Reaction-Based Fluorescent Probes for the Detection and Imaging of Reactive Oxygen, Nitrogen, and Sulfur Species

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CONSPECTUS: This Account describes a range of strategies for the development of fluorescent probes for detecting reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive (redox-active) sulfur species (RSS). Many ROS/RNS have been implicated in pathological processes such as Alzheimer’s disease, cancer, diabetes mellitus, cardiovascular disease, and aging. These species are involved in many biological signaling pathways, overproduction of ROS/RNS (oxidative stress) can result in oxidative damage to a wide range of biomolecules such as nucleic acids, carbohydrates, lipids, and proteins, which can lead to loss of molecular and cellular functions. Importantly, excess production of ROS/RNS has been implicated in pathological processes such as Alzheimer’s disease (AD), cancer, diabetes mellitus, cardiovascular disease, and aging.

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are groups of reactive neutral and anionic small molecules that are produced within many cell types. Common biologically relevant ROS/RNS include singlet oxygen (\(1^\text{O}_2\)), superoxide (\(\text{O}_2^-\)), hydroxyl radical (\(\cdot\text{OH}\)), peroxyl radical (\(\cdot\text{RO}\)), hydrogen peroxide (\(\text{H}_2\text{O}_2\)), hypochlorous acid/hypochlorite (\(\text{HOCl}/\text{ClO}^-\)), nitric oxide (\(\text{NO}\)), nitroxyl (\(\text{HNO}\)), and peroxynitrite (\(\text{ONOO}^-\)). Although ROS/RNS are involved in many biological signaling pathways, overproduction of ROS/RNS (oxidative stress) can result in oxidative damage to a wide range of biomolecules such as nucleic acids, carbohydrates, lipids, and proteins, which can lead to loss of molecular and cellular functions. Importantly, excess production of ROS/RNS has been implicated in pathological processes such as Alzheimer’s disease (AD), cancer, diabetes mellitus, cardiovascular disease, and aging.

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Biological thiols such as glutathione (GSH) and cysteine (Cys) play important roles in maintaining redox homeostasis, serving as antioxidants and acting as free radical scavengers (e.g., GSH acts as a ONOO\(^{-}\) scavenger).\(^3\) As a result, elevated levels of GSH are often produced to protect cells that are exposed to oxidative stress. Therefore, the susceptibility of a cell toward ONOO\(^{-}\) is largely dependent upon the concentration of intracellular GSH present. As a consequence, imbalances in thiol concentration have been implicitly linked to a number of diseases, such as inflammatory diseases, cancer, and neurodegenerative disorders.\(^4\),\(^5\)

The development of fluorescent small-molecule probes to monitor the intracellular concentrations of bioanalytes is a powerful tool to monitor specific cellular processes related to certain diseases.\(^6\)–\(^16\) Because biological pathways often involve multiple reactive chemical species, dual-responsive fluorescent
probes (AND-logic probes) have been developed for two different analyte inputs in order to communicate a detectable signal output. At this point we should raise a note of caution: in the design of fluorescent probes, it is important to consider the reaction mechanisms involved, products and intermediates formed, and their cellular distribution and concentration in addition to environmental factors that may influence the performance and utility of the probes.21,22

1. FLUORESCENT PROBES FOR ROS/RNS

1.1. Fluorescent Probes for H$_2$O$_2$

Hydrogen peroxide can diffuse freely across cell membranes and into and out of cells, where it is known to play critical roles in several physiological processes, such as apoptosis, cell proliferation, cell signaling, and differentiation. Therefore, the accumulation of excess H$_2$O$_2$ has also been implicated in...
numerous pathological conditions, including aging, neurodegenerative diseases, and cancer.24,25

Background knowledge accumulated from over 26 years of research on boronic acid-based molecular fluorescent saccharide sensors in the James group inspired our first fluorescent sensor system for detecting H2O2. This probe design relies on the increased Lewis acidity of boronate esters over their corresponding boronic acids.26,27

As illustrated in Scheme 1, the rates of reaction of boronic acids 1 and 2 with H2O2 in the presence/absence of D-fructose are opposed. Boronic acid 1 displays enhanced reactivity toward H2O2 in the presence of D-fructose. Since the boronate ester formed contains a more electrophilic boron center, it reacts more rapidly with H2O2, resulting in accelerated oxidative cleavage to afford the corresponding naphthol. Alternatively, boronic acid 2 displays reduced reactivity toward H2O2 in the presence of D-fructose because it forms a boronate ester whose reactivity toward H2O2 is reduced by the presence of a water-inserted N–B bond that reduces the electrophilicity of its boron center. The Anslyn group has spent many years unraveling the nature of solvent-inserted N–B bonds and has established that they are best represented as species containing a negatively charged sp3 boron atom (cf. reduced electrophilicity), a positively charged tertiary ammonium group, and a hydrogen bond between the solvent-inserted oxygen and ammonium group (see Scheme 1b).28,29 The sensing performance of boronic acid 2 was particularly interesting since addition of D-fructose results in an increase in the intensity of its fluorescence response in the visible region. The dual fluorescence response of probe 2 suggests that using similar systems with more soluble probes may enable mapping of both intracellular H2O2 AND saccharide concentrations.30

“Turn on” fluorescent sensors are generally more useful for intracellular sensing applications because “turn off” fluorescent sensors can be quenched (nonspecifically) by off-target endogenous species to afford false-positive results. This led us to start developing sensors based on turn-on intramolecular charge transfer (ICT) fluorescence systems, which was achieved by the synthesis of a series of H2O2-responsive boronate ester-based fluorescence probes (Scheme 2). Fluorophores that alter their emission via changes in ICT usually have an electron donor (D) on one end of the fluorophore and an electron acceptor (A) on the other end.31 The probes DSTBPin and MSTBPin exhibited an off–on fluorescence response when exposed to H2O2, with phenol formation resulting in loss of an ICT excited state. Conversely, probe CSTBPin exhibited an on–off reduction in fluorescence when treated with H2O2, caused by the presence of an ICT excited state in its phenol cleavage product. Interestingly, small reductions in fluorescence intensity were observed when the long-wavelength probe NDSTBPin was treated with H2O2. This was attributed to the extended conjugation in that system, which reduced the ICT effect. These changes in fluorescence output demonstrate the importance of electron-withdrawing and electron-donating groups for determining the fluorescent output of these types of probes.32

The development of a sensor for a bioanalyte often requires appropriate selection of both an efficient receptor/reactive group and a matched chromophore, but this approach often requires long synthetic routes, and once the chromophore fragment has been prepared, it is not easy to change its properties. One approach for overcoming the limitations of this type of receptor-based system is to use the indicator displacement assay (IDA) approach championed by Anslyn and co-workers.33 In an IDA system, an indicator is allowed to bind reversibly to a receptor. Then a competitive analyte is
introduced into the system, resulting in displacement of the indicator from the host, which in turn modulates the optical signal. We extended the IDA approach to include reaction-based systems and developed a reaction-based indicator displacement assay (RIA) suitable for use in detecting H$_2$O$_2$. This system relies on complexation of phenylboronic acid (PBA) with the indicator Alizarin Red S (ARS) to afford a fluorescent complex whose aryl unit is oxidatively cleaved upon exposure to H$_2$O$_2$ (turn off) in aqueous media. The reaction of boronic acid receptors with ARS was recently investigated by Ishihara, who reported that all “systems examined are the chelated tetrahedral anions” over a 5–10 pH range. Therefore, Scheme 3 has been updated to reflect the formation of a tetrahedral ARS–PBA complex as the major species present in solution. These RIA systems can be used to detect the presence of H$_2$O$_2$ through monitoring of changes to their colorimetric, fluorometric, and electrochemical outputs. For example, reaction of the ARS–PBA probe with H$_2$O$_2$ resulted in a red-shifted visible color change, an on–off fluorescence response (due to ARS release), and a turn-on electrochemical signal caused by the formation of phenol. Our research has shown that this type of RIA system is effective for monitoring of H$_2$O$_2$ (and H$_2$O$_2$-related species) in environmental and physiological scenarios.

1.2. Fluorescent Probes for HOCl/ClO$^-$$^-$$^-$$^-$$^-D$Fructose

The reaction between chloride anions and H$_2$O$_2$ is catalyzed by myeloperoxidase (MPO) in leukocytes to generate HOCl/ClO$^-$$^-$$^-$$^-$$^-$, which is deployed for its microbiocidal properties. Unfortunately, excess production of HOCl/ClO$^-$$^-$$^-$$^-$$^-$ can also lead to uncontrolled damage to a range of biomolecules, such as amino acids, proteins, carbohydrates, and lipids.

We recently became interested in exploiting the properties of excited-state intramolecular proton transfer (ESIPT) to develop fluorescent sensors. Briefly, ESIPT fluorophores normally exist in an enolic (E) form in the ground state, and photoexcitation results in redistribution of their electronic charge, increasing the acidity of the hydrogen-bond-donor group and enhancing the basicity of the hydrogen-bond-acceptor unit. This results in extremely fast enol-to-keto phototautomerization, with the photoexcited enol form (E*) rapidly converting into the excited keto form (K*), which displays increased fluorescence. Therefore, we decided to develop an ESIPT-based fluorescent probe, TCBT-OMe, containing a HCIO/ClO$^-$$^-$$^-$$^-$$^-$-responsive dimethylthiocarbamate linker for HCIO/ClO$^-$$^-$$^-$$^-$$^-$ detection (Scheme 4) over biologically relevant concentration ranges (limit of detection (LOD) = 0.16 nM). Excellent selectivity was obtained for HCIO/ClO$^-$$^-$$^-$$^-$$^-$ over other ROS/RNS (ONOO$^-$, H$_2$O$_2$, ROO$^-$, OH, O$_2$•$^-$$^-$$^-$, 1O$_2$, NO) and a range of free amino acids (glycine, asparagine, cysteine, homocysteine, glutathione, arginine, histidine, serine, glycine,
and threonine), and TCBT-OMe could be used to monitor endogenous and exogenous HClO/ClO\(^{-}\) in HeLa cells.\(^{37}\)

More recently, a series of turn-on benzathiazole probes C1–C7 containing reactive dimethylthiocarbamate units for the in vivo detection of HClO/ClO\(^{-}\) at biologically relevant concentrations (nanomolar) were developed (Scheme 5).\(^{38}\) These probes displayed a range of blue-to-red “fluorescence rainbow” emissions, thus facilitating a broad choice of potential colors for in vivo colorimetric imaging of HClO/ClO\(^{-}\). The fluorescence of probe C7 can target mitochondria and displays different fluorescence responses to low and high concentrations of HOCl: an obvious turn-on signal in response to nanomolar concentrations of HOCl (biologically relevant concentrations, LOD\(_1 = 18\) nM) and a subsequent ratiometric response to HOCl at the micromolar level (high-risk pathogenic concentrations, LOD\(_2 = 0.47\) \(\mu\)M). Importantly, the ratiometric response of the fluorescent output of these sensors provides enhanced reliability compared with simple fluorescence and avoids any cross-talk caused when several probes are combined for detection.\(^{39}\)

1.3. Fluorescent Probes for O\(_2\)\(^{2-}\)

Often known as the “primary” reactive oxygen species, superoxide is responsible for the production of other ROS/RNS species such as H\(_2\)O\(_2\) and ONOO\(^{-}\). In addition, O\(_2\)\(^{2-}\) is recognized as a potential envoy that regulates the cell-signaling network.\(^{40}\) The production of O\(_2\)\(^{2-}\) occurs primarily in mitochondria, caused by leakage of electrons to O\(_2\) from the electron transport chain, resulting in high reactivity and a short half-life, making O\(_2\)\(^{2-}\) difficult to detect. However, because of its high reactivity, it is associated with a range of pathological conditions such as aging, ischemia–reperfusion injury, and inflammation.

The importance of O\(_2\)\(^{2-}\) in biological systems led us to explore the possibility of developing an ESIPT-based fluorescent probe...
for its rapid detection both in vitro and in vivo. We developed HMBT-LW as a simple ESIPT-based fluorescent probe that was shown to exhibit good sensitivity and selectivity for the rapid detection of low concentrations of $O_2^{••}$ (LOD = 7.4 μM) (Scheme 6).41

1.4. Fluorescent Probes for ONOO−

Peroxynitrite, a reactive nitrogen species, is produced via the rapid reaction of $O_2^{••}$ with NO and is known for its destructive properties in cellular systems, where it readily causes irreversible damage to lipids, proteins, and DNA. As a result, uncontrolled generation of ONOO− has been implicated as a key pathogenic factor in numerous diseases, including ischemia–reperfusion injury, neurodegenerative diseases, inflammation, and cancer. Consequently, the development of a fluorescent probe for the selective cellular detection of ONOO− in the presence of other ROS/RNS may potentially facilitate advances in diagnostics resulting in improved treatments for numerous medical conditions.

Expanding on our original approach previously described in Scheme 1, we employed a similar approach for the intracellular detection of ONOO− using boronic acid42,43 as a sensing motif in the presence of monosaccharides (e.g., D-fructose). Once again, the presence of ONOO− results in oxidative cleavage of the boronic ester fragment of the probe 3–D-fructose complex, which triggers a significant on–off fluorescence response (Scheme 7). The probe 3–D-fructose complex demonstrated excellent selectivity over most other ROS and RNS (H2O2, NO3−, NO2−, ROO−, $O_2^{••}$, HO•, and NO), enabling it to be used to successfully visualize exogenous (HeLa) and endogenous (RAW 264.7) ONOO− in living cells, thus providing the opportunity to investigate diseases such as inflammation that are known to generate increased levels of ONOO−.44

ARIA probe containing a boronic acid (NBA) as the receptor and ARS as the reporter fluorophore was also developed for the fluorescent detection of ONOO− (Scheme 8).45 The parent ARS–NBA system displayed a negligible response toward H2O2 and other ROS/RNS as a result of protection of the boron center by the N–B solvent–insertion interaction.28 However, a significant UV–vis absorption and fluorescence response was observed in the presence of more reactive ONOO−, illustrating that an RIA system could be developed and employed for the in vitro and in vivo sensing of ROS and RNS.45

Our initial sensors were designed to incorporate a N–B solvent-insertion interaction to reduce the reactivity of the boronic acid unit in order to enable selectivity for more reactive ONOO− over less nucleophilic ROS. However, during our investigations we discovered that the reactivity of ONOO− compared with other ROS (i.e., H2O2) was sufficiently greater that this additional protection element was not necessary. Therefore, while the N–B solvent insertion does enhance the selectivity of the probes for ONOO− over other ROS, we have found that simple boronate esters react significantly faster with low concentrations of ONOO− to make this additional protecting group strategy unnecessary. Therefore, we set out to develop an ESIPT-based fluorescent sensor for detecting ONOO− where rapid ONOO−-facilitated oxidation of a boronate ester “protecting” unit of probe 4 is used to trigger the ESIPT turn-on process (Figure 1). Oxidative cleavage of the boronate ester fragment of probe 4 results in an increase in fluorescence, allowing this probe to be used for the visualization of ONOO− in the RAW 264.7 and HeLa cell lines.46 More recently, we developed ABAH-LW as an ESIPT ratiometric boronate-based probe that was used for the successful visualization of ONOO− in the endoplasmic reticulum of HeLa cells (Scheme 9).47

An important feature of turn-on fluorescent sensors used for detection of bioanalytes in cellular systems is the excitation and emission wavelengths of the fluorescence process. In particular, long-wavelength systems in the near-infrared (NIR) region are desirable because they have lower background fluorescence and enable the use of low-energy light sources that result in less tissue damage during imaging. In addition, long-wavelength fluorophores also allow for deeper penetration and visualization of cellular masses and tissues, making them more suitable for whole-animal imaging experiments. Because of these factors, we developed two long-wavelength boronate fluorescent probes, TCFB1 and TCFB2, for detecting ONOO− in cellular systems (Figure 2). TCFB1 was shown to have a low sensitivity toward ONOO− and exhibited poor solubility in aqueous solution, but TCFB2 could be used to monitor exogenous and endogenous ONOO−, producing a significant fluorescence turn-on response to ONOO− in numerous cell lines (Hep-G2, RAW 264.7, HeLa, and A431).48

The Chang group developed PR1, a seminal long-wavelength probe for detecting H2O2 (Scheme 10).49 We recently improved the synthesis of PR1 and demonstrated that it can be used as a
sensitive and selective sensor for ONOO$^-$ as well as H$_2$O$_2$. As part of our research, we carried out detailed investigations of the ability of PR1 to visualize pathways responsible for the generation of ONOO$^-$, with fluorescence imaging demonstrating the cellular location of PR1 in polarized J774.2 macrophages.$^{50}$

1.5. Fluorescent Probes for NO/HNO

Nitric oxide is a signaling molecule produced from L-arginine by a family of nitric oxide synthases (NOSs). Many physiological processes are mediated by NO, including neurotransmission, immune regulation, smooth muscle relaxation, and blood pressure regulation, and uncontrolled production of NO can lead to nitrosative stress with the formation of ONOO$^-$. The one-electron reduction of NO produces nitroxy radical (HNO), which has a unique chemical and biological profile compared with NO. Since it is a small neutral molecule ($pK_a \approx 11.4$), it can freely cross cell membranes and be involved in redox reactions with a range of biological oxidants and reductants. Since HNO has been shown to have some beneficial physiological properties, it is considered to be a possible pharmacological agent in applications to increase cardiac output, afford protective effects against myocardial ischemia injury, and act as an anticancer agent.

Consequently, we designed a selective and sensitive fluorescent probe with a turn-on fluorescence response for NO and HNO under physiological conditions (Scheme 11). The fluorescence response of this water-soluble complex 1−copper(II) is caused by NO$^+$-mediated nitration of its amino group, which stops photoinduced electron transfer (PeT)$^{31}$ through release of copper(II) from the receptor. This biocompatible probe could be used for imaging of exogenous and endogenous NO and HNO in live cells (HeLa and RAW 264.7, respectively).$^{51}$

2. FLUORESCENT PROBES FOR REACTIVE (REDOX-ACTIVE) SULFUR SPECIES

2.1. Fluorescent Probes for Biological Thiols (GSH, Cys, and HCys)

Glutathione is a natural tripeptide (γ-L-glutamyl-L-cysteinylglycine) that is present as an antioxidant in cells at millimolar concentrations; it exists mainly as the reduced form (GSH) rather than the oxidized disulfide form (GSSG). However, under conditions of oxidative stress, elevated levels of GSH are often seen, and the susceptibility of cells toward damage from ROS/RNS species is strongly correlated to the intracellular GSH level. In this respect, dysregulation of GSH homeostasis has been identified as an indicator of numerous diseases, including the onset of AIDS, cancer, neurodegenerative diseases, and liver damage.

Cysteine is a biological-thiol-containing α-amino acid that plays an important part in several biological processes, including cellular detoxification, protein synthesis, and metabolism.
HeLa cells. TCFCl-GSH could be used to evaluate changing levels of GSH in live cells. For example, the addition of H$_2$O$_2$ or cisplatin, which are known to deplete endogenous GSH, reduced the fluorescence intensity, whereas addition of N-acetylcysteine, a known GSH-generating drug, restored the biological thiol levels and fluorescence intensity.\textsuperscript{53}

Expanding the use of the DCM chromophore, the potential of using a novel NIR theranostic prodrug, DCM-S-CPT, for cancer chemotherapy in living animals was explored (Figure 4).

Connection of the DCM fragment to the camptothecin (CPT) drug fragment through a covalent disulfide linker turns off the fluorescence and cytotoxicity of DCM-S-CPT. However, the presence of excess GSH in cancer cells can result in selective cleavage of the disulfide bond of the linker of DCM-S-CPT, which releases the cytotoxic drug cargo and the fluorophore from PEG−PLA-loaded nanoparticles. This tumor-cell-specific release mechanism enabled NIR fluorescent monitoring to be used to track the ability of this DCM-S-CPT prodrug system to deliver its cytotoxic payload to tumor-bearing nude mice in vivo. PEG−PLA nanoparticles loaded with DCM-S-CPT were found to exhibit enhanced antitumor activity and greater plasma half-life in mice than CPT alone.\textsuperscript{54}

The excellent photophysical properties of boron–dipyrrromethene (BODIPY)\textsuperscript{55} were exploited to develop molecular probe 6 as a red-emitting fluorescence resonance energy transfer (FRET) sensor for the selective detection of Cys and HCys (Figure 5). The BODIPY unit of probe 6 (shown in blue) acts as a FRET donor, while its O-protected 4-hydroxyphenyl-BODIPY fragment (shown in green) acts as a FRET acceptor to modulate its fluorescence response. Treatment of 6 with an exogenous thiol results in cleavage of the DNBS unit from the fluorophore fragment, which then affords a turn-on fluorescence response. Addition of Cys (or HCys) turns on red emission at 590 nm upon excitation at 505 nm, thus producing a pseudo-Stokes shift of 77 nm that is significantly greater than the small Stokes shift.
2.2. Fluorescent Probes for H₂S/Na₂S

Hydrogen sulfide has been reported as an endogenous gaseous transmitter that regulates several physiological and pathological processes, including neurotransmission, vasodilation, inflammation, atherosclerosis, oxidative stress, and inhibition of insulin signaling.

The biological importance of H₂S prompted us to develop the galactosyl(azid0)naphthalimide-based fluorogenic probe DT-Gal, in which the galactosyl unit is used as a targeting group for liver cells and the azido unit acts as a functional group for selective reaction with H₂S (Scheme 13). The potential of DT-Gal to act as a target-specific probe for imaging of H₂S in HepG2 liver cancer cells has been demonstrated, as abnormal H₂S levels are known to be important in the pathogenesis of a number of liver diseases.

1-Oxo-1H-phenalen-2,3-dicarbonitrile (OPD) was also developed as a selective and sensitive fluorescent probe for detection of aqueous Na₂S over other thiols and inorganic sulfur compounds (Scheme 14). The fluorescence increased with the sulfide concentration from 1.0 to 30 μM with an LOD of 52 nM.

3. AND-LOGIC-BASED FLUORESCENT PROBES FOR DETECTING ROS/RNS, RSS, AND OTHER SPECIES

Standard fluorescent probes require a single analyte to produce a fluorescence response. However, biological pathways are complex and dependent on the presence/action of multiple reactive chemical species. As a result, a number of fluorescent probes for dual or multianalyte detection have been developed that can be used as molecular logic gates for medical diagnostic applications. Consequently, we are interested in the development of AND-logic-based fluorescent probes that require the simultaneous or sequential action of two or more bioanalytes to produce a fluorescence response. Importantly, dual-analyte probes have the advantage that they are capable of simultaneously detecting short-lived species that may be produced only transiently in a cell, which is often not possible if two different sensing probes are employed for the independent sensing of each analyte. In addition, we believe that these dual-probe systems are particularly interesting since they provide an unequivocal method to monitor bimolecular events that may be responsible for progression of a specific disease.

Our initial aim was to develop an AND-logic-based fluorescent probe for the simultaneous detection of ONOO⁻ and GSH that would allow monitoring of intracellular GSH.
levels in cells under oxidative stress. The probe GSH-PF3 containing a cleavable DNBS linker, which was prepared from commercially available fluorescein in three steps, produced a minimal fluorescence response when GSH and ONOO$^-$ were added independently. However, when both analytes were present simultaneously, GSH-PF3 produced a significant fluorescence enhancement (40-fold) (Scheme 15). GSH-PF3 exhibited exceptional selectivity for detection of GSH AND ONOO$^-$ analytes in cellular systems, producing a strong fluorescence response only when both GSH AND ONOO$^-$ are present.60

The ESIPT-based AND-logic fluorescent probe GSH-ABAH with good cellular permeability was next prepared for the simultaneous detection of ONOO$^-$ AND biological thiols (Scheme 16). A fluorescence response was not observed when either SIN-1 (ONOO$^-$ donor) or GSH was added to RAW264.7 cells, but simultaneous addition of both analytes resulted in a strong fluorescence response.61

Since Chang’s PRI sensor was a good system for ONOO$^-$, we decided to add additional cleavable linkers to that probe to develop a new series of AND probes for ONOO$^-$ and other selected bioanalytes. Accordingly, a new fluorescent resorufin-based “pinkment probe”62 was used as an easily functionalizable scaffold to produce a range of AND-based fluorescent probes for detecting ONOO$^-$ and a second analyte (Scheme 17). A pinkment-OH core unit was used to prepare pinkment-OTBS
and pinkment-OAc as proof-of-concept systems that were shown to function as dual probes for detecting ONOO⁻ AND F⁻ or H₂O₂ AND esterase activity, respectively.63

More recently, an enzyme-mediated fluorescein-based dual-analyte probe, PF3-Glc, consisting of β-glucosidase (β-glc) and H₂O₂ trigger units was developed as part of an AND-based system. β-glc catalyzes hydrolysis of the glycosidic bond of PF3-Glc to a nonfluorescent boronic ester fragment of PF3 (nonfluorescent) and glucose, which is oxidized by glucose oxidase (GOx) to afford D-glucono-δ-lactone with the concomitant generation of H₂O₂. The H₂O₂ that is produced then facilitates oxidative cleavage of the boronic ester fragment of PF3 to afford fluorescein, whose formation results in an 80-fold increase in the fluorescence intensity of the system (Scheme 18).64

A series of environmentally sensitive 3-hydroxyflavone (3-HF) ESIPT boronate-based fluorescent probes were developed that exhibit a ratiometric response toward ONOO⁻ in a micellar environment (Figure 6a). Environmental sensitivity of a fluorescence probe is often considered to be an unwelcome property for sensing applications, but we have used the sensitivity of 3-HF toward hydrophobic environments to differentiate between micellar and aqueous environments. In addition the progression of AD is known to be associated with the formation of insoluble amyloid-β (Aβ) plaques, so we have used our ESIPT-based 3-HF probes to image different aggregation states of Aβ in the presence of ONOO⁻. For example, the probe 3-HF-OMe was found to produce a ratiometric fluorescence response when bound to Aβ aggregates.
in the presence of ONOO\textsuperscript{−}, thus affording a novel protein-based host–guest system. Fluorescence imaging studies of 3-HF-OMe revealed high N-state fluorescence in mice brain sections (Figure 6b, blue channel) that were shown to contain Aβ aggregates through correlation studies with anti-Aβ42 antibodies (Figure 6b, red channel). Subsequent treatment of these brain sections with ONOO\textsuperscript{−} generated the T\textsuperscript{*} state of 3-HF-OMe (Figure 6c, green channel) with regions of fluorescence once again correlated with anti-Aβ antibody fluorescence studies (Figure 6c, red channel). These highly promising biomacromolecular imaging results demonstrate how other ESIPT-based probes might potentially be used for simultaneous sensing of fibrous proteins/peptides AND environmental ROS/RNS. This system can be considered as a “reactive species” AND “environment” based fluorescent probe.\textsuperscript{65}

■ CONCLUSIONS

This Account has described research carried out in our research groups over the past six years toward the development of fluorescent sensors to probe redox biology and provide a better understanding of the various disease states that generate excess ROS. In section 3 we have described the development of dual-activated sensors, which we believe will prove instrumental in providing an understanding of the complex role that different ROS species play in the complex pathways that underpin disease-specific redox processes.

We anticipate that the use of dual-activated probes to evaluate cellular changes associated with many different disease states will become increasingly important, with probes that are responsive to different environmental conditions present in cells becoming more prevalent. We hope that this Account of the research carried out in our groups has demonstrated that responsive fluorescent probes can serve as effective tools to investigate dynamic redox chemistry in living systems and will provide inspiration to develop fluorescent tools that will facilitate improved disease diagnostics and therapies for the treatment of diseases.

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Figure 6. (a) ESIPT probes 3-HF-X (X = OMe, Me, H) for detecting ONOO\textsuperscript{−}. The normal (N) and phototautomeric (T\textsuperscript{*}) forms are shown. (b, c) Fluorescence imaging of a brain section of a transgenic mouse treated with 3-HF-OMe (20 μM) (b) without and (c) with ONOO\textsuperscript{−} (30 μM). The excitation/emission wavelengths for the blue (N-state), green (T\textsuperscript{*}-state), and red (anti-Aβ antibody) channels are 404/425–475, 404/500–550, and 561/640–730 nm, respectively. The white arrows indicate stained Aβ aggregates. Reprinted from ref 65. Copyright 2018 American Chemical Society.
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Notes

The authors declare no competing financial interest.

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