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Review

Probing oxidative stress: Small molecule fluorescent sensors of metal ions, reactive oxygen species, and thiols

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ABSTRACT

Oxidative stress is a common feature shared by many diseases, including neurodegenerative diseases. Factors that contribute to cellular oxidative stress include elevated levels of reactive oxygen species, diminished availability of detoxifying thiols, and the misregulation of metal ions (both redox-active iron and copper as well as non-redox active calcium and zinc). Deciphering how each of these components interacts to contribute to oxidative stress presents an interesting challenge. Fluorescent sensors can be powerful tools for detecting specific analytes within a complicated cellular environment. Reviewed here are several classes of small molecule fluorescent sensors designed to detect several molecular participants of oxidative stress. We focus our review on describing the design, function and application of probes to

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Metal ions
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detect metal cations, reactive oxygen species, and intracellular thiol-containing compounds. In addition, we highlight the intricacies and complications that are often faced in sensor design and implementation.

1. Components of oxidative stress and their implication in neurodegenerative disease

Neurodegenerative diseases have multifactorial causes and various pathological characteristics, but a common feature shared by many of these diseases is an elevation of oxidative stress [1–4]. Oxidative stress results from an imbalance in a cell's production and detoxification of reactive oxygen species (ROS), a catch-all term that encompasses a diverse range of species, including superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$), among others [2,5,6]. In addition to antioxidant enzymes like catalase, superoxide dismutase, and glutathione peroxidase, intracellular thiols like glutathione (GSH) help to maintain a healthy redox balance by scavenging superfluous oxidant species. Alterations in GSH concentration and antioxidant enzymes are markers for diseased systems where the cellular equilibrium favors an oxidative environment [7,8]. Under these conditions, levels of ROS are elevated and can produce irreparable cellular damage by degrading lipids, proteins, and nucleic acids [9,10].

The hydroxyl radical is the most reactive and damaging ROS, and its propagation is exacerbated by redox-active metal ions. Iron and copper notably undergo Fenton reactivity wherein reduced Cu^+ or Fe^{2+} reacts with H_2O_2 to produce $OH^{\bullet}$ and OH^- and the corresponding oxidized metal center, either Cu^{2+} or Fe^{3+} [11]. Other variations of the classic Fenton reaction are also possible, although a key point is that redox cycling of the metal is requisite for catalytic production of damaging radicals and exacerbation of oxidative stress [11].

Metal ions that do not redox cycle, however, are also linked to oxidative stress. Zinc deficiency, for example, has been recognized for many years to cause an elevation in ROS [12]. Zinc is therefore sometimes referred to as an antioxidant, although its antioxidant properties cannot be the result of direct electron exchange between redox-inactive Zn^{2+} ions and oxidant species, but are rather mediated via indirect pathways. While zinc deficiency can be toxic, excessive cellular zinc, which may be released in response to oxidative stress, can also be toxic [13]. Similarly to Zn^{2+}, Ca^{2+} does not directly interact with oxidants, yet fluxes in cellular Ca^{2+} concentrations appear to be regulated by oxidative stress via complex pathways in which Ca^{2+} networks and ROS networks may mutually influence each other [14,15]. Organisms therefore maintain highly coordinated and regulated pathways for acquiring, distributing, and recycling both redox-active and redox-inactive metal ions in order to maintain requisite metal levels for function without causing damage. Defects and breakdowns in metal homeostasis and trafficking are increasingly being linked to neurodegenerative diseases [16–26].

How all of these various components, ROS, thiols, and metal ions, interact and contribute to oxidative stress is a current area of intense research. Fluorescence imaging is one tool that enables interrogation of the connections and implications arising from the misregulation of each of these factors, provided that the limitations of the probes are taken into account [27,28]. This review article provides an overview of small molecule fluorescent sensors designed to detect factors associated with oxidative stress and neurodegenerative disease, in particular metal ions, ROS, and intracellular thiols, as well as advanced multifunctional sensors that respond to multiple stimuli.

2. Introduction of small molecule fluorescent sensors

Fluorescence detection is a highly sensitive analytical tool and an obvious choice for visualizing cellular function through the use of fluorescent small molecules engineered to respond specifically to a cellular event or analyte. Upon excitation, a fluorescent molecule or sensor emits a bright signal that is measured against a dark background. This response allows for easy visualization of the molecule within cell culture or tissue samples even at low concentrations. High sensitivity is a key requirement for studying cellular environments where the concentration of a particular analyte may be in the low nanomolar or picomolar range. In addition, most fluorescence imaging techniques display precise spatial and temporal resolution, as demonstrated by the fact that confocal and widefield microscopy can resolve cellular organelles as small as one micrometer and acquire images on the millisecond timescale [29].

Numerous small molecule fluorescent sensors have been designed for a variety of purposes, including labeling of cellular organelles and membranes, indication of pH, assessment of cell viability, and detection of metal cations and small molecules. In some examples, a fluorophore is modified with a recognition component that enables the dye to localize and therefore image a specific area of the cell [30]. In other designs, the sensor's recognition site explicitly reacts with an intracellular small molecule, as with the pH and metal indicators, to elicit a change in its fluorescence emission [30]. It is the latter of these two examples that will be the focus of this review.

3. Requirements for an effective fluorescent sensor

In order to successfully utilize small molecules as fluorescent sensors for cellular processes, several criteria must be considered. In general, they should be water soluble, cell-permeable and non-toxic. In some cases, cell-permeability can be achieved by esterifying a functional group (e.g. carboxylate) to eliminate any charged species. The neutral molecule is more likely to pass through the cellular membrane where internal esterases transform the sensor to its charged form usually trapping it inside the cell. Ideally, the subcellular localization of the probe should be understood.

In addition, the sensor should be specific for the analyte of interest in order to limit off-target effects. In the case of metal ion sensors, selectivity implies that the sensor binds to one metal over a host of others commonly found in a cellular system. To ensure the binding is specific, the ligand denticity and geometry, the hardness or softness of the donor atoms, and the size of the metal ion must be considered. In addition, the chelating ligand should possess a dissociation constant (K_d) that is within the same order of magnitude as the concentration of the metal ion of interest within a particular cellular location. A chelator with a binding affinity that is too strong for a given area may disrupt the metal cation's intracellular equilibrium by removing it from storage proteins or essential enzymes. Likewise, a chelator with a weak binding affinity might not chelate any of the available metal cation. In both scenarios, the sensor would provide a false report on the intracellular metal concentration.

In the case of ROS sensors, specificity implies that the probe gives a unique response to specific species of ROS. This is a difficult challenge that is not often met by most probes. Rather, it is