Intracellular Imaging of Metal Ions

Subjects: Chemistry, Analytical

Contributor: Yang Shi , Wenxian Zhang , Yi Xue , Jingjing Zhang

Metal ions are known to play indispensable roles in many critical biochemical processes. The amount and dispersion of metal ions in body fluids have a significant impact on the normal physiological function of the human body. In terms of their effects in biosystems, the general public generally classifies metals into two categories: essential and non-essential. It is widely recognized that there are ten essential metal ions for life, and the body must have appropriate amounts of them, including potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), cobalt (Co), molybdenum (Mo), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn). Among them, K, Na, Ca, and Mg make up over 99% of the total metal elements in the human body, while the remaining six elements are present in small amounts. When essential metal ions are maintained at normal levels, they play a crucial role in various physiological functions, including catalyzing enzymes, participating in oxidative metabolism, and contributing to DNA synthesis.

fluorescent sensors metal ions biosystems

1. Fluorescent Sensors for Essential Metal Ions

1.1. Na^+

Schiff base ligands possess excellent photochemical properties and can be used to create fluorescent probes, but the use of sodium (I) complexes based on Schiff base ligands is rare [1]. Tamilselvi et al. recently designed a sodium ion sensor based on a pyridoxal-bearing triazole ring Schiff base [2], which exhibits strong blue-green emission in the solid state and emits a yellow light when Na^+ is present. They further studied the proportional fluorescence response of this probe with sodium ions in the U87 cell line, indicating its potential application in cell biology protocols.

Potassium and sodium play crucial roles in various biological processes, but their synergies also have important implications for various biological processes. Yang et al. designed the first cell-surface fluorescent probe that can simultaneously detect Na^+ and K^+ in the microenvironment of cells [3]. The probe utilizes a Y-shaped DNA sensor composed of three distinct DNA sequences: a Na^+-specific enzyme strand hybridized with the substrate strand and a GQ strand that binds to K^+. The use of this probe to detect Na^+/K^+ concurrently provides a more comprehensive understanding of the dynamic changes of the targets than single-ion assays. The design and use of this probe have great significance in further understanding Na^+ and K^+-related cellular events and biological processes.

1.2. K^+

For imaging intracellular K^+, it is vital to develop a molecular recognition element that can achieve high affinity and selectivity to K^+. Tian et al. presented the first polymer-based ratiometric fluorescent K^+ indicator (PK1), which was modified with a water-soluble polymer skeleton to enable high-throughput monitoring of K^+ fluctuations in living cells [4]. Subsequently, they further enhanced the detection of potassium ions in cells by incorporating a small-molecule K^+ fluorescent probe into a hydrophilic F127 block and then binding it to cationic liposomes to create modified nanoparticles with enhanced cellular affinity [5]. A pioneering chemosensor for the accurate intracellular ratiometric imaging of potassium using a dual fluorophore strategy was introduced by Chang et al. [6]. Furthermore, Chen et al. innovated a remotely operated “lock-unlock” nanosystem [7]. This nanosystem utilizes a dual-stranded aptamer precursor (DSAP) as the recognition molecule and a SiO_2-based gold nanoshell (AuNS) as the nanocarrier, with NIR light as the stimulus for remote application. AuNS generates an increased local temperature upon receiving NIR light, which induces the dehybridization of DSAP, activates the binding capability of the aptamer, and enables the monitoring of intracellular K^+ via changes in the fluorescence signal. This DSAP-AuNS nanosystem provides a new means to visualize endogenous K^+ in living cells.

1.3. Ca^{2+}

Fluorescent sensing and imaging have become useful tools to investigate the signaling pathways of calcium ions, which act as a widespread secondary messenger and play an essential role in neurodegenerative diseases. Modified with a specific Ca^{2+} chelator ligand with two formaldehyde groups, a copper nanocluster ratiometric fluorescent probe was developed for real-time sensing and imaging of Ca^{2+} in neurons [8]. In another example, an inner-filter-mediated luminescence probe was developed by using biomass quantum dots as a fluorescent reporter. This probe was initially quenched by a Ca^{2+} chelator alizarin red S yet turned on after binding to intracellular Ca^{2+}. Despite fluorescent nanoclusters and quantum dots, green fluorescent protein could also be combined with the specific chelator for Ca^{2+} imaging [9]. Mitochondrial Ca^{2+} concentration in living cells is also of great importance. Mt-fura-2, the first ratiometric chemical Ca^{2+} probe for mitochondria, was developed by coupling two triphenylphosphonium cations to the molecular backbone of the ratiometric Ca^{2+} indicator fura-2 [10]. Mt-fura-2 binds calcium ions in vitro with a dissociation constant of ≈1.5 μM and exhibits correct mitochondrial localization and precise measurement of matrix [Ca^{2+}] changes in cells.

Ca^{2+}-specific DNAzyme, which was first reported in 2017 [11], was used to construct a SERS-fluorescence dual-mode probe for Ca^{2+} imaging in living cells in 2021 by Li and co-workers [12]. The Ca^{2+}-responsive nanoprobe was constructed by modifying DNAzyme and a Cy5-labelled substrate strand on gold nanostars. When the two chains hybridize, the fluorescence of Cy5 is quenched, which enhances the SERS signal concurrently. The substrate chains could be cleaved and freed from the surface of the gold nanopillar by the catalytic induction of Ca^{2+}, which leads to the weakening of the SERS signal, as well as the fluorescence signal recovery. The nanosensor has been successfully used in HeLa cells under the treatment of T-2 toxin, which increased the intracellular free Ca^{2+} concentration and caused cell apoptosis. Moreover, the calmodulin domain and its cognate M13 peptide have also been widely used in biological research. Rhodamines are highly bright and photostable fluorophores, and one of their key properties is that they exist in a balance between the non-fluorescent, spirocyclic form and the fluorescent, amphoteric form. The remarkable ability of HaloTag7 to affect rhodamine spirocyclization was used to

develop biosensors in which the analyte affects the conformation of HaloTag7 and thus the balance of spirocyclization. The Johnsson group developed a ratiometric biosensor based on spirocyclization in an environmentally sensitive discolored fluorophore that reversibly switches between the green and red fluorescent forms, successfully imaging calcium ions in living cells [13]. This biosensor combines a HaloTag7 and a Ca^{2+}-sensing structural domain (rHCaMP) to enable reversible switching between green and red fluorescent forms through intramolecular spirocyclization by using a color-shifted fluorophore. The biosensor provides precise ratiometric measurements of Ca^{2+} both in vitro and intracellularly. Furthermore, by coupling the CSFs to various protein ligands, the biosensor achieves exceptional sensitivity, with some probes demonstrating up to 2400-fold changes in fluorescence ratios upon binding to the target. The Campbell group developed a NIR genetically encoded indicator using a biliverdin-binding fluorescent protein for multi-color imaging [14]. BAPTA could form a chemigenetic indicator due to the interaction between the BAPTA moiety and the GFP chromophore, which provided creative guidance in the design of chemigenetic indicators. In addition to fluorescent intensity-based readout, the Goedhart group also proposed a turquoise fluorescence lifetime-based biosensor for quantitative imaging of intracellular Ca^{2+} with a low sensitivity for pH, which was a teaser for traditional intensity-based indicators [15].

1.4. Zn^{2+}

Accurately monitoring the zinc profile and levels in living cells is crucial for various biological studies. Among various developed methods, DNAzyme-based sensors are at the forefront of zinc ion imaging studies. However, their application in cells is limited due to the difficulty in maintaining the activity of RNA-cleaving DNAzymes during delivery and poor biological imaging performance [16][17]. To address this issue, Zhang et al. constructed a TP imaging probe based on an RNA-cleaving DNAzyme by modifying the Zn^{2+}-specific DNAzyme with TP fluorophores and utilized gold nanoparticles (AuNPs) for efficient intracellular delivery [18]. This NIR light-excited probe exhibits exceptional imaging capabilities for Zn^{2+} in viable cells and tissues, with remarkable in-depth penetration of tissues reaching depths of 160 μm. The Lu group developed a new fluorescent imaging technique that allows for ratiometric imaging of Mg^{2+} and Zn^{2+} in living cells [19], using DNAzyme-mediated, genetically encoded fluorescent proteins. The merit of this approach is that Mg^{2+}-dependent multi-round cleavage of the target mRNA by DNAzyme activity allows for a correlation between the expression level of fluorescent proteins and the concentration of the target metal ion. This sensor can utilize a variety of metal-specific DNAzyme, greatly expanding the range of metal ions that can be imaged with genetically encoded proteins. Additionally, Luo et al. developed a DNAzyme-based normalized strategy for direct quantification of endogenous zinc in living cells [20]. Recently, Li's team described the first example of DNAzyme-based sensors for subcellular metal-ion imaging [21], which combines a photoactivatable DNAzyme sensor probe with upconversion nanotechnology and organelle-localized strategies. Except for DNAzyme-based sensors [22], Schiff base based chemosensors [23][24], peptide-based sensors [25] and single red fluorescent protein-based sensors [26] also offer a variety of options for researchers when studying the distribution and concentration of zinc ions in living cells.

1.5. Mg^{2+}

As the second largest intracellular cation after potassium, the magnesium ion is essential for various biological processes and physiological functions. However, traditional fluorescent chemosensors for the detection of magnesium ions currently face the limitations of low selectivity and poor fluorescence signal enhancement. Yuki et al. overcame this limitation by synthesizing the first highly selective and NIR fluorescent probes for the detection of Mg^{2+} [27]. These probes consisted of charged β-diketones as specific bound spots for Mg^{2+} and Si-rhodamine remnants as NIR fluorophores. They are primarily located in the cytoplasm and are localized partially in the lysosomes and mitochondria of cultured rat hippocampal neurons. Moreover, the Aharon group reported the first example of aqueous CDs with high selectivity for intracellular Mg^{2+} detection [28]. Furthermore, Ashok's team successfully synthesized novel chromone-based chemosensors (La and Lb) that are highly sensitive to Mg^{2+} [29], with La also showing potential for Mn^{2+} detection through absorption studies. On the other hand, Lb was found to sense Cu^{2+} through absorption studies and also showed sensitivity to Mg^{2+} via emission studies. These ligands were successfully used for Mg^{2+} imaging in HeLa cancer cells.

1.6. Cu^{2+}

The development of copper ion sensors in recent years has still primarily relied on the design of organic fluorescent probes [30][31] and nanomaterials [32]. In the previous section, it was mentioned that the Cu(I)-catalyzed click reaction has a high selectivity for Cu^{2+}, making it ideal for complex intracellular environments. Bu et al. developed an innovative "OFF–ON" fluorescent biosensor by combining the Cu(I)-catalyzed click reaction with a 3D DNA walker based on spherical nucleic acid [33]. In the initial "OFF" state, the fluorophore (Cy3) on the hairpin is close to the surface of AuNPs, resulting in quenched fluorescence. Cu is rapidly produced in situ from Cu^{2+} in the presence of ascorbic acid, triggering the click reaction-based 3D DNA walker. The activated swing arm hybridizes with the neighboring Cy3-hairpin and drives the 3D DNA walker by endonuclease to produce several Cy3-labeled DNA fragments away from the AuNP surface, resulting in a restored fluorescence response (transitioning to the "ON" state). The utilization of this assay provides a means for transducing signals and assessing intracellular Cu^{2+} at picomolar concentrations.

1.7. Fe^{2+}/Fe^{3+}

CDs have garnered significant attention in metal ion detection in living cells. To this end, double-emission CDs (NS-CDs) with varying dimensions of nitrogen/sulfur doping were synthesized by solubilizing sodium alginate (SA) and glutathione (GSH) in formamide for heat treatment [34]. Since Fe^{3+} induces the aggregation of NS-CDs, which enhances the fluorescence signal. Therefore, this nanosensor enables ratiometric measurements of iron ions and exhibits remarkable detectability and sensitivity, with detection limits as low as 0.56 μM. Moreover, NS-CDs display unparalleled capability for localized, specific cell membrane imaging. Similarly, the Guo group proposed the synthesis of N-CDs utilizing fresh tea leaves and urea [35], while Zhang's team fabricated deferoxamine-inspired CDs using L-aspartic acid (Asp) and 2,5-diaminobenzenesulfonic acid (DABSA) as reactants in a single-boiler hydrothermal synthesis [36]. Both N-CDs and deferoxamine-inspired CDs were applied for cell imaging of Fe^{3+} and exhibited promising results. Hou et al. developed an innovative "turn-on" fluorescent probe using rhodamine 6G derivatives and spirolactam ring-opening reactions [37]. This probe is highly effective in detecting Fe^{3+} for

fluorescence imaging in living cells. In addition, Wang et al. designed dual-targeting fluorescent probes that combine galactose and imidazole to detect Fe^{3+} in the hepatic lysosome [38], enabling both hepatic and lysosomal targeting. Although indole-based fluorescent sensors for Fe^{3+} detection are rare, Nantanit reported three new sensors with fluorescence responses to Fe^{2+} and Fe^{3+} sensing in aqueous buffer systems [39]. One of these isomers is an excellent candidate for tracking Fe^{3+} in biological systems.

2. Fluorescent Sensors for Non-Essential Metal Ions

2.1. Li^+

The lithium-based complex is a widely used and effective drug for the treatment of bipolar disorder (BD) for more than 70 years. The distribution of lithium ions in the patient's cells is crucial to optimize the therapeutic effect. However, imaging lithium selectively in the biomedically relevant concentration range (0.5–2.0 mM) in living cells remains a major challenge. A major breakthrough was recently reported by Lu's team, which developed a lithium-specific DNAzyme with a selectivity exceeding 100-fold that of other biologically relevant metal ions [40]. This novel sensor allows for the visualization of lithium in HeLa cells, neurons from BD patients, healthy controls and human neuronal progenitor cells for comparison, making it a promising tool for investigating the therapeutic effects of lithium.

2.2. Ag^+

Dong et al. developed a label-free N-CDs system for the detection of silver ions and GSH through intrinsic ratiometric fluorescence [41]. The N-CDs emit a single long-wavelength light at 618 nm when excited at 478 nm. When silver ions are present, this nanosensor shows a rising peak at 532 nm and a decrease in emission at 618 nm, enabling the detection of silver ions in the range of 0–140 μM. Yu et al. presented a groundbreaking approach to visualize Ag^+ in living bacterial cells by utilizing a genetically encoded biosensor [42]. The sensor incorporates a cytosine-Ag^+-cytosine metal base pair into a fluorogenic RNA aptamer, known as Broccoli, which folds and emits a fluorescent signal upon binding to Ag^+. This unique RNA sensor can be further adapted for cellular imaging of other metal ions by implementing a similar design principle based on specific metal base pairs.

The Guo group synthesized a novel ratiometric chemosensor called CHa based on the hydrolysis of hydrazone derivatives of coumarin fluorescent moieties induced by Ag^+ [43]. When CHa encounters silver ions, it undergoes hydrolysis, resulting in the release of a 3-formyl-substituted coumarin derivative that acquires blue emission at short wavelengths from yellow emission. Additionally, a phenanthro [9,10-d] imidazole-based fluorescent probe with AIE activity was designed by Bu et al. [44], for simultaneous sensing of Ag^+ and SCN^-. In another example, a sustainably modifiable 1,2-alternating thiacalix[4]arene was synthesized by Yu et al. [45], which displayed a highly sensitive ratio recognition for Ag^+.

2.3. Ni^{2+}

Nickel ions play a vital role as a cofactor for a variety of microbial enzymes, supporting essential cellular functions necessary for prokaryotic survival. A FRET-based genetically encoded biosensor was developed by Neha [46], taking enhanced cyan fluorescent protein and Venus (a variant of yellow fluorescent protein) respectively into account as donor and acceptor fluorescent molecules. Such sensors permit concentration-dependent monitoring of nickel ion fluxes within viable cells with a high spatial and temporal resolution to provide in-depth insight into the distribution of nickel ions physiologically at the cellular and subcellular levels.

Reports on fluorescent probes for nickel ions are relatively scarce, and there is still a need to develop simple and effective detection methods. Wang et al. constructed a molecular probe FA-Ni to achieve highly selective and ultrasensitive rapid detection of Ni^{2+}, avoiding the interference of other ions [47]. Nickel ions were converted to elemental nickel under the reducing conditions of $NaBH_4$, and then triphenylphosphine was used as a ligand to detach allyl from the probe FA-Ni, thereby generating a fluorescent signal. They also successfully applied the probe FA-Ni for in situ imaging of nickel ions in living cells. Additionally, Shahzad's team reported AIE active sensors for the detection of Ni^{2+} in live cells and acid/base sensing [48]. Gu et al. developed a new chemosensor based on a pyrazolopyrimidine core that can simultaneously detect Cu^{2+} and Ni^{2+}, which has good imaging properties for both Cu^{2+} and Ni^{2+} in living cells [49].

2.4. Pb^{2+}

The interaction between metals and biomolecules can be found throughout nature and provides a wealth of resources and principles of design in the search for novel, recognizable ligands. Peptides are promising candidates for designing metal-binding ligands due to their rich coordination chemistry, high stability, and availability of optional building blocks. Additionally, their high biocompatibility makes them well-suited for detecting Pb^{2+} in biological systems. The Zhao group designed a biomimetic peptide-based fluorescent sensor GSSH-2TPE inspired by the structure of glutathione [50]. Mechanistic studies confirmed that there is a delicate balancing between the chelating groups and the molecular configuration responsible for the highly selective complexation of Pb^{2+} by the sensor. Additionally, the ion-induced supramolecular assembly generates a bright fluorescence signal. Featuring good biocompatibility and the lowest possible disturbance to both endogenous biothiols and background fluorescence, the sensor allows precise imaging of Pb^{2+} in vivo. Similarly, Mehta et al. designed a proportional fluorescent peptide-based sensor by coupling the peptide receptor of Pb(II) with an excimer-forming benzothiazolylcyanovinylene fluorophore [51].

2.5. Pd^{2+}

Palladium is a scarce internal transition metal that is widely employed as an extremely potent catalyst in various areas. However, it should be noted that Pd species can disrupt many biological processes and pose significant health hazards. Of particular concern is Pd^{2+}, which is the most abundant oxidation state in alive cells, thus the potential for developing validated Pd^{2+} detection and imaging approaches are vital for environmental safety and the health of humans. Wen et al. developed a naphthofluorescein-based NIR fluorescent probe called M-PD [52], which boasts exceptional sensing properties for the detection of Pd^{2+}. The lower limit of detection for this sensor was

found to be 10.8 nM, a value significantly below the drug in threshold values (5–10 ppm). Additionally, M-PD has been successfully utilized for the near-infrared fluorescence imaging of Pd^{2+} in living cells. A chemosensor, DCF-MPYM-Pd, featuring a wide stokes shift and an ability for lysosomal targeting was synthesized by Wang et al. [53]. It was shown that this sensor can accurately sense palladium (II) in living cells and can specifically accumulate in lysosomes. To avoid the problem of the multi-step synthesis of a probe, Mareeswaran's group found a simple and well-known organic molecule, coumarin-460 (C460), that can selectively sense palladium ions in aqueous media [54]. They went on to validate the binding and sensing properties of C460 for Pd^{2+} by absorption and fluorescence spectroscopy techniques, thus demonstrating that the C460 molecule can be used as an "off" probe for Pd^{2+} for real-time detection and biological applications.

While most researchers have designed probes to detect only one form of palladium, the ability to discriminate between Pd (0) and Pd (II) has been rarely reported. In this regard, Zhang et al. designed Umb-Pd2, a corymbone-derived sensor that can be used as a tiny, robust, reliable, and detective sensor for the detection of Pd (II) [55]. In both the stand-alone and co-existing systems, it is distinguished from typical research by its unique selectivity for Pd (II) and Pd (0), which is commonly referred to as Pd (0)-selective. This differentiation capability was further used in the case of living cell imaging.

2.6. Hg^{2+}

Sarah et al. developed highly NIR fluorescent graphene quantum dots (GQDs) by pyrolyzing biomass-derived CBDA-2 in basic conditions [56]. When treated with mercury ions, the fluorescence of GQDs was quenched. Exploiting the intramolecular charge transfer (ICT) mechanism, Duan's team introduced a new phenothiazine-based sensor [57], PHE-Ad, for monitoring Hg^{2+}. With its excellent fluorescent signaling behavior and low cytotoxicity, PHE-Ad proved to be successful in detecting and imaging Hg^{2+} in living cells. Furthermore, the same team reported another two novel PET fluorescent probes, CH3-R6G and CN-R6G, rationally synthesized by partial doping of rhodamine 6G fluorophore with a triazolyl benzaldehyde moiety and applied with great success for Hg^{2+} imaging in breast cancer cells [58]. Ding et al. reported the synthesis of ethyl 2,5-diphenyl-2H-1,2,3-triazole-4-carboxylate [59], which was then introduced into rhodamine B to produce a novel derivative, REDTC. This probe exhibited remarkable selectivity for Hg^{2+} through a chromogenic reaction, without interference from other metal ions. Lastly, two novel NIR monosulfide probes, MTSQ-1 and MTSQ-2, were designed and used for Hg^{2+} imaging based on a mercury deuteration strategy [60]. Particularly, the MTSQ-2 was packaged in a β-CD and showed excellent performance for imaging Hg^{2+} in HeLa cells as well as a high signal-to-background ratio.

2.7. Cd^{2+}

Cadmium ions, one of the most dangerous heavy metals, can affect various cellular physiological effects. Toxic cadmium ions may lead to acute or chronic toxicity, causing cancer and other diseases. The development of highly sensitive and selective methods for the detection of cadmium ions in cells is still challenging. Lin et al. developed a highly selective probe, (E)-4-(4-([2,2′:6′,2″-terpyridin]-4′-yl)styryl)-1 octadecylpyridin-1-ium bromide (ZC-F8) [61]. The fluorescence spectra of ZC-F8 showed an excellent response to Cd^{2+} through both an intramolecular charge

transfer effect and an AIE effect. Moreover, the results of cell imaging experiments showed that the probe has ideal membrane permeability and a labeled property for Cd^{2+}, indicating its promising application in the detection and tracking of metal ions in living cells. In addition, Liao's team successfully synthesized a new peptide-based probe (DSC) with good water solubility and biocompatibility, showing a fluorescent "turn-on" response to Cd^{2+} based on the PET principle [62]. Fluorescence imaging experiments showed that DSC can selectively monitor Cd^{2+} in living cells.

2.8. Au^{3+}

Gold has a broad range of uses in chemistry, catalysis, and medicine. However, the binding of Au^{3+} with certain DNA and enzymes poses severe health risks, causing damage to organs. Guo et al. synthesized carbon dots by a simple solvothermal method using the acetic acid-treated peel of red dragon fruits, called ACDs [63]. With their high sp2-hybrid carbon and carboxyl group contents, ACDs can efficiently convert Au^{3+} to Au^{0} and stabilize the resulting AuNPs. Electron transfer from ACD to Au^{3+} and the inner filtering effect from ACD to AuNPs synergistically quenched the fluorescence within 30 s. In addition, ACDs possessed promising photo-stability, low cytotoxicity, and favorable biological compatibility, enabling their successful application in intracellular Au^{3+} sensing and imaging.

2.9. Al^{3+}

The Nikhil group synthesized a new compound, (E)-2-(benzamido)-N′-((2-hydroxynaphthalen-1-yl) methylene) benzohydrazide (BBHAN) [64], which belongs to the Schiff base derivative family and contains a hydrazine-bridged anthranilic acid-naphthalene conjugate. BBHAN is a highly sensitive Al^{3+} detection probe with a limit of detection of 1.68×10^{-9} M. Its detection mechanism is based on the chelation-enhanced fluorescence phenomenon, as demonstrated by time-resolved fluorescence measurements. Moreover, BBHAN is capable of detecting Al^{3+} in MDA-MB-468 cells. Later, Jessica et al. designed and synthesized a new series of Schiff base chemosensors to sense Al^{3+} [65]. The molecular solubility and compatibility of the amino acid Schiff base (A) in the presence and absence of Al^{3+} were well demonstrated. Furthermore, in human epithelial cells Hs27, the fluorescent bioimaging applications were demonstrated. In the same way, Wang et al. designed an innovative nanoprobe by co-self-assembling an amphiphilic polymer containing a Schiff-base fluorescent unit [66]. This novel nanoprobe not only provides high sensitivity for Al^{3+} imaging but also has the potential to be applicable to other ions or biomolecules by adjusting the fluorescent unit incorporated into the amphiphilic polymer.

References

1. García-Valle, F.M.; Tabernero, V.; Cuenca, T.; Mosquera, M.E.G.; Cano, J. Intramolecular C–F Activation in Schiff-Base Alkali Metal Complexes. Organometallics 2019, 38, 894–904.
2. Ananthan Karthick, K.; Shankar, B.; Kubendran Aravind, M.; Ashokkumar, B.; Tamilselvi, A. Small-Molecule Fluorescent Probe: Ratiometric and Selective Detection of Sodium Ions for Imaging and Solid-State Sensing Applications. ChemistrySelect 2022, 7, e202203235.

3. Deng, Z.; Gao, P.; Liu, H.; He, Y.; Zhong, S.; Yang, Y. Cell-Surface-Anchored DNA Sensors for Simultaneously Monitoring Extracellular Sodium and Potassium Levels. Anal. Chem. 2021, 93, 16432–16438.
4. Ning, J.; Liu, H.; Sun, X.; Song, G.; Shen, M.; Liao, J.; Su, F.; Tian, Y. Rational Design of a Polymer-Based Ratiometric K+ Indicator for High-Throughput Monitoring Intracellular K+ Fluctuations. ACS Appl. Bio Mater. 2021, 4, 1731–1739.
5. Pan, T.; Shen, M.; Shi, J.; Ning, J.; Su, F.; Liao, J.; Tian, Y. Intracellular potassium ion fluorescent nanoprobes for functional analysis of hERG channel via bioimaging. Sens. Actuator B-Chem. 2021, 345, 130450.
6. Wang, Z.; Detomasi, T.C.; Chang, C.J. A dual-fluorophore sensor approach for ratiometric fluorescence imaging of potassium in living cells. Chem. Sci. 2021, 12, 1720–1729.
7. Cui, M.-R.; Chen, L.-X.; Li, X.-L.; Xu, J.-J.; Chen, H.-Y. NIR Remote-Controlled “Lock–Unlock” Nanosystem for Imaging Potassium Ions in Living Cells. Anal. Chem. 2020, 92, 4558–4565.
8. Liu, Z.; Jing, X.; Zhang, S.; Tian, Y. A Copper Nanocluster-Based Fluorescent Probe for Real-Time Imaging and Ratiometric Biosensing of Calcium Ions in Neurons. Anal. Chem. 2019, 91, 2488–2497.
9. Chen, D.; Zhao, J.; Zhang, L.; Liu, R.; Huang, Y.; Lan, C.; Zhao, S. Capsicum-Derived Biomass Quantum Dots Coupled with Alizarin Red S as an Inner-Filter-Mediated Illuminant Nanosystem for Imaging of Intracellular Calcium Ions. Anal. Chem. 2018, 90, 13059–13064.
10. Pendin, D.; Norante, R.; De Nadai, A.; Gherardi, G.; Vajente, N.; Basso, E.; Kaludercic, N.; Mammucari, C.; Paradisi, C.; Pozzan, T.; et al. A Synthetic Fluorescent Mitochondria-Targeted Sensor for Ratiometric Imaging of Calcium in Live Cells. Angew. Chem. Int. Ed. 2019, 58, 9917–9922.
11. Zhou, W.; Saran, R.; Huang, P.-J.J.; Ding, J.; Liu, J. An Exceptionally Selective DNA Cooperatively Binding Two Ca2+ Ions. ChemBioChem 2017, 18, 518–522.
12. Li, C.; Chen, P.; Wang, Z.; Ma, X. A DNAzyme-gold nanostar probe for SERS-fluorescence dual-mode detection and imaging of calcium ions in living cells. Sens. Actuator B-Chem. 2021, 347, 130596.
13. Wang, L.; Hiblot, J.; Popp, C.; Xue, L.; Johnsson, K. Environmentally Sensitive Color-Shifting Fluorophores for Bioimaging. Angew. Chem. Int. Ed. 2020, 59, 21880–21884.
14. Zhu, W.; Takeuchi, S.; Imai, S.; Terada, T.; Ueda, T.; Nasu, Y.; Terai, T.; Campbell, R.E. Chemigenetic indicators based on synthetic chelators and green fluorescent protein. Nat. Chem. Biol. 2023, 19, 38–44.

15. van der Linden, F.H.; Mahlandt, E.K.; Arts, J.J.G.; Beumer, J.; Puschhof, J.; de Man, S.M.A.; Chertkova, A.O.; Ponsioen, B.; Clevers, H.; van Buul, J.D.; et al. A turquoise fluorescence lifetime-based biosensor for quantitative imaging of intracellular calcium. Nat. Commun. 2021, 12, 7159.
16. Wu, P.; Hwang, K.; Lan, T.; Lu, Y. A DNAzyme-Gold Nanoparticle Probe for Uranyl Ion in Living Cells. J. Am. Chem. Soc. 2013, 135, 5254–5257.
17. Li, L.; Feng, J.; Fan, Y.; Tang, B. Simultaneous Imaging of Zn2+ and Cu2+ in Living Cells Based on DNAzyme Modified Gold Nanoparticle. Anal. Chem. 2015, 87, 4829–4835.
18. Yang, C.; Yin, X.; Huan, S.-Y.; Chen, L.; Hu, X.-X.; Xiong, M.-Y.; Chen, K.; Zhang, X.-B. Two-Photon DNAzyme–Gold Nanoparticle Probe for Imaging Intracellular Metal Ions. Anal. Chem. 2018, 90, 3118–3123.
19. Xiong, M.; Yang, Z.; Lake, R.J.; Li, J.; Hong, S.; Fan, H.; Zhang, X.-B.; Lu, Y. DNAzyme-Mediated Genetically Encoded Sensors for Ratiometric Imaging of Metal Ions in Living Cells. Angew. Chem. Int. Ed. 2020, 59, 1891–1896.
20. Zhang, X.; Song, Z.-L.; Chao, Q.; Li, Q.; Kong, R.; Fan, G.-C.; Luo, X. A DNAzyme-based normalized fluorescence strategy for direct quantification of endogenous zinc in living cells. Chem. Commun. 2022, 58, 577–580.
21. Yi, D.; Zhao, H.; Zhao, J.; Li, L. Modular Engineering of DNAzyme-Based Sensors for Spatioselective Imaging of Metal Ions in Mitochondria. J. Am. Chem. Soc. 2023, 145, 1678–1685.
22. Zhan, J.; Liu, Z.; Liu, R.; Zhu, J.-J.; Zhang, J. Near-Infrared-Light-Mediated DNA-Logic Nanomachine for Bioorthogonal Cascade Imaging of Endogenous Interconnected MicroRNAs and Metal Ions. Anal. Chem. 2022, 94, 16622–16631.
23. Jayaraj, A.; Gayathri, M.S.; Sivaraman, G.; Swamy, P.C.A. A highly potential acyclic Schiff base fluorescent turn on sensor for Zn2+ ions and colorimetric chemosensor for Zn2+, Cu2+ and Co2+ ions and its applicability in live cell imaging. J. Photochem. Photobiol. B Biol. 2022, 226, 112371.
24. Zhang, G.; Zhao, Y.; Peng, B.; Li, Z.; Xu, C.; Liu, Y.; Zhang, C.; Voelcker, N.H.; Li, L.; Huang, W. A fluorogenic probe based on chelation–hydrolysis-enhancement mechanism for visualizing Zn2+ in Parkinson's disease models. J. Mat. Chem. B 2019, 7, 2252–2260.
25. Wang, P.; Wu, X.; Wu, J.; Liao, Y. Highly selective and sensitive peptide-based fluorescent chemosensor for detection of Zinc(II) ions in aqueous medium and living cells. J. Photochem. Photobiol. A Chem. 2019, 382, 111929.
26. Dischler, A.M.; Maslar, D.; Zhang, C.; Qin, Y. Development and Characterization of a Red Fluorescent Protein-Based Sensor RZnP1 for the Detection of Cytosolic Zn2+. ACS Sens. 2022, 7, 3838–3845.

27. Murata, O.; Shindo, Y.; Ikeda, Y.; Iwasawa, N.; Citterio, D.; Oka, K.; Hiruta, Y. Near-Infrared Fluorescent Probes for Imaging of Intracellular Mg2+ and Application to Multi-Color Imaging of Mg2+, ATP, and Mitochondrial Membrane Potential. Anal. Chem. 2020, 92, 966–974.
28. Sadhanala, H.K.; Aryal, S.; Sharma, K.; Orpaz, Z.; Michaeli, S.; Gedanken, A. Nitrogen-doped carbon dots as a highly selective and sensitive fluorescent probe for sensing Mg2+ ions in aqueous solution, and their application in the detection and imaging of intracellular Mg2+ ions. Sens. Actuator B-Chem. 2022, 366, 131958.
29. Yadav, N.; Kumar, R.; Singh, A.K.; Mohiyuddin, S.; Gopinath, P. Systematic approach of chromone skeleton for detecting Mg2+ ion: Applications for sustainable cytotoxicity and cell imaging possibilities. Spectroc. Acta Part A-Molec. Biomolec. Spectr. 2020, 235, 118290.
30. Ranee, S.J.; Sivaraman, G.; Pushpalatha, A.M.; Muthusubramanian, S. Quinoline based sensors for bivalent copper ions in living cells. Sens. Actuator B-Chem. 2018, 255, 630–637.
31. Chailek, N.; Kaewnok, N.; Petdum, A.; Sirirak, J.; Chaneam, S.; Kamkaew, A.; Girdthep, S.; Wanichacheva, N. Near infrared and colorimetric fluorescence sensor for ultra-selective detection of Cu2+ level with applications in diverse water samples, brain tumor cell and flow injection analysis. J. Photochem. Photobiol. A Chem. 2021, 421, 113533.
32. Chang, D.; Shi, L.; Zhang, Y.; Zhang, G.; Zhang, C.; Dong, C.; Shuang, S. Smilax China-derived yellow-fluorescent carbon dots for temperature sensing, Cu2+ detection and cell imaging. Analyst 2020, 145, 2176–2183.
33. Wang, Z.; Jia, N.; Zhou, X.; Han, J.; Bu, H. Cu(I)-Catalyzed Click Reaction-Triggered 3D DNA Walker for Constructing an “OFF–ON” Fluorescent Biosensor for Cu2+ Detection. ACS Appl. Bio Mater. 2021, 4, 3571–3578.
34. Liang, C.; Xie, X.; Shi, Q.; Feng, J.; Zhang, D.; Huang, X. Nitrogen/sulfur-doped dual-emission carbon dots with tunable fluorescence for ratiometric sensing of ferric ions and cell membrane imaging. Appl. Surf. Sci. 2022, 572, 151447.
35. Ge, G.; Li, L.; Chen, M.; Wu, X.; Yang, Y.; Wang, D.; Zuo, S.; Zeng, Z.; Xiong, W.; Guo, C. Green Synthesis of Nitrogen–Doped Carbon Dots from Fresh Tea Leaves for Selective Fe3+ Ions Detection and Cellular Imaging. Nanomaterials 2022, 12, 986.
36. Cai, H.; Xu, H.; Chu, H.; Li, J.; Zhang, D. Fabrication of multi-functional carbon dots based on “one stone, three birds” strategy and their applications for the dual-mode Fe3+ detection, effective promotion on cell proliferation and treatment on ferric toxicosis in vitro. J. Mat. Chem. B 2021, 9, 767–782.
37. Diao, Q.; Guo, H.; Yang, Z.; Luo, W.; Li, T.; Hou, D. A rhodamine-6G-based “turn-on” fluorescent probe for selective detection of Fe3+ in living cells. Anal. Methods 2019, 11, 794–799.

38. Wang, Y.; Liu, F.; Pu, C.; Tong, Z.; Wang, M.; Wang, J. Galactose-imidazole mediated dual-targeting fluorescent probe for detecting Fe3+ in the lysosomes of hepatocytes: Design, synthesis and evaluation. Biosens. Bioelectron. 2022, 204, 114083.
39. Rattanopas, S.; Piyanuch, P.; Wisansin, K.; Charoenpanich, A.; Sirirak, J.; Phutdhawong, W.; Wanichacheva, N. Indole-based fluorescent sensors for selective sensing of Fe2+ and Fe3+ in aqueous buffer systems and their applications in living cells. J. Photochem. Photobiol. A Chem. 2019, 377, 138–148.
40. McGhee, C.E.; Yang, Z.; Guo, W.; Wu, Y.; Lyu, M.; De Long, C.J.; Hong, S.; Ma, Y.; McInnis, M.G.; O'Shea, K.S.; et al. DNAzyme-Based Lithium-Selective Imaging Reveals Higher Lithium Accumulation in Bipolar Disorder Patient-Derived Neurons. ACS Central Sci. 2021, 7, 1809–1820.
41. Jiao, Y.; Gao, Y.; Meng, Y.; Lu, W.; Liu, Y.; Han, H.; Shuang, S.; Li, L.; Dong, C. One-Step Synthesis of Label-Free Ratiometric Fluorescence Carbon Dots for the Detection of Silver Ions and Glutathione and Cellular Imaging Applications. ACS Appl. Mater. Interfaces 2019, 11, 16822–16829.
42. Yu, Q.; Shi, J.; Mudiyanselage, A.P.K.K.K.; Wu, R.; Zhao, B.; Zhou, M.; You, M. Genetically encoded RNA-based sensors for intracellular imaging of silver ions. Chem. Commun. 2019, 55, 707–710.
43. Tang, H.-Y.; Gao, Y.; Li, B.; Li, C.-W.; Guo, Y. Reaction-based colorimetric and ratiometric fluorescent probe for highly selective detection of silver ions. Sens. Actuator B-Chem. 2018, 270, 562–569.
44. Bu, F.; Zhao, B.; Kan, W.; Wang, L.; Song, B.; Wang, J.; Zhang, Z.; Deng, Q.; Yin, G. A phenanthroimidazole-based AIE active fluorescence probe for sequential detection of Ag+/AgNPs and SCN− in water and saliva samples and its application in living cells. Spectroc. Acta Part A-Molec. Biomolec. Spectr. 2019, 223, 117333.
45. Yu, M.; Liu, B.; Guo, J.; Wu, F. A sustainable modificatory 1, 2- alternate thiacalixarene for detection of silver ion. Dyes Pigment. 2023, 210, 110983.
46. Soleja, N.; Mohsin, M. Real time quantification of intracellular nickel using genetically encoded FRET-based nanosensor. Int. J. Biol. Macromol. 2019, 138, 648–657.
47. Wang, X.; Liu, C.; Zhu, H.; Cheng, S.; Zhang, Y.; Su, M.; Rong, X.; Yu, M.; Sheng, W.; Zhu, B. An ultra-sensitive and highly selective fluorescent probe for nickel ions and its environmental and biological applications. Sens. Actuator B-Chem. 2022, 369, 132300.
48. Assiri, M.A.; Junaid, H.M.; Waseem, M.T.; Hamad, A.; Shah, S.H.; Iqbal, J.; Rauf, W.; Shahzad, S.A. AIEE active sensors for fluorescence enhancement based detection of Ni2+ in living cells: Mechanofluorochromic and photochromic properties with reversible sensing of acid and base. Anal. Chim. Acta. 2022, 1234, 340516.

49. Gu, Y.-Q.; Shen, W.-Y.; Zhou, Y.; Chen, S.-F.; Mi, Y.; Long, B.-F.; Young, D.J.; Hu, F.-L. A pyrazolopyrimidine based fluorescent probe for the detection of Cu2+ and Ni2+ and its application in living cells. Spectroc. Acta Part A-Molec. Biomolec. Spectr. 2019, 209, 141–149.
50. Gui, S.; Huang, Y.; Zhu, Y.; Jin, Y.; Zhao, R. Biomimetic Sensing System for Tracing Pb2+ Distribution in Living Cells Based on the Metal–Peptide Supramolecular Assembly. ACS Appl. Mater. Interfaces 2019, 11, 5804–5811.
51. Mehta, P.K.; Jeon, J.; Ryu, K.; Park, S.-H.; Lee, K.-H. Ratiometric fluorescent detection of lead ions in aquatic environment and living cells using a fluorescent peptide-based probe. J. Hazard. Mater. 2022, 427, 128161.
52. Wen, J.; Lv, Y.; Xia, P.; Liu, F.; Xu, Y.; Li, H.; Chen, S.-S.; Sun, S. A water-soluble near-infrared fluorescent probe for specific Pd2+ detection. Biorg. Med. Chem. 2018, 26, 931–937.
53. Wang, D.; Hou, H.; Chen, W.; Wu, Y.; Peng, X.; Song, F. A turn-on fluorescent probe for palladium(II) detection with a large Stokes shift and lysosomes-targeting ability. Tetrahedron Lett. 2022, 102, 153932.
54. Ashwin, B.C.M.A.; Sivaraman, G.; Stalin, T.; Yuvakkumar, R.; Muthu Mareeswaran, P. Selective and sensitive fluorescent sensor for Pd2+ using coumarin 460 for real-time and biological applications. J. Photochem. Photobiol. B Biol. 2018, 183, 302–308.
55. Zhang, X.-P.; Yuan, Q.; Qi, Y.-L.; Zheng, D.-J.; Liu, Q.-X.; Wang, B.-Z.; Yang, Y.-S.; Zhu, H.-L. An umbelliferone-derivated fluorescent sensor for selective detection of palladium(II) from palladium(0) in living cells. Spectroc. Acta Part A-Molec. Biomolec. Spectr. 2019, 220, 117134.
56. Reagen, S.; Wu, Y.; Liu, X.; Shahni, R.; Bogenschuetz, J.; Wu, X.; Chu, Q.R.; Oncel, N.; Zhang, J.; Hou, X.; et al. Synthesis of Highly Near-Infrared Fluorescent Graphene Quantum Dots Using Biomass-Derived Materials for In Vitro Cell Imaging and Metal Ion Detection. ACS Appl. Mater. Interfaces 2021, 13, 43952–43962.
57. Sun, Y.; Wang, L.; Zhou, J.; Qin, D.; Duan, H. A new phenothiazine-based fluorescence sensor for imaging Hg2+ in living cells. Appl. Organomet. Chem. 2020, 34, e5945.
58. Zhong, W.; Wang, L.; Qin, D.; Zhou, J.; Duan, H. Two Novel Fluorescent Probes as Systematic Sensors for Multiple Metal Ions: Focus on Detection of Hg2+. ACS Omega 2020, 5, 24285–24295.
59. Ding, G.; Wu, L.; Feng, H.; Liu, Y.; Li, J.; Si, H.; Yao, X.; He, M.; He, W. The specific binding of a new 1,2,3-triazole to three blood proteins and it's appended rhodamine complex for selective detection of Hg2+. Spectroc. Acta Part A-Molec. Biomolec. Spectr. 2020, 228, 117728.
60. Wang, G.; Xu, W.; Liu, Y.; Fu, N. Ultrasensitive detection and high-contrast bioimaging of Hg2+ using monothiosquaraine-based fluorescent probe via hydrogen bond promoted desulfurization. Microchem. J. 2022, 179, 107481.

61. Lin, W.; Xie, X.; Wang, Y.; Chen, J. A New Fluorescent Probe for Selective Cd2+ Detection and Cell Imaging. Z. Anorg. Allg. Chem. 2019, 645, 645–648.
62. Wang, P.; An, Y.; Liao, Y. A novel peptide-based fluorescent chemosensor for Cd(II) ions and its applications in bioimaging. Spectroc. Acta Part A-Molec. Biomolec. Spectr. 2019, 216, 61–68.
63. Guo, Y.; Li, T.; Xie, L.; Tong, X.; Tang, C.; Shi, S. Red pitaya peels-based carbon dots for real-time fluorometric and colorimetric assay of Au3+, cellular imaging, and antioxidant activity. Anal. Bioanal. Chem. 2021, 413, 935–943.
64. Bhattacharyya, A.; Makhal, S.C.; Guchhait, N. CHEF-Affected Fluorogenic Nanomolar Detection of Al3+ by an Anthranilic Acid–Naphthalene Hybrid: Cell Imaging and Crystal Structure. ACS Omega 2018, 3, 11838–11846.
65. Berrones-Reyes, J.; Muñoz-Flores, B.M.; Gómez-Treviño, A.; Treto-Suárez, M.A.; Páez-Hernández, D.; Schott, E.; Zarate, X.; Jiménez-Pérez, V.M. Novel fluorescent Schiff bases as Al3+ sensors with high selectivity and sensitivity, and their bioimaging applications. Mater. Chem. Phys. 2019, 233, 89–101.
66. Wang, C.-X.; Ai, S.-L.; Wu, B.; Huang, S.-W.; Liu, Z. Biotinylated and fluorophore-incorporated polymeric mixed micelles for tumor cell-specific turn-on fluorescence imaging of Al3+. J. Mat. Chem. B 2020, 8, 3557–3565.