Review Article Open Access

Xian Zheng, Wenyu Cheng, Chendong Ji, Jin Zhang*, and Meizhen Yin*

Detection of metal ions in biological systems: A review

https://doi.org/10.1515/revac-2020-0118
received September 17, 2020; accepted October 27, 2020

Abstract: Metal ions are widely present in biological systems and participate in many critical biochemical processes such as material transportation, energy conversion, information transmission and metabolic regulation, making them indispensable substance in our body. They can cause health problems when deficiency or excess occurs. To understand various metabolic processes and facilitate diseases diagnosis, it is very important to measure the content and monitor the distribution of metal ions in individual cells, tissues and whole organisms. Among the various methods for metal ion detection, fluorescent sensors with organic dyes have attracted tremendous attention due to many advantages such as high fluorescence quantum yield, facile modification approaches and biocompatibility in addition to operation ease, high sensitivity, fast detection speed, and real-time detection. This review summarizes the recent progress on the detection and imaging of the metal ions in biological systems including Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}/Fe^{3+}, Zn^{2+}, and Cu^{2+} provides an opinion on remaining challenges to be addressed in this field.

Keywords: metal ions sensing, fluorescent detection, fluorescent imaging, organic dyes

*** Corresponding author: Jin Zhang,** Beijing Advanced Innovation Center for Soft Matter Science and Engineering, State Key Laboratory of Chemical Resource Engineering, Ministry of Education, Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, 100029 Beijing, China, e-mail: zjin@mail.buct.edu.cn
*** Corresponding author: Meizhen Yin**, Beijing Advanced Innovation Center for Soft Matter Science and Engineering, State Key Laboratory of Chemical Resource Engineering, Ministry of Education, Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, 100029 Beijing, China, e-mail: yinmz@mail.buct.edu.cn
Xian Zheng, Wenyu Cheng, Chendong Ji: Beijing Advanced Innovation Center for Soft Matter Science and Engineering, State Key Laboratory of Chemical Resource Engineering, Ministry of Education, Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, 100029 Beijing, China

1 Introduction

Metal ions play crucial structural or functional roles in all life. They are required in essential biological processes such as osmotic regulation, catalysis, metabolism, and cell signaling. As the major metal ions in our body fluid, alkali metals ions, Na^+ and K^+ are responsible for maintenance of fluid and electrolyte balance, acid-base homeostasis, and maintenance of cell membrane potential allowing the generation of action potentials that are critical for neurotransmission, muscle contraction, and heart function [1]. Alkaline earth metal ions, Ca^{2+} and Mg^{2+}, play an important role in cell signaling including neurotransmission as well as their structural role and involvement in enzyme function [2,3]. Transition metals such as Fe^{2+}/Fe^{3+}, Zn^{2+}, Cu^{2+}, generally serving as a component of proteins and enzymes or activator of enzymes, are considered to be the critical players in many vital biological processes including oxygen transport, energy production, neurotransmission, regulation of gene expression and synthesis of essential molecules [4,5].

Although metal ions are critical for sustaining life, their concentration in organisms must be maintained within a proper range for optimal cellular function. Deficiency of these essential elements can cause serious health problems [6–8]. Excessive levels of these metal ions in our body or even extended exposure to them can also result in many health issues or toxicity [9,10]. Understanding the distribution and concentration fluctuation of metal ions in cells gives information on cell signaling, metabolic engineering and helps medical diagnosis. Thus, detection and imaging of metal ions in living organisms became a central topic in the bioanalytical and biomedical sciences. In the past decades, several spectrophotometric methods and electroanalytical techniques for metal ions detection have been developed, such as atomic absorption/emission spectroscopy [11,12], inductively coupled plasma mass spectroscopy (ICP-MS) [13,14], electrochemical assays [15,16], and colorimetric methods [17,18]. However, the application of these techniques is limited as they need expensive instrumentation and complicated pretreatments, which shows slow detection speed and does

Open Access. © 2020 Xian Zheng *et al.*, published by De Gruyter. This work is licensed under the Creative Commons Attribution 4.0 International License.

not permit the on-site instant detection and inspection of cells and living tissues. Fluorescent probes have become versatile tools due to their promising photophysical properties including high sensitivity and selectivity, low detection limit, fast response, operational simplicity, real-time monitoring and low cost [19,20]. Moreover, benefited from the new fluorescence imaging techniques, great progress has been made in the development of probes derived from organic fluorescent dyes (such as AIEgen, cynine and rylene) [21–26] as they possess features such as good biocompatibility, high spatio-temporal resolution, facile chemical modification and they are capable of monitoring the subcellular localization and dynamics of biological targets [27–29]. This review summarized the recent advances of fluorescence sensing and imaging of metal ions in biological systems with the focus on probe design, responding mechanism and biological applications (Scheme 1).

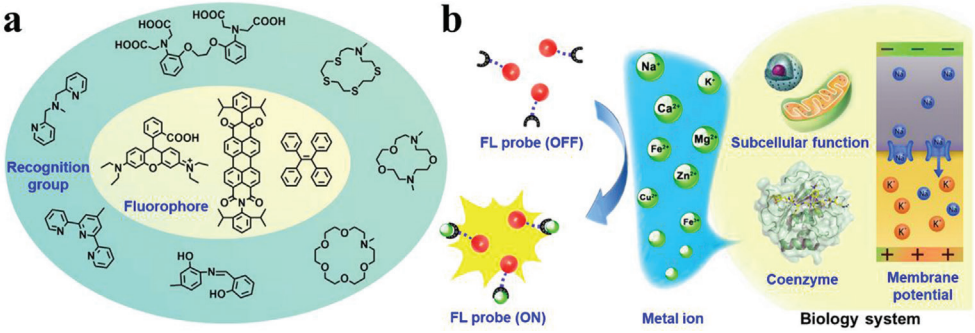
Typical organic fluorescent probes are mainly composed of at least one fluorescent core and a metal chelating or binding moiety that recognizes different metal ions. There are several types of fluorescent cores such as rylenecarboximide (RI), cyanine (Cy), rhodamine, difluoro-boron-dipyrromethene (BODIPY), etc. Upon metal binding, the electronic and/or molecular structure of the probes alters and it induces changes in fluorescence properties of the fluorophore, indicating the presence of metal ions. Fluorescent probes can be classified according to different criteria or characteristics. For instance, according to the responding mechanism, the probes can be divided into photoinduced electron transfer (PET) probes, fluorescence resonance energy transfer (FRET) probes, intramolecular charge transfer (ICT) probes, twisted intramolecular charge transfer (TICT) probes, through bond energy transfer (TBET) probes and aggregation-induced emission (AIE) probes. According to the optical performance, the probes can be divided into "off-on" probes, "on-off" probes and ratiometric probes. Fluorescent "off-on" probes and ratiometric probes constitute the biggest group of fluorescent probes. The former features a fluorescent enhancement upon mental ions binding, the latter is based on the use of a ratio between two fluorescence intensities that allows correction of artifacts due to bleaching, changes in focus, and variations in laser intensity. Different types of fluorescent probes have been designed to adapt to various samples and systems, and can be used as powerful tools to study the function of metal ions in subcellular function, enzyme activity and dynamic change of membrane potential [30].

2 Detection of metal ions in biological systems

2.1 Probes for sodium ions (Na^+)

Sodium is the most abundant cation in extracellular fluid. It plays major roles in maintenance of fluid and electrolyte balance and regulates blood volume, blood pressure, osmotic equilibrium and pH values. The difference between the concentrations of sodium ions and potassium ions in extracellular and intracellular fluid causes the cell membrane potential which allows the cell to generate an action potential. Action potential is critical for body functions such as neurotransmission, muscle contraction, and heart function [31]. Absorption of sodium in the small intestine plays an important role in the absorption of chloride, amino acids, glucose, and water. Sodium is also an important component of gastric juice which aids the digestion and absorption of many nutrients. Therefore, the detection of Na^+ ions is quite crucial for both clinical diagnosis and biochemical applications.

To achieve Na^+ sensing and imaging in living cells, various types of fluorescent probes were developed. For instance, a DNAzyme-based sensor was coupled with a catalytic hairpin assembly (to detect endogenous Na^+



Scheme 1: Schematic illustration of the composition, (a) sensing mechanism and (b) biological applications of metal ions probe.

inside cells without the requirement for Na^+ concentration increase by influx of the ions [32]. In their strategy, NaA43 DNAzymes cleaved the substrate strands in the presence of Na^+ and released initiator DNA that triggered the following CHA signal amplification reaction. In another case, a highly selective and robust Na^+ aptamer was developed (Figure 1a). This probe contained two strands derived from the Ce13d DNAzyme and a fluorescent adenine analog, 2-aminopurine (2AP) at the cleavage site of the substrate strand [33]. It was shown that the fluorescence was enhanced by ~40% upon Na^+ binding by introducing a 2AP at the cleavage site of the substrate strand and fluorescence quenching was observed upon Na^+ binding by replacing a non-conserved adenine in the enzyme strand by 2AP. The fluorescence changes allowed for Na^+ detection with a detection limit of 0.4 mM and signal saturation in less than 10 s. Apart from DNA based sensors, a protein-coupled Na^+-sensitive fluorescent probe, HaloNa-1, was developed [34] by conjugating a Na^+ chelator, fluorescein, and a HaloTag ligand moiety (Figure 1b). The obtained probe could selectively label HaloTag-fusion proteins both *in vitro* and in cells, and its fluorescence was increased in response to concentration elevation of Na^+. Another fluorophore (mCherry) was introduced as an internal standard to eliminate signal artifacts. Two-photon fluorescence imaging with advantages such as minimized tissue autofluorescence background, a larger penetration depth, and reduced photodamage in biotissues has received enormous attention in both clinical diagnostics and basic biological research. Two-photon transition has strong excitation selectivity, which is conducive to imaging research on some special substances in biological tissues. Two-photon sensors were also prepared for the detection and imaging of Na^+. To achieve the quantification of Na^+, a fluorescent dye Asante NaTRIUM Green-2 (ANG-2) with Na^+ sensitivity was reported and evaluated both *in vitro* and in situ with two-photon coupled fluorescence lifetime imaging microscopy [35]. It was found that the biexponential fluorescence decay behaviour of the dye in situ could be successfully analyzed in terms of quantitative [Na^+] recordings, indicating that ANG-2 is a promising new sodium indicator applicable for diverse biological systems. Moreover, two-photon microscopy was used for Na^+ imaging to directly report the opening of voltage-gated sodium channels due to action potential propagation in mitral cells and the axonless granule cells [36].

2.2 Probes for potassium ions (K^+)

While sodium is the major cation outside animal cells, potassium is the major cation (positive ions) inside animal cells. Similar to sodium, potassium also has a major role in maintenance of fluid and electrolyte balance, acid-base homeostasis, systemic blood pressure control, hormone secretion and action, glucose and insulin metabolism and neurotransmission. Potassium deficiency and excess can each result in numerous signs and symptoms, including an abnormal heart rhythm and various electrocardiographic abnormalities. Highly selective and sensitive detection and imaging of physiological K^+ is urgently needed for monitoring K^+ related diseases and understanding the K^+ involved physiological and pathological processes.

A TPE derivative modified DNA oligonucleotide fluorescent probe was reported for cellular K^+ detection and imaging [37]. The probe exhibits K^+ triggered AIE effect, and outstanding sensitivity with more extended photostability than most reported probes, and, thus, facilitates the long-lasting fluorescence imaging of K^+ in living cells. To obtain probes with higher binding affinity to K^+, phenylaza-18-crown-6, a well-known ligand for K^+ was adopted. Two molecular fluorescent probes based on the o-(2-methoxyethoxy) phenylaza-18-crown-6 lariat ether unit were reported [38]. These ratiometric fluorescent

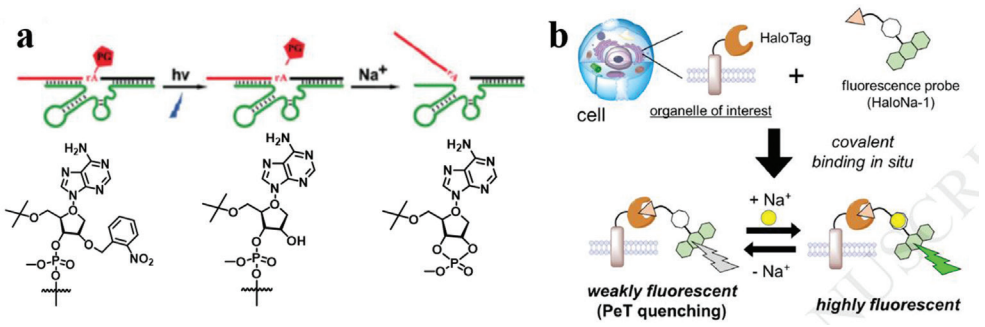


Figure 1: (a) Synthesis of DNAzyme-based Na^+ probes. Reprinted with permission from reference [32]. (b) Probes covalently bound to HaloTag protein for Na^+ imaging. Reprinted with permission from reference [34].

probes show high K^+-triggered fluorescence enhancement in water samples with physiologically relevant levels of K^+. A set of highly K^+-selective fluorescent probes were prepared through varying the lariat-alkoxy unit of a phenylaza-18-crown-6 lonophore to obtain improved K^+/Na^+ selectivity [39]. Among all the probes, an isopropyl group substituted probe showed high K^+/Na^+ selectivity and can serve as a suitable fluorescent probe to measure physiological K^+ levels in the range of 10-80 mM *in vitro*. In addition, a hydrogen-bonded supramolecular framework based on 3,3'-((6-hydroxy-1,3,5-triazine-2,4-diyl) bis(azanediyl))dibenzoic acid and Zn(II) was prepared [40]. The 2D layer architecture is further stacked via hydrogen bond and N···N van der Waals interactions to form a 3D supramolecular framework. Because of its matched ionic size and charge number, K^+ effectively facilitates the energy transfer process. Apart from the detection of K^+, the parallel monitoring of K^+ and PPIX in living animals was achieved by a functional nucleic acid (FNA)-based technique [41]. A K^+ induced G-rich FNA probe formed a parallel G-quadruplex with enhanced fluorescence, and thus allowed the detection of K^+ and PPIX. In another case, multi-functional fluorescent probes with high K^+ selectivity were constructed. Using N-(2-methoxyethoxyphenyl) aza-18-crown-6 ionophore, Holdt et.al, developed a highly K^+-selective two-photon fluorescent probe for *in vitro* monitoring of physiological K^+ levels (1-100 mM) [42]. The two-photon excited fluorescence probe shows three times fluorescence enhancement in the presence of 160 mM K^+. To achieve higher K^+ sensitivity, a fluorescence and resonance Rayleigh scattering (RRS) di-model probe was developed to monitor K^+ coupled N-doped carbon dot (CDUN) and aptamer (Apt) with a detection limit of 0.3 nmol/L [43]. The CDUN encapsulated by Apt reduced its fluorescence and RRS intensities. When K^+ was added, it reacts with Apt to form a stable G-quadruplex and free CDUN. The fluorescence and RRS di-model intensity increased linearly with the concentration of K^+. Due to its involvement in maintaining membrane potential, monitoring of K^+ around cell membranes is of particular importance. For the first time, a membrane-anchored fluorescent probe for real-time detecting K^+ in the cell microenvironment was reported [44]. This probe exhibits high sensitivity and selectivity to K^+ with a ratiometric fluorescent signal. Especially, the reversible coordination between K^+ and the probe could monitor dynamic changes of K^+ in complex physiological systems. In another study, a protein-coupled fluorescent probe TLSHalo was designed by covalently conjugating the probe with HaloTag protein, which is sensitive to K^+ in the physiological range [45]. The real-time change of localized extracellular K^+ with TLSHalo was observed under a fluorescence microscope (Figures 2 a-d).

2.3 Probes for calcium ions (Ca^{2+})

Calcium is the most abundant metal and the fifth-most abundant element in the human body. 99% of the calcium in the body is found in bones and teeth, while the other 1% is found in the blood and soft tissue. As electrolytes, they act as a second messenger in cell-signaling pathways [46,47] where they are involved in mediating the constriction and relaxation of blood vessels (vasoconstriction and vasodilation), nerve impulse transmission, muscle contraction, and the secretion of hormones like insulin. In addition, many enzymes require calcium ions as a cofactor, including several of the coagulation factors. For example, calcium ions are required for the activation of the seven "vitamin K-dependent" clotting factors that control the clotting of blood. For normal physiological functioning, calcium concentrations in the blood and extracellular fluid are tightly controlled by the parathyroid hormone and vitamin D at the expense of the skeleton when dietary calcium intakes are inadequate. The rise or fall of calcium is the molecular clock that times the execution of important processes, therefore, it is crucial to measure [Ca^{2+}] changes at the cellular level.

A red-emitting BODIPY-based fluorophore was developed for Ca^{2+} detection [48]. The probe demonstrated good selectivity towards Ca^{2+} over other metal ions. Upon Ca^{2+} binding, the probe exhibited 43-fold fluorescence enhancement with the detection limit of 39 μM. In another case, a coumarin and pyrazoline derived Ca^{2+} probe was developed on the basis of PET strategy [49]. The probe could detect Ca^{2+} rapidly (within 5 s) with a low detection limit (2.7×10^{-7} M) and can be used for Ca^{2+} imaging in living A549 cells. Probes with different dyes derived from naphthalene diimide [50], rhodamines, and fluorescein [51,52] enriched the library of Ca^{2+} sensors and enabled sensitive detection and imaging of biological Ca^{2+}. Urano et al. synthesized silicon-substituted rhodamines with enhanced permeability and far-red emission for Ca^{2+} imaging in lysosomes and cytosol in living cells and brain [53]. To develop near-infrared (NIR) probe with better bio-compatibility especially *in vivo* applications, Urano et al. prepared phosphorus-substituted rhodamines [54]. Results indicated that phosphorus-substituent effectively red shifted the emission of rhodamine to 712 nm, and better sensitivity was achieved compared to

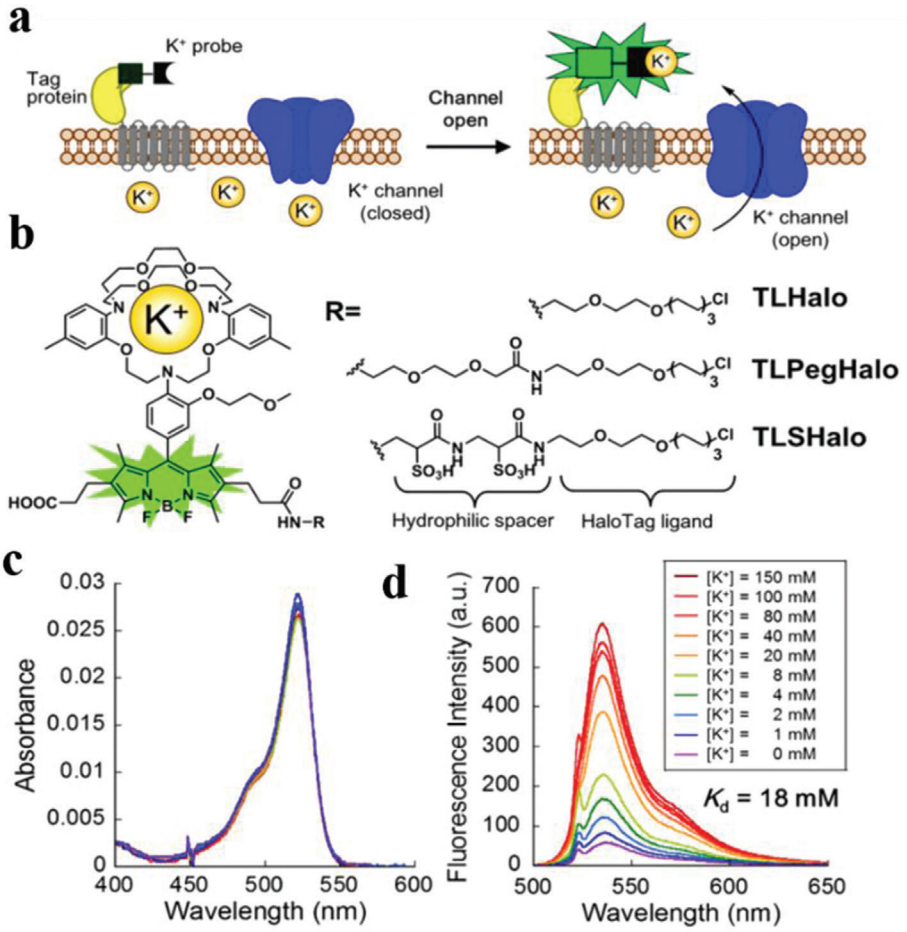


Figure 2: (a) Illustration of the probe that detects K^+ channel activity at the cell surface. (b) Chemical structures of the K^+ probes. (c,d) Absorption (c) and emission (d) spectra of TLSHalo measured in HEPES buffer containing [K^+]. Reprinted with permission from reference [45].

that of silicon-substituted rhodamines. The new probe can be also used to image Ca^{2+} at dendrites and spines in brain slices, indicating its potential use in neuroscience research. Besides metal ion concentrations, pH values are another important concern in biological systems. The simultaneous quantification of Ca^{2+} and pH values can be useful in clinic diagnosis and disease intervention. A Ca^{2+} fluorescent probe was assembled onto a DNA nanostructure together with pH-sensitive, mitochondria targeted and inner-reference molecules to carry on dual function (Figure 3a). With this probe in hand, ROS and Aβ triggered transitory cytoplasmic acidosis and activated acid-sensing ion channels in the mitochondrial membrane were observed (Figure 3b) [55,56]. Two-photon fluorescent probe is favorable for *in vivo* applications due to their long absorption wavelength. A Ca^{2+} probe was prepared with a two-photon dye and an internal reference dye. The probe could directly and quantify Ca^{2+} concentration within live neurons and various tissues including rat spinal cord tissue [57].

2.4 Probes for magnesium ions (Mg^{2+})

Magnesium is the fourth-most-abundant metal ions in cells (per mole). It is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in our body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation [58]. Magnesium is required for energy production, oxidative phosphorylation, and glycolysis. It contributes to the structural development of bone and is required for the synthesis of DNA, RNA, and the antioxidant glutathione. Magnesium also plays a role in the active transport of calcium and potassium ions across cell membranes, a process that is important to nerve impulse conduction, muscle contraction, and normal heart rhythm [59]. However, the understanding of the molecular mechanisms of cellular Mg^{2+} homeostasis remains a challenge and demands molecular tools that can detect the ions with high sensitivity. A set of BODIPY-based fluorescence probes was developed for Mg^{2+} detection [60].

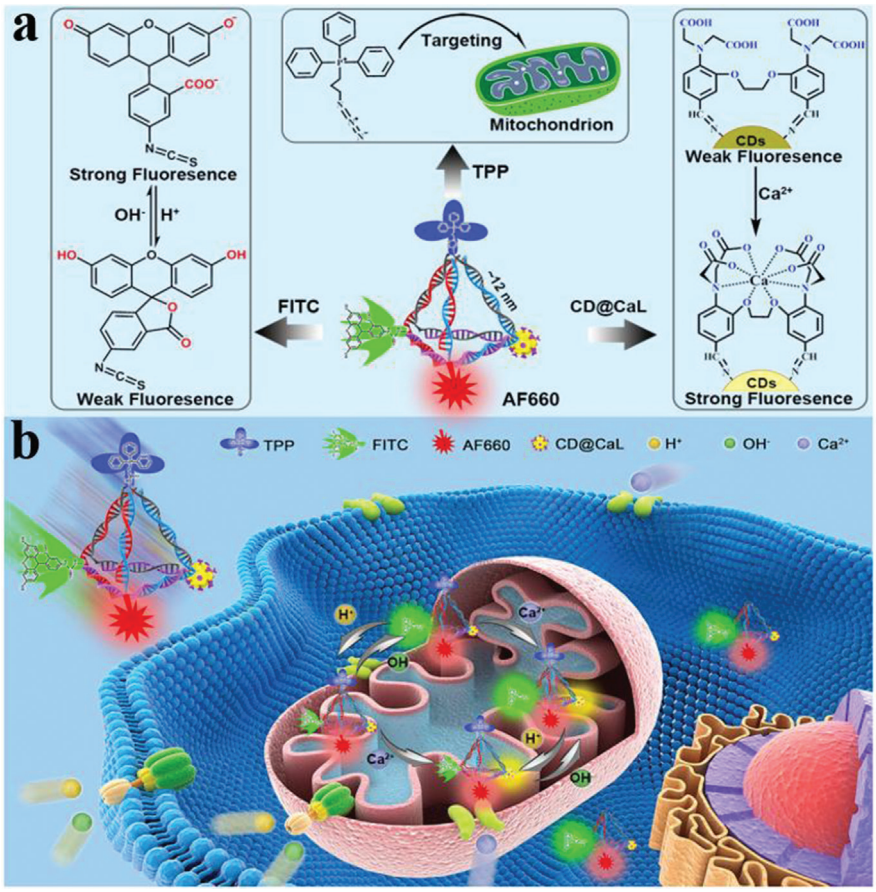


Figure 3: (a) Design of tetrahedron DNA-based Ca^{2+} nanoprobe. (b) Mechanism of DNA nanosensor for Ca^{2+} and pH sensing. Reprinted with permission from reference [55].

The probes showed 29-fold fluorescence enhancement upon Mg^{2+}binding both *in vitro* and in cell, demonstrating high selectivity for Mg^{2+}. A natural product, thymol derivatives, were prepared with simple synthetic procedures and used as a fluorescent indicators for Mg^{2+} in living cells [61]. Due to the small size and lipophilic character of thymol, the probe exhibits good cell penetration ability that allows the probe to get into subcellular organelles and enables intracellular free Mg^{2+} imaging in living RAW 264.7 and HeLa cells. Most fluorescent probes are not reusable. A three-dimensional (3D) MOF with one-dimensional (1D) hexagonal channels was synthesized for Mg^{2+} detection (Figure 4a) [62]. This probe can be recycled without signal attenuation. After absorbing Mg^{2+}, the fluorescence of MOF increased by 3.2-fold. Futhermore, multi-color imaging [63] and two-photon fluorescent imaging [64] was also used for Mg^{2+} detection. Murata et al. developed Si-rhodamine probe with PET-type "off-on" response in the presence of Mg^{2+}. In addition, it is possible to visualize the interaction of signals within the cell by using four chromophores for simultaneous multicolor imaging [65]. Two coumarin-based fluorescent probes, OC7 (methoxy phenyl alkynyl) and NC7 (dimethylamino phenyl alkynyl), were prepared as two-photon fluorophore for Mg^{2+} detection [64]. OC7 and NC7 demonstrated significant signals with 9.05-fold and 23.8-fold fluorescence enhancement and large two-photon absorption cross sections (340 and 615 GM) at the NIR wavelengths (740 and 860 nm). Metal ion detection in a specific organelle is often desirable in disease dignosis [66–68]. A probe with a tetrazine-functionalized pre-sensor and a genetically encoded HaloTag fusion protein conjugate was developed to target a specific cellular localization [65]. The probe was successfully applied for Mg^{2+} imaging in different organelles such as the nucleus and Golgi apparatus of HEK 293T cells. A series of fluorescent probes were designed for targeted detection of free Mg^{2+} in specific intracellular organelles and their application in the study of programmed cell death were evaluated [66,67]. The triazole-based probe can detect free Mg^{2+} levels in mitochondria. Moreover, fluorescence imaging study of Staurosporine-treated HeLa cells directly revealed that the free Mg^{2+} levels increase in mitochondria during early apoptosis (Figures 4c,d).

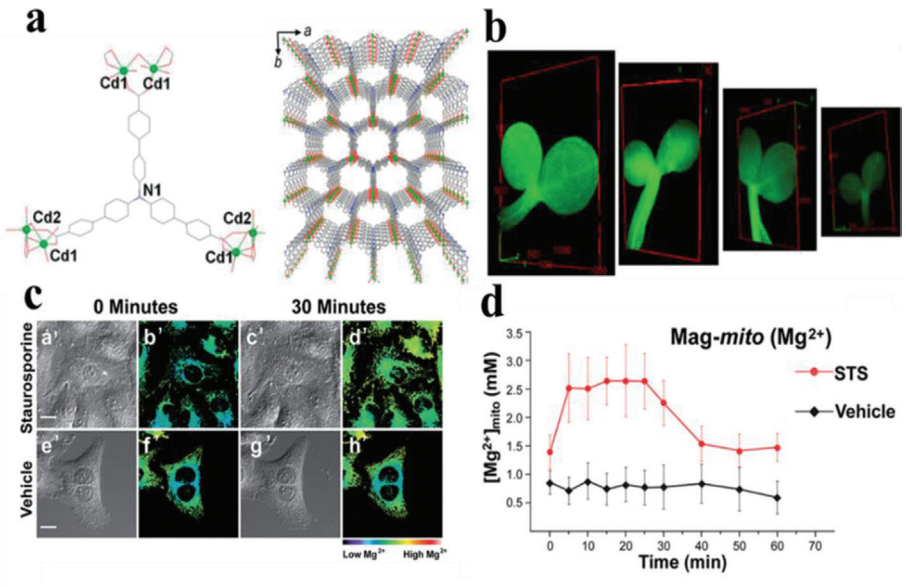


Figure 4: (a) Coordination of the ligand to Cd^{2+} ions and 3D network structure of 1 with one-dimensional channels along the c axis. Reprinted with permission from reference [62]. (b) 3D fluorescence images of plants with different concentrations of Mg^{2+} treatment. Reprinted with permission from reference [68]. (c) Widefield fluorescence imaging of mitochondrial free Mg^{2+} in HeLa cells treated with 1 mM apoptosis-inducing Staurosporine (a'-d'), or vehicle (e'-h') and (d) corresponding quantified changes. Reprinted with permission from reference [67].

2.5 Probes for iron ions (Fe^{2+} and Fe^{3+})

In the human body, iron is the most abundant transition metal. It exists in two biologically relevant oxidation states: the ferrous form (Fe^{2+}) and the ferric form (Fe^{3+}). Iron plays an essential role in many biological processes [69], such as oxygen transport, energy production, DNA synthesis, and cell growth and replication through iron-dependent proteins. For example, Globin-heme proteins such as hemoglobin, myoglobin, and neuroglobin are involved in oxygen transport and storage. Heme enzymes (e.g. cytochromes a, b, f oxidase) are involved in electron transfer and/or with oxidase activity. Iron-sulfur (Fe-S) cluster proteins are involved in energy production, DNA synthesis or DNA replication and repair. Nonheme enzymes such as phenylalanine and lysine hydroxylases require iron as a cofactor for their catalytic activities. Nonheme proteins such as ferritin and transferrin are responsible for iron transport and storage [70]. In addition to iron-dependent proteins, iron also exists in 'transit labile iron pools' where iron is loosely bound to low molecular-weight compounds such as phosphate, carbonate and citrate. Labile iron ions are toxic to cells, as they promote the production of ROS, which is associated with the aging process and a few degenerative diseases [71]. In addition, the 'transit' iron pool contributes to the cellular iron uptake via transferrin. In order to gain better understanding of the intracellular iron transport mechanisms and the biological functions of labile irons, effective tools to monitor iron ions in living cells are required. In the past decades, many efforts have been made to develop iron sensors with redox state selectivity, sensitivity, and organelle specificity. An "off-on" Fe^{2+} selective fluorescent probe was constructed by incorporating an Fe^{2+}-induced N–O cleavage of acylated hydroxylamine moiety into the naphthalimide fluorophore (Figure 5b) [72]. It was demonstrated that the probe can be used to detect endogenous, basal level of labile Fe^{2+} pools in living cells and Fe^{2+} involved in biological processes, such as the Zn^{2+}-induced Fe^{2+} flux and the elevated level of Fe^{2+} in the brain tissue of a rat undergoing ischemic stroke at the ischemic site. Ion sensors targeting specific cellular localization have emerged in recent years. A Mem-RhoNox probe was developed to monitor local Fe^{2+} at the surface of the plasma membrane of living cells [73]. The probe consists of the N-oxygenated rhodamine scaffold with two arms, both of which are tethered with palmitoyl groups as membrane-anchoring domains (Figure 5a). With a model compound Ac-RhoNox in an aqueous buffer, the probe shows a fluorescence turn-on response to the Fe^{2+} redox state-selectively and the capability of monitoring endosomal Fe^{2+} in primary cultured neurons during endocytotic uptake. To specifically target endogenous mitochondrial Fe^{2+} in living cells, a fluorescent probe MtFluNox/Ac-MtFluNox was developed [74]. The deacetylated form, MtFluNox, showed a turn-on response towards Fe^{2+} with high metal selectivity in cuvette

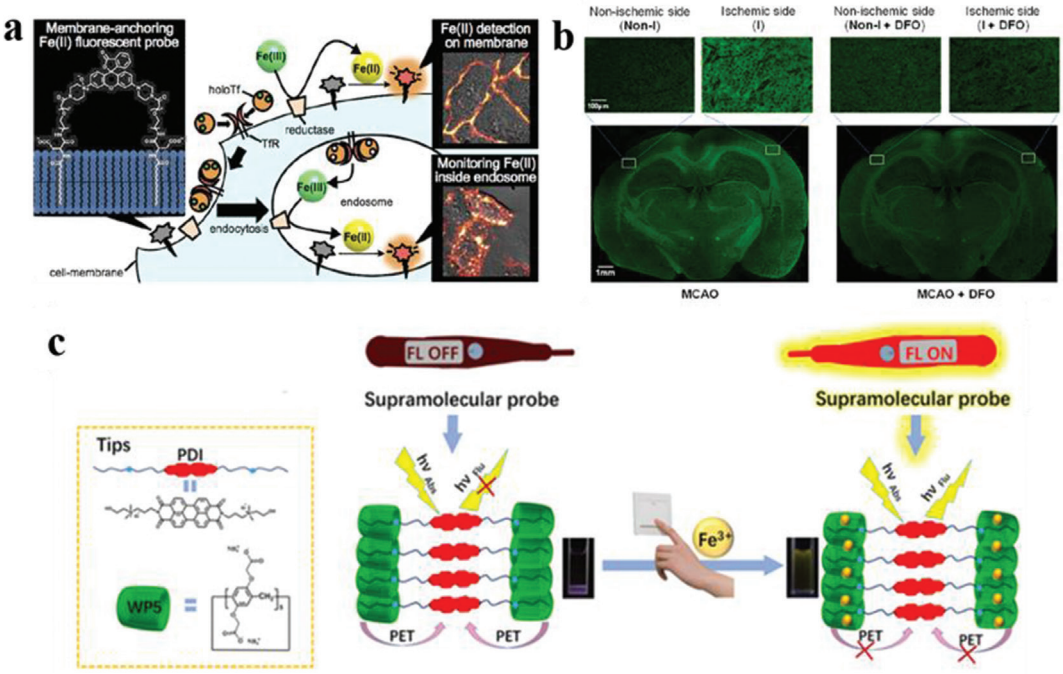


Figure 5: (a) Schematic illustration of Fe^{2+} ions release imaging during endocytotic uptake process. Reprinted with permission from reference [73]. (b) Fluorescent images of labile Fe^{2+} in rat's ischemic brain tissue. Reprinted with permission from reference [72]. (c) supramolecular system for Fe^{3+} ions sensing. Reprinted with permission from reference [86].

experiments, and an imaging study using its cell-compatible analogue, Ac-MtFluNox, demonstrated mitochondria-specific fluorescence enhancement in response to Fe^{2+} in living cells. They also demonstrated that the probe was able to detect endogenously accumulated Fe^{2+} induced as a result of the inhibition of heme synthesis. In addition, an organic-inorganic hybrid is reported for ratiometric fluorescent biosensing of Fe^{2+} [75]. The probe contained nanoclusters (AuNCs) with a ligand that recognized Fe^{2+} and a reference element comprised of water-soluble sulfocyanine 7 Nhydroxysuccinimide ester (Cy7 NHS ester). The fluorescent nansosensor showed great selectivity and accuracy with a detection limit of 210 nM, as well as quick response (detecting time of less than 1.23 s) and long-term stability. Specifically, it displayed good linearity with the concentration of Fe^{2+} in the range of $1\text{-}10^5$ μM. Furthermore, this probe was successfully applied in real-time biosensing and bioimaging of Fe^{2+} in neurons and HepG2 cells. Moreover, a two-photon fluorescence probe for Fe^{2+}, DCM-Fe was developed [76]. The probe incorporated dicyanomethylene-4H-pyran (DCM) and a Fe^{2+} reduction group (N-oxide) for Fe^{2+} sensing. The probe was considered as a "naked-eye colorimetric sensor" as the NIR fluorescence emission (λ_{em} = 690 nm) increased remarkably with ICT enhanced when the N-oxide is reduced by Fe^{2+}. Similarly, a naphthoquinone-based chemosensor was synthesized and applied in human cancer cells and zebrafish [77]. The probe also featured the ability to sense Fe^{2+} via naked-eye detection. The sensing property was evaluated and the detection limit of the ligand 2HPN to Fe^{2+} was calculated to be 0.272 μM in aqueous acetonitrile medium by a fluorescence emission method. Another Fe^{2+} sensor, Phen-MDI-CA with naked-eye detection mode, was synthesized via chemically bonding 1,10-phenanthroline-5-amine (Phen) onto cellulose acetate (CA) [78]. The sensor displayed excellent fluorescence properties in both solution and solid state. Due to the good solubility and easy processing of Phen-MDI-CA in common organic solvents, it can be used in different material forms. In addition to its fluorescence detection mode, the probe can work in instrument-free visual mode because a red, insoluble, and nonfluorescent Fe-(Phen-MDI-CA) complex appears immediately upon binding to Fe^{2+} ions. Recently, great progress has been made in the development of iron probes suitable for *in vivo* application. A live cell-specific probe Racy was reported [79]. The probe was equipped with an acetate group as a switch and showed a highly sensitive and selective response to Fe^{2+} ions in the presence of esterase in live cells. The probe was also used to evaluate the esterase activity in live animals. A fluorescent probe DCI-Fe(II) based on the N-oxide chemistry was prepared for real-time detection and imaging of Fe^{2+} both in cells and *in vivo* [80]. The probe showed Fe^{2+}concentration-dependent NIR fluorescence turn-on response at 700 nm with a

desirably large Stokes shift and a rapid response (5 min) and detection limit of 51 nM for Fe^{2+}. In this study, the probe was successfully applied to visualize Fe^{2+} in lipid droplets of living cells and living animals.

For the detection and imaging of Fe^{3+}, molecular and supramolecular probes, and even 2D/3D materials are prepared as sensors. As a group of molecular probes, rhodamine based dyes were widely used in Fe^{3+} sensors. For example, a rhodamine-phenanthroline dye based fluoresecent probe was prepared and used to selectively visualize endogenous Fe^{3+} levels under stress conditions [81]. The probe can also be used for real time imaging of endogenous Fe^{3+} in living C6 cell lines. In another study, a rhodamine-B armed fluorescent chemosensor was designed and synthesized via condensation reaction between rhodamine B hydrazide and naphthyl as a probe for Fe^{3+} detection [82]. The probe showed selective turn-on fluorescent change for Fe^{3+} over other cations even in the presence of competing trivalent metal ions and gave a detection limit of 0.16 μM. This probe was applied to monitor and image Fe^{3+} ions in HeLa cell line and zebrafish. Another Rhodamine B-based fluorescent chemosensor (RBTM) for Fe^{3+} was designed [83]. Upon addition of Fe^{3+} in aqueous ethanol, the probe displayed significant fluorescence enhancement and distinct color change. The detection limit was calculated to be 0.256 μM. It was also showed that RBTM probe can be used to detect Fe^{3+} in MKN-45 cells and dorsal root ganglia. In addition, a rhodamine 6G derivative fluorescent probe named RG5NC was designed [84]. The probe showed high selectivity with a detection limit of 8.2 nM towards Fe^{3+} in the presence of various other metal ions and exhibited negligible cytotoxicity in human blood samples and cells. An "off-on" colorimetric and fluorescent probe for Fe^{3+} was designed and synthesized [85]. The probe could sensitively respond to Fe^{3+} among other tested metal cations in THF-H_2O solution with a calculated detection limit of 0.0231 μM. The fluorescence intensity of the probe showed a good linearity with the concentration of Fe^{3+} in the range of 0.1 to 26 μM. It was shown that the probe can be used to monitor Fe^{3+} level in human blood serum and water samples, and for fluorescence imaging of Fe^{3+} in living cell. Supramolecular systems present another promising system for metal ion sensors. Recently, a supramolecular host-guest system (WP5⊃G) comprised of water-soluble pillar[5]arene (WP5) and quaternized perylene diimide derivative (G) showed great potential as a "turn-on" fluorescent probe (Figure 5c) with a detection limit of 0.213 μM for Fe^{3+} ions [86]. 2 D material can also be used for ions sensing. For example, Wang et al. demonstrated that two porous organic polymer nanotubes (PNT-2 and PNT-3) could both serve as luminescent probes for Fe^{3+} due to the distinct luminescent quenching upon addition of Fe^{3+}, while the luminescent intensities of PNTs have no obvious change after adding other metal ions [87]. Fluorescent Ti_3C_2 MXene quantum dots (MQDs) of the size of 1.75 nm were synthesized and used for Fe^{3+} detection since the fluorescence of the MQDs can be significantly suppressed by Fe^{3+} due to the oxidation–reduction reaction between the MQDs and Fe^{3+} and the IFE [88]. The sensor showed high sensitivity with a detection limit of 310 nM and was successfully used to detect Fe^{3+} in serum and sea water. Furthermore, a study by Cui et al. described the first example of a 3D MOF-based turn-on Fe^{3+} sensor, ZJU-109 [89]. The sensor contained Co as the metal center and 6-(4-pyridyl)-terephthalic acid (H2pta) and 4,40-bis(imidazolyl)-biphenyl (4,40-bimbp) as double linkers, a detection limit of 0.053 μM was reported. In addition, another MOF-based potential sensor for Fe^{3+} ions was reported by Chang et al. [90]. In this study a channel-type fluorescent Eu (III)-containing metal-organic framework (MOF) {$[Eu_2L_2]\cdot 2NH_2(CH3)_2DMA$} with a flexible tetracarboxylic acid ligand was successfully prepared. The sensor operated based on a luminescence quenching effect and gave a detection limit of 23 μM in a water solution. In addition, it can be used to treat PD rats, presenting its potential as a candidate for PD treatment.

2.6 Probes for zinc ions (Zn^{2+})

Zinc is the second most abundant trace metal in humans after iron, and it plays important roles in growth and development, immune function, neurotransmission, vision, reproduction, and intestinal ion transport [91]. Zinc is the only metal ion that appears in all six enzyme classes and over 300 enzymes [92] and 1000 transcription factors require zinc for their function [93]. In addition, zinc serves a structural role in many proteins. For example, zinc finger motifs in the structure of nuclear receptors allow them to bind to DNA and act as transcription factors to regulate gene expression. Zinc has been found to influence hormone release [94] and nerve impulse transmission [95]. Dietary zinc deficiency has been associated with impaired growth and development in children, pregnancy complications, and immune dysfunction with increased susceptibility to infections [96]. Due to the importance of Zn^{2+} in biology and the growing evidence that Zn^{2+} levels are both heterogeneous and dynamic, Zn^{2+} attracted the most attention regarding the development of fluorescent sensors for

transition metals. Recently, a reversible fluorescence Zn^{2+} sensor [97] based on 8-aminoquioline was reported. The probe showed a remarkable low detection limit (2.15 nM) in aqueous solution. A fluorescein based colorimetric turn-on fluorescent sensor was prepared and applied to detect Zn^{2+} in apoptotic HEK 293 cells [98]. This probe can be used as an instrument-free sensor as drastic color changes was observed upon Zn^{2+} binding. To minimize the background interference, a NIR fluorescent Zn^{2+} probe based on dicyanoisophorone was developed [99]. This probe showed a very large stokes shift (λ_{em} - λ_{ex} = 168 nm), which provides high contrast for Zn^{2+} sensing. A naphthalene imide-based probe was developed for plasma membrane-specific Zn^{2+} detection by introducing membrane-anchoring domains of hydrophobic alkyl chains [100]. The probe assembled into aggregates and exhibited ACQ (Figure 6a). When anchoring on the membrane surface that led to the probe disaggregate, the probe exhibited a fluorescence-enhanced response upon recognition of zinc ions. Another novel strategy for detecting Zn^{2+} through a so-called multiple steps process was presented [101]. A bis-Schiff base fluorescent probe based on 2-(benzo[d]oxazol-2-yl) phenol was designed and synthesized. The as-prepared probe can be used to monitor [Zn^{2+}] levels in a Parkinson's disease model *in vitro* via chelation-hydrolysis interaction. In order to detect Zn^{2+} *in vivo* and in tissue, a cyanine-based fluorescent probe was constructed [102]. This probe exhibited a high fluorescence quantum yield upon binding to Zn^{2+} and was successfully used to detect endogenous Zn^{2+} in living zebrafish. Furthermore, to enhance the sensitivity of Zn^{2+} sensor in organisms, a fluorescent probe based on dye-assembled lanthanide-doped upconversion nanoparticles was prepared and used to detect Zn^{2+} in mouse brain slice with Alzheimer's disease and zebrafish [103]. This nano system showed a low detection limit (78 nM) and quick response (within 5 s) via blocking the FRET process. To further improve tissue depth penetration of probes, Okamoto et al. reported a two-photon, fluorescent Zn^{2+} probe that enables the monitoring of presynaptic Zn^{2+} dynamics at the single-synapse level in mossy fiber termini of neurons in adult mice hippocampal tissue [104]. Similarly, a two-photon, fluorescence probe was developed with intramolecular charge transfer, that allows the ratiometric determination of Zn^{2+} [105] (Figure 6b). This probe features high temporal resolution and a low detection limit (~15 nM), and can be used for Zn^{2+} detection in hippocampal tissue with Alzheimer's disease and zebrafish.

2.7 Probes for copper ions (Cu^{2+})

Copper is essential to all living organisms as a trace dietary mineral. In humans, copper is found mainly in the liver, muscle, and bone [106], and it is necessary for the proper growth, development, and maintenance of bone, connective tissue, brain, heart, and many other body organs. It is involved in the formation of red blood cells, iron metabolism, the metabolism of cholesterol and glucose, and the synthesis and release of life-sustaining proteins and enzymes. These enzymes, in turn, produce cellular energy and regulate nerve transmission, blood clotting, and oxygen transport. Copper also stimulates the immune system to fight infections, to repair injured tissues, and to promote healing [107]. Acquired copper deficiency is linked to symptoms including deficiencies in blood cells, bone and connective tissue abnormalities, and neurologic disorders. Many detection methods of copper have provided valuable information for a better understanding of the complex handling of copper in cells.

A novel "off-on" fluorescence probe was designed using two rhodamine B moieties [108]. Upon the addition of Cu^{2+}, the probe showed almost 40-fold enhancement in fluorescence intensity at 580 nm. The probe

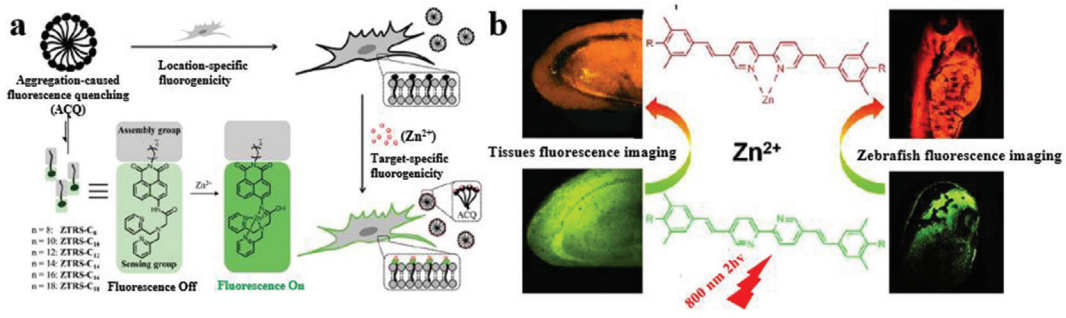


Figure 6: (a) Fluorescence probe for imaging Zn^{2+} at the surface of the plasma membrane. Reprinted with permission from reference [100]. (b) Two-photon responses of Zn^{2+} probe for imaging in brain tissue and zebrafish. Reprinted with permission from reference [105].

exhibited high selectivity and sensitivity (detection limit is 38 nM) for Cu^{2+}. A coumarin-salicylidene Schiff based "on-off" probe was constructed [109]. The probe exhibits fluorescence quenching caused by Cu^{2+} through a chelation-enhanced quenching process. The probe showed a lower fluorescence detection limit of 24 nM, and was successfully used to measure Cu^{2+} in tap water and pharmaceutical samples. In addition, an ultrasensitive naphthalimide-derived probe was designed and used in living cells [110]. The probe showed fluorescence intensity decrease at 541 nm as the concentration of Cu^{2+} increased due to the suppression of ICT process. A new aggregation-induced ratiometric fluorescent probe was prepared for Cu^{2+} detection [111]. Upon Cu^{2+} binding, the fluorescence of the probe showed a color change due to aggregation and ICT process. The probe was successfully used to detect Cu^{2+} in HeLa cells. Most ratiometric probes operate on the FRET mechanism. A FRET-based ratiometric Cu^{2+} probe was synthesized using rhodamine 6G and 2-formylaniline [112]. The binding between the probe and Cu^{2+} induced the opening of the spiro-ring, which enabled the FRET process (Figure 7b), resulting in an apparent yellow fluorescence at 546 nm. In another study, a benzimidazole-quinoline based ratiometric probe for Cu^{2+} was prepared [113]. The probe showed an obvious red shift (132 nm), which can be visualized by the "naked-eye". In recent years, many fluorescence probes with high sensitivity, improved biocompatibility and penetrability, have been developed for Cu^{2+} detection in living systems (Table 1). For example, a NIR ratiometric fluorescent probe exhibited an obvious fluorescent color change from blue to red upon the addition of Cu^{2+}. The fluorescent ratiometric change (F_{red}/F_{blue}) was analyzed to quantify free Cu^{2+} levels in cells [114] (Figure 7a). In addition, the probe showed fast response to Cu^{2+} and resisted pH values change, indicating the great potential for studying Cu^{2+} in cell biology.

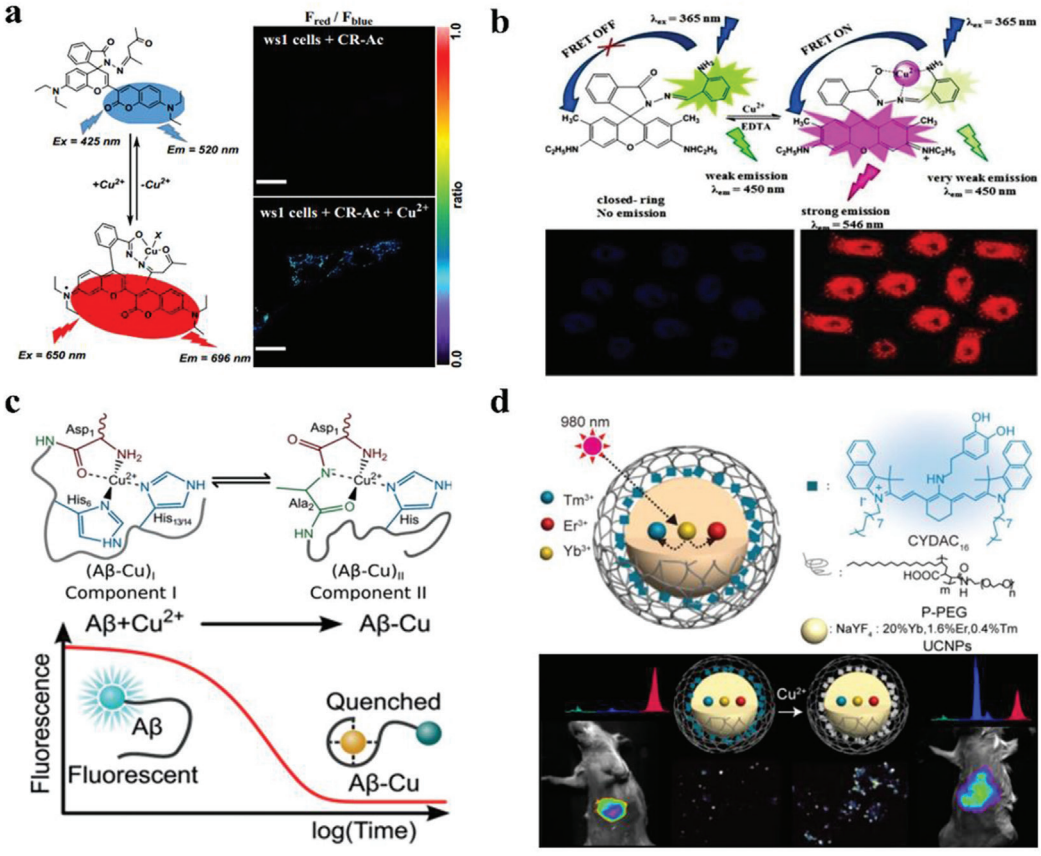
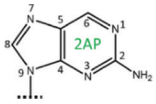
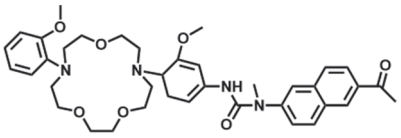
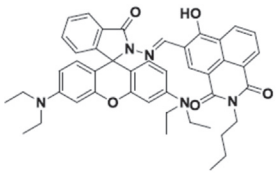
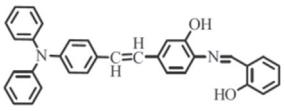
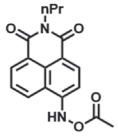
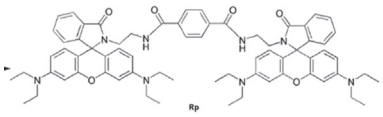
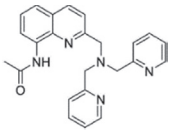
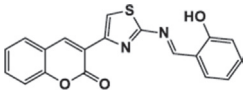


Figure 7: (a) NIR ratiometric fluorescent probe for monitoring the dynamic changes of subcellular Cu^{2+}. Reprinted with permission from reference [114]. (b) Cu^{2+} detection based on FRET mechanism. Reprinted with permission from reference [112]. (c) Cu^{2+} coordination to Aβ at physiological pH. Reprinted with permission from reference [115]. (d) Dye-upconversion nanocomposite for ratiometric fluorescence Bioimaging of Cu^{2+} ions *in vivo*. Reprinted with permission from reference [116].

Table 1: Typical fluorescence probes for metal ions

Mental icons	Probes	Detection limit	Fluorescence peak	Reference
Na^+		0.4 mM	370 nm	[34]
K^+		160 mM	860 nm	[42]
Ca^{2+}		2.7×10^{-7} M	537 nm	[50]
Mg^{2+}		1.47×10^{-6} M	590 nm	[68]
Fe^{2+}		0.5 μM	544 nm	[80]
Fe^{3+}		0.0231 μM	550 nm	[85]
Zn^{2+}		2.15 nM	510 nm	[97]
Cu^{2+}		24 nM	544 nm	[109]

Cu^{2+} was considered as one of the metal ions that promotes Amyloid-β (Aβ) aggregation, which was proposed as a highly relevant factor in Alzheimer's disease. A fluorescent probe consisting of clioquino and Aβ peptides was used to investigate the kinetics of the interaction between Aβ and Cu^{2+} in a physiological environment, as Cu^{2+} can cause fluorescent quenching of the probe [115] (Figure 7c). Another NIR probe with cyanine conjugated Lanthanide-doped upconversion nanoparticles (UCNPs) was prepared to detect Cu^{2+} in living cells and animal tissues [116] (Figure 7d). It was demonstrated that the UCNPs conjugate decreased detection limit of cyanine probe from 78 to 37 nM. In addition, the probe provided real-time detection that is suitable for biological application.

3 Perspective

We briefly reviewed the recent advances in the detection and imaging of metal ions in biological systems using organic dye-based fluorescent sensor. With various modifications, these organic dyes can be used to specifically detect different metal ions in various organisms and cells and provide useful information on the concentration, distribution and even dynamics of metal ions in cells. This information helps us gain insight into biological pathways and metabolic processes, and benefits diseases diagnose. Despite all the progress that has been made in the development of organic dyed based fluorescent probes, several issues

still remain in this field, which has been summarized as follows:

(1) In addition to their role of static cofactors buried within protein active sites, most transition metal ions are also present in loosely bound, labile pools that can participate in critical biological processes such as signaling pathways. Metal ion homeostasis is tightly regulated by cells and tissues. Disrupting the homeostasis can cause disorders and vice versa. Therefore, developing metal ions sensors that could provide real-time monitoring and reveal the dynamics of metal ions will be desirable.
(2) Fluorescent probes have been widely used for metal ions detection *in vitro*. However, the *in vivo* applications of fluorescence probes were hampered by the limitation of the penetration depth of the excitation light. Therefore, development of probe with red-shifted absorption, especially in NIR-II region, will be the future direction for fluorescent probe design.
(3) Despite their advantages, most organic dyes suffer from photobleaching. In addition, the lesion site might have low pH values, high reactive oxygen species or enzyme concentrations. All of these factors will affect the stability of organic dyes. Therefore, improving the stability of organic dyes is another problem to be solved.

Acknowledgements: This work was financially supported by Beijing Natural Science Foundation (2202043), the National Natural Science Foundation of China (51903014, 21908005 and 21774007), China Postdoctoral Science Foundation (2020T130044), and the Fundamental Research Funds for the Central Universities (ZY2024 and PT1811).

References

[1] Carter KP, Young AM, Palmer AE. Fluorescent Sensors for Measuring Metal Ions in Living Systems. Chem Rev. 2014;114:4564–601.
[2] Weaver CM. Calcium. In Present Knowledge in Nutrition; 10th Ed. Erdman Jr. JW, Macdonald IA, Zeisel SH, Ed. Wiley-Blackwell, 2012.
[3] Rude RK. Magnesium. In Encyclopedia of Dietary Supplements; 2nd Ed. Coates PM, Betz JM, Blackman MR, Cragg GM, Levine M, Moss J, et al., Ed. CRC Press: New York, 2010.
[4] Aggett PJ. Iron. In Present Knowledge in Nutrition; 10th Ed. Erdman Jr. JW, Macdonald IA, Zeisel SH, Ed. Wiley-Blackwell, 2012.
[5] Nielsen F. Ultratrace minerals. In Modern Nutrition in Health and Disease; 11th Ed. Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, Ed. Williams and Wilkins: Baltimore, 1999.
[6] Prasad AS. Zinc Deficiency: Has Been Known of for 40 Years but Ignored by Global Health Organisations. BMJ. 2003;326(7386):409–10.
[7] Geiger H, Wanner C. Magnesium in Disease. Clin Kidney J. 2012;5:25-38.
[8] Stern BR. Essentiality and Toxicity in Copper Health Risk Assessment: Overview, Update and Regulatory Considerations. J Toxicol Environ Health A. 2010;73(2):114–27.
[9] Basketter DA, Angelini G, Ingber A, Kern PS, Menne T. Nickel, Chromium and Cobalt in Consumer Products: Revisiting Safe Levels in the New Millennium. Contact Dermatitis. 2003;49(1):1–7.
[10] Avila DS, Puntel RL, Aschner M. Manganese in Health and Disease. Met Ions Life Sci. 2013;13:199–227.
[11] Ghaedi M, Ahmadi F, Shokrollahi A. Simultaneous Preconcentration and Determination of Copper, Nickel, Cobalt and Lead Ions Content by Flame Atomic Absorption Spectrometry. J Hazard Mater. 2007;142(1–2):272–8.
[12] Arienzo M, Capasso R. Analysis of Metal Cations and Inorganic Anions in Olive Oil Mill Waste Waters by Atomic Absorption Spectroscopy and ions Chromatography. Detection of Metals Bound Mainly to the Organic Polymeric Fraction. J Agric Food Chem. 2000;48(4):1405–10.
[13] Houk RS, Fassel VA, Flesch GD, Svec HJ. Inductively Coupled Argon Plasma as an Ion Source for Mass Spectrometric Determination of Trace Elements. Anal Chem. 1980;52(14):2283–9.
[14] Liu KL, Xu ZJ, Yin MZ, Yang WT, He BC, Wei W, et al. A multifunctional perylenediimide derivative (DTPDI) can be used as a recyclable specific Hg^{2+} ion sensor and an efficient DNA delivery carrier. J Mater Chem B. 2014;2:2093–6.
[15] Ouyang R, Zhu Z, Tatum CE, Chambers JQ, Xue Z. Simultaneous Stripping Detection of Pb (II), Cd (II) and Zn (II) Using a Bimetallic Hg-Bi/Single-Walled Carbon Nanotubes Composite Electrode. J Electroanal Chem. 2011;656(12):78–84.
[16] Ma LJ, Liu KL, Yin MZ, Chang J, Geng YT, Pan K. Fluorescent nanofibrous membrane (FNFM) for the detection of mercuric ion (II) with high sensitivity and selectivity. Sensor Actuat B Chem. 2017;238:102–7.
[17] Zhang JF, Zhou Y, Yoon J, Kim JS. Recent Progress in Fluorescent and Colorimetric Chemosensors for Detection of Precious Metal Ions (Silver, Gold and Platinum Ions). Chem Soc Rev. 2011;40(7):3416–29.
[18] Hung Y, Hsiung T, Chen Y, Huang Y, Huang C. Colorimetric Detection of Heavy Metal Ions Using Label-Free Gold Nanoparticles and Alkanethiols. J Phys Chem C. 2010;114(39):16329–34.
[19] Lü BZ, Chen YF, Li PY, Wang B, Müllen K, Yin MZ. Stable Radical Anions Generated from a Porous Perylenediimide Metal-Organic Framework for Boosting Near-Infrared Photothermal Conversion. Nat Commun. 2019;10:767.
[20] Xu Z, Han SJ, Lee C, Yoon J, Spring DR. Development of Off-On Fluorescent Probes for Heavy and Transition Metal Ions. Chem Commun. 2010;46(10):1679–81.

[21] Cheng WY, Chen HT, Liu C, Ji CD, Ma GP, Yin MZ. Functional Organic Dyes for Health-Related Applications. View, 2020, published ahead of print; DOI: 10.1002/VIW.20200055.
[22] Cheng WY, Chen HT, Ji CD, Yang R, Yin MZ. Dual-Stimulus-Responsive Fluorescent Supramolecular Prodrug for Antitumor Drug Delivery. Polym Chem. 2019;10:255–8.
[23] Cheng WY, Chen HT, Wan SS, Zhang XZ, Yin MZ. Dual-Stimulus-Responsive Fluorescent Supramolecular Prodrug for Antitumor Drug Delivery. Chem Mater. 2017;29:4218–26.
[24] Liu C, Zhang SB, Li JH, Wei J, Müllen K, Yin MZ. A Water-Soluble, NIR-Absorbing Quaterrylenediimide Chromophore for Photoacoustic Imaging and Efficient Photothermal Cancer Therapy. Angew Chem Int Ed. 2019;58:1638–42.
[25] Ji CD, Cheng WY, Yuan QP, Müllen K, Yin MZ. From Dyestuff Chemistry to Cancer Theranostics: The Rise of Rylenecarboximides. Acc Chem Res. 2019;52(8):2266–77.
[26] Ji CD, Gao Q, Dong XH, Yin WY, Gu ZJ, Gan ZH, et al. A Size-Reducible Nanodrug with an Aggregation-Enhanced Photodynamic Effect for Deep Chemo-Photodynamic therapy. Angew Chem Int Ed. 2018;57:11384–8.
[27] Zhu H, Fan J, Du J, Peng X. Fluorescent Probes for Sensing and Imaging within Specific Cellular Organelles. Acc Chem Res. 2016;49(10):2115–26.
[28] Xu W, Zeng Z, Jiang J, Chang Y, Yuan L. Discerning the Chemistry in Individual Organelles with Small-Molecule Fluorescent Probes. Angew Chem Int Ed. 2016;55(44):13658–99.
[29] Gao P, Pan W, Li N, Tang B. Fluorescent Probes for Organelle-Targeted Bioactive Species Imaging. Chem Sci. 2019;10(24):6035–71.
[30] Zhang JJ, Cheng FF, Li JJ, Zhu JJ, Lu L. Fluorescent Nanoprobes for Sensing and Imaging of Metal Ions: Recent Advances and Future Perspectives. Nano Today. 2016;16:309–29.
[31] Ding YB, Tang YY, Zhu WH, Xie YS. Fluorescent and Colorimetric Ion Probes Based on Conjugated Oligopyrroles. Chem. Soc. Rev. 2015;44:1101–12.
[32] Wu ZK, Fan HH, Satyavolu NSR, Wang WJ, Lake R, Jiang HJ, et al. Imaging Endogenous Metal Ions in Living Cells Using a DNAzyme-Catalytic Hairpin Assembly Probe. Angew Chem Int Ed. 2017;56(30):8721–5.
[33] Zhou WH, Ding JS, Liu JW. A Highly Specific Sodium Aptamer Probed by 2-Aminopurine for Robust Na^+ Sensing. Nucleic Acids Res. 2016;44:10377–85.
[34] Taguchi R, Terai TT, Ueno T, Komatsu T, Hanaoka K, Urano Y. A Protein-Coupled Fluorescent Probe for Organelle-Specific Imaging of Na^+. Sensor Actuat B: Chem. 2018;265: 575–81.
[35] Roder P, Hille C. ANG-2 for Quantitative Na^+ Determination in Living Cells by Time-Resolved Fluorescence Microscopy. Photochem Photobiol Sci. 2014;13:1699–710.
[36] Jodar TO, Gerkau NJ, Aghvami SS, Rose CR, Egger V. Two-Photon Na^+ Imaging Reports Somatically Evoked Action Potentials in Rat Olfactory Bulb Mitral and Granule Cell Neurites. Front Cell Neurosci. 2017;11:50.
[37] Lu D, He L, Wang Y, Xiong M, Hu M, Liang H, et al. Tetraphenylethene Derivative Modified DNA Oligonucleotide for in Situ Potassium Ion Detection and Imaging in Living Cells. Talanta. 2017;167:550–6.
[38] Schwarze T, Riemer J, Holdt HJ. A Ratiometric Fluorescent Probe for K^+ in Water Based on a Phenylaza-18-Crown-6 Lariat Ether. Chem Eur J. 2018;24:10116–21.
[39] Schwarze T, Schneider R, Riemer J, Holdt HJ. A Highly K^+-Selective Fluorescent Probe-Tuning the K^+-Complex Stability and the K^+/Na^+ Selectivity by Varying the Lariat-Alkoxy Unit of a Phenylaza[18]crown-6 Ionophore. Chem Asian J. 2016;11:241–7.
[40] Han ZB, Xiao ZZ, Hao M, Yuan DQ, Liu L, Wei N, et al. Functional Hydrogen-Bonded Supramolecular Framework for K^+ Ion Sensing. Cryst Growth Des. 2015;15:531–3.
[41] Cheng H, Qiu X, Zhao X, Meng W, Huo D, Wei H. Functional Nucleic Acid Probe for Parallel Monitoring K^+ and Protoporphyrin IX in Living Organisms. Anal. Chem. 2016;88:2937–43.
[42] Schwarze T, Riemer J, Eidner S, Holdt HJ. A Highly K^+-Selective Two-Photon Fluorescent Probe. Chem Eur J. 2015;21:11306–10.
[43] Wang X, Jiang X, Zhang Z, Li J, Liang A, Jiang Z. A fluorescence and resonance Rayleigh scattering di-model probe was developed for trace K^+ coupled N-doped carbon dot and aptamer. J Lumin. 2019;214:16559.
[44] Xiong M, Zhu H, Rong Q, Yang C, Qiu L, Zhang XB, et al. A membrane-anchored fluorescent probe for detecting K^+ in the cell microenvironment. Chem Commun. 2016;52:4679–82.
[45] Hirata T, Terai T, Yamamura H, Shimonishi M, Komatsu T, Hanaoka K, et al. Protein-Coupled Fluorescent Probe to Visualize Potassium Ion Transition on Cellular Membranes. Anal Chem. 2016;88:2693–700.
[46] Brini M, Cali T, Ottolini D, Carafoli E. Intracellular Calcium Homeostasis and Signaling. Met Ions Life Sci. 2013;12:19–68.
[47] Brini M, Ottolini D, Cali T, Carafoli E. Calcium in Health and Disease. Met Ions Life Sci. 2013;13:81–137.
[48] Liu R, Cai X, Li Y, Zhu S, Hu J, Zhu H. Highly Efficient and Selective Red-Emitting Ca^{2+} Probe Based on a BODIPY Fluorophore. Tetrahedron. 2017;73:5091–5.
[49] Hua Y, Wei Q, Wu G, Sun ZB, Shang YJ. Fluorescent Determination of Calcium Ion Using a Coumarinyl Pyrazoline Scaffold and Its Application in Living Cells. Anal Lett. 2019;6:1–13.
[50] Hughes W, Rananaware A, La DD, Jones LA, Bhargava S, Bhosale SV. Aza-Crown Ether-core Substituted Naphthalene Diimide Fluorescence "Turn-On" Probe for Selective Detection of Ca^{2+}. Sensors and Actuators, B: Chemical. 2017;244:854–60.
[51] Zhang H, Yin C, Liu T, Zhang Y, Huo F. "Turn-On" Fluorescent Probe Detection of Ca^{2+} Ions and Applications to Bioimaging. Spectrochim Acta A. 2017;180:211–6.
[52] Ogasawara H, Grzybowski M, Hosokawa R, Sato Y, Taki M, Yamaguchi S. A Far-Red Fluorescent Probe Based on a Phospha-Fluorescein Scaffold for Cytosolic Calcium Imaging. Chem Commun. 2018;4:299–302.
[53] Hirabayashi K, Hanaoka K, Egawa T, Kobayashi C, Takahashi S, Komatsu T, et al. Development of Practical Red Fluorescent Probe for Cytoplasmic Calcium Ions with Greatly Improved Cell-Membrane Permeability. Cell Calcium. 2016;60(4):256–65.
[54] Takahashi S, Hanaoka K, Okubo Y, Echizen H, Ikeno T, Komatsu T, et al. Rational Design of a Near-Infrared Fluorescence Probe for Ca^{2+} Based on Phosphorus-substituted Rhodamines Utilizing Photoinduced Electron Transfer. Chem Asian J. 2020;15(4):524–30.
[55] Liu Z, Pei H, Zhang L, Tian Y. Mitochondria-Targeted DNA Nanoprobe for Real-Time Imaging and Simultaneous

Quantification of Ca^{2+} and pH in Neurons. ACS Nano. 2018;12:12357–68.
[56] 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;91:2488–97.
[57] Kim HJ, Lim CS, Lee HW, Um YJ, Kumar H, Han I, et al. A Ratiometric Two-Photon Probe for Ca^{2+} in Live Tissues and its Application to Spinal Cord Injury Model. Biomaterials. 2017;141:251–9.
[58] Rude, RK. Magnesium. In Encyclopedia of Dietary Supplements; 2nd Ed. Coates PM, Betz JM, Blackman MR, Cragg GM, Levine M, Moss J, et al., Ed. CRC Press: New York, 2010.
[59] Rude, RK. Magnesium. In Modern Nutrition in Health and Disease; 11th Ed. Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, Ed. Williams and Wilkins: Baltimore, 1999.
[60] Lin Q, Buccella D. Highly Selective, Red Emitting BODIPY-Based Fluorescent Indicators for Intracellular Mg^{2+} Imaging. J Mater Chem B. 2018;6:7247–56.
[61] Adhikari S, Ghosh A, Guria S, Sarkar S, Sahana A. Naturally Occurring Thymol Based Fluorescent Probes for Detection of Intracellular Free Mg^{2+} Ion. Sensor Actuat B: Chem. 2016;236(29):512–9.
[62] Eom S, Park S, Song JH, Lee WR, Lee HG, Kang DW, et al. Synthesis, Structure, and Photoluminescence Properties of a Metal-Organic Framework with Hexagonal Channels: Selective Turn-On Sensing for Mg^{2+} Ion. Eur. J. Inorg Chem. 2019;2:330–5.
[63] Murata O, Shindo Y, Ikeda Y, Iwasawa N, Citterio D, Oka K, et al. Near-Infrared Fluorescent Probes for Imaging of Intracellular Mg^{2+} and Application to Multi-Color Imaging of $Mg2^{+}$, ATP, and Mitochondrial Membrane Potential. Anal Chem. 2020;92(1):966–74.
[64] Yin HJ, Zhang BC, Yu HZ, Zhu L, Feng Y, Zhu MZ, et al. Two-Photon Fluorescent Probes for Biological Mg^{2+} Detection Basedon 7-Substituted Coumarin. J Org Chem. 2015;80(9):4306–12.
[65] Gruskos JJ, Zhang GQ, Buccella D. Visualizing Compartmentalized Cellular Mg^{2+} on Demand with Small-Molecule Fluorescent Sensors. J Am Chem Soc. 2016;138:14639–49.
[66] Sargenti A, Farruggia G, Zaccheroni N, Marraccini C, Sgarzi M, Cappadone C, et al. Synthesis of a Highly Mg^{2+}-Selective Fluorescent Probe and its Application to Quantifying and Imaging Total Intracellular Magnesium. Nat protoc. 2017;12(3):461–71.
[67] Zhang G, Gruskos JJ, Afzal MS, Buccella D. Visualizing Changes in Mitochondrial Mg^{2+} during Apoptosis with Organelle-Targeted Triazole-Based Ratiometric Fluorescent Densors. Chem Sci. 2015;6:6841–6.
[68] Yu TT, Sun P, Hu YJ, Ji YG, Zhou HP, Zhang BW, et al. A Novel and Simple Fluorescence Probe for Detecting Main Group Magnesium Ion in HeLa Cells and Arabidopsis. Biosen Bioelectron. 2016;86:677–82.
[69] Aggett PJ. Iron, in Present Knowledge in Nutrition, 10th Edition; Wiley-Blackwell, 2012.
[70] Winter WE, Bazydlo LA, Harris NS. The Molecular Biology of Human Iron Metabolism. Lab Med. 2014;45(2):92–102.
[71] Xu JZ, Jia ZH, Knutson MD, Leeuwenburgh C. Impaired Iron Status in Aging Research. Int J Mol Sci. 2012;13(2):2368–86.
[72] Xuan W, Pan R, Wei YY, Cao YT, Li HQ, Liang FS, et al. Reaction-Based "Off-On" Fluorescent Probe Enabling Detection of Endogenous Labile Fe^{2+} and Imaging of Zn^{2+}-induced Fe^{2+} Flux in Living Cells and Elevated Fe^{2+} in Ischemic Stroke. Bioconjug Chem. 2016; 27(2):302–8.
[73] Niwa M, Hirayama T, Oomoto I, Wang DO, Nagasawa H. Fe(II) Ion Release during Endocytotic Uptake of Iron Visualized by a Membrane-Anchoring Fe(II) Fluorescent Probe. ACS Chem Biol. 2018;13:1853–61.
[74] Hirayama T, Kadota S, Niwa M, Nagasawa H. A Mitochondria-Targeted Fluorescent Probe for Selective Detection of Mitochondrial Labile Fe(II). Metallomics. 2018;10(6):794–801.
[75] Liu ZC, Wang SN, Li WY, Tian Y. Bioimaging and Biosensing of Ferrous Ion in Neurons and HepG2 Cells upon Oxidative Stress. Anal Chem. 2018;90(4):2816–25.
[76] Yang XP, Wang YS, Liu R, Zhang YR, Tang J, Yang EB, et al. A novel ICT-Based Two Photon and NIR Fluorescent Probe for Labile Fe^{2+} Detection and Cell Imaging in Living Cells. Sensor Actuat B Chem. 2019;288:217–24.
[77] Nawaz H, Tian WG, Zhang JM, Jia RN, Chen ZY, Zhang J. Cellulose-Based Sensor Containing Phenanthroline for the Highly Selective and Rapid Detection of Fe^{2+} Ions with Naked Eye and Fluorescent Dual Modes. ACS Appl Mater Interfaces. 2018;10(2):2114–21.
[78] Ravichandiran P, Boguszewska-Czubara A, Masłyk M, Bella AP, Subramaniyan SA, Johnson PM, et al. Naphthoquinone-Based Colorimetric and Fluorometric Dual-Channel Chemosensor for the Detection of Fe^{2+} Ion and Its Application in Bio-Imaging of Live Cells and Zebrafish. ACS Sustain Chem Eng. 2019;7(20):17210–9.
[79] Dong BL, Song WH, Lu YR, Tian MG, Kong XQ, Mehmood AH, et al. Live Cell-Specific Fluorescent Probe for the Detection of Labile Fe(II) and the Evaluation of Esterase Activity in Live Animals. Sensor Actuat B Chem. 2020;305:127470.
[80] Zheng JR, Feng SM, Gong SY, Xia QF, Feng GQ. In Vivo Imaging of Fe^{2+} Using an Easily Obtained Probe with a Large Stokes Shift and Bright Strong Lipid Droplet-Targetable Near-Infrared Fluorescence. Sensor Actuat B Chem. 2020;309:127796.
[81] Sharma N, Reja SI, Gupta N, Bhalla V, Kaur D, Arora S, et al. A Highly Selective Fluorescent Probe for Fe^{3+} in Living Cells: a Stress Induced Cell Based Model Study. Org Biomol Chem. 2017;15(4):1006–12.
[82] Vijay N, Wu SP, Velmathi S. Turn on Fluorescent Chemosensor Containing Rhodamine B Fluorophore for Selective Sensing and in Vivo Fluorescent Imaging of Fe^{3+} Ions in HeLa Cell Line and Zebrafish. J Photoch Photo A. 2019;384:112060.
[83] Qian YY, Suo JS, Gao ZG, Liu HB, Hua QH, Lua YT, et al. A Selective and Sensitive Turn-On Chemosensor for Detection of Fe^{3+} in Aqueous Solution and its Cell Imaging in Dorsal Root Ganglia Neurons and MKN-45 Cells. Bioorg Med Chem. 2020;28(4):115309.
[84] Vishaka VH, Saxena M, Geetha BR, Latiyan S, Jain S. Remarkably Selective Biocompatible Turn-On Fluorescent Probe for Detection of Fe^{3+} in Human Blood Aamples and Cells. RSC Adv. 2019;9:27439–48.
[85] Liu PY, Luo AH. Wang YY. Hu JK. Huang Q, Wang HQ. A Bis-(Rhodamine)-Based Off-On Colorimetric and Fluorescent Probe for Fe^{3+} Ion Detection in Serum and Bioimaging. Chem Pap. 2018;72(10):2575–87.
[86] Yao QF, Lü BZ, Ji CD, Cai Y, Yin MZ. Supramolecular Host-Guest System as Ratiometric Fe^{3+} Ion Sensor Based on

Water-Soluble Pillar[5]arene. ACS Appl Mater Interfaces. 2017;9(41):36320–6.
[87] Wang M, Guo L, Cao D. Porous Organic Polymer Nanotubes as Luminescent Probe for Highly Selective and Sensitive Detection of Fe^{3+}. Sci China Chem. 2017;60:1090–7.
[88] Zhang Q, Sun Y, Liu ML, Liu Y. Selective Detection of Fe^{3+} Ions Based on Fluorescence MXene Quantum Dots Via a Mechanism Integrating Electron Transfer and Inner Filter Effect. Nanoscale. 2020;12(3):1826–32.
[89] Cui, Y, Yue D, Huang Y, Zhang J, Wang Z, Yang D, et al. Photo-Induced Electron Transfer in a Metal-Organic Framework: a New Approach towards a Highly Sensitive Luminescent Probe for Fe (III). Chem Commun. 2019;55(75):11231–4.
[90] Chang Q, Du Ka, Chen LQ, Xu NN, Liu CX, Wang FC, et al. A Fluorescent Channel-Type Eu (III)-Organic Framework for Selective Detection of Fe^{3+} ion and Protective Effect against Parkinson Disease by Increasing Mitochondrial Complex Activity. J Mol Struct. 2020;1203:127439.
[91] Maret W. Zinc and human disease. Met Ions Life Sci. 2013;13:389–414.
[92] Vallee BL, Falchuk KH. The Biochemical basis of Zinc Physiology. Physiol Rev. 1993;73(1):79–118.
[93] King JC. Zinc: an Essential but Elusive Nutrient. Am J Clin Nutr. 2011;94(2):679–84.
[94] Ruz M, Carrasco F, Rojas P, Basfifer K, Hernandez MC, Perez A. Nutritional Effects of Zinc on Metabolic Syndrome and Type 2 Diabetes: Mechanisms and Main Findings in Human Studies. Biol Trace Elem Res. 2019;188(1):77–188.
[95] Takeda A, Tamano H. The Impact of Synaptic Zn^{2+} Dynamics on Cognition and Its Decline. Int J Mol Sci. 2017;18(11):2411.
[96] Prasad AS. Zinc deficiency. BMJ 2003;326(7386):409–10.
[97] Chen X, Ma Y, Zhang Y, Chen Q, Wang H,Wang Z. A Selective and Reversible Fluorescent Probe for Zn^{2+} Detection in Living Cells. ChemistrySelect. 2020;5(13):4017–27.
[98] Vidya B, Sivaraman G, Sumesh RV, Chellappa D. Fluorescein-Based ''Turn On'' Fluorescence Detection of Zn^{2+} and Its Applications in Imaging of Zn^{2+} in Apoptotic Cells. ChemistrySelect. 2016;1(13):4024–9.
[99] Zhang W, Jin X, Chen W, Jiang C, Lu H. A Turn-On, Near-Infrared Fluorescent Probe with Rapid Response, Large Stokes Shift for Selective and Sensitive Detection of Zinc (II) and its Application in Living Cells. Anal Methods. 2019;11(18):2396–403.
[100] Deng F, Liu L, Qiao Q, Huang C, Miao L, Xu Z. A General Strategy to Develop Cell Membrane Fluorescent Probes with Location and Target-Specific Fluorogenicities: a case of a Zn^{2+} Probe with Cellular Selectivity. Chem Commun. 2019;55(100):15045–8.
[101] Zhang G, Zhao Y, Peng B, Li Z, Xu C, Liu Y, et al. Fluorogenic Probe Based on Chelation-Hydrolysis-Enhancement Mechanism for Visualizing Zn^{2+} in Parkinson's Disease Models. J Mater Chem B. 2019;7(14):2252–60.
[102] Guo Z, Kim G, Yoon1 J, Shin I. Synthesis of a Highly Zn^{2+}-Selective Cyanine-Based Probe and its Use for Tracing Endogenous Zinc Ions in Cells and Organisms. Nat Protoc. 2014;9(6):1245–54.
[103] Peng J, Xu W, Teoh CL, Han S, Kim B, Samanta A, et al. High-efficiency In Vitro and In Vivo Detection of Zn^{2+} by Dye-assembled Upconversion Nanoparticles. J Am Chem Soc. 2015;137(6):2336–42.
[104] Khana M, Goldsmithb CR, Huang Z, Georgioua J, Luybena TT, Rodera JC, et al. Two-Photon Imaging of Zn^{2+} Dynamics in Mossy Fiber Boutons of Adult Hippocampal Slices. PNAS. 2014;111(18):6786–91.
[105] Li W, Fang B, Jin M, Tian Y. Two-Photon Ratiometric Fluorescence Probe with Enhanced Absorption Cross Section for Imaging and Biosensing of Zinc Ions in Hippocampal Tissue and Zebrafish. Anal Chem. 2017;89(4):2553–60.
[106] Porter RS, Kaplain MD, Homeier BP. Merck Manual Home Health Handbook; Merck & Company, 2009.
[107] Stern BR. Essentiality and Toxicity in Copper Health Risk Assessment: Overview, Update and Regulatory Considerations. J Toxicol Environ Health A. 2010;73(2):114–27.
[108] Meng X, Xu Y, Liu J, Sun L, Shi L. A New Fluorescent Rhodamine B Derivative as "Off-On" Chemosensor for Cu^{2+} with High Selectivity and Sensitivity. Anal Methods. 2016;8(5):1044–51.
[109] Padhan SK, Murmu N, Mahapatra S, Dalai M, Sahu SN. Ultrasensitive Detection of Aqueous Cu^{2+} Ions by a Coumarin-Salicylidene Based AIEgen. Mater Chem Front. 2019;3(11):2437–47.
[110] Zhang J, Fan CC, Zhu M, Jiang DY, Zhang H, Li LY, et al. An Ultra-Sensitive Naphthalimide-Derived Fluorescent Probe for the Detection of Cu^{2+} in Water Samples and Living Cells. ChemistrySelect. 2019;4:11920–5.
[111] Wang Y, Wu H, Wu WN, Yu YP, Zhao XL, Xu ZH, et al. Aggregation-Induced Ratiometric Emission Active Monocarbazone: Ratiometric Fluorescent Probe for Cu^{2+} in Either Solution or Aggregation States. Journal of Luminescence. 2018;204:289–95.
[112] Sikdar A, Roy S, Mahto RB, Mukhopadhyay SS, Haldar K, Panja SS. Ratiometric Fluorescence Sensing of Cu(II): Elucidation of FRET Mechanism and Bio-Imaging Application. ChemistrySelect. 2018;3:13103–9.
[113] Liu L, Dan F, Liu W, Lu X, Han Y, Xiao S, et al. A High-Contrast Colorimetric and Fluorescent Probe for Cu^{2+} Based on Benzimidazole-Quinoline. Sensors and Actuators B: Chemical. 2017;247:445–50.
[114] Aydin Z, Yan B, Wei Y, Guo M. A Tovel Near-Infrared Turn-On and Ratiometric Fluorescent Probe Capable of Copper (II) Ion Determination in Living Cells. Chem Commun. 2020;56(45):6043–6.
[115] Branch T, Girvan P, Barahona M, Ying L. Introduction of a Fluorescent Probe to Amyloid-β to Reveal Kinetic Insights into Its Interactions with Copper (II). Angew Chem Int Ed. 2014;53:1–5.
[116] Shi Y, Liu Q, Yuan W, Xue M, Feng W, Li F. Dye-assembled Upconversion Nanocomposite for Luminescence Ratiometric In-Vivo Bioimaging of Copper ions. ACS Appl Mater Interfaces. 2019;11(1):430–6.