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Review—Intracellular Sensors Based on Carbonaceous Nanomaterials: A Review

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In recent years, carbon nanomaterials and their derivatives/composites have attracted much attention for their role in new developments in the field of biosensors due to their unique electronic, optical, thermal and mechanical properties in biosensors, which inspires us to compile this review. To focus on the relationship between cell biology and some diseases (e.g., cancer or diabetes), this review describes the applications of various types of carbon nanomaterials in intracellular sensors. We also introduce four kinds of intracellular sensors based on carbon nanomaterials, including intracellular pH sensors, intracellular thermal sensors, intracellular metal ions sensors, intracellular biomolecule sensors. Then, we briefly summarize the applications of carbon nanomaterials based intracellular sensors for diagnosis or treatment of various diseases. Finally, a future perspective and the challenges of intracellular sensors based on carbon nanomaterials are briefly rendered.

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Carbon nanomaterials refer to carbon materials having at least one dimension of less than 100 nm and are composed of sp^2 and sp^3 -bonded carbon atoms or heterogeneous components (non-carbon atoms),¹ which generally include carbon dots (also known as carbon quantum dots, CDs), fullerene, carbon nanotube, graphene, graphene oxide and graphitic carbon nitride ($g-C_3N_4$).^{2–5} Due to excellent physical, chemical, mechanical and electrical properties, carbon nanomaterials have attracted intensive interest and have been widely used to fabricate sensors, such as gas or vapor sensors,^{6–12} electrochemical sensors,^{13–30} biological sensors,^{31–37} strain sensors,^{38–44} optical chemical sensors.^{45,46} In particular, the fascinating biocompatibility and chemical inertness of carbon nanomaterials and their composites make them attractive candidates for perception intracellular microenvironment (e.g., pH value and temperature),^{13,47–49} sensing molecules or ions inside cells.^{50–53}

Cell biology aims to study the structures and functions of millions of biomolecules in single cells, and to discover the relationship between abnormal cell metabolism and some diseases such as cancer or diabetes.^{54,55} Intracellular microenvironment (e.g., pH value and temperatures) and the spatiotemporal distribution of molecules or ions in cells play important roles in cell metabolism.⁵⁶ Abnormal microenvironment parameters and inappropriate concentrations of molecules or ions are intuitional indicators for these intractable diseases.⁵⁷ Thus, recent advances in the development of cell biology and sensing systems have been achieved, which allow for the precise and rapid detection of intracellular species and the complex microenvironment in live cell for biomedical applications.^{49,58–62} However, using common sensing system, the determination of some given analytes or specific microenvironments in cells is easily interfered by the complicated cellular matrices and unavoidable background signals. Here carbon nanomaterials-based sensors may offer great opportunities to monitor intracellular microenvironment and analyzed disease-related species in complicated live cells due to their excellent properties.

In this review, we will summarize the most recent advance on the applications of intracellular sensors fabricated by carbon nanomaterials and their composites (Scheme 1) was summarized. Firstly, we will briefly introduce various kinds of carbon nanomaterials, including CDs or graphene quantum dots, carbon nanotubes, graphene or graphene oxide, $g-C_3N_4$ and fullerene, and their possibility as intracellular sensors. Then, we will discuss the application of intracellular sensors and summarize four kinds of

intracellular sensors based on carbon nanomaterials, i.e., intracellular pH sensors, intracellular thermal sensors, intracellular metal ions sensors, and intracellular biomolecule sensors. We also briefly summarize the applications of carbon nanomaterials based intracellular sensors for diagnosis or treatment of various diseases. Lastly, the future perspectives and challenges of intracellular sensors based on carbon nanomaterials are briefly rendered.

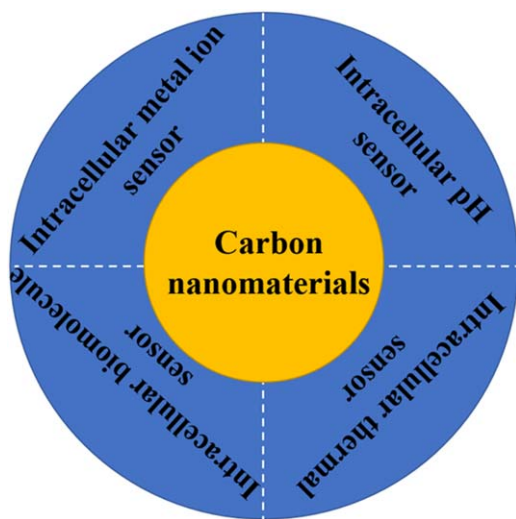
Carbon Nanomaterials and Their Possibility as Intracellular Sensors

Carbon nanomaterials and its derivatives have unique electronic, optical, thermal, and mechanical properties and have attracted considerable attention in the field of biosensors or intracellular sensor in recent years. In this section, we will summarize and discuss the properties of various kinds of carbon nanomaterials and the possibility of application in intracellular sensor for each of carbon nanomaterials.

CDs or graphene quantum dots are zero-dimensional carbon nanomaterials.^{63,64} Considerable attention has been paid to fluorescent CDs or graphene quantum dots owing to their small sizes (~ 5 nm), low toxicity, good biocompatibility, stable photoluminescence and chemical inertness, which are advantageous over other fluorescence probes used for intracellular sensors.^{20,65} In the application of intracellular sensor, CDs generally enter living cells by incubating living cells with CDs at 37 °C and then intracellular microenvironment (e.g., pH value and temperatures) or intracellular molecules or ions are analyzed by CDs through confocal fluorescence microscopic technique or electrochemistry methods.

Carbon nanotubes, including single-walled carbon nanotubes and multi-walled carbon nanotubes, are one kind of carbon nanomaterials with relatively large surface area, unique chemical property (including mechanical, electrical, thermal and catalytic properties). Especially, the excellent catalytic effect between multi-walled carbon nanotubes and metal/metal oxide or sulfides have attracted intensive interest.^{66–72} The single-walled carbon nanotubes have a strong photoluminescence and have been used to analyze intracellular microenvironment or intracellular molecules or ions by entering the living cell in the intracellular sensing system. Multi-walled carbon nanotubes with excellent electronic properties and functional surface structures, are not only used to prepare electrode to detect substances secreted by living cells in extracellular sensing system,^{73,74} but also modified with designed biomolecules or dyes via functional groups (such as, carboxyl groups) to achieve sensing functions in intracellular sensing system.^{74,75}

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Scheme 1. A summary of carbon nanomaterials used as intracellular sensors.

Graphene is another kind of carbon nanomaterial, which is composed of sp^2 -hybridized carbon atom and exhibits no fluorescence due to a zero optical bandgap.^{76–79} Graphene oxide is obtained by introducing functional groups to graphene, and shows fluorescence. Graphene or graphene oxide has shown great potentials for biosensing applications due to its good water solubility, large surface area, unique surface properties, low cytotoxicity and high cellular uptake efficiency.^{80–82} Graphene is usually used to detect substances secreted by living cells or cell lysates in extracellular sensing system through electrochemical methods.^{83,84} In sensing system, graphene oxide has been used to sense intracellular microenvironment⁸⁵ and determine the molecules or ions inside/outside cells.^{86,87}

$g-C_3N_4$, including bulk $g-C_3N_4$, $g-C_3N_4$ nanosheets, and $g-C_3N_4$ quantum dots, possesses superior optical properties, thermostability, chemical stability, biocompatibility and low toxicity.^{88–92} Based on its excellent properties, $g-C_3N_4$ has been used to detect pH, metal ions, biomolecules in living cells. In sensing system, $g-C_3N_4$ has been used to sense intracellular microenvironment and determine the intracellular molecules or ions by entering living cells or outside the living cells.

Fullerene is also one of kind carbon nanomaterials. Recent studies^{93,94} have proved that fullerene was toxicity to bacteria due to direct oxidation of the cell, therefore the application of fullerene in intracellular sensor was still be explored.

Intracellular Sensor Based on Carbon Nanomaterials

Carbon nanomaterials have been widely studied for intracellular sensor. In this section, we will summarize and discuss the application of carbon nanomaterials in pH sensing, thermal sensing, metal sensing and biomolecule sensing in living cells, respectively.

Intracellular pH sensors.—Intracellular pH is an important parameter of intracellular microenvironment. It is directly related to protein conformational transformation and enzyme catalytic efficiency.^{95,96} In order to maintain normal physiological functions, the pH of intracellular organelles (e.g., cytoplasm, mitochondria and lysosomes) are located in a very narrow range.⁹⁷ Abnormal intracellular pH causes cytopathic and even apoptosis, and is one of the important indicators of many diseases including cancer.^{98–101} Due to the importance of pH in the cell, there are a variety of intracellular pH sensors fabricated by carbon nanomaterials described in this section.

Chen et al.¹⁰² have reported an intracellular pH sensor, which has been prepared by multi-walled carbon nanotubes decorated with gold/silver core-shell nanoparticles (Au@Ag NPs) labeled with 4-mercaptobenzoic acid (4MBA) and fluorescein isothiocyanate

(FITC), for detecting pH variation through exploit the surface enhanced Raman scattering (SERS) spectra features of 4MBA molecules during intracellular process such as endocytosis (Fig. 1a). Shao et al.⁸⁵ have developed an intracellular pH sensor based on graphene oxide-DNA nanosystem fabricated by coupling a hybrid comprising dye-labeled i-motif forming oligonucleotide (IFO) and its cDNA with one mismatch to graphene oxide to sensitively detect pH variations in live cells (Fig. 1b). Shi et al.¹⁰³ have proposed an intracellular pH sensor based on nitrogen-rich functional groups carbon nanoparticles (N-CNs) for pH fluorescence imaging of live T24 cells (Fig. 1c). Furthermore, Zhang et al.¹⁰⁴ even have prepared a lysosome pH sensor with CDs (Fig. 1d).

Intracellular thermal sensors.—Intracellular temperature is another important parameter of intracellular microenvironment. First, intracellular temperature affects and regulates chemical and biological reactions in cytoplasm and in organelles, such as endoplasmic reticulum, mitochondria and nucleus.^{105–107} For example, the intracellular temperature affects and regulates the synthesis of DNA in nucleus, and also regulates the formation of adenosine triphosphate (ATP) in mitochondria. Second, temperature variation in cells can be an indicator for some disease. For example, the temperature in cancer cells is higher than normal cells owing to excessive metabolic activities or reactive oxygen species (ROS) scavenging in cancer cells, therefore temperature increase in cell can be an evidence for cancer diagnosis.¹⁰⁸ Consequently, there is a need for seeking methods based on new materials that enable reliable and accurate monitoring intracellular temperature. Carbon nanomaterials have attracted increasing interest in monitoring intracellular temperature because of their fascinating biocompatibility and chemical inertness.^{49,109,110}

Kumawat et al.⁵⁸ have synthesized a graphene quantum dots using ethanolic extracts of *mangifera indica* (mango) leaves with excitation-independent near-infrared (NIR) fluorescence emission. The graphene quantum dots have intracellular temperature sensing properties and have been used to analyze intracellular temperature (25 °C–45 °C) of live L929 cells (Fig. 2a).

Shi et al.¹¹¹ have used green fluorescence N, S co-doped CDs as a stable bio-imaging probe for sensing of intracellular temperature. Specifically, Hela cells are stained by the N, S co-doped CDs and the green fluorescence of Hela cells in the temperature of 35 °C is weaker than in the temperature of 25 °C (Fig. 2b).

We et al.¹¹² have prepared a ratiometric intracellular temperature sensor by a fluorescence probe (a CDs-RhB probe) fabricated by conjugating Rhodamine B (RhB) with CDs via a covalent bond. The CDs-RhB probe has two noticeable peaks, 482 and 586 nm, which could be assigned to the emission from CDs and RhB. Therefore, the intracellular temperature of cell is quantified by calculating the ratio between these two peaks (I_{586}/I_{482}) (Fig. 2c).

Macairan et al.¹¹³ also have proposed a ratiometric intracellular temperature sensor by a temperature-dependent fluorescence CDs synthesized via a microwave method. The CDs have a blue band and a red fluorescence band when excited at 405 nm. With increasing temperature, red-to-blue fluorescence ratio are increased. The ratiometric change in fluorescence has been also observed in cells incubated at different temperatures, based on which the ratiometric intracellular temperature sensor has been prepared (Fig. 2d).

Kavitha et al.¹¹⁴ have prepared a thermometer for sensing intracellular temperatures by a fluorescent thermo-responsive polymer consisting of poly(N-vinylcaprolactam) (PVCL) coupled with CDs (PVCL-CDs). Figure 2e shows the grey-scale intensity of the fluorescence microscopy images of NIH 3T3 cells incubated in PVCL-CDs solution for 24 h obviously decreases as temperature increases. Therefore, the biocompatible PVCL-CDs could be used as an intracellular thermometer.

Intracellular metal ions sensors.—Metal ions are required for all cells by serving as essential structural cofactor, redox centers and oxygen porter of chemical and biological reactions in intracellular or

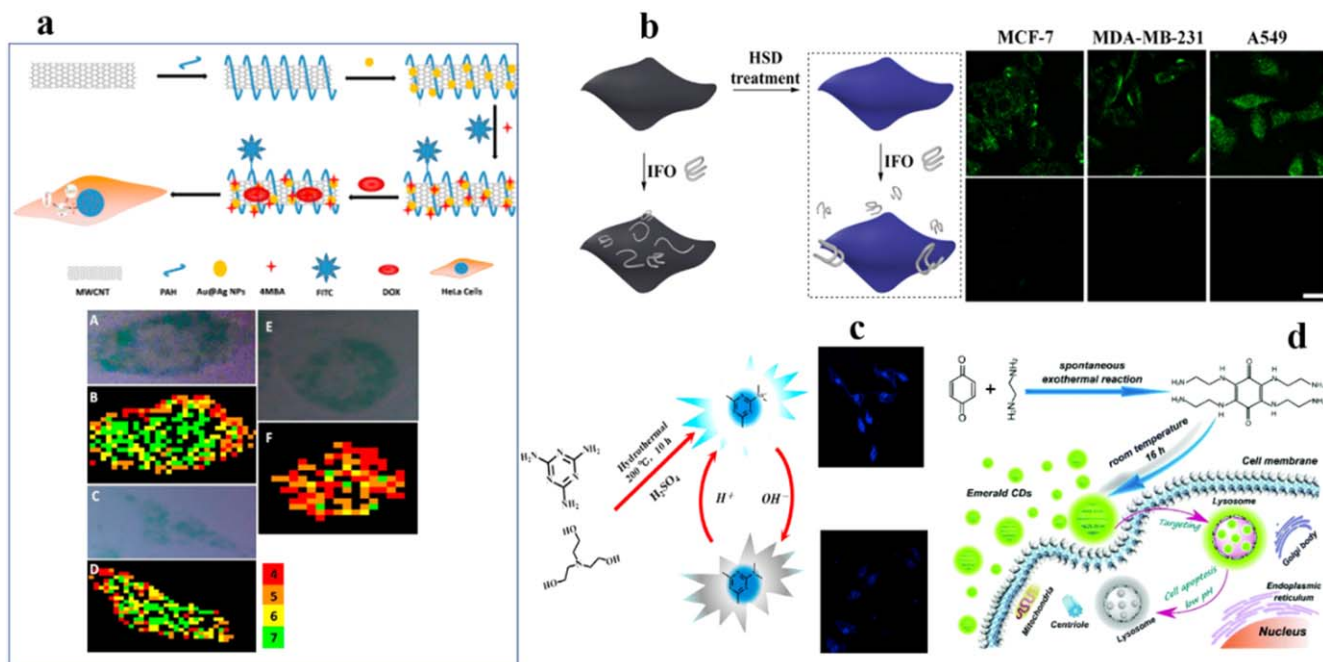


Figure 1. (a) The synthesis and functionalization procedure of multifunctional nanocarrier based on multi-walled carbon nanotubes (MWCNT) (up); The bright field image of HeLa cells incubated with multi-walled carbon nanotubes for (A) 2 h, (C) 6 h, (E) 24 h. The SERS mapping image of the ratio of the SERS intensity at 1390 cm^{-1} and 1183 cm^{-1} , (B) 2 h, (D) 6 h, (F) 24 h (down).¹⁰² Reproduced with permission Copyright 2015, Elsevier B.V. (b) Illustration of physical adsorption between IFO and pristine graphene oxide (Left) or herring sperm DNA-treated graphene oxide (Right) under acidic conditions; Fluorescence images (scale bar = $25\text{ }\mu\text{m}$) of MCF-7, MDA-MB-231, and A549 cells probed by the graphene oxide-cDNA/IFO nanosystem with herring sperm DNA passivation at pH 5.0 and 7.3.⁸⁵ Reproduced with permission Copyright 2017, American Chemical Society. (c) Schematic illustrations for the synthesis process of N-CN and pH-responsive.¹⁰³ Reproduced with permission Copyright 2016, Elsevier B.V. (d) Schematic representation of CDs preparation and lysosomal pH monitoring.¹⁰⁴ Reproduced with permission Copyright 2018, The Royal Society of Chemistry.

subcellular regions.^{115–117} Therefore, there is great interest in accurate monitoring of the distribution of intracellular metal ions to study the cell biology of metals.^{118–121} As nanomaterials, carbon nanomaterials have been widely studied for determination metal ions and possessed promising potential in metal ion sensor. Much research effort has been devoted to develop intracellular metal ions sensors for understanding physiological or pathological functions of metal ions in living cells. In recent years, carbon nanomaterials were used to probe various types of metal ions, such as Fe^{3+} , Ag^{+} , Cu^{2+} , Al^{3+} , Zn^{2+} , Pb^{2+} , and Cr^{3+} .^{122–126}

Cao et al.¹²⁷ have successfully prepared an intracellular Ag^{+} sensor based on acetaldehyde-modified $\text{g-C}_3\text{N}_4$ ultrathin nanosheets (ACNNSs). The ACNNSs are low cytotoxicity and good biocompatibility. HeLa cells after incubating with ACNNSs for 6 h show bright blue fluorescence, whereas negligible fluorescence in HeLa cells with ACNNSs is observed followed by incubating with Ag^{+} for 2 h. The above results proved that ACNNSs can react with intracellular Ag^{+} , therefore intracellular Ag^{+} sensor has been established (Fig. 3a).

Zou et al.¹²⁸ have proposed an intracellular Cu^{2+} ratiometric nanosensor based on a satellite hybrid nanoprobe composed by CdTe/CdS quantum dots capped around the silica microsphere and blue-emitting CDs. Intracellular Cu^{2+} could quench the CdTe/CdS quantum dots emission (at 650 nm) effectively but have no ability for reduction of the CDs emission (at 425 nm). The variation of intracellular Cu^{2+} concentration is in accordance with linearly with decrease of the F_{650}/F_{425} ratio, and satellite hybrid nanoprobe is utilized to the ratiometric fluorescence sensing for Cu^{2+} in HeLa cells (Fig. 3b).

Shi et al.¹²⁹ have developed an intracellular Fe^{3+} nanosensor based on multi heteroatoms (nitrogen and phosphorus) co-doped carbon nanodots (N, P-CDs). The N, P-CDs could enter into intracellular region with high cell viability. Consequently, the usefulness of N, P-CDs in sensing intracellular Fe^{3+} are demonstrated by confocal fluorescence images. Experimental results prove

that the fluorescence brightness of cells became weaker as Fe^{3+} into cells treated with N, P-CDs. All observations indicate that the proposed N, P-CDs could be used to effectively semiquantitative sensing Fe^{3+} in live cells (Fig. 3c).

Xiong et al.¹³⁰ have developed an intracellular Pb^{2+} sensor via electrochemiluminescence method using N doped CDs (N-CDs) in situ electro-polymerized onto a glassy carbon electrode (GCE) as luminophores, and Pd-Au hexoctahedrons (Pd@Au HOHs) as enhancers (Fig. 3d). Yan et al.¹³¹ have described an intracellular Hg^{2+} sensor using photoluminescent CDs (Fig. 3e).

Intracellular biomolecule sensors.—Intracellular biomolecules, including biomacromolecules (e.g., proteins, nucleic acids and polysaccharides) and small biomolecules (e.g., cysteines, cholesterol, glutathione and ascorbic acids), are crucial for cell metabolic processes. Intracellular bioenzyme, as one kind of proteins or ribonucleic acids (RNA), ensures biological reactions and chemical reactions in living cells perform efficiently and specifically.¹³² Intracellular nucleic acids, including deoxyribonucleic acids (DNA) or RNA, exist in all cells and create, encode, store, transmit and express information inside/outside the cells.¹³³ Some small biomolecules, such as cysteines, play considerable roles in peptide and protein synthesis, and some small biomolecules, such as ascorbic acid, are basic vitamins for biological systems and participate in amino acid metabolism, collagen formation and ion absorption.^{134,135} Despite the necessity and importance of biomolecules for intracellular life processes, rapid and accurate determination of intracellular biomolecules is essential. Up to now, several approaches based on carbon nanomaterials have been explored for determination of intracellular biomolecules, such as based on carbon nanospheres, CDs, $\text{g-C}_3\text{N}_4$, graphene, graphene oxide.^{136,137}

Wang et al.¹³⁸ have constructed an intracellular telomerase sensor based a single patchy gold/carbon nanosphere (PG/CNS). The surface of the PG/CNS is decorated with hybridized-DNA (HS-

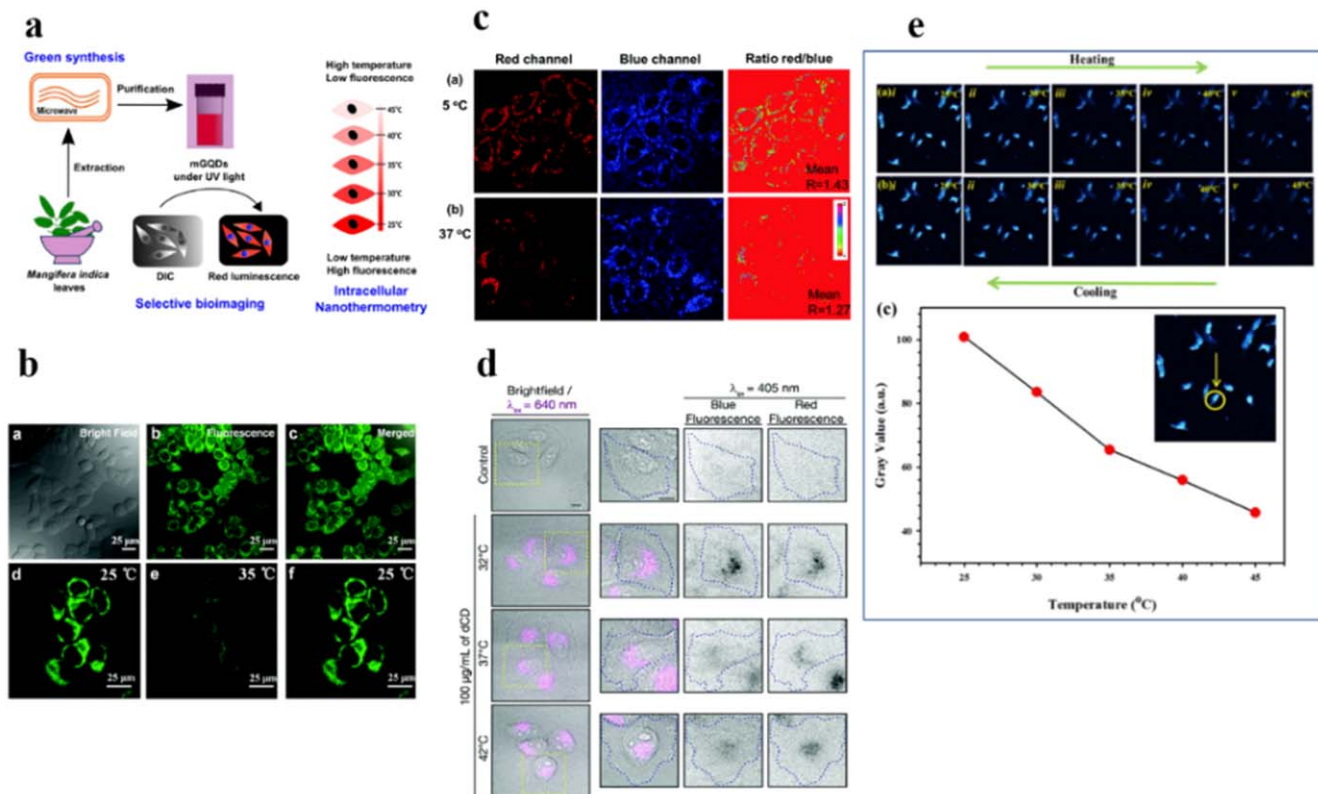


Figure 2. (a) Schematic representation of graphene quantum dots (mGQDs) preparation and live intracellular temperature sensing using graphene quantum dots in L929 cells.⁵⁸ Reproduced with permission Copyright 2017, American Chemical Society. (b) (a)–(c) Confocal microscopy images of HeLa cells (at 25 °C); (d)–(f) Confocal microscopy images of N, S co-doped CDs-stained cells with corresponding fluorescence field at 25, 35 and 25 °C, respectively. (Emission was collected at 415–550 nm and excited at 405 nm.)¹¹¹ Reproduced with permission Copyright 2017, The Royal Society of Chemistry. (c) Fluorescent microscopic images of HeLa cells stained with CDs-RhB at 5 °C and 37 °C (b) respectively; From left to right: the red, blue channel fluorescence images and the red/blue ratio image respectively.¹¹² Reproduced with permission Copyright 2017, The Royal Society of Chemistry. (d) Fluorescence microscopy images of CD-treated HeLa cells incubated at the different temperatures; The scale bars: 10 μm; The red-to-blue fluorescence ratios are 1.8 at 32 °C, 2.0 at 37 °C and 2.3 at 42 °C; The control shows untreated HeLa cells at 42 °C with no fluorescence signal as expected.¹¹³ Reproduced with permission Copyright 2019, The Royal Society of Chemistry. (e) Fluorescence microscopy images of the NIH 3T3 cells incubated with the PVCL-CDs during (a) heating and (b) cooling at (i) 25, (ii) 30, (iii) 35, (iv) 40, and (v) 45 °C; (c) Grey-scale intensity of the images of the circled area during heating as a function of temperature.¹¹⁴ Reproduced with permission Copyright 2015, Elsevier B.V.

DNA/Primer-DNA/Flare-DNA) via Au–S bond and H1/H2-DNA (a pair of cross complementary DNA hairpins) via electrostatic interaction. In the presence of telomerase, the primer-DNA (telomerase primer) is released from the surface PG/CNS, and which initiates cross hybridization chain reaction (HCR) with H1-DNA and H2-DNA resulting in amplifying the fluorescence signal (Fig. 4a). Li et al.¹³⁹ have developed an intracellular protein kinase A sensor based on g-C₃N₄ and titanium dioxide (TiO₂) complex via a novel photoelectrochemical (PEC) assay. The protein kinase A (PKA) can catalyze phosphorylation reaction in solution to obtain phosphorylated peptide (P-peptide). The P-peptide is immobilized on electrode surface which was modified with g-C₃N₄ and TiO₂ in advance for providing PEC signal and conjugating phosphate groups of P-peptide, respectively. Subsequently, PAMAM dendrimers are captured on the P-peptide to immobilize Alkaline Phosphatase (ALP) which can catalyze L-ascorbic acid 2-phosphate trisodium salt (AAP) to produce electron donor of ascorbic acid (AA), resulting in an increased photocurrent (Fig. 4b).

There also exists some intracellular small biomolecule sensor. Liu et al.¹⁴⁰ have reported an intracellular adenosine triphosphate (ATP) sensor based on fluorescent aptamer probes covalently linked to graphene oxide (Fig. 4c). Zhang et al.⁸³ have established an intracellular glutathione sensor based on magnetic nanoporous graphene nanocomposites synthesized by graphene oxide and ferric chloride. In cells, the oxidation of glutathione and thiamine are competitive, and a novel and simple intracellular glutathione sensor

are established on the basis of the inhibition effect of glutathione on the oxidation of thiamine (Fig. 4d). Liu et al.¹⁴¹ have prepared an intracellular microRNA-21 based on lysozyme-modified gold nanoclusters modified with the ss-DNA probe (a 22-mer) for microRNA-21 and carbon nanotubes. There exists a fluorescence resonance energy transfer between gold nanoclusters and carbon nanotubes. However, after addition of microRNA-21, the fluorescence resonance energy transfer between gold nanoclusters and carbon nanotubes is blocked because of combination of ss-DNA and microRNA-21 (Fig. 4e). Liu et al.¹⁴² have prepared an intracellular H₂O₂ sensor based on porous graphene network (Fig. 4f).

Intracellular gaseous signaling molecules including carbon monoxide (CO), hydrogen sulfide (H₂S) and nitric oxide (NO) generate in biological reactions in living cells with complex interrelationships and are of great significance in many biological processes.^{143–145} Unusual concentrations of CO, H₂S and NO are associated with some diseases, such as asthma, sickle cell anemia, diabetes,¹⁴³ a variety of neurodegenerative pathologies,¹⁴⁴ Huntington's, Parkinson's and Alzheimer's diseases.¹⁴⁶ From these perspectives, it is worthy to develop sensitive, specific and fast techniques for these intracellular gaseous signaling molecules detection. Recent studies have focused on intracellular gaseous signaling molecules sensor based on carbon nanomaterials.

Li et al.¹⁴⁷ have developed an intracellular H₂S sensor based on (2,4-dinitrophenoxy) tyrosine functionalized graphene quantum dots via fluorescent techniques to detect H₂S in MCF-7 cells. Ulissi et al.¹⁴⁸ have constructed an intracellular NO sensor based on

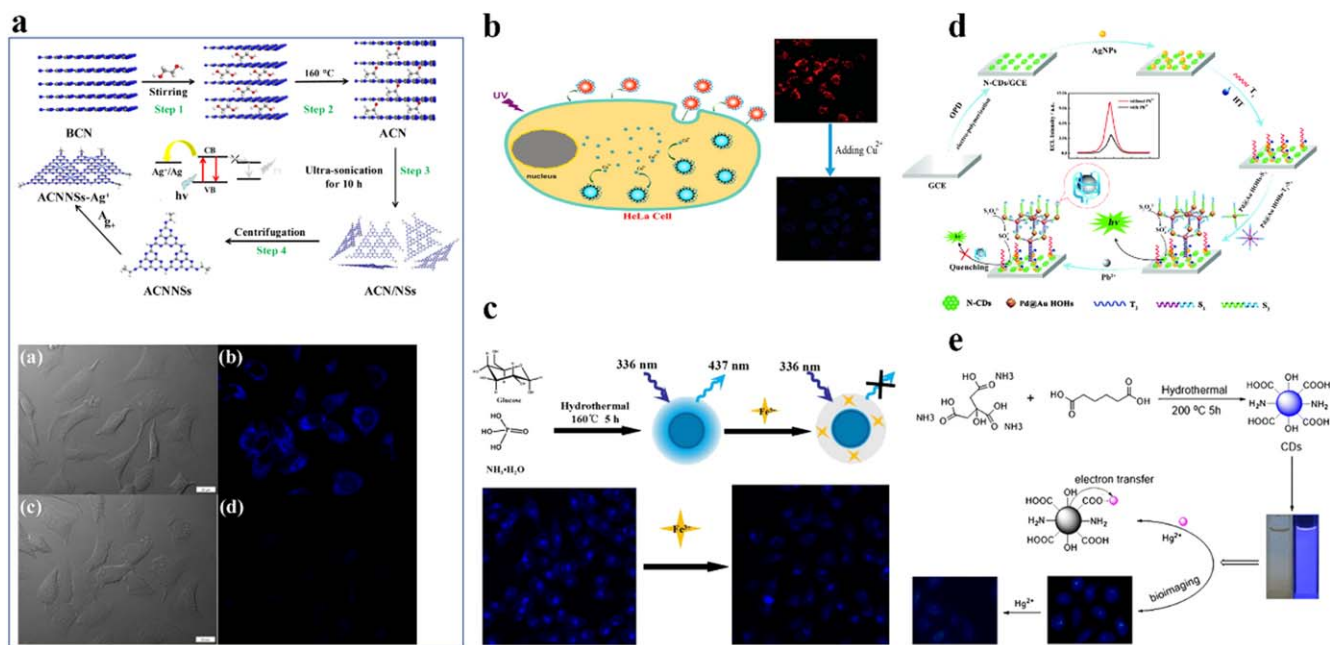


Figure 3. (a) General scheme for the preparation of ACNNSs from graphitic carbon nitride (BCN) and their application in sensing Ag^+ (up); Fluorescence images of cells: (a) control cells treated with no ACNNSs or Ag^+ and (b) control cells treated with 100 μl ACNNSs for 6 h only; (c) contrast and (d) fluorescence images of cells treated with both 100 μl ACNNSs and 2.0 ml Ag^+ (20 μM) for 2 h (down).¹²⁷ Reproduced with permission Copyright 2018, American Chemical Society. (b) scheme of carbon-dot and quantum-dot-coated dual-emission core-satellite silica nanoparticles for ratiometric intracellular Cu^{2+} sensing.¹²⁸ Reproduced with permission Copyright 2016, American Chemical Society. (c) Synthesis of N, P-CDs and Fe^{3+} detection in human serum and living cells.¹²⁹ Reproduced with permission Copyright 2016, American Chemical Society. (d) The fabrication and reaction mechanism of the proposed electrochemiluminescence (ECL) biosensor for the detection of intracellular lead ions.¹³⁰ Reproduced with permission Copyright 2016, The Royal Society of Chemistry. (e) Schematic illustration for the synthesis of CDs and their applications in the determination and intracellular imaging $\text{Hg}(\text{II})$.¹³¹ Reproduced with permission Copyright 2016, Springer-Verlag Wien.

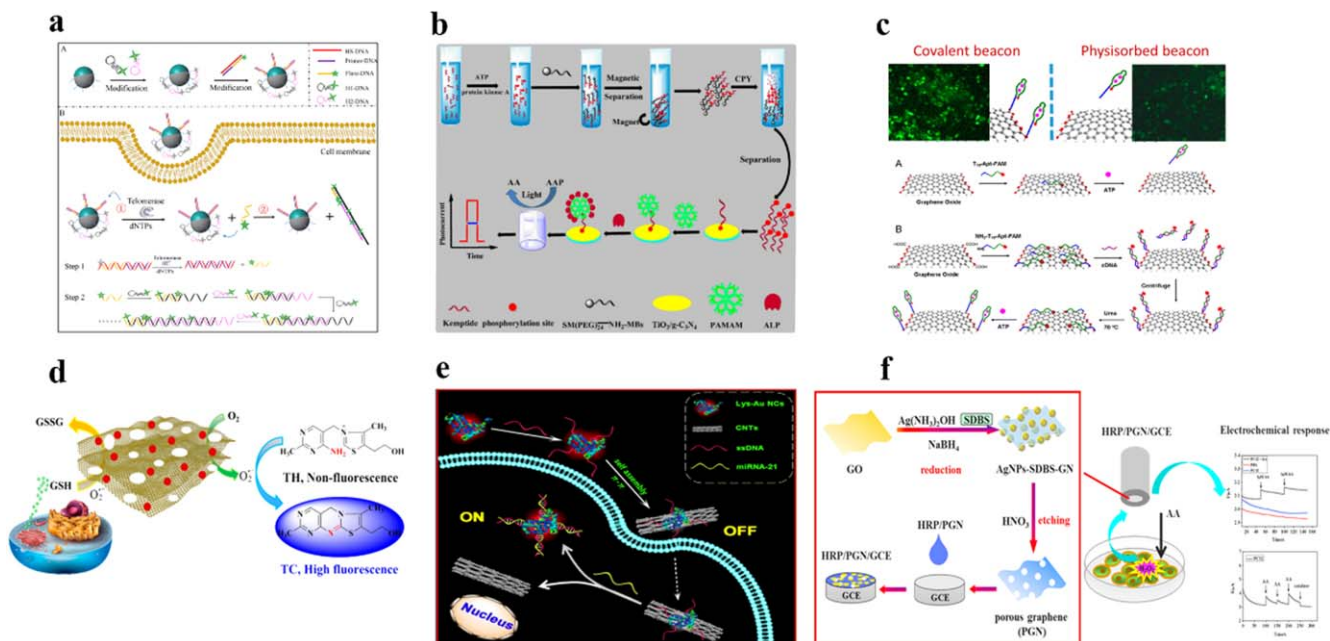


Figure 4. (a) Schematic diagram of the preparation process of PG/CNS-based sensor.¹³⁸ Reproduced with permission Copyright 2019, Elsevier B.V. (b) Schematic illustration of PG/CNSs-based mimic-HCR sensor for in situ detection of intracellular telomerase.¹³⁸ Reproduced with permission Copyright 2019, Elsevier B.V. (c) Schematic representation of the fabricated PEC biosensor for assay of PKA activity based on PKA-Catalyzed phosphorylation reaction in solution and signal amplification of $\text{TiO}_2/\text{g-C}_3\text{N}_4$, PAMAM-COOH, and ALP.¹³⁹ Reproduced with permission Copyright 2017, American Chemical Society. (d) Schematic diagram of intracellular detection of ATP using an aptamer beacon covalently linked to graphene oxide resisting nonspecific probe displacement.¹⁴⁰ Reproduced with permission Copyright 2014, American Chemical Society. (e) Schematic diagram of discriminative detection of glutathione in cell lysates based on oxidase-like activity of magnetic nanoporous graphene.⁸³ Reproduced with permission Copyright 2019, American Chemical Society. (f) Schematic presentation of the FRET fluorescent assay for microRNA-21 analysis.¹⁴¹ Reproduced with permission Copyright 2019, Springer-Verlag GmbH Austria, part of Springer Nature. (f) Schematic of horseradish peroxidase/porous graphene (HRP/PGN) modified glassy carbon electrode (GCE) used for detecting H_2O_2 release from cells stimulated with ascorbic acid (AA).¹⁴² Reproduced with permission Copyright 2016, Elsevier B.V.

near-infrared fluorescent intracellular single-walled carbon nanotubes to determine NO in A375 melanoma cells.

Carbon Nanomaterials Based Intracellular Sensors for Diagnosis or Treatment of Diseases

The early diagnosis of diseases, especially various types of cancers, plays a critical role in clinical treatment. As already discussed above, abnormal intracellular microenvironmental parameters and inappropriate concentrations of molecules or ions are intuitive indicators for various types of diseases. Therefore, intracellular sensors have become essential tools in achieving diagnosis and treatment of diseases. Until now, diseases diagnosed and/or treated by carbon nanomaterials based intracellular sensor include cancers (e.g., cervical cancer, liver cancer, breast cancer, bladder cancer),^{127,129,149,150} disease caused by pathogenic bacteria,¹⁵¹ and neurological disease.^{152,153} For examples, Shu et al.¹⁴⁹ have prepared intracellular metal ions sensors based on CDs to diagnose breast cancer. Mohapatra et al.¹⁵⁰ have established intracellular ions sensors based on Fe₃O₄@SiO₂@carbon quantum dot to diagnose colon cancer. Shi et al.¹²⁹ have established intracellular metal ions based on nitrogen and phosphorus co-doped CDs to diagnosed bladder cancer. Cao et al.¹²⁷ have successfully prepared intracellular thermal sensor based on g-C₃N₄ to diagnosed cervical cancer. These works may pave a new possible way to rapid screening and accurate treatment of these intractable diseases.

Conclusions and Outlooks

In summary, this paper provides a brief overview of recent researches on the applications of carbon nanomaterials, including CDs or graphene quantum dots, carbon nanotubes, graphene or graphene oxide, g-C₃N₄ and fullerene, in pH sensing, thermal sensing, metal ion sensing and biomolecule sensing in living cells. Though many achievements have been obtained for intracellular sensors based on carbon nanomaterials, there are still significant challenges that need to be solved.

- (1) CDs are the main materials in the application of fabricating intracellular sensors. Therefore, other carbon nanomaterials (such as, carbon nanotubes, graphene or graphene oxide, g-C₃N₄ and fullerene) should be further exploited in intracellular sensors urgently.
- (2) During the use of intracellular sensor, confocal fluorescence microscopic technique and electrochemistry methods are more commonly used. Thus, other advanced techniques (such as, Raman spectroscopy method) should be tried.
- (3) Some given analytes or microenvironments in intracellular organelles (e.g., cytoplasm, mitochondria and lysosomes) are also important in measurement of the cells status. Therefore, we must focus on the detection of analytes or parameters of microenvironments in cell organelles in future.

Acknowledgments

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