REACTION-BASED SMALL-MOLECULE FLUORESCENT PROBES

Reported by Adina Badea March 11, 2013

INTRODUCTION

Living organisms contain a variety of molecules and ions that play many important roles within cells and tissues. Their concentrations are tightly regulated by the cell and deregulation often occurs in diseased states. Traditional methods for detecting reactive chemical species, including colorimetric assays, polarographic sensors, and gas chromatography, often result in sample destruction or are limited to extracellular detection.[1] A reaction-based approach to sensing these species would be a powerful tool to investigate their physiology and pathology. Small-molecule fluorescent probes can undergo selective, bioorthogonal transformations in the presence of specific analytes in cells, tissues, and organisms. This review will focus on recent advances in developing fluorescent probes for two types of biologically important reactive small molecules.

PROBES FOR REACTIVE NITROGEN SPECIES

Reactive nitrogen species (RNS), including nitric oxide (NO), nitroxyl (HNO), and peroxynitrite ($ONOO^-$), are involved in cell signaling in a variety of physiological processes.[2a] NO has a role in vasodilatation and functions both as a neurotransmitter and an antimicrobial agent.[2b,c] Many disorders related to NO signaling impairment have been reported, including cancer and neurodegenerative diseases.[2d]

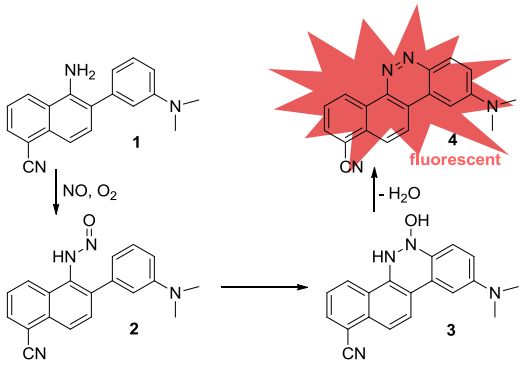

Figure 1. Probe NO_{550} and proposed reaction with NO

Therefore, visualizing NO is crucial in analyzing its signaling mechanisms and may be useful in diagnosis. The first NO fluorescent probes, diaminofluoresceins (DAFs), were developed by Nagano and co-workers,[3] and were widely used for real-time NO imaging.[4] DAFs are nonfluorescent, but react with NO in the presence of oxygen to form highly fluorescent triazoles. The main shortcoming of these probes is the low signal they emit in living cells. Using the same principle of reactivity, Nagano and co-workers later proposed a set of boron dipyrromethane (BODIPY)-based fluorescent NO probes,[5] and a series of near IR cyanine-based probes (DACs) with improved signal intensity in rat kidneys.[6] Shear, Anslyn and co-workers developed probe NO_{550} (**1**, Figure 1), which relies on a reaction cascade involving the electrophilic substitution of an NO-derived *N*-nitrosoaniline intermediate (**2**). This rapid reaction initiated by NO increases the specificity and speed in response to NO. This probe was shown to be suitable for both extracellular and intracellular NO detection in cells.[7]

PROBES FOR REACTIVE SULFUR SPECIES

Hydrogen sulfide (H_2S), originally thought to be a toxin for biological systems, was recently found to be produced in a controlled fashion in a variety of tissues, suggesting an important role in signaling and metabolic processes. Additionally, a variety of diseases have been linked to abnormal levels of H_2S, including Alzheimer's disease and the impaired cognitive ability in cystathionine β-synthase-deficient patients.[1] The ability to selectively visualize H_2S is critical for understanding its complex biological role. In 2011, Chang and co-workers took advantage of the H_2S-mediated reduction of an azide to an amino group and synthesized azide-caged rhodamine analogs as selective, live-cell imaging probes for H_2S.[8] He, Zhao and co-workers developed selective probes based on the reaction of H_2S with a benzaldehyde containing an ortho acrylate group[9] (**5**, Figure 2). A 1,2-carbonyl tandem Michael addition yields a trapped thio hemiacetal (**6**), which then leads to PET-induced fluorescence of the neighboring aromatic system. These probes are highly selective for H_2S over other reactive thiol groups, such as those in cysteine or glutathione, and over reactive oxygen and nitrogen species. The probes are capable of imaging free sulfide in living cells and monitoring enzymatic H_2S biogenesis.

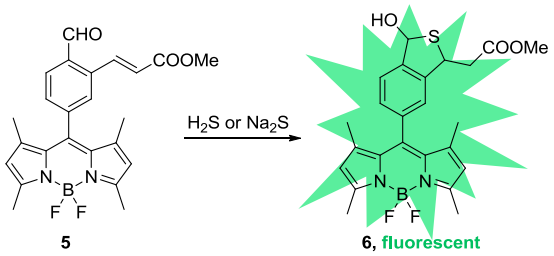

Figure 2. 1,2-Carbonyl tandem Michael addition strategy for visualizing hydrogen sulfide

CONCLUSION

Selective molecular imaging via reaction-based small-molecule fluorescent probes is a rapidly expanding field. Traditional methods for detecting reactive chemical species are often invasive and limited in scope and accuracy. Thus, fluorescent molecular probes are an appealing alternative. This review highlights probes that are highly selective and effective in living cells. Future directions for the field include reversible probes for dynamic imaging, and targeted delivery and retention of probes.

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