Luminescent Silica Nanosensors for Lifetime Based Imaging of Intracellular Oxygen with Millisecond Time Resolution

Longjiang Ding, Wei Zhang, Yinglu Zhang, Zhenzhen Lin, and Xu-dong Wang* 

Department of Chemistry, Fudan University, 200433 Shanghai, P. R. China

ABSTRACT: Intracellular oxygen concentration was quantitatively imaged and rapidly traced with millisecond time resolution. We have demonstrated a new kind of oxygen nanosensors based on a ruthenium complex doped solid silica nanoparticles, which showed high oxygen sensing performance (I0/I100 = 3.29, t50 < 3 s) and ease of surface functionalization. Their sensing performance can be tuned by changing types of oxygen-sensitive probes and particle morphology. The nanosensors showed excellent control in both sensor size (from 30 to 200 nm), monodispersity, morphology, surface chemistry, and batch to batch consistency. Their uniform size distribution and good biocompatibility made them suitable for intracellular studies. Because the sensor surface can be easily functionalized with arbitrary units (such as transmembrane motifs, drugs, organelle-targeting groups, imaging reagent, and multiple sensor probes), these nanosensors provide a general platform to build easy-to-use tools for intracellular applications. The ease of surface functionalization was demonstrated by modifying the sensors outer surface with morpholinopropylamine and (3-carboxypropyl) triphenyl phosphonium, to actively target intracellular lysosomes and mitochondria of the tested cell lines (HeLa, MCF-7, and MCF-10A). Applying the mitochondria-targeting oxygen nanosensor together with our custom-built rapid phosphorescent lifetime imaging system, variations of intracellular oxygen have been quantitatively imaged and traced (in millisecond intervals) in real time and in situ.

Intracellular metabolism of molecular oxygen is the major route to provide basic energy for cellular activities and functionalities. Excess supply and insufficient delivery of intracellular oxygen would result in both damage to cell behavior and viability.1−5 Tracing variations of intracellular oxygen concentration and studying how cells (especially brain, neuronal, and immune cells) utilize oxygen provides invaluable information for biology and medicine. Thus, it is important to monitor and quantify oxygen delivery, diffusion, and distribution inside cells online and in situ.

Oxygen sensing based on luminescence-quenching attracted much attention in recent two decades due to their ease of usage, noninvasiveness, capability of intracellular imaging (high throughput), remote signal readout, and miniaturized size down to the nanosize range.3−5 It has become the major method of choice in studying intracellular oxygen variation.6 However, most oxygen-sensitive probes (OSPs) possess a large conjugated π-system in their chemical structures, and are hydrophobic in nature, which make them difficult to be directly used for intracellular studies.5,6 Therefore, it is not a surprise to witness that encapsulating hydrophobic OSPs in compatible gas-permeable nanomaterials to form nanosensor becomes the most popular approach for intracellular oxygen sensing.8 Water-soluble OSPs have been reported and successfully applied for intracellular studies.9−14 These probes have much smaller size compared with polymeric nanosensors and offer high spatial resolution. However, their applications were limited by complicated synthesis and difficulties in both functionalization and cellular internalization. The hydrophobic luminescent core of these water-soluble probes should be fully protected, for example, by dendritic structure.15 Otherwise, their luminescence can be influenced by many factors, including solvent effect, ions, biomolecules, etc. Modification of hydrophobic fluorinated OSPs with hydrophilic glucose and galactose provided another solution.16 These conjugates had minimum aggregation and self-quenching and were successfully applied as oxygen imaging probes for 3D tissue models. However, their hydrophobic luminescent core was not fully protected and may still suffer from influences of potential quenchers.

Modern optical oxygen nanosensors were mostly prepared using commercially available polymers, such as polystyrene,17−20 polyacrylamide,21,22 Eudragit RL-100,23,24 poly(styrene-b-2-vinylpyrrolidone),25 luminescent conjugated polymer dots,26−30 and micelles31−34 because they are readily available with distinct oxygen permeability. However, polymer functionalization is difficult, since most polymers are chemi-
cally inert after production, and introduction of functional groups will influence both polymerization process and polydispersity of obtained nanomaterials. Nanoprecipitation of binary polymer composites provides an alternative approach to produce nanosensors with chemically functional surface.\(^{35}\) However, it is nearly impossible to precisely control and tune surface chemistry of polymer nanomaterials while keeping their monodispersity. Techniques for particle size control of polymer-based nanosensors are not well established, and most of them always exhibit poor dispersity and broad size distribution. Established techniques for monodispersed polymer nanoparticles (such as poststaining, microemulsion polymerization, dispersed polymerization, and mini-emulsion solvent evaporation) are limited to several polymers (e.g., polystyrene). Numerous in vivo researches have revealed that the size, surface chemistry and morphology of nanomaterials determine their biological fate.\(^{36−39}\) Therefore, the biodistribution of polymer-based nanosensors is hardly to be anticipated and controlled. Although the organic–inorganic hybrid nanomaterials developed by Kopelman and co-workers provided improved surface functionalities, they still suffer from their poor size control (100−600 nm in diameter),\(^{40}\) and their hydrophobic shell makes further chemical modification difficult.\(^{41}\)

Silica nanoparticles have excellent size and morphology control (from several nanometer to micrometer) and well-established functionalization techniques for surface chemistry modulation.\(^{42}\) They are optically transparent, highly stable and monodispersed in aqueous solution, and have reproducible composition. All these features made them attractive and suitable to build nanosensors for intracellular applications.\(^{43,44}\) However, due to their dense structure and poor gas adsorption,\(^{45}\) they were excluded from constructing nano-sensor for oxygen. Attempts have been made to immobilize oxygen-sensitive probes in silica gel (with large size 9.5−11 \(\mu\)m) and bulky silica xerogel.\(^{46−48}\) Obviously, these materials are too large in size for in vivo applications. Chu et al.\(^{49}\) reported that Pt(II) complex can be entrapped in core−shell silica nanoparticles (average size 170 nm). Embedding these particles in sol−gel matrix resulted in a planar sensor film for oxygen. In the reported silica nanoparticle based oxygen nanosensors,\(^{40−52}\) OSPs were either physically absorbed or chemically immobilized on silica surface rather than encapsulated into the interiors. Physically absorbed OSPs can leach out, and requires hydrophobic silica surface to firmly hold the OSPs, which hinders the biocompatibility of the nanosensors. Covalently immobilization of OSPs on silica surface requires chemical modification of OSPs, and immobilized OSPs cannot be fully protected from potential quenchers.

In an attempt to dope OSPs inside the interior of solid silica nanoparticles, we have observed that OSPs-doped solid silica nanoparticles show good response to molecular oxygen. They not only show good sensitivity and fast response to oxygen but also exhibit excellent size and morphology control and good biocompatibility. Interestingly, oxygen-sensing performance can be tuned by changing the types of OSPs and silica morphologies. Because of their “clean” outer surface, these nanosensors can be further functionalized with arbitrary units for intracellular active-targeting and multiple purposes of applications. We have demonstrated the ease of functionalization by modifying the surface of nanosensors with organelle-targeting groups and used them for sensing oxygen concentration at subcellular level. Owing to the long luminescent lifetime of the nanosensors, we are able to image intracellular oxygen concentration using our custom-built microscopic rapid lifetime imaging system. The combination of rapid lifetime imaging technique with solid-silica based nanosensors shows superior accuracy and reproducibility, which provides important tools for intracellular oxygen studies, and allows us quantitatively imaging and tracing intracellular oxygen concentration over time and in situ with millisecond time resolution.

**EXPERIMENTAL SECTION**

**Characterization.** The morphology and size of nanosensors were characterized using a Field Emission Transmission Electron Microscopy (TEM, FEI Tecnai G2 F20 S-Twin). The concentration of oxygen was controlled by two mass-flow controllers (Red-Y, www.voegtlins.com, Switzerland). Luminescence spectra was recorded using a fluorospectrometer (Hitachi F-7000, Japan). Zeta potential was measured using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). Cell viability was studied on a Synergy H1 Hybrid Multi-Mode Microplate Reader. Fluorescence images of HeLa cells were obtained using a wide-field fluorescence microscope (Leica DMi8, Germany). The filter cube sets are shown in Table 1.

**Table 1. Filter Cube Sets for the Fluorescence Images Using the Wide-Field Fluorescence Microscopy**

<table>
<thead>
<tr>
<th>name</th>
<th>excitation (nm)</th>
<th>emission (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru(dp2)</td>
<td>yellow channel</td>
<td>425−475</td>
</tr>
<tr>
<td>lysotracker green</td>
<td>green channel</td>
<td>340−380</td>
</tr>
<tr>
<td>mito-tracker deep red</td>
<td>red channel</td>
<td>595−645</td>
</tr>
</tbody>
</table>

The rapid phosphorescent lifetime imaging (rPLIM) system consists of a PI-MAx4 Gen III ICCD camera (Princeton Instruments, USA.), a fiber-optic laser (FC-ML, Changchun, China), and the Leica DMi8 wide-field fluorescence microscope. Signal synchronization of the laser and the camera were controlled by a built-in timing generator of the ICCD camera. The laser beam was guided using an optical fiber into the microscope. The excitation pulse duration was set at 100 \(\mu\)s, and gate width was optimized at 8 \(\mu\)s. The first gate was opened 600 ns after the laser was tuned off. Lifetime image acquisition and data processing were performed using the LightField software (v6.3.1) from Princeton Instruments and Matlab (v2015b) from MathWorks.

**Synthesis of Oxygen-Sensitive Nanosensors.** Nonporous Ru(dp2)@silica nanoparticles (RuNPSiNPs) were synthesized following a modified Stöber method.\(^{53}\) Typically, 815 \(\mu\)L of deionized water, 100 \(\mu\)L of ammonium hydroxide (28%); 0.14 M,\(^{36}\) and 8.4 mL of ethanol were mixed at room temperature. Then, different amounts of tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) bis(perchlorate) complex (Ru(dp2))\(_2\)(ClO\(_4\))\(_2\) denoted as Ru(dp2)) were dissolved in 300 \(\mu\)L of tetraethyl orthosilicate (TEOS; 0.14 M) and added in the mixture. The solution was stirred in dark for 24 h. The obtained brownish silica nanoparticles were harvested by centrifuging at a g-force of 17 000g. The produced nanosensors were washed with ethanol for at least three times to remove unbounded dyes and unreacted chemicals, and stored in ethanol at a concentration of 10.0 mg mL\(^{-1}\).
Mesoporous Ru(dpp)@silica nanoparticles (RuMPSiNPs) were synthesized in an aqueous buffer solution with pH 7.00.\(^\text{54}\) Initially, 0.25 g of hexadecyltrimethylammonium bromide (CTAB) and 0.092 g of polyoxylethylene (20) cetyl ether (Brij-58) were dissolved in 25 mL of phosphate-buffered saline (pH 7.00) at 95 °C under vigorous stirring. Then, 450 μL of TEOS with dissolved Ru(dpp)\(_2\)(ClO\(_4\))\(_2\) was added slowly in the buffer solution. After continuous stirring for 8 h, synthesized nanoparticles were collected by centrifuging at 17,000 g, washed three times by ethanol, and stored in ethanol.

Dendritic mesoporous Ru(dpp)@silica nanoparticles (RuDPSiNPs) were prepared via the sol–gel approach.\(^\text{55}\) In a typical process, 0.375 g of CTAB was mixed with 50 μL of triethanolamine (TEOA) solution (TEOA/H\(_2\)O (w/w) = 1/3), 7.5 mL of deionized water and stirred at 60 °C for 30 min. Subsequently, 2.0 mL of cyclohexane was added and continuously stirred for 5 min. Then 500 μL of TEOS with Ru(dpp)\(_2\)(ClO\(_4\))\(_2\) was added and stirred for 6 h. After that, the mixture was added into 10 mL of ethanol, followed by centrifuging at 17,000 g to isolate the particles. After washed with ethanol for three times, the obtained RuDPSiNPs were stored in ethanol.

**Surface Modification with Organelle-Targeting Groups.** First, a solution of 3-morpholinopropylamine (MPA; 1.0 μM in ethanol) or (3-carboxypropyl)-triphenylphosphonium bromide (TPP-COOH; 1.0 μM in DMSO) was reacted with N,N′-dicyclohexylcarbodiimide (DCC; 3.75 μM) and N-Hydroxysuccinimide (NHS; 3.75 μM) at room temperature for 2 h to give active-ester. Then, 10 μmol 3-aminopropyltriethoxysilane (APTES) was added into the active-ester solution. The mixture was kept in dry argon atmosphere, and stirred for 12 h to give silanes with active-ester groups. Finally, the obtained silanes were added into 1.0 mL 10.0 mg mL\(^{-1}\) RuNPSiNPs and incubated on a rotary shaker for 20 h. The solid was collected by centrifuging at 17,000 g, followed by washing three times with ethanol and water. The surface-modified RuNPSiNPs were dispersed in ethanol at a concentration of 10.0 mg mL\(^{-1}\).

**Oxygen Responses of the Nanosensors Based on the Measurement of Luminescent Lifetime.** The oxygen response of the nanosensors was studied directly on the microscope. Typically, 1.5 mL oxygen-sensitive nanosensors dispersed in water at a concentration of 1.0 mg/mL was placed in a plastic microscopic dish. The solution was then saturated with synthetic gas containing different concentrations of oxygen for 15 min. The oxygen concentration in the synthetic gas was adjusted using two mass-flow controllers. The nanosensors were imaged on the Leica DMi8 fluorescent microscope equipped with the filter cube set for Ru(dpp) (Table 1). The lifetimes of nanosensors at different particle size, chemical composition and morphology with excellent property control and tunability.\(^\text{54,56,57}\) In order to encapsulate luminescent OSPs inside silica nanoparticles, OSPs should have good solubility and chemically fit the physicochemical properties of silica matrix. There are residual silanol groups (−Si−OH) inside silica nanoparticle,\(^\text{58}\) which makes the interior of silica negatively charged. To chemically fit the special microenvironment inside silica, we have selected a positively charged OSP, tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) perchlorate (referred as Ru(dpp)), to prepare nanosensors for oxygen.\(^\text{59,60}\) The Ru(dpp) dissolved well in silica precursor tetraethyl orthosilicate (TEOS), and directly encapsulated inside silica nanoparticles during particle formation. Attempts had been made to encapsulate photostable fluorinated metallophorphyrins (such as PtTFPP) inside silica nanoparticles, but failed. Although they were soluble in silica precursor TEOS, continuously hydrolyzation of TEOS produces hydrophilic oligomers and reduces the solution’s hydrophobicity. The reduction in hydrophobicity caused precipitation and aggregation of PtTFPP. Therefore, condensation of hydrophilic oligomers and formation of nanoparticles occurred in the absence of PtTFPP, which produced silica nanoparticles without oxygen-sensitive probes.

The Ru(dpp)-doped nonporous solid silica nanoparticles (RuNPSiNPs) were produced via the Stöber method. Transmission electron microscopic (TEM) image (Figure
showed the RuNPSiNPs were monodispersed and had a narrow size distribution (75 ± 5 nm, Figure S1). The nanosensors showed excellent size control by simply adjusting ammonia concentration in the synthetic media, and nanosensors with diameter from 30 to 200 nm can be massively produced (at gram level) via the one-pot reaction (Figure S2). Elemental mapping of RuNPSiNPs (Figure 1b) shows that Ru(dpp) were uniformly distributed throughout silica nanoparticles. The RuNPSiNPs offer high luminescent brightness even in pure oxygen environment (Figure S3), suggesting sufficient number of photons emitted when excited at 445 nm. Calculation based on UV/vis absorption spectra reveals that there are ∼4680 Ru(dpp) molecules encapsulated inside a single nanosensor, which warrants that it is bright enough for microscopic imaging.

The quenchability of RuNPSiNPs (expressed as $I_0/I_{100}$ where $I_0$ and $I_{100}$ are the intensities at 0 and at 100% oxygen) reaches 3.29 (Figure 1c,d), which is even higher than that of Ru(dpp) in polystyrene particles ($I_0/I_{100} = ∼2.3$) indicating the nonporous silica matrix have good oxygen permeability. The oxygen response is fully reversible, and the response time ($t_{95}$) is less than 3 s (including gas diffusion time from the gas controllers to the measurement cell). Moreover, the RuNPSiNPs showed excellent batch to batch consistency in terms of size, morphology, monodispersity and oxygen sensing performance, which ensures repeatable and reliable sensor properties in real applications (Figure S4).

Figure 1. (a) TEM image of RuNPSiNPs (insert: the structure model). (b) EDX element mapping images of the elements Si, O, and Ru of RuNPSiNPs. (c) The reversible oxygen response of the RuNPSiNPs. (d) The corresponding Stern–Volmer plot. The calibration curve fits well with the Stern–Volmer equation, and the two-site model was applied to calculate Stern–Volmer parameters. Stern–Volmer equation:

$$I = I_0 \frac{1}{1 + K_1 O_{100} + \frac{1-f}{1 + K_2 O_{100}}}$$

Tunable Sensing Performance. The oxygen response can be tuned by modulating the morphology of the nanosensors. Two different kinds of Ru(dpp)-doped porous silica nanoparticles have been synthesized. One is mesoporous (denoted as RuMPSiNPs), and the other is dendritic and porous (referred as RuDPSiNPs). Their oxygen responses were studied and compared to that of nonporous nanosensors. Figure 2 shows these porous nanosensors have well-defined, uniform and regular pore structures, which are beneficial for gas molecules diffusing in and out. For RuMPSiNPs, TEM showed their average diameter is 80 ± 5 nm (Figure 2a). Oxygen response study reveals RuMPSiNPs have a quenchability of 2.14 (Figure 2b). The typical oxygen response time ($t_{95}$) is only 2 s, which can be attributed to the porous structure and high gas accessibility (Figure S5). Nanosensors made from dendritic porous silica nanoparticle had larger pores (∼10 nm). They were uniform in size and had an average size of 72 ± 8 nm (Figure 2c). The larger inner pores are beneficial for the diffusion of oxygen molecules in and out, and RuDPSiNPs exhibited higher sensitivity, especially at low oxygen concentration (<20%; Figure 2d). Compared with nonporous nanosensors, the porous structure does not significantly improve sensor quenchability, which is out of our expectation. Detailed studies have shown that the existence of structure-directing surfactant influences oxygen diffusion (data not shown).

Analytical Chemistry Article

 DOI: 10.1021/acs.analchem.9b03726

Figure 2. Two different kinds of Ru(dpp)-doped porous silica nanoparticles have been synthesized. One is mesoporous (denoted as RuMPSiNPs), and the other is dendritic and porous (referred as RuDPSiNPs). (a) TEM image of RuMPSiNPs. (b) Oxygen response study reveals RuMPSiNPs have a quenchability of 2.14. The typical oxygen response time ($t_{95}$) is only 2 s, which can be attributed to the porous structure and high gas accessibility. (c) Nanosensors made from dendritic porous silica nanoparticle had larger pores (∼10 nm). They were uniform in size and had an average size of 72 ± 8 nm. (d) Compared with nonporous nanosensors, the porous structure does not significantly improve sensor quenchability, which is out of our expectation. Detailed studies have shown that the existence of structure-directing surfactant influences oxygen diffusion (data not shown).
The oxygen response can be further tuned by varying types of OSPs. To chemically fit the microenvironment of silica nanoparticles, hydrophilic OSPs are preferred and should be chemically immobilized inside silica nanoparticles to prevent dye leakage. The hydrophilic probe, Pt(II) meso-tetra (4-carboxyphenyl) porphine (PtTCPP), was selected as an example to show the capability of tuning sensor response. PtTCPP was covalently immobilized inside solid silica nanoparticles using the same procedure as RuNPSiNPs. Results showed PtTCPP-doped silica nanosensors show much higher quenchability ($I_0/I_{100} = 18.67$; Figure S6), because the Pt(II) porphyrins have much longer luminescence lifetime. However, the poor photostability of PtTCPP limited their applications in long-term intracellular imaging.

**Measuring Dissolved Oxygen via Luminescence Lifetime.** The long luminescence lifetime of OSP-doped silica nanoparticles offers another attractive feature that intracellular oxygen can be imaged via lifetime measurement. Luminescence lifetime is an intrinsic parameter of a luminophore, and its value is not depending on dye concentration and excitation light intensity. Lifetime measurements are self-referenced, and immune to photobleaching and auto fluorescence, which are notorious error sources for quantitative analysis, especially under intense illumination on a fluorescence microscope. The use of highly stable luminescent materials together with
...domain imaging system with high frame rate. Dissolved oxygen in the range from 0 to 40 mg L$^{-1}$ since the rPLIM measurement is independent of probe and it takes only 118 ms to image a sample at a spatial resolution of 1024 pixels. The current system is limited in di...meters, 7 these new nanosensors showed uniform performance in different cell lines, including the tested breast cancer MCF-7 cells (Figure S12a) and normal breast MCF-10A cells (Figure S12b). These results proved the ease of surface-functionalization of the new nanosensor, which provided an easy-to-use platform for intracellular studies. The outer surface of nanosensors can be further explored for intracellular multiple sensing, drug delivery, sense and treat of diseases, imaging guide surgeries, etc.

**Organelle-Targeted Imaging.** Before applying the RuNPSiNPs for intracellular studies, their cytotoxicity in HeLa cells was studied using CCK-8 assay. Results showed that cell viability remains above 90% when concentration of nanosensors was below 150 μg mL$^{-1}$ (Figure S9), demonstrating their low cytotoxicity and good biocompatibility. Incubation of HeLa cells with RuNPSiNPs for 12 h showed that bare RuNPSiNPs cannot pass through cell membranes and remain at extracellular space (Figure S10). This is mainly due to the negatively charge outer surface (zeta potential, $-41.2$ mV) and results in low interaction with negatively charged cell membranes. 18,69

The well-established silane technique allows us to easily functionalize nanosensors’ surface with required features. We can simply feature the nanosensors with lysosome- and mitochondria-targeting capabilities by covalently immobilizing MPA and TPP groups on nanosensor surface, respectively. Colocalization experiments showed that they had excellent organelle-targeting capability, with Spearman’s rank correlation coefficient above 0.8 for both lysosome and mitochondria targeting (Figure 4 and S11). Figure 4a showed that RuNPSiNPs-Lyso was mainly located in lysosome ($\rho = 0.86$, Figure S11b), and barely found in lysosomes ($\rho = 0.3$, Figure S11b). Moreover, unlike the polymer-based nanosensors whose intracellular internalization is highly depending on cell types, these new nanosensors showed uniform performance in different cell lines, including the tested breast cancer MCF-7 cells (Figure S12a) and normal breast MCF-10A cells (Figure S12b).
The mitochondria-targeted nanosensors were further applied for tracing variations of intracellular oxygen concentration over time. To better demonstrate the performance of the sensors and the high speed and accuracy of the rPLIM approach, local oxygen concentration of HeLa cells was modulated using glucose and glucose oxidase. When glucose oxidase catalytically oxidized glucose, oxygen was consumed and caused decrease in local oxygen concentration. Figure 5b (black line) showed that the brightness of nanosensors decreased significantly over time, because of dye photobleaching under intensive incident light (Figure S13). It is difficult to quantitatively measure oxygen concentration via luminescence intensity (Figure 5a and red line in Figure 5b). In contrast, local intracellular oxygen concentration can be measured using our rPLIM technique, which offers much more stable and reproducible data (Figure 5c). As shown in Figure 5d, the luminescence lifetime increased rapidly and reached maximum after 5 min of adding glucose and glucose oxidase. The lifetime decreased again after 5 min, because molecular oxygen in the air dissolved again in the media and quenched sensor luminescence. After calculating the oxygen concentration according to the calibration plot (Figure S14), it is obvious that intracellular oxygen concentration can completely recover to its initial value (around 8 mg mL⁻¹, Figure 5e), which proves that the lifetime measurement is much more reliable and accurate. Because of the high brightness of the nanosensors, it takes only 118 ms to
record a microscopic lifetime image, which makes tracing rapid intracellular events (such as calcium burst, neuron activities) possible. The conventional laser scanning lifetime imaging techniques need several minutes or even half of an hour to obtain a lifetime image, which will lose the temporal information and are not suitable for tracing fast intracellular events. Owing to its good temporal resolution and high accuracy, the combination of rPLIM with the new nanosensors provide important tools for intracellular oxygen studies.

■ CONCLUSION

It is shown that OSPs-doped solid silica nanoparticles have good sensitivity and fast fluorometric response to oxygen. The sensors exhibit excellent control in size, morphology, monodispersity, surface chemistry, and batch to batch consistency. Oxygen responses can be tuned by changing silica morphologies and types of OSPs. Their large surface area and ease of surface modification enable sensor surface to be functionalized with arbitrary components (such as targeting, drug delivery, multiple sensing, imaging, etc.), which provide a versatile platform to build multifunctional nanosensors for intracellular applications. After modifying nanosensor surface with lysosome and mitochondria active-targeting groups, they can be internalized by cells and precisely located in these organelles. By employing our custom-built rPLIM system, variations of intracellular oxygen concentration can be traced in real time (with milliseconds interval) and quantitatively with superior accuracy and reproducibility. Because of its high temporal resolution, this system is extremely suitable for studying fast processes, such as photosynthesis, mitochondria function, and utilization of oxygen of neuron cells, response of cellular metabolism to drugs, and so on.

■ ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.9b03726.

Source of materials, particle size distribution, luminescence spectra, size control, batch to batch consistency, luminescence lifetime, and cytotoxicity of RuNPSiNPs; response of the RuNPSiNPs; response of PtTCP doped solid silica nanoparticles; fluorescence images of HeLa cells, MCF-7 cells, and MCF-10A cells treated with nanosensors; the photobleaching of the nanosensors inside cells during the monitoring process and the luminescence lifetime calibration plot (PDF).

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: wangxudong@fudan.edu.cn.

ORCID

Xu-dong Wang: 0000-0002-3402-7995

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the National Key R&D Program of China (2017YFC0906800), National Natural Science Foundation of China (21775029), the Recruitment Program of Global Experts (1000 Talent program) in China, and the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning (No. TP2014004).

■ REFERENCES
