD surface, resulting in FRET quenching of the QD photoluminescence. Added maltose displaces the β-cyclodextrin–QSY9, and the QD photoluminescence increases in a systematic manner. In another configuration, QDs were coupled with Cy3-labeled MBPs bound to β-cyclodextrin-Cy3.5. In this case, the QD donor drives the sensor function through a two-step FRET mechanism that overcomes inherent QD donor–acceptor distance limitations. A ratiometric measurement was made on the basis of the emission peaks of Cy3 and Cy 3.5. In these two designs, the loss of displaceable quenchers may cause errors. A QD–MBP-based maltose sensor was developed without quencher molecules.[45,46] In this design,

a ruthenium complex ([(tetraamine)(5-maleimido-phenanthroline)ruthenium]-[PF$_6$]$_2$) is covalently linked to MBP. The interaction (distance) between the Ru complex and QD changes in accordance with the conformational change of MBP upon binding with maltose, resulting in a concentration-dependent increase in QD fluorescence.

## Metronidazole Sensor

A Type 1 nanosensor for detecting metronidazole, a drug for the treatment of anaerobic protozoan and bacterium infections, was developed by covalent immobilization of indicator dye, 3-amino-9-ethylcarbazole (AEC), in poly(methacrylate) NP of the size less than 100 nm in diameter.[26] The obtained sensors have higher photostability and lower toxicity in comparison with free AEC. The results revealed that the probe showed good selectivity and had a linear response to the analyte in the range from $2.0 \times 10^{-5}$ to $1.0 \times 10^{-3}$ mol L$^{-1}$ with a detection limit of $9.0 \times 10^{-6}$ mol L$^{-1}$

## NP SENSORS FOR LARGE BIOLOGICAL MOLECULES

NP sensors have been used for detecting large biological molecules such as DNAs and proteins. The basic design is composed of NPs functionalized with receptors (antibodies,[52] DNAs,[48] or aptamers[77]) for target-specific detection. The analysis was done with various approaches including optical methods such as fluorescence, SERS, and SPR. These sensors have been developed for diagnostic assay, i.e. laboratory measurements of the analytes in biological samples like blood, which is not really relevant for this review article focused on direct intracellular measurements. Readers interested in these sensors are referred to the literature for reviews on NP-based diagnostic assay.[49,78–80]

## NP SENSORS FOR CELLULAR ACTIVITY

### Apoptosis Sensor

A nanosensor for detecting apoptosis of cells was developed by conjugating a caspase-specific FRET-based apoptosis reagent (PhiPhiLux G1D2) to the G5 poly(amidoamine) (PAMAM) dendrimer for apoptosis detection and folic acid for specific targeting.[31] The nanosensors were applied for apoptosis measurements in two different cell lines: KB cell (folate receptor positive) and UMSCC-38 cell (folate receptor negative). The cells were first incubated with either 0.45μM NP

sensors or phosphate-buffered saline (PBS) (untreated cells) for 30 min, added with either the apoptosis-inducing agent staurosporine at a concentration of 0.5μM or PBS (control), and incubated again for an additional 3 h. The apoptosis was observed on the basis of the fluorescence of the detached cells using a flow cytometer. The cell death by apoptosis was not monitored. The apoptotic KB cells increased the fluorescence intensity to a much greater degree, while the apoptotic UMSCC-38 cells did not show any increase in fluorescence intensity over the background fluorescence of stained control cells. These results suggest that the sensor can measure the intracellular activities or analytes in the specific location selected by the targeting moieties linked to the NP surface.

## NP Sensor for Lipid Peroxidation

A nanosensor for detecting lipid peroxidation by chemiluminescence was designed by conjugating Coumarin C343 (C$_{16}$H$_{15}$NO$_4$) to silica NPs (15 nm) and then entrapping these dye-linked silica in a sol–gel silica NP ($\sim$ 100 nm).[39] Coumarine C343 is known to enhance the weak chemiluminescence associated with lipid peroxidation. The produced nanosensor enhanced low-level chemiluminescence by approximately 100%.

## NP SENSORS FOR INTRACELLULAR PHYSICAL PROPERTIES

### NP Sensors for Electric Field

Intracellular electric fields have been measured by voltage dyes or patch/voltage clamps. These techniques frequently require lengthy calibration steps for each cell or cell type measured, and the measurements are confined to cellular membranes. A nanodevice to determine electric field inside any live cell or cellular compartment, called E-PEBBLE, was developed using polymerized micelles.[41] The E-PEBBLE is prepared by encasing the fast-response, voltage-sensitive dye di-4-ANEPPS inside the hydrophobic core of a silane-capped (polymerized) mixed micelle, which provides a uniform environment for the molecules and therefore allows for universal calibration.

The E-PEBBLEs are calibrated externally and applied for *in vitro* E-field determinations, with no further calibration steps. The PEBBLEs were introduced into immortalized rat astrocytes, DITNC cells, by endocytosis and enabled, for the first time, complete three-dimensional electric field profiling throughout the entire volume of living cells (not just inside membranes). This new ability is expected to greatly enhance the understanding of the role

of cellular E-fields in influencing and/or regulating biological processes, with wider implications for cellular biology, biophysics, and biochemistry.

## NP Sensor for Local Viscosity Measurements

A new type of sensors for local viscosity measurements has been developed with so called 'MOONs' (MOdulated Optical Nanoprobes). The MOONs are half-metal-capped fluorescent NPs whose fluorescence signals can be modulated according to their orientations, as the metal-coated side reflects the excitation light. The Brownian rotation or the rotational behaviors of the MOONs under an external magnetic field have been utilized to measure the local viscosity, which affects the rotation rate of the MOONs.[81,82] We note that the same rotational behavior of MOONs also allows the sensor's signal-to-noise (background) ratios (SNR) to be enhanced by up to 4000 times.[83] So far, MOON-based sensors have been developed using a micron size particle owing to the size-related difficulties for efficient magnetization or high fluorescent intensity of individual sensor particle. With recent progress on nanotechnology and coating technology, such as molecular beam epitaxy, nanometer-sized MOONs are being developed. This sensor design is quite attractive, as adding a metal coating on one hemisphere of any NP sensor containing a fluorescent indicator allows the simultaneous measurement of the local viscosity as well as the concentration of a chemical analyte. It also increases tremendously the SNR of the chemical sensing part.

## CONCLUSION

A variety of NP-based opical sensors have been developed in concurrence with advances in nanomaterials. These sensors provide minimal physical as well as chemical interferences owing to the combination of their small size and their protective NP matrix, or surface coatings. Some, but not all of these NP sensors have been successfully utilized for real-time measurements of important intracellular analytes. It has been reported that single-cell analysis has the potential for diagnosing diseases at an early stage, at which changes on a tissue level are not yet evident but chemical changes within cells are observable.[84] Getting chemical or physical information from a single cell or a specific location within a single cell would be one of the important future applications of NP sensors. The following issues must be considered in order to improve the performance of the NP sensors for wider and more effective future intracellular applications:

### Sensitivity and Signal-to-Noise

The goal will eventually be to enable single analyte molecule (ion) detection, in a single cell, *in vitro* or *in vivo*, despite the large background. Owing to the limited numbers of analyte molecules (ions) within a small volume single cell, instrumentation and sensing technology must meet stringent detection limits. One of the promising future sensor designs for enhanced sensitivity may be based on 'MOONs' that provide a background-free detection. This technique can be useful for samples with highly scattering and/or fluorescent backgrounds, or for experiments with several fluorescent probes.

### Selectivity

The selectivity of the sensors toward the analytes of interest is mainly determined by that of the molecular probes or receptors. A higher level of selectivity can be obtained by locating the NP sensors at a specific location in a live cell, either through molecular targeting groups conjugated to the NP surface, or through remote steering means such as magnetic or laser tweezers. A recent study demonstrates the potential use of magnetic tweezers for remote control of the orientation and position of the NP sensors.[85]

### Multiplexing Capability

Detection of multiple analytes can be made possible by a properly designed single NP sensor. An NP sensor containing multiple molecular probes or receptors that are specific to different analytes could be, for example, one in which the various optical signals are well resolved. The MOON-based fluorescent NP sensors provide another example of multitasking sensors that can measure the chemical property simultaneously with a physical property, such as local temperature or viscosity.[73,74,81,82] A third example could be given in which confocal microscopy resolves the individual signals from a number of cell-embedded nanosensors.

# REFERENCES

1. Buck SM, Xu H, Brasuel M, Philbert MA, Kopelman R. Nanoscale probes encapsulated by biologically localized embedding (PEBBLEs) for ion sensing and imaging in live cells. *Talanta* 2004, 63(1):41–59.

2. Graber ML, Dilillo DC, Friedman BL, Pastoriza-munoz E. Characteristics of fluoroprobes for measuring intracellular pH. *Anal Biochem* 1986, 156(1):202–212.

3. Clark HA, Hoyer M, Parus S, Philbert MA, Kopelman R. Optochemical nanosensors and subcellular applications in living cells. *Mikrochim Acta* 1999, 131(1–2):121–128.

4. Harrell JA, Kopelman R. Biocompatible probes measure intracellular activity. *Biophotonics Int* 2000, 7:22–24.

5. Montalti M, Prodi L, Zaccheroni N. Fluorescence quenching amplication in silica nanosensors for metal ions. *J Mater Chem* 2005, 15(27–28):2810–2814.

6. Montet X, Funovics M, Montet-Abou K, Weissleder R, Josephson L. Multivalent effects of RGD peptides obtained by nanoparticle display. *J Med Chem* 2006, 49(20):6087–6093.

7. Sasaki K, Shi ZY, Kopelman R, Masuhara H. Three-dimensional pH microprobing with an optically-manipulated fluorescent particle. *Chem Lett* 1996, (2):141–142.

8. Clark HA, Barker SLR, Brasuel M, Miller MT, Monson E, et al. Subcellular optochemical nanobiosensors: probes encapsulated by biologically localised embedding (PEBBLEs). *Sens Actuators, B* 1998, 51(1–3):12–16.

9. Kneipp K, Kneipp H, Itzkan I, Dasari RR, Feld MS. Surface-enhanced Raman scattering and biophysics. *J Phys Condens Matter* 2002, 14(18):R597–R624.

10. Kneipp J, Kneipp H, McLaughlin M, Brown D, Kneipp K. In vivo molecular probing of cellular compartments with gold nanoparticles and nanoaggregates. *Nano Lett* 2006, 6(10):2225–2231.

11. Bosnick KA, Jiang J, Brus LE. Fluctuations and local symmetry in single-molecule rhodamine 6G Raman scattering on silver nanocrystal aggregates. *J Phys Chem B* 2002, 106(33):8096–8099.

12. Nabiev IR, Morjani H, Manfait M. Selective analysis of antitumor drug-interaction with living cancer cells as probed by surface-enhanced Raman-spectroscopy. *Eur Biophys J* 1991, 19(6):311–316.

13. Ramanavièius A, Herberg FW, Hutschenreiter S, Zimmermann B, Lapënaitë I, et al. Biomedical application of surface plasmon resonance biosensors (review). *Acta Med Lit* 2005, 12(3):1–9.

14. Clark HA, Hoyer M, Philbert MA, Kopelman R. Optical nanosensors for chemical analysis inside single living cells. 1. Fabrication, characterization, and methods for intracellular delivery of PEBBLE sensors. *Anal Chem* 1999, 71(21):4831–4836.

15. Park EJ, Brasuel M, Behrend C, Philbert MA, Kopelman R. Ratiometric optical PEBBLE nanosensors for real-time magnesium ion concentrations inside viable cells. *Anal Chem* 2003, 75(15):3784–3791.

16. Sumner JP, Aylott JW, Monson E, Kopelman R. A fluorescent PEBBLE nanosensor for intracellular free zinc. *Analyst* 2002, 127(1):11–16.

17. Sumner JP, Westerberg N, Stoddard AK, Fierke CA, Kopelman R. $Cu^+$ and $Cu^{2+}$ sensitive PEBBLE fluorescent nanosensors using Ds red as the recognition element. *Sens Actuators, B* 2005, 113(2):760–767.

18. Sumner JP, Kopelman R. Alexa Fluor 488 as an iron sensing molecule and its application in PEBBLE nanosensors. *Analyst* 2005, 130(4):528–533.

19. Xu H, Aylott JW, Kopelman R. Fluorescent nano-PEBBLE sensors designed for intracellular glucose imaging. *Analyst* 2002, 127(11):1471–1477.

20. King M, Kopelman R. Development of a hydroxyl radical ratiometric nanoprobe. *Sens Actuators, B* 2003, 90(1–3):76–81.

21. Brasuel M, Kopelman R, Miller TJ, Tjalkens R, Philbert MA. Fluorescent nanosensors for intracellular chemical analysis: decyl methacrylate liquid polymer matrix and ion-exchange-based potassium PEBBLE sensors with real-time application to viable Rat C6 glioma cells. *Anal Chem* 2001, 73(10):2221–2228.

22. Brasuel M, Kopelman R, Kasman I, Miller TJ, Philbert MA. Ion concentrations in live cells from highly selective ion correlations fluorescent nanosensors for sodium. *Proc IEEE Sens* 2002, 1:288–292.

23. Brasuel MG, Miller TJ, Kopelman R, Philbert MA. Liquid polymer nano-PEBBLES for $Cl^-$ analysis and biological applications. *Analyst* 2003, 128(10):1262–1267.

24. Cao YF, Koo YE, Kopelman R. Poly (decyl methacrylate)-based fluorescent PEBBLE swarm nanosensors for measuring dissolved oxygen in biosamples. *Analyst* 2004, 129(8):745–750.

25. Kim SH, Kim B, Yadavalli VK, Pishko MV. Encapsulation of enzyme within polymer spheres to create optical nanosensors for oxidative stress. *Anal Chem* 2005, 77(21):6828–6833.

26. Tan SZ, Jiang JH, Yan BN, Shen GL, Yu RQ. Preparation of a novel fluorescence probe based on covalent immobilization by emulsion polymerization and its application to the determination of metronidazole. *Anal Chim Acta* 2006, 560(1–2):191–196.

27. Ruedas-Rama MJ, Hall EAH. $K^+$-selective nanospheres: maximizing response range and minimizing response time. *Analyst* 2007, 131(12):1282–1291.

28. Pringsheim E, Zimin D, Wolfbeis OS. Fluorescent beads coated with polyaniline: a novel nanomaterial for optical sensing of pH. *Adv Mater* 2001, 13(11):819–822.

29. Kuang YN, Walt DR. Detecting oxygen consumption in the proximity of saccharomyces cerevisiae cells using self-assembled fluorescent nanosensors. *Biotechnol Bioeng* 2007, 96(2):318–325.

30. Schmälzlin E, van Dongen JT, Klimant I, Marmodée B, Steup M, et al. An optical multifrequency phase-modulation method using microbeads for measuring intracellular oxygen concentrations in plants. *Biophys J* 2005, 89(2):1339–1345.

31. Myc A, Majoros IJ, Thomas TP, Baker JR. Dendrimer-based targeted delivery of an apoptotic sensor in cancer cells. *Biomacromolecules* 2007, 8(1):13–18.

32. Meallet-Renault R, Pansu R, Amigoni-Gerbier S, Larpent C. Metal-chelating nanoparticles as selective fluorescent sensor for $Cu^{2+}$. *Chem Commun* 2004, 20:2344–2345.

33. Koo YE, Cao Y, Kopelman R, Koo SM, Brasuel M, et al. Real-time measurements of dissolved oxygen inside live cells by ormosil (organically modified silicate) fluorescent PEBBLE nanosensors. *Anal Chem* 2004, 76(9):2498–2505.

34. Cao Y, Koo YEL, Koo S, Kopelman R. Ratiometric singlet oxygen nano-optodes and their use for monitoring photodynamic therapy nanoplatforms. *Photochem Photobiol* 2005, 81(6):1489–1498.

35. Xu H, Aylott JW, Kopelman R, Miller T, Philbert M. A Real-time ratiometric method for the determination of molecular oxygen inside living cells using sol gel-based spherical optical nanosensors with applications to rat C6 glioma. *Anal Chem* 2001, 73(17):4124–4133.

36. Brasola E, Mancin F, Rampazzo E, Tecilla P, Tonellato U. A fluorescence nanosensor for $Cu^{2+}$ on silica particles. *Chem Commun* 2003, 24:3026–3027.

37. Rampazzo E, Brasola E, Marcuz S, Mancin F, Tecilla P, et al. Surface modification of silica nanoparticles: a new strategy for the realization of self-organized fluorescent chemosensors. *J Mater Chem* 2005, 15(27–28):2687–2696.

38. Arduini M, Marcuz S, Montolli M, Rampazzo E, Mancin F, et al. Turning fluorescent dyes into Cu(II) nanosensors. *Langmuir* 2005, 21(20):9314–9321.

39. Baker N, Greenway GM, Wheatley RA, Wiles C. A chemiluminescence nanosensor to monitor lipid peroxidation. *Analyst* 2007, 132(2):104–106.

40. Cheng ZL, Aspinwall CA. Nanometre-sized molecular oxygen sensors prepared from polymer stabilized phospholipid vesicles. *Analyst* 2006, 131(2):236–243.

41. Tyner KM, Kopelman R, Philbert MA. "Nano-sized voltmeter" enables cellular-wide electric field mapping. *Biophys J* 2007, 93:1163–1174.

42. Chen YF, Rosenzweig Z. Luminescent CdS quantum dots as selective ion probes. *Anal Chem* 2002, 74(19):5132–5138.

43. Gattas-Asfura KM, Leblane RM. Peptide-coated CdS quantum dots for the optical detection of copper(II) and silver(I). *Chem Commun* 2003, 21:2684–2685.

44. Medintz IL, Clapp AR, Mattoussi H, Goldman ER, Fisher B, et al. Self-assembled nanoscale biosensors based on quantum dot FRET donors. *Nat Mater* 2003, 2(9):630–638.

45. Sandros MG, Gao D, Benson DE. A modular nanoparticle-based system for reagentless small molecule biosensing. *J Am Chem Soc* 2005, 127(35):12198–12199.

46. Sandros MG, Shete V, Benson DE. Selective, reversible, regentless maltose biosensing with core-shell semiconducting nanoparticles. *Analyst* 2006, 131(2):229–235.

47. Maxwell DJ, Taylor JR, Nie S. Self-assembled nanoparticle probes for recognition and detection of biomolecules. *J Am Chem Soc* 2002, 124:9606–9612.

48. Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL, Mirkin CA. Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science* 1997, 277(5329):1078–1081.

49. Thaxton CS, Georganopoulou DG, Mirkin CA. Gold nanoparticle probes for the detection of nucleic acid targets. *Clin Chim Acta* 2006, 363(1–2):120–126.

50. Talley CE, Jusinski L, Hollars CW, Lane SM, Huser T. Intracellular pH sensors based on surface-enhanced Raman scattering. *Anal Chem* 2004, 76(23):7064–7068.

51. Bishnoi SW, Rozell CJ, Levin CS, Gheith MK, Johnson BR, et al. All-optical nanoscale pH meter. *Nano Lett* 2006, 6(8):1687–1692.

52. Hirsch LR, Jackson JB, Lee A, Halas NJ, West J. A whole blood immunoassay using gold nanoshells. *Anal Chem* 2003, 75(10):2377–2381.

53. Kumar MNVR. Nano and microparticles as controlled drug delivery devices. *J Pharm Pharm Sci* 2000, 3(2):234–258.

54. Koo YEL, Fan W, Hah HJ, Kopelman R, Xu H, et al. Photonic explorers based on multifunctional nanoplatforms for biosensing and photodynamic therapy. *Appl Opt* 2007, 46(10):1924–1930.

55. Bailey RE, Smith AM, Nie S. Quantum dots in biology and medicine. *Physica E* 2004, 25(1):1–12.

56. Sun YH, Liu YS, Vernier PT, Liang CH, Chong SY, et al. Photostability and pH sensitivity of CdSe/ZnSe/ZnS quantum dots in living cells. *Nanotechnology* 2006, 17(17):4469–4476.

57. Clapp AR, Medintz IL, Mauro JM, Fisher BR, Bawendi MG, et al. Fluorescence resonance energy transfer between quantum dot donors and dye-labeled protein acceptors. *J Am Chem Soc* 2004, 126(1):301–310.

58. Willard DM, Van Orden A. Quantum dots: resonant energy-transfer sensor. *Nat Mater* 2003, 2(9):575–576.

59. Alivisatos P. The use of nanocrystals in biological detection. *Nat Biotechnol* 2004, 22(1):47–52.

60. Kneipp K, Haka AS, Kneipp H, Badizadegan K, Yoshizawa N, et al. Surface-enhanced Raman spectroscopy in single living cells using gold nanoparticles. *Appl Spectrosc* 2002, 56(2):150–154.

61. Buck SM, Koo YEL, Park E, Xu H, Philbert MA, et al. Optochemical nanosensor PEBBLEs: photonic explorers for bioanalysis with biologically localized embedding. *Curr Opin Chem Biol* 2004, 8(5):540–546.

62. Sun H, Scharff-Poulsen AM, Gu H, Almdal K. Synthesis and characterization of ratiometric, pH sensing nanoparticles with covalently attached fluorescent dyes. *Chem Mater* 2006, 18(15):3381–3384.

63. Clark HA, Kopelman R, Tjalkens R, Philbert MA. Optical nanosensors for chemical analysis inside single living cells. 2. Sensors for pH and calcium and the intracellular application of PEBBLE sensors. *Anal Chem* 1999, 71(21):4837–4843.

64. Monson E, Brasuel M, Philbert M, Kopelman R. PEBBLE nanosensors for in vitro bioanalysis. In: Vo-Dinh T: ed. *Biomedical Photonics Handbook*. Boca Raton, FL: CRC Press LLC; 2003:, 59–1–59–14.

65. Martin-Orozco N, Touret N, Zaharik ML, Park E, Kopelman R, et al. Visualization of vacuolar acidification-induced transcription of genes of pathogens inside macrophages. *Mol Biol Cell* 2006, 17(1):498–510.

66. Vescovi EG, Soncini FC, Groisman EA. $Mg^{2+}$ as an extracellular signal: environmental regulation of Salmonella virulence. *Cell* 1996, 84(1):165–174.

67. Brown JQ, McShane MJ. Core-referenced ratiometric fluorescent potassium ion sensors using self-assembled ultrathin films on europium nanoparticles. *IEEE Sens J* 2005, 5(6):1197–1205.

68. Bühlmann P, Pretsch E, Bakker E. Carrier-based ion-selective electrodes and bulk optodes, 2. Ionophores for potentiometric and optical sensors. *Chem Rev* 1998, 98(4):1593–1687.

69. Bakker E, Simon W. Selectivity of Ion-sensitive bulk optodes. *Anal Chem* 1992, 64(17):1805–1812.

70. Méallet-Renault R, Hérault A, Vachon JJ, Pansu RB, Amigoni-Gerbier S, et al. Fluorescent nanoparticles as selective Cu(II) sensors. *Photochem Photobiol Sci* 2006, 5(3):300–310.

71. Changela A, Chen K, Xue Y, Holschen J, Outten CE, et al. Molecular basis of metal-ion selectivity and zeptomolar sensitivity by CueR. *Science* 2003, 301(5638):1383–1387.

72. Finney LA, O'Halloran TV. Transition metal speciation in the cell: insights from the chemistry of metal ion receptors. *Science* 2003, 300(5621):931–936.

73. Bush AI. Metals and neuroscience. *Curr Opin Chem Biol* 2000, 4(2):184–191.

74. Guice KB, Caldorera ME, McShane MJ. Nanoscale internally referenced oxygen sensors produced from self-assembled nanofilms on fluorescent nanoparticles. *J Biomed Opt* 2005, 10(6):1–10.

75. Schmälzlin E, Walz B, Klimant I, Schewe B, Löhmannsröben HG. Monitoring hormone-induced oxygen consumption in the salivary glands of the blowfly, Calliphora vicina, by use of luminescent microbeads. *Sens Actuators, B* 2006, 119(1):251–254.

76. Reddy GR, Bhojani MS, McConville P, Moody J, Moffat BA, et al. Vascular targeted nanoparticles for imaging and treatment of brain tumors. *Clin Cancer Res* 2006, 12(22):6677–6686.

77. Levy M, Cater SF, Ellington AD. Quantum-dot aptamer beacons for the detection of proteins. *Chembiochem* 2005, 6(12):2163–2166.

78. Penn SG, Hey L, Natan MJ. Nanoparticles for bioanalysis. *Curr Opin Chem Biol* 2003, 7(5):609–615.

79. Kubik TK, Bogunia-Kubik K, Sugisaka M. Nanotechnology on duty in medical applications. *Curr Pharm Biotechnol* 2005, 6(1):17–33.

80. Fischer NO, Tarasow TM, Tok JBH. Heightened sense for sensing: recent advances in pathogen immunoassay sensing platforms. *Analyst* 2007, 132(3):187–191.

81. Behrend CJ, Anker JN, McNaughton BH, Kopelman R. Microrheology with modulated optical nanoprobes (MOONs). *J Magn Magn Mater* 2005, 293(1):663–670.

82. McNaughton BH, Agayan RR, Wang JX, Kopelman R. Physiochemical microparticle sensors based on nonlinear magnetic oscillations. *Sens Actuators, B* 2007, 121(1):330–340.

83. Anker JN, Kopelman R. Magnetically modulated optical nanoprobes. *Appl Phys Lett* 2003, 82(7):1102–1104.

84. Soper SA, Brown K, Ellington A, Frazier B, Garcia-Manero G, et al. Point-of-care biosensor systems for cancer diagnostics/prognostics. *Biosens Bioelectron* 2006, 21(10):1932–1942.

85. Anker JN, Koo YE, Kopelman R. Magnetically controlled sensor swarms. *Sens Actuators, B* 2007, 121(1):83–92.

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