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A REVIEW ON THE FLORESCENT SENSORS AND THEIR BIOLOGICAL USES

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ABSTRACT

All life forms have an absolute requirement for metals, as metals play critical roles in fundamental processes, including osmotic regulation, catalysis, metabolism, biomineralization and signaling. Group I and II metals (alkali and alkaline earth metals such as sodium, potassium, calcium and magnesium) are highly abundant in most biological organisms. Gradients of group I and II metals across membranes represent a classical way to store potential energy and these ions play roles in osmotic regulation, generation of action potentials and signaling. Transition metals that are generally recognized as playing critical roles in biology include iron, zinc, copper, manganese, cobalt, nickel, molybdenum, tungsten, chromium and vanadium.[1] These elements are often referred to as trace elements because they are present at much lower levels than the group I and II metals, although it is important to note that iron and zinc are often found in substantial amounts and hence their characterization as trace elements is sometimes misleading. Transition metal abundance and usage differs notably across different superkingdoms. For example, eukaryotes devote a higher proportion of their proteome to binding zinc than bacteria or archaea, but the reverse is true for iron, manganese and cobalt.[2] A growing number of comparative genomics studies suggest that iron and zinc are widely used in biology, whereas other metals such as copper, molybdenum, tungsten, nickel, and cobalt are used more sporadically across groups of organisms. To add an additional level of complexity, a recent proteomics study suggested the microbial metallome, that is, the full distribution of metals used by an organism, is largely uncharacterized and there may be additional uses of transition metals, such as cadmium, uranium, arsenic and lead not commonly recognized as being beneficial biometals.

KEYWORDS: Transition metal, Biomineralization, Sensors, Florescent.

INTRODUCTION

Elemental mapping of metals involves measurement of the distribution of metals in a biological sample in a spatially resolved manner. One method for accomplishing this is to adapt mass spectrometry techniques to permit spatial resolution of total metal content in fixed biological specimens at the cellular and subcellular levels.[1-5] Some of the more widely used techniques include secondary ion mass spectrometry (SIMS), nano-SIMS[6-8] and laser ablation coupled with ICP-MS (LA-ICP-MS).[9-11] Additional analytical techniques that permit mapping of total metal content with high sensitivity and spatial resolution involve synchrotron or focused ion-beam microprobes.[12-13] Many of these techniques have recently been comprehensively reviewed elsewhere and will not be the focus of this Review.[14-16]

As a complement to the above techniques, it is important to define the chemical form or speciation of metal ions in biological samples and the distribution between free hydrated ions, loosely bound ions and a tightly bound, largely inaccessible, pool. Currently, there is no single technique available that permits measurement of all of these different species within the same specimen. Yet there are some techniques that permit measurement of different subsets of these pools, for example, the use of fluorescent sensors as detailed below. Thus, combinations of complementary methods will be required for a comprehensive view of cellular metal regulation. Another important factor is the measurement of metal ions in live samples. Life is by definition dynamic, and this dynamism is key to understanding the mechanisms between cause and effect for biological processes. Analytical methods that permit examination of accessible metal pools in live samples would enable identification of metal ion fluxes, dynamics and movements in response to environmental perturbations, a critical step in defining how metals are regulated and used in cells. An analogy that has often been used to emphasize the importance of visualization of living specimens is that reconstructing the basic rules and their consequences of a sports game such as football from a series of still images taken at different times from different games would be exceedingly challenging, if not impossible.[17-19] This is because events are not simply a factor of time, but are also a consequence of factors that happened earlier within the same game.

Light microscopy is an indispensible tool for cell and molecular biology and is compatible with visualization of living specimens. The human eye can only resolve objects on the order of 0.1 mm, but cells are orders of magnitude smaller, often ranging from 5 to 30 μm.

Moreover, bacteria (1 μm), viruses (10–100 nm), and subcellular structures such as the nucleus (10 μm), mitochondrion (2–5 μm), or microvilli (1 μm) are smaller still.[20-22] Because a traditional light microscope can resolve objects on the order of 250 nm, it has been an instrumental tool for studying the microscopic world. Recent advances in super-resolution microscopy have extended the resolution limit, permitting visualization and analysis of nanoscale structures.[23-25] The biggest challenge with microscopy is differentiating the interesting (i.e., a specific object, structure, molecule, or metal) from the uninteresting (i.e., the background).

One of the most exciting and powerful possibilities of fluorescence microscopy is that it can provide a window into the intracellular metabolism of metals in live intact systems. Fluorescence microscopy permits visualization of an object of interest in unicellular organisms, individual cells from multicellular organisms, cells encapsulated in 3D matrices, organotypic cultures, ex vivo models, and, with the right instrumentation, whole organisms (bacteria, yeast, plants, flies, worms, fish, and mice).[26-28] The application of fluorescent sensors and fluorescence microscopy, in combination with other analytical techniques for mapping total metal content, offers researchers the opportunity to address fundamental questions about cellular metal homeostasis. Some of these basic unanswered questions include: What is the amount and speciation of metals in cells? Where are metals located? How do metal ion concentrations change in response to cellular events, environmental changes, or onset of disease? Finally, how do cells regulate metal dynamics and how do metal dynamics impact cellular function?.

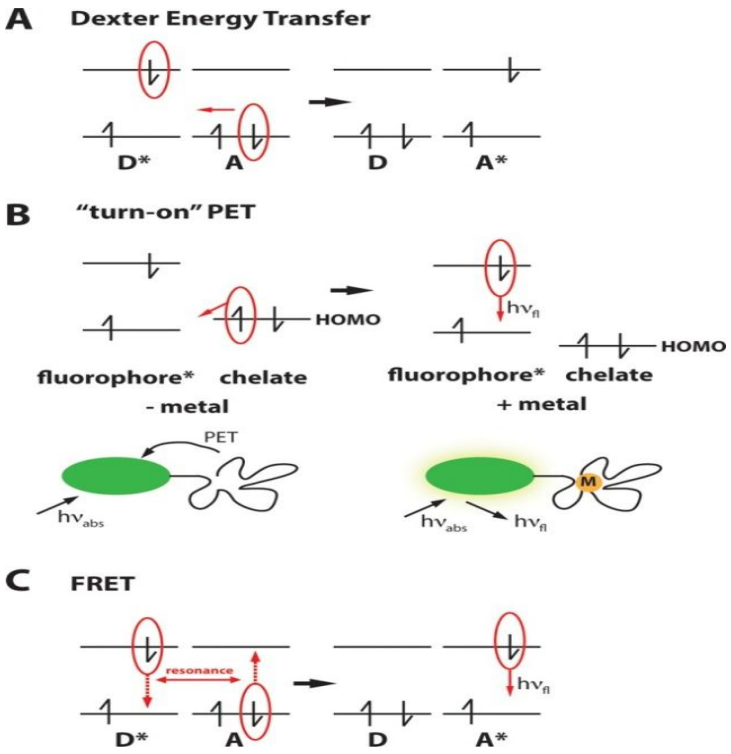
Fluorescence involves the emission of photons that occurs nanoseconds after an absorption event. A fluorescence microscope takes advantage of the shift in wavelength between the absorbed and emitted light by filtering out light due to the excitation source without blocking the emitted light.[30-32] Fluorescent sensors for metals contain two essential features: a metal chelating or binding moiety and at least one fluorophore capable of absorbing and emitting light. To function as a sensor, metal binding must alter either the electronic structure or the molecular structure of the sensor. Changes in the electronic structure can lead to a change in the intensity or wavelength of light absorption or emission, while changes in the molecular structure can alter the distance or orientation between a pair of fluorophores that serve as a donor–acceptor pair. A fluorescence microscope permits visualization of changes in

fluorescence, and hence the target of a particular sensor, which in this case is a specific metal ion of interest, in a spatially resolved manner.

Mechanisms of Altering a Fluorescence Signal

As stated above, metal binding must alter the electronic and/or molecular structure of the sensor to induce changes in fluorescence properties that can be detected by a fluorescence microscope. Two common mechanisms by which a metal can modulate the electronic structure and hence fluorescence are energy transfer or electron transfer between the metal and photo excited fluorophore.[33-35] Both processes can give rise to either a "turn-off" or a "turn-on" fluorescence response, due to fluorescence quenching or enhancement, respectively. A variety of clever approaches have been used to manipulate these properties to design platforms for optical detection of metal ions. There is an extensive body of literature on chemosensors whose optical properties are altered by analyte binding, and that make use of small-molecule fluorophores, polymers, solids and gels, material surfaces (quantum dots, glass or gold surfaces, carbon nanotubes) and mesoporous materials.[36-37] Such probes exploit a variety of different mechanisms for chemical or environmental detection of metal ions. In some cases, such probes have been used for biological detection of transition metals. This Review focuses on fluorescent sensors for metals that have been applied to biology, and so the discussion below focuses on the mechanisms that are prevalent in the subset of probes that have been applied for biological detection of transition metals.

Energy transfer can occur between transition metals with partially filled d-orbitals of appropriate energy and a photoexcited fluorophore by a double electron exchange process (Figure A). This type of energy transfer, first postulated by Dexter, is also referred to as short-range or collision[38-41] it is a form of quenching whereby an excited electron from one molecule (the donor) is transferred to another molecule (the acceptor). Displays a schematic of Dexter energy transfer. The process is active only at very short distances, typically less than 10 Å, because it requires wave function overlap. This electron exchange is one of the primary mechanisms by which the emission of organic fluorophores can be quenched by metal ions[42-46] While this quenching property means that most metal ions are capable of directly modulating fluorescence emission, it also poses a challenge in distinguishing between different metals if multiple metals capable of quenching are present in a complex sample. It also complicates the design of "turn-on" sensors in which a fluorescence signal is increased in response to metal ions.

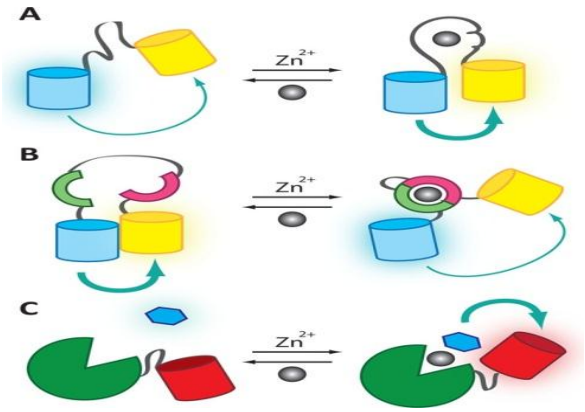


Fluorescence quenching by metal ions does not have to be deleterious and the right sensor design can turn it into a benefit. As one example, Kool and co-workers created polyfluorophore sensors on a DNA backbone that take advantage of quenching properties.[47] The molecular design of these sensors incorporates fluorophores and metal binding ligands into DNA-like oligomers. A variety of fluorescence responses were observed including fluorescence enhancement and red- and blue-shifts. A panel of sensors was then used to differentiate eight metal ions that are typically implicated in fluorescence quenching, including Hg^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Ag^{+}, Cr^{3+} and Fe^{3+}. While this approach was only employed for chemical detection of metals in solution, recent efforts by the same research group have demonstrated that polyfluorophores can be fused to a protein of interest in a mammalian cell using the HaloTag technology, opening the possibility that this sensor platform could be adapted for cellular detection of metal ions.

Reviews for Zinc

Recently, significant work has led to the generation of Zn^{2+} sensors based entirely on protein or peptide motifs. Such constructs can be introduced into cells, tissues, or whole organisms as DNA by transient transfection or viral transduction. The sensors are then transcribed and translated by the machinery of the cell and do not require the addition of any exogenous cofactors for functionality. Currently, all genetically encoded Zn^{2+} sensors operate by Förster

resonance energy transfer (FRET) between donor and acceptor fluorescent proteins (FPs). As a general design, the donor and acceptor FPs are joined by a domain that binds Zn^{2+} and changes conformation in such a way that the FRET efficiency is altered (Figure .9A,B). Thus, changes in Zn^{2+} levels can be monitored by changes in FRET efficiency. Experimentally, researchers excite the donor fluorophore and measure the resulting emission from the acceptor fluorophore and then take the ratio (R) of FRET emission intensity to donor emission intensity. The ratiometric nature of these sensors means they can allow for more accurate quantification of labile Zn^{2+} levels than intensity-based sensors. The overall sensitivity and dynamic range are defined by the ΔR and R_{max}/R_{min} parameters.



The first genetically encoded sensors to monitor Zn^{2+} in cells were developed by the Eide laboratory and consisted of pairs of Zn^{2+} fingers from the yeast transcription factor Zap1 between CFP and YFP.[48] These sensors were expressed in yeast and demonstrated that manipulation of Zn^{2+} levels could induce a change in FRET signal, thus demonstrating the feasibility of such a sensor platform. Merkx and co-workers introduced an alternative design strategy in their CALWY family of sensors. Instead of a Zn^{2+} finger motif that folds into a compact three-dimensional structure in the presence of Zn^{2+}, these sensors rely on Zn^{2+}-induced association of metal-binding domains from the copper ATPase ATP7B (fourth domain referred to as WD4) and the copper chaperone protein Atox1. The name of these sensors derives from the molecular components: *C*FP-*A*tox1-*L*inker-*W*D4-*Y*FP. Through engineering of the metal binding domains and linker region, the Merkx group was able to generate a panel of sensors that was specific for Zn^{2+} and had a wide range of affinities. While these first generation sensors were never tested in cells, they showed the functionality of this platform. By enhancing the dynamic range, the Merkx laboratory created the eCALWY family and used these improved sensors to measure cytosolic Zn^{2+} levels in a variety of mammalian cell types.[49] The Palmer lab has continued work on the Zn^{2+} finger-

based sensor platform. Current sensors include the ZifCY and ZapCY family that feature single or double Zn^{2+} fingers derived from the transcription factors Zif268 or Zap1, respectively, and a cyan-yellow FRET pair (hence the designation "CY") comprised of a truncated CFP and citrine variant of YFP or circularly permuted Venus FP. By mutating the metal ion-coordinating residues, the lab has generated sensors with affinities for Zn^{2+} that range from a K_d of 2.5 pM to hundreds of micromolarand used the sensors to measure Zn^{2+} in a variety of cell types. Both the Palmer and the Merkx laboratories have enhanced the dynamic range and other properties of their sensors by optimizing the linker between the FPs and Zn^{2+} binding domains, manipulating the dimerization tendency of the FPs, and exploring alternate FP FRET pairs.

Reviews for Copper

Copper is a trace metal nutrient essential for most forms of life and is the third most abundant transition metal in humans. Copper serves as a structural and catalytic cofactor for many proteins and enzymes including important metabolic factors such as cytochrome *c* oxidase and copper–zinc superoxide dismutase. Copper occurs in two oxidation states within biological systems, either oxidized (Cu^{2+}) or reduced (Cu^{+}). Cu^{+} is thought to be the dominant oxidation state of labile copper in cells, where this speciation is largely ascribed to the function of membrane reductases that reduce extracellular Cu^{2+} prior to import as well as the reducing environment maintained within the cytosol. The redox activity of copper is critical for several key physiological processes; however, unregulated levels of copper can induce oxidative stress and toxicity in cells. Like zinc, dysregulation of copper homeostasis is associated with disease, including the following neurodegenerative disorders: Alzheimer's disease, amyotrophic lateral sclerosis, Menkes disease, Parkinson's disease, and Wilson's disease. Cells must maintain optimal concentrations and speciation of copper by tightly regulating the uptake, distribution, storage, mobility, and efflux of this ion. Much of the total cellular copper is associated with high affinity binding proteins, and what is considered labile copper is effectively buffered by a plethora of cellular ligands that minimize free copper ions.

Live-cell fluorescence microscopy using copper selective sensors provides a valuable method to better understand the complex handling of copper in cells. However, there are added challenges posed by targeting copper ions over Zn^{2+} due to the need for selectivity between different oxidation states, the fluorescence quenching activity of Cu^{2+} and the fact that sensors must have high enough affinities to compete for copper within its biological window

(10^{-21}–10^{-17} M). As a result, only a handful of copper sensors have been generated for biologically accessible copper. Most of the probes designed for biological systems target Cu^{+}. It is noteworthy that a substantial body of work has been devoted toward production of small molecule, nucleic acid, and protein-based fluorescent sensors for both mono- and divalent copper; however, this Review will focus only on the sensors applied to imaging Cu^{+} in biological systems.

CONCLUSION

We have witnessed an explosion in the number of probes available, an expansion in the range of metals that can be detected, increasing sophistication in the types of measurements that can be performed, and remarkably creative design platforms to ensure specificity (reaction-based probes) and overcome traditional challenges associated with detecting paramagnetic ions. While many existing tools could benefit from improved brightness, higher dynamic range, and increased specificity, the current toolbox has nevertheless provided an unprecedented view of accessible metal pools in live cells and organisms. These tools have been used to quantify accessible metal ion pools, map their location, and monitor dynamics and fluxes of metal ions. Such studies have revealed that metal ion pools are more widespread and dynamic than previously imagined, that such pools can be systematically perturbed in disease states, and that metal ions are intimately connected to canonical signaling pathways, suggesting a rich connection between transition metals and cell physiology.

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