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Perspective

Exploring the Structural Space of Chemiluminescent 1,2-Dioxetanes

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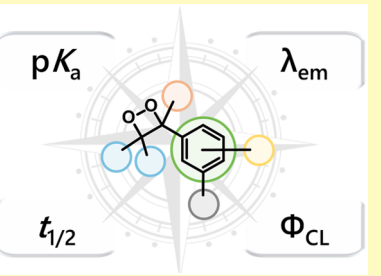
ABSTRACT: Chemiluminescent molecules which emit light in response to a chemical reaction are powerful tools for the detection and measurement of biological analytes and enable the understanding of complex biochemical processes in living systems. Triggerable chemiluminescent 1,2-dioxetanes have been studied and tuned over the past decades to advance quantitative measurement of biological analytes and molecular imaging in live cells and animals. A crucial determinant of success for these 1,2-dioxetane based sensors is their chemical structure, which can be manipulated to achieve desired chemical properties. In this Perspective, we survey the structural space of triggerable 1,2-dioxetane and assess how their design features affect chemiluminescence properties including quantum yield, emission wavelength, and decomposition kinetics. Based on this appraisal, we identify some structural modifications of 1,2-dioxetanes that are ripe for exploration in the context of chemiluminescent biological sensors.

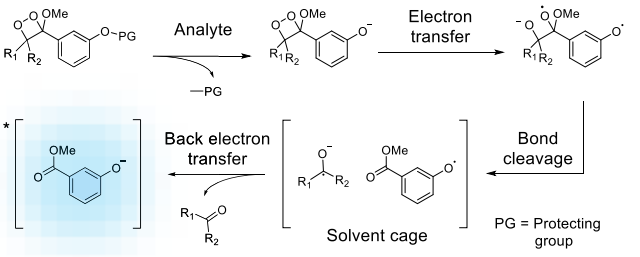


KEYWORDS: *chemiluminescence, 1,2-dioxetanes, chemically initiated electron exchange luminescence, molecular imaging, chemiluminescence quantum yield*

Chemiluminescence is the emission of light from a molecular excited state accessed not through photon absorption, but rather from the energy released in a chemical reaction.[1] Nature has harnessed chemiluminescence using enzyme/substrate pairs in a process referred to as bioluminescence, which can be observed in fireflies, jellyfish, mushrooms, and many other organisms.[2−4] Chemists have sought to understand and harness several classes of nonenzymatic chemiluminescent systems including ozone-based chemiluminescence, luminol, peroxyoxalate, and the decomposition of 1,2-dioxetanes.[1,5−7] These systems are advantageous for bioanalysis and imaging because no light is needed to reach the molecular excited state. This drastically reduces background from autofluorescence and light scattering, eliminates photobleaching, provides high sensitivity, and enables imaging deeper into tissue than typically achieved with fluorescence imaging.[8−10]

1,2-Dioxetanes have been the focus of extensive research for development of triggerable biosensors. The thermal decomposition of 1,2-dioxetanes produces high yields of the triplet excited state, but only low yields of the emissive singlet excited state, making them less suitable for bioanalysis.[1] However, a nonthermal chemically initiated electron exchange luminescence (CIEEL) mechanism, first described by Koo and Schuster, leads to high chemiluminescent quantum yields of the singlet excited state.[11] Because the reaction is initiated by an electron transfer (or possibly charge transfer) from an electron-rich donor to a peroxide acceptor,[12] Schaap and coworkers were able to develop a 1,2-dioxetane linked to a phenolate that could undergo an intramolecular CIEEL mechanism.[13−15] This mechanism proceeds via an electron transfer from the phenolate to the 1,2-dioxetane, followed by bond cleavage, and then another back electron transfer to form the singlet excited state of the phenolate, which emits a photon of light (Scheme 1).[1] Intramolecular and intermolecular back

Scheme 1. Mechanism of CIEEL



electron transfer steps,[16,17] as well as other mechanisms like charge transfer induced luminescence (CTIL), wherein partial charge transfer from the phenolate to the peroxide bond is thought to occur as opposed to the full transfer and generation of radical ion pairs assumed in CIEEL, have been proposed.[18] Importantly, chemiluminescence emission via a CIEEL mechanism can be triggered by masking the phenolate with

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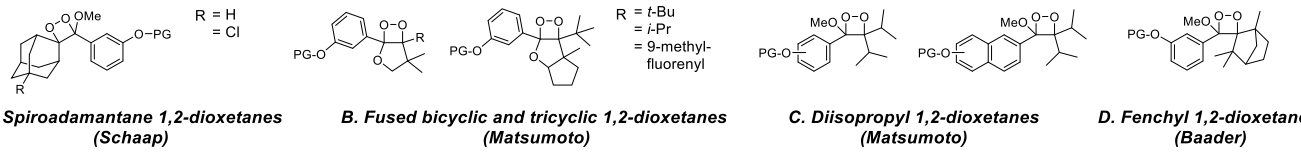

Figure 1. Selected sterically hindering groups for stabilizing 1,2-dioxetanes. (A) Schaap's dioxetanes with spiroadamantane stabilization. (B) Matsumoto's stabilized fused bicyclic and tricyclic systems with bulky R groups. (C) Diisopropyl stabilized dioxetanes developed by Matsumoto. (D) Fenchone stabilized dioxetanes reported by Baader. PG = Protecting group.

a protecting group that is only deprotected in the presence of an analyte. Removal of the protecting group releases the phenolate, which spontaneously initiates CIEEL (Scheme 1).

These types of triggerable dioxetanes were used in commercially available *in vitro* assays for several decades. However, there has recently been a flurry of renewed research activity using chemiluminescent 1,2-dioxetanes for cellular and *in vivo* imaging following demonstrations that these molecules were biocompatible and had bright enough emission for use in whole animal imaging.[19−21] Shabat and co-workers also introduced important structural modifications that increased quantum yield in aqueous systems without the need for polymeric encapsulation,[9,22] leading to improved applicability for bioanalysis and imaging. These innovations have enabled a new field of activity-based chemiluminescence imaging agents for analytes including galactosidase,[22] phosphatase,[22] proteases,[23] tyrosinase,[24] nitroreductase,[21,25,26] carboxyl esterase,[27] H_2S,[20,28] $ONOO^-$,[29−31] H_2O_2,[22,32] HNO,[33] HOCl,[34] formaldehyde,[35] pH,[36,37] a genetically engineered esterase/ester pair,[38] and O_2,[39,40] to name a few.[41−45] Central to advancing the capabilities of these types of 1,2-dioxetane imaging agents is understanding how modifications of the structural design impact the chemiluminescence properties including quantum yields, kinetics, and emission wavelengths. In this Perspective, we will do a selective survey of the landscape of triggerable 1,2-dioxetane structures and consider how their design features affect chemiluminescent properties. We will then try to identify structural design features that are ripe for future exploration.

STRUCTURES THAT STERICALLY STABILIZE THE 1,2-DIOXETANE

The first important design consideration is that the thermal mechanism of chemiluminescence decomposition, which preferentially forms oxygen-quenched triplet states, needs to be attenuated so that the CIEEL mechanisms that produce singlet emission will dominate.[1,11,12] This has generally been accomplished by incorporating structural motifs with high steric strain bonded to the carbon atoms of the 1,2-dioxetane. A prominent example is Schaap's spiroadmantane dioxetane,[13−15] which uses an adamantane group connected to the 1,2-dioxetane in a spirocyclic ring (Figure 1A) and emits light centered at 470 nm in acetonitrile.[14] This scaffold has been widely adopted, and most of the recent work in activity-based probes for cellular and whole animal imaging uses a spiroadamantane stabilizing group.[7,41−45] This is because spiroadamantane 1,2-dioxetanes have very slow thermal decomposition rates,[46] and their synthetic procedures are now well established.[25] A chlorine modification on the adamantane ring leads to dioxetanes with improved properties and has been used in commercially available agents like CDP-Star for biochemical assays.[47] While chlorine modification of the phenol is well understood, it is not completely clear why chlorine modification of the adamantane has such a dramatic effect. One could propose that secondary orbital interactions between the σ^* orbital of the C−Cl bond and the adjacent C−C bonds of the adamantane structure alter its geometry and puts additional strain on the 1,2-dioxetane ring. This chlorine modification has not been fully explored in conjunction with other recent structural modifications, possibly due to the increased expense and lower thermal stability of the chlorospiroadamantane 1,2-dioxetane.

Another structural design strategy to sterically stabilize 1,2-dioxetanes was developed by Matsumoto and co-workers and consists of fusing the dioxetane ring to a five-membered ring in a bicyclic system (Figure 1B).[48−50] Adding a *tert*-butyl group (or isopropyl or methylfluorenyl) to one of the dioxetane carbons and a dimethyl group to the five-membered ring results in a sterically stabilized 1,2-dioxetane with slow thermal decomposition. The bicyclic structure may also have important mechanistic consequences due to limiting diffusion from a solvent cage and increasing the probability of back electron transfer to produce the excited state.[51] A tricyclic derivative has also been communicated to have even higher chemiluminescent quantum yield.[50] Matsumoto's bicyclic dioxetane has been demonstrated to have a triggered emission similar to Schaap's spiroadamantane dioxetane when appended to a *meta*-phenol using fluoride as a trigger to deprotect a silane protecting group. Other stabilizing groups include a diisopropyl group (Figure 1C), which interestingly displays higher quantum yields than the spiroadamantane dioxetanes in some cases,[52] and a dioxetane derived from fenchone, developed by Baader and co-workers (Figure 1D).[53,54] The fenchone derivative can be made from cheap starting materials using an accessible synthetic protocol, and Baader's fenchyl dioxetane displays similar triggerable chemiluminescence as Schaap's spiroadamantane dioxetane and Matsumoto's bicyclic dioxetane.

STRUCTURAL MODIFICATIONS OF THE HETEROATOMS BONDED TO DIOXETANE CARBONS

Another set of design features includes the addition of heteroatoms bonded to the carbons of the dioxetane, which have been used as structural linkers and to tune emission properties upon decomposition. In the commonly used Schaap's spiroadamantane dioxetanes, there is usually a methyl ether with the oxygen bonded to the carbon of the dioxetane.[14] Thioether substitutions have been reported with moderate stability and emission in the yellow to red region of the spectrum (Figure 2A).[55] Interesting trends in heteroatom substitutions at carbons of the dioxetane have been observed in Matsumoto's bicyclic dioxetane scaffold.[56] Substitution of a nitrogen atom for the oxygen atom leads to a series of compounds with emission red-shifted to 571 nm (Figure 2B). A further shift into the NIR region at 688 nm can be achieved when using this nitrogen substitution in combination with replacing the phenol with a naphthol unit. These scaffolds have

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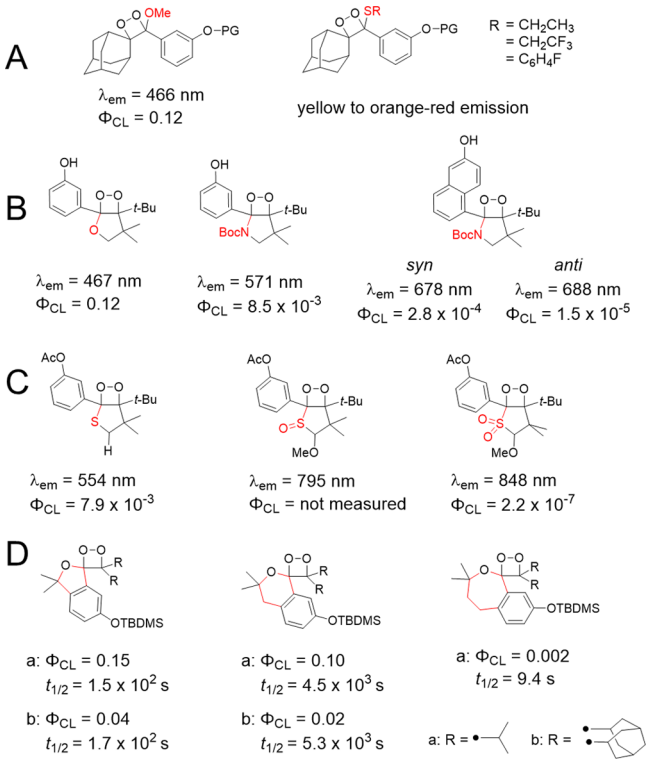

Figure 2. Modifications at carbon of dioxetane. (A) Replacement of methyl ether with thioether on spiroadamantane 1,2-dioxetanes. Incorporation of (B) oxygen, nitrogen, and (C) sulfur five-membered heterocycles in Matsumoto's bicyclic system for modulation of emission wavelength and chemiluminescence quantum yield, and (D) five-to-seven-member ether heterocycles in diisopropyl and spiroadamantane stabilized 1,2-dioxetanes to tune decomposition rate. λ_{em} and Φ_{CL} in organic media.

two rotameric isomers with different emission properties. While red-shifted emission was achieved, chemiluminescent quantum yields were somewhat lower than the oxygen-substituted counterparts: $\Phi_{CL} = 8.5 \times 10^{-3}$, $\lambda_{em} = 571$ nm and $\Phi_{CL} = 2.8 \times 10^{-4}$, $\lambda_{em} = 688$ nm for the nitrogen-substituted dioxetanes versus a $\Phi_{CL} = 0.12$, $\lambda_{em} = 467$ nm for the oxygen substituted dioxetane. Sulfur atom substitution in a similar position of Matsumoto's dioxetane with further substitution on the ring to increase stability has also been investigated (Figure 2C).[57] Sulfanyl, sulfinyl, and sulfonyl groups red-shifted emission to 554, 795, and 848 nm with $\Phi_{CL} = 7.9 \times 10^{-3}$ for the sulfide and a lower Φ_{CL} below 1×10^{-6} for the sulfinyl and sulfonyl derivatives. Another modification with this group was made by linking ether as a ring to the appended phenol (Figure 2D).[58] This had the effect of slowing the rate of decomposition, while keeping a high chemiluminescent quantum yield, at least for five-membered ring and six-membered ring derivatives.

MODIFICATION OF THE *META*-PHENOL AND *ORTHO* GROUPS

Substitution of the oxygen of the *meta*-phenol with other elements has been reported to effect emission and quantum yields. A *meta*-thiol on Schaap's spiroadamantane dioxetane has been shown to be emissive in the context of a probe for cholinesterase[59] and *beta*-lactamase activity,[60] with the intent of improved performance at physiological pH due to the lower pK_a of the thiophenol versus the phenol (Figure 3A).

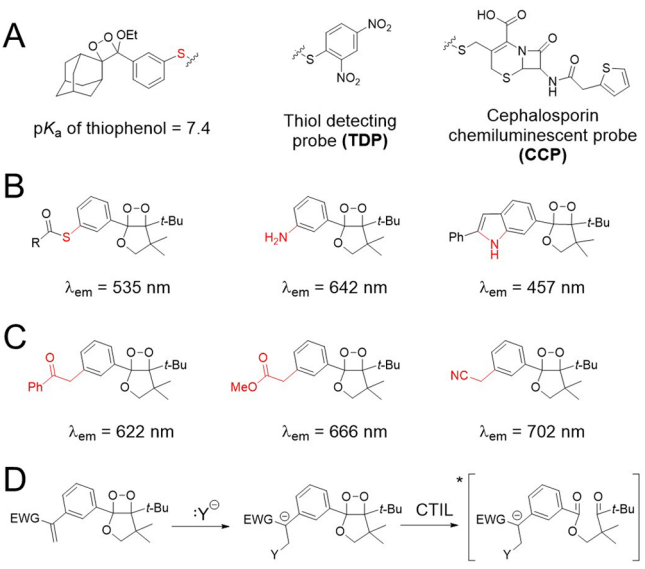

Figure 3. Modifications to *meta*-hydroxy group of the phenol. (A) Lower pK_a of thiophenol enables probes for detection of cholinesterase (**TDP**) and *beta*-lactamase (**CCP**) at neutral pH. (B) Sulfur, amine, and indole modifications in fused bicyclic systems for wavelength modulation. (C) Incorporation of activated methylene groups with low pK_a for triggered chemiluminescence. (D) Chemiluminescence initiated at an activated benzylic carbon via a Michael addition reaction. EWG = electron withdrawing group; CTIL = charge transfer induced luminescence. λ_{em} in organic media.

Modifications with other heteroatoms have also been explored on Matsumoto's bicyclic dioxetane (Figure 3B). A sulfur modification red-shifted the chemiluminescence emission to 535 nm (Figure 3B).[49] An amine modification also further red-shifted the emission to 642 nm, although the quantum yield was reported to be very low and a strong base needed to deprotonate the amine.[49] An indole-based nitrogen group has also been prepared, which emits blue light at 457 nm.[61] An interesting series of carbon substitutions were also explored in this position (Figure 3C).[62−64] Replacing the phenol with an active methylene unit using −CN, $-CO_2Me$, or −COPh groups decreases the pK_a of the methylene and enables fluoride induced chemiluminescence emission at 702, 666, and 622 nm, respectively.[62] Realization that a negatively charged carbon at this position could induce chemiluminescence enabled a clever Michael addition trigger (Figure 3D),[63,64] which one can imagine could be used for the detection of thiols, hydrogen sulfide, and other molecules that participate in 1,4-addition reactions.

Chlorination *ortho* to the phenol has been used as a strategy to enhance chemiluminescence emission,[47] and Lippert and co-workers directly compared structures with hydrogen, fluorination, and chlorination *ortho* to the phenol, showing that the increase in response to the probes correlated with a lowering of the pK_a (Figure 4A).[20] A similar comparison was made for a tumor homing *beta*-galactosidase probe, where again an increase in efficacy was observed in the chlorinated structures.[65] An *ortho* acetamide group was also shown by Higuchi and co-workers to lower the pK_a of the phenol, leading to improved chemiluminescence under aqueous conditions and neutral pH (Figure 4B).[66] Another example of how a structural modification *ortho* to the phenol improved chemiluminescent properties was reported by Pu and co-workers, who explored *ortho* benzoxazole derivatives for

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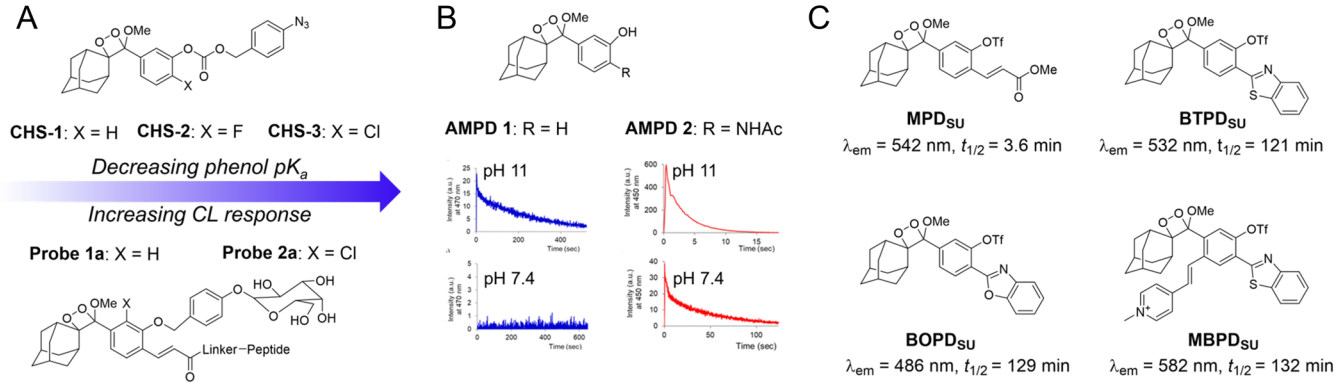

Figure 4. Modifications of the group *ortho* to phenol. (A) Addition of *ortho* fluoro and chloro substituents results in increased chemiluminescent response for H_2S probes **CHS 1−3** and *beta*-galactosidase probe **1a** and **2a**. (B) *Ortho* acetamide group in **AMPD 2** lowers pK_a of phenol for efficient chemiluminescent response at pH 7.4. Adapted with permission from ref 66. Copyright 2019 American Chemical Society. (C) Addition of benzoxazole or benzothiazole *ortho* to phenol enables intramolecular hydrogen bonding and increased chemiluminescence half-life. λ_{em} and decay data in aqueous media.

intramolecular hydrogen bonding with the phenol, slowing the rate of the phenol's deprotonation to the emissive phenolate and effectively increasing the half-life of the chemiluminescent decay (Figure 4C).[67]

ELECTRON WITHDRAWING GROUPS TO INCREASE AQUEOUS QUANTUM YIELDS AND RED-SHIFT EMISSION

A key advance for chemiluminescence imaging with 1,2-dioxetanes in recent years was the discovery by Shabat and co-workers that simple addition of an acrylate or acrylonitrile group to the scaffold could drastically increase chemiluminescence emission in aqueous systems without the need for an additional polymeric enhancer, by virtue creating a push−pull system and extending conjugation.[22] Addition of an acrylonitrile or methyl acrylate via a Heck coupling reaction provides dioxetanes with emission red-shifted to 525−540 nm and chemiluminescent quantum yields in water approaching 0.1, which is more than a 3000-fold increase over the unmodified structure (Figure 5A). The benzoate decomposition products were independently synthesized, and it was shown that a large part of the increase in the chemiluminescent quantum yield was due to increases in the fluorescence quantum yield. Many examples of cellular and *in vivo* imaging have adopted this strategy in recent years.[25,29,33,44,45] An interesting extension of this work showed that a carboxylate linked via an acrylate or through a styryl linker increased the rate of decomposition in a way that correlated to the computationally determined electron spin density on the phenol (Figure 5B).[68] Lippert and co-workers also showed that masking the carboxylate as an acetoxymethyl ester (AM ester) drastically improved the signal for cellular experiments monitoring oxygen-dependent enzyme activity (Figure 5C).[69] This is likely due to a combination of the cellular "trapping" effect of the acetoxymethyl ester,[70] characterized by the scaffold's restricted diffusion out of cells following cleavage of the ester by cellular esterases, and the rapid chemiluminescent decomposition of the product carboxylate.

A related development is the appendage of a dicyanochromone to increase the conjugation in a push−pull system starting with the phenol and ending on the cyano groups of the dicyanochromone (Figure 5D).[71] This scaffold grants near-infrared emission at 660 nm and has been used for H_2O_2,[71]

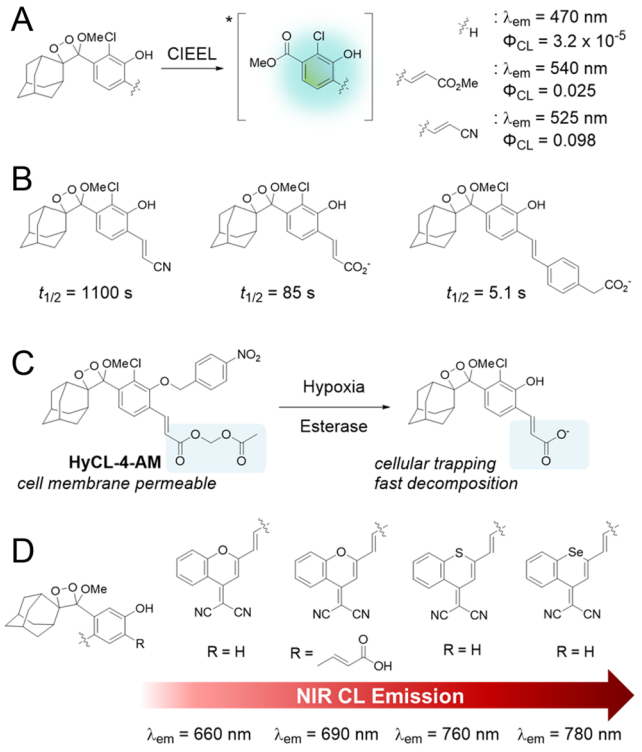

Figure 5. Push−pull systems for enhanced chemiluminescence of spiroadamantane 1,2-dioxetanes. (A) Addition of acrylate and acrylonitrile groups on the luminescent scaffold enhances aqueous quantum yield and red-shifts emission. (B) Conjugation of the luminophore with a carboxylate group results in faster decomposition. (C) Cell trappable hypoxia probe **HyCL-4-AM** with an AM ester for enhanced chemiluminescence, accumulation in cells, and fast decomposition kinetics. (D) Conjugation of dicyanochromone derivatives with the chemiluminophore for NIR chemiluminescence emission. λ_{em}, Φ_{CL}, and $t_{1/2}$ in aqueous media.

singlet oxygen,[72] and formaldehyde probes.[35] Pu and co-workers demonstrated that substitution of sulfur or selenium heteroatoms can further red-shift these emissions to 760 nm for sulfur substitution and 780 nm for selenium substitution.[31] We do note that in our hands, the emission intensity and wavelengths of these systems seems to be dependent on the

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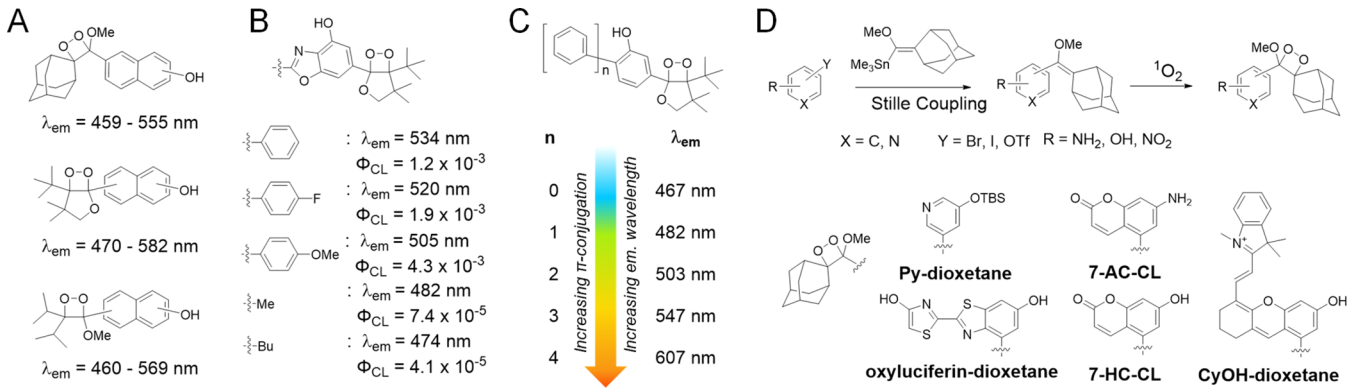

Figure 6. Modifications to luminescent scaffold. Red shift in emission wavelengths achieved by (A) replacing the phenol unit with a naphthol in Schaap's dioxetanes, bicyclic dioxetanes, and diisopropyl dioxetanes (λ_{em} in organic media), (B) replacement of the phenol unit with benzoxazole derivatives (λ_{em} and Φ_{CL} in aqueous media), and (C) increasing π conjugation of the scaffold by appending additional phenyl units (λ_{em} in organic media). (D) Novel synthetic route for preparation of chemiluminophore precursors using Stille cross-coupling between haloaryl substrates and stannane adamantyl enolethers allows generation of synthetically challenging dioxetanes.

addition of fetal bovine serum, perhaps due to protein binding, which could be an interesting avenue for future exploration.

■ ALTERNATE LUMINESCENT SCAFFOLDS

While a phenol *meta* to the dioxetane (including modifications described above) has arguably been the most used scaffold for cellular monitoring and whole animal imaging, there are many elegant examples of using alternatives to this type of phenol. Replacing the phenyl unit with a naphthol has the effect of red-shifting the emission. This has been used with Schaap's spiroadamantane dioxetane,[13,73] diisopropyl dioxetanes,[74] and Matsumoto's bicyclic dioxetane (Figure 6A).[75] The phenol has also been replaced with a benzoxazole, which has the effect of red-shifting emission as far 534 nm in aqueous media and to 543 nm in organic solvent, with a quantum yield of greater than 4×10^{-3} under aqueous conditions (Figure 6B).[76] A recent, systematic approach appended oligophenyl groups to Matsumoto's bicyclic dioxetane, red-shifting emission from 467 to 607 nm, depending on the number of phenyl groups (Figure 6C).[77] Addition of cyclodextrin or surfactants enhanced the aqueous quantum yields of these derivatives.

Direct linkage of traditional fluorophores to the dioxetane is a promising strategy to develop high performing chemiluminophores for biological imaging. Renard, Romieu, and co-workers reported the synthesis and properties of a coumarin fluorophore directly linked to Matsumoto's bicyclic dioxetane, which displayed an emission maximum at 470 nm.[78] Shabat also synthesized the coumarin fluorophore linked this time to Schaap's spiroadamantane dioxetane, which exhibited similar chemiluminescence properties.[79] An exciting development was recently reported in a collaboration between Shabat, Baran, and co-workers to develop a convergent synthesis of luminophore dioxetanes using a Stille coupling to append a dioxetane to a fluorophore at a late stage (Figure 6D).[80] A range of dioxetane luminophores were prepared including coumarin, aminocoumarin, a luciferin analogue, a NIR cyanin, and a pyridine based scaffold, demonstrating how this strategy can be used to tune the kinetics, emission wavelengths, and quantum yields of dioxetane chemiluminophores. Lastly, Lippert and co-workers have reported using a spiropyran photoswitch as the phenol unit of the dioxetane, furnishing **Spiro-CL** as a unique example of a photoswitchable chemiluminescent dioxetane (Figure 7).[81] This compound

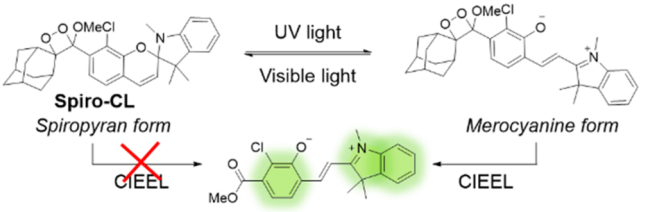

Figure 7. Photoswitchable chemiluminescence of **Spiro-CL**. UV light and visible light irradiation allow toggling between emissive merocyanine form and nonemissive spiropyran form.

can be switched from an ether spiropyran into a phenolate merocyanine form using ultraviolet light. In the merocyanine open form, it steadily decomposes in a CIEEL mechanism, but can be switched back to the spiropyran form with visible light, where it is stable and nonemissive. Photoswitchable chemiluminescent molecules could have interesting applications for advanced types of photoactivatable 3D displays[82,83] and photochemical micropatterning techniques.[84]

■ ENERGY TRANSFER CASSETTES

Another strategy to tune the chemiluminescent properties of dioxetanes is to link the dioxetane to a fluorophore in a way that intramolecular energy transfer mechanisms can occur.[45] Schaap showed in a patent that a fluorophore could be linked via the methoxy group on the dioxetane (Figure 8A)[85] and Matsumoto also designed and synthesized a specialized linker for appending fluorophores at the *tert*-butyl stabilizing group of bicyclic dioxetanes (Figure 8B).[86] Both of these strategies were able to red-shift the emission wavelength and provide higher quantum yields under aqueous conditions. Later, Shabat designed a linker to link fluorophores to the phenol unit of the dioxetane using a benzylic connection (Figure 8C).[87] Lippert and co-workers also developed a strategy to link luminophores to the dioxetane through a diamino or piperazine linker using the carboxylic acid of an acrylate group attached to the phenol (Figure 8D). It was shown that this approach could link responsive dyes like a pH-sensitive carbofluorescein for pH sensing,[37] building on a previous intermolecular energy transfer approach to pH sensing.[36] The strategy was also used for energy transfer to nontraditional luminophores; for example, Beharry and co-workers linked the

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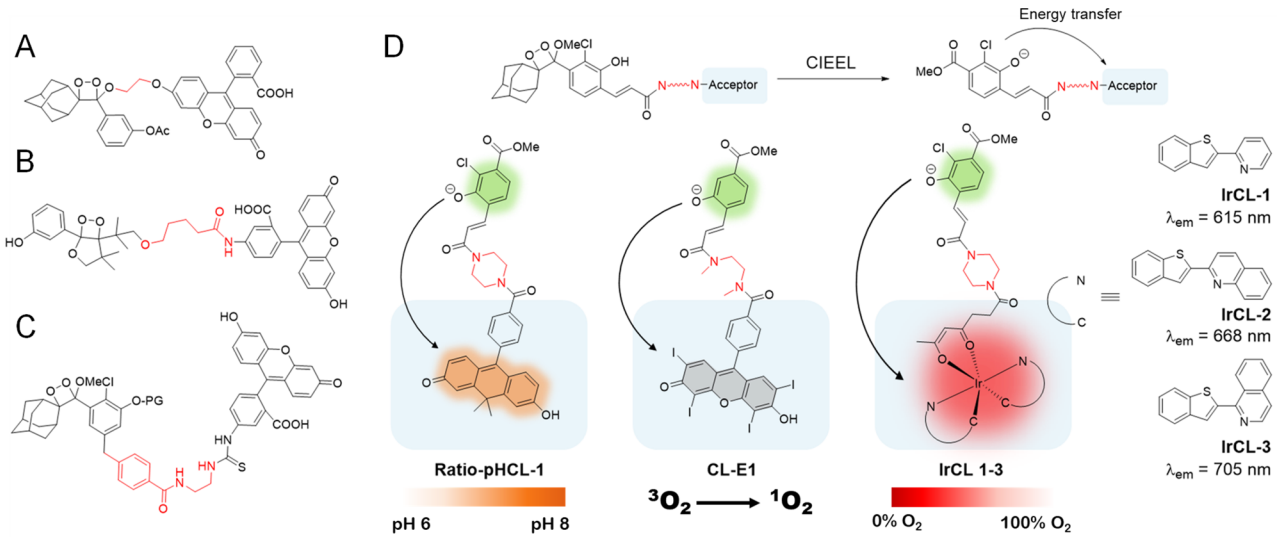

Figure 8. Energy transfer chemiluminescence. Fluorophore appended to the 1,2-dioxetane scaffold (A) *via* an ether linkage on the carbon of the dioxetane, (B) on the *tert*-butyl group on the carbon of a bicyclic dioxetane, and (C) using a linker at the benzylic position. (D) A carbofluorescein fluorophore, an erythrosin B photosensitizer, and an iridium phosphore appended to spiroadamantane 1,2-dioxetanes using piperazine or diamine linkers at the acrylate functionality to furnish a ratiometric pH probe, a "dark" photodynamic therapeutic, and a NIR ratiometric oxygen sensor. λ_{em} in organic media.

dioxetane to a photosensitizer to develop a new class of "dark photodynamic therapy" agents that generated cytotoxic singlet oxygen in the absence of light as a result of intramolecular energy transfer to the erythrosin B sensitizer.[88] Kagalwala and co-workers used a piperazine linker to accomplish chemiluminescence resonance energy transfer to an iridium complex for ratiometric oxygen sensing.[39] Recently, the same team also reported that a Suzuki coupling can be employed to directly link an iridium luminophore to the phenol unit to achieve highly efficient through bond energy transfer.[40] We do note that efficiency of energy transfer between the excited state phenolate and appended fluorophore can be dependent on the precise molecular structure. For example, Pu and co-workers observed almost no energy transfer between the chemiluminescent excited state phenolate and an appended cyanine near-infrared fluorophore, perhaps due to supramolecular interactions with covalently linked cyclodextrin molecules.[89] A systematic study of supramolecular interactions and energy transfer could lead to a deeper understanding of these effects.

PERSPECTIVE

As discussed in this Perspective, a wide area of chemical space has been explored for chemiluminescent 1,2-dioxetanes. Figure 9 summarizes design features we have identified that can be used to tune the chemiluminescent properties of dioxetanes. The emission wavelength can be adjusted by extending the conjugation on the phenol, energy transfer to an appended fluorophore, or substitution of heteroatoms bonded to the dioxetane. The kinetics of the chemiluminescence decay can be tuned by changing the electron density on the phenol or dioxetane or by linking the ether group as a ring to the phenol unit. Adjusting the fluorescence quantum yield of the decomposition product allows tuning of chemiluminescent quantum yields. Further rational tuning of quantum yield remains challenging, and deeper mechanistic insights may help with this.

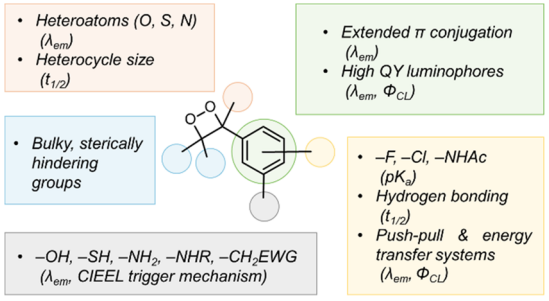

Figure 9. Structural modifications for tuning chemiluminescent properties of 1,2-dioxetanes.

Looking forward, it is interesting to note that most of the recent cellular and *in vivo* imaging studies have focused on using Schaap's spiroadamantane dioxetane, but there may very well be advantages to using Matsumoto's bicyclic dioxetane including potentially more efficient back electron-transfer and the ability to tune the emission properties with heteroatom substitutions. It may prove worthwhile to explore this scaffold in the context of whole animal imaging. Baader's fenchyl dioxetane is also well positioned for further exploration. Substitutions on these scaffolds, for example, the chloroadamantane, may also yield interesting and unexpected results. A phenol in the *meta* position is a requirement for high chemiluminescent quantum yields, but sulfur,[59−61] nitrogen,[49] or even carbon[62−64] in this position have also been shown capable of chemiluminescence emission and present an intriguing route to tune kinetics and chemiluminescence quantum yields, as well as creative new triggering strategies. A prominent advantage of using synthetic chemiluminescent compounds versus their bioluminescent counterparts for imaging and sensing applications is having the ability to specifically modify each structural aspect of the molecule to impact its chemiluminescence properties. This chemical space is vast, and bold explorers will surely be rewarded with marvelous new discoveries in the years to come.

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REFERENCES

(1) Vacher, M.; Fdez; Galván, I.; Ding, B.; Schramm, S.; Berraud-Pache, R.; Naumov, P.; Ferré, N.; Liu, Y.; Navizet, I.; Roca-Sanjuán, D.; Baader, W.; Lindh, R. Chemi- And Bioluminescence of Cyclic Peroxides. *Chem. Rev.* **2018**, *118*, 6927−6974.

(2) Adams, S. T.; Miller, S. C. Enzymatic promiscuity and the evolution of bioluminescence. *FEBS J.* **2020**, *287*, 1369−1380.

(3) Stevani, C. V.; Oliveira, A. G.; Mendes, L. F.; Ventura, F. F.; Waldenmaier, H. E.; Carvalho, R. P.; Pereira, T. A. Current Status of Research on Fungal Bioluminescence: Biochemistry and Prospects for Ecotoxicological Application. *Photochem. Photobiol.* **2013**, *89*, 1318−1326.

(4) Widder, E. A. Bioluminescence in the Ocean: Origins of Biological, Chemical, and Ecological Diversity. *Science* **2010**, *328*, 704−708.

(5) Delafresnaye, L.; Bloesser, F. R.; Kockler, K. B.; Schmitt, C. W.; Irshadeen, I. M.; Barner-Kowollik, C. All Eyes on Visible-Light Peroxyoxalate Chemiluminescence Read-Out Systems. *Chem.—Eur. J.* **2020**, *26*, 114−127.

(6) Ciscato, L. F. M. L.; Augusto, F. A.; Weiss, D.; Bartoloni, F. H.; Albrecht, S.; Brandl, H.; Zimmermann, T.; Baader, W. J. The chemiluminescent peroxyoxalate system: state of the art almost 50 years from its discovery. *Arkivoc* **2012**, *2012*, 391−430.

(7) Li, B.; Kim, Y.; Lippert, A. R. Chemiluminescence Measurement of Reactive Sulfur and Nitrogen Species. *Antioxid. Redox. Signal.* **2022**, *36*, 337−353.

(8) Zhen, X.; Zhang, C.; Xie, C.; Miao, Q.; Lim, K. L.; Pu, K. Intraparticle Energy Level Alignment of Semiconducting Polymer Nanoparticles to Amplify Chemiluminescence for Ultrasensitive *In Vivo* Imaging of Reactive Oxygen Species. *ACS Nano* **2016**, *10*, 6400−6409.

(9) Lippert, A. R. Unlocking the Potential of Chemiluminescence Imaging. *ACS Cent. Sci.* **2017**, *3*, 269−271.

(10) Ryan, L. S.; Lippert, A. R. Ultrasensitive Chemiluminescent Detection of Cathepsin B: Insights into The New Frontier of Chemiluminescent Imaging. *Angew. Chem., Int. Ed.* **2018**, *57*, 622−624.

(11) Koo, J.; Schuster, G. Chemically Initiated Electron Exchange Luminescence. A New Chemiluminescent Reaction Path for Organic Peroxides. *J. Am. Chem. Soc.* **1977**, *99*, 6107−6109.

(12) Ciscato, L. F. M. L.; Bartoloni, F. H.; Weiss, D.; Beckert, R.; Baader, J. Experimental Evidence of the Occurrence of Intramolecular Electron Transfer in Catalyzed 1,2-Dioxetane Decomposition. *J. Org. Chem.* **2010**, *75*, 6574−6580.

(13) Schaap, A. P.; Handley, R. S.; Giri, B. P. Chemical and Enzymatic Triggering of 1,2-Dioxetanes. 1: Aryl Esterase-catalyzed Chemiluminescence from a Naphthyl Acetate-Substituted Dioxetane. *Tetrahedron Lett.* **1987**, *28*, 935−938.

(14) Schaap, A. P.; Chen, T. S.; Handley, R. S.; DeSilva, R.; Giri, B. P. Chemical and Enzymatic Triggering of 1,2-Dioxetanes. 2: Fluoride-induced Chemiluminescence from *tert*-Butyldimethylsilyloxy-substituted Dioxetanes. *Tetrahedron Lett.* **1987**, *28*, 1155−1158.

(15) Schaap, A. P.; Sandison, M. D.; Handley, R. S. Chemical and Enzymatic Triggering of 1,2-Dioxetanes. 3: Alkaline Phosphatase-catalyzed Chemiluminescence from an Aryl Phosphate-substituted Dioxetane. *Tetrahedron Lett.* **1987**, *28*, 1159−1162.

(16) Bastos, E. L.; da Silva, S. M.; Baader, W. J. Solvent Cage Effects: Basis of a General Mechanism for Chemiluminescence. *J. Org. Chem.* **2013**, *78*, 4432−4439.

(17) Khalid, M.; Souza, S. P.; Cabello, M. C.; Bartoloni, F. H.; Ciscato, L. F.; Bastos, E. L.; Seoud, O. A. A.; Baader, W. J. Solvent Polarity Influence on Chemiexcitation Efficiency of Inter and Intramolecular Electron-Transfer Catalyzed Chemiluminescence. *J. Photochem. Photobiol. A Chem.* **2022**, *433*, 114161.

(18) Catalani, L. H.; Wilson, T. Electron transfer and chemiluminescence. Two inefficient systems: 1,4-dimethoxy-9,10-diphenylanthracene peroxide and diphenoyl peroxide. *J. Am. Chem. Soc.* **1989**, *111*, 2633−2639.

(19) Liu, L.; Mason, R. Imaging B-Galactosidase Activity in Human Tumor Xenografts and Transgenic Mice Using a Chemiluminescent Substrate. *PLoS. One.* **2010**, *5*, e12024.

(20) Cao, J.; Lopez, R.; Thacker, J.; Moon, J.; Jiang, C.; Morris, S.; Bauer, J.; Tao, P.; Mason, R.; Lippert, A. R. Chemiluminescent Probes for Imaging H2S In Living Animals. *Chem. Sci.* **2015**, *6*, 1979−1985.

(21) Cao, J.; Campbell, J.; Liu, L.; Mason, R.; Lippert, A. R. In Vivo Chemiluminescent Imaging Agents for Nitroreductase and Tissue Oxygenation. *Anal. Chem.* **2016**, *88*, 4995−5002.

(22) Green, O.; Eilon, T.; Hananya, N.; Gutkin, S.; Bauer, C.; Shabat, D. Opening A Gateway for Chemiluminescence Cell Imaging: Distinctive Methodology for Design of Bright Chemiluminescent Dioxetane Probes. *ACS Cent. Sci.* **2017**, *3*, 349−358.

(23) Roth-Konforti, M.; Bauer, C.; Shabat, D. Unprecedented Sensitivity in a Probe for Monitoring Cathepsin B: Chemiluminescence Microscopy Cell-Imaging of a Natively Expressed Enzyme. *Angew. Chem., Int. Ed.* **2017**, *129*, 15839−15844.

(24) Shelef, O.; Sedgwick, A. C.; Pozzi, S.; Green, O.; Satchi-Fainaro, R.; Shabat, D.; Sessler, K. L. Turn-On Chemiluminescence-Based Probes for Monitoring Tyrosinase Activity in Conjunction with Biological Thiols. *Chem. Commun.* **2021**, *57*, 11386−11389.

(25) Ryan, L. S.; Gerberich, J. L.; Cao, J.; An, W.; Jenkins, B. A.; Mason, R. P.; Lippert, A. R. Kinetics-Based Measurement of Hypoxia in Living Cells and Animals Using an Acetoxymethyl Ester Chemiluminescent Probe. *ACS Sens.* **2019**, *4*, 1391−1398.

(26) Sun, J.; Hu, Z.; Wang, R.; Zhang, S.; Zhang, X. A Highly Sensitive Chemiluminescent Probe for Detecting Nitroreductase and Imaging in Living Animals. *Anal. Chem.* **2019**, *91*, 1384−1390.

(27) Ozsan, C.; Kailass, K.; Digby, E. M.; Almammadov, T.; Beharry, A. A.; Kolemen, S. Selective detection of carboxylesterase 2 activity in cancer cells using an activity-based chemiluminescent probe. *Chem. Commun.* **2022**, *58*, 10929−10932.

(28) Levinn, C. M.; Pluth, M. D. Direct Comparison of Triggering Motifs on Chemiluminescent Probes for Hydrogen Sulfide Detection in Water. *Sens. Actuators B Chem.* **2021**, *329*, 129235.

(29) Cao, J.; An, W.; Reeves, A. G.; Lippert, A. R. A Chemiluminescent Probe for Cellular Peroxynitrite Using a Self-

Immolative Oxidative Decarbonylation Reaction. *Chem. Sci.* **2018**, *9*, 2552−2558.
(30) Huang, J.; Huang, J.; Cheng, P.; Jiang, Y.; Pu, K. Near-Infrared Chemiluminescent Reporters for In Vivo Imaging of Reactive Oxygen and Nitrogen Species in Kidneys. *Adv. Funct. Mater.* **2020**, *30*, 2003628.
(31) Huang, J.; Jiang, Y.; Li, J.; Huang, J.; Pu, K. Molecular Chemiluminescent Probes with a Very Long Near-Infrared Emission Wavelength for in Vivo Imaging. *Angew. Chem., Int. Ed.* **2021**, *133*, 4045−4049.
(32) Ye, S.; Hananya, N.; Green, O.; Chen, H.; Zhao, Q.; Shen, J.; Shabat, D.; Yang, D. A Highly Selective and Sensitive Chemiluminescent Probe for Real-Time Monitoring of Hydrogen Peroxide in Cells and Animals. *Angew. Chem., Int. Ed.* **2020**, *59*, 14326.
(33) An, W.; Ryan, L. S.; Reeves, A. G.; Bruemmer, K. J.; Mouhaffel, M.; Gerberich, J. L.; Winters, A.; Mason, R. P.; Lippert, A. R. A Chemiluminescent Probe for HNO Quantification and Real-time Monitoring in Living Cells. *Angew. Chem., Int. Ed.* **2019**, *58*, 1361−1365.
(34) Ye, S.; Yang, B.; Wu, M.; Chen, Z.; Shen, J.; Shabat, D.; Yang, D. Recurring Real-Time Monitoring of Inflammations in Living Mice with A Chemiluminescent Probe for Hypochlorous Acid. *CCS Chem.* **2022**, *4*, 1871.
(35) Bruemmer, K. V.; Green, O.; Su, T. A.; Shabat, D.; Chang, C. J. Chemiluminescent Probes for Activity-Based Sensing of Formaldehyde Released from Folate Degradation in Living Mice. *Angew. Chem., Int. Ed.* **2018**, *57*, 7508−7512.
(36) An, W.; Mason, R. P.; Lippert, A. R. Energy Transfer Chemiluminescence for Ratiometric pH Imaging. *Org. Biomol. Chem.* **2018**, *16*, 4176−4182.
(37) Ryan, L. S.; Gerberich, J.; Haris, U.; Nguyen, D.; Mason, R. P.; Lippert, A. R. Ratiometric pH Imaging Using a 1,2-Dioxetane Chemiluminescence Resonance Energy Transfer Sensor in Live Animals. *ACS Sens.* **2020**, *5*, 2925−2932.
(38) Jones, K. A.; Kentala, K.; Beck, M. W.; An, W.; Lippert, A. R.; Lewis, J. C.; Dickinson, B. C. Development of a Split Esterase for Protein-Protein Interaction-Dependent Small-Molecule Activation. *ACS Cent. Sci.* **2019**, *5*, 1768−1776.
(39) Kagalwala, H. N.; Gerberich, J.; Smith, C. J.; Mason, R. P.; Lippert, A. R. Chemiluminescent 1,2-Dioxetane Iridium Complexes for Near-Infrared Oxygen Sensing. *Angew. Chem., Int. Ed.* **2022**, *61*, e202115704.
(40) Kagalwala, H. N.; Bueno, L.; Wanniarachchi, H.; Unruh, D. K.; Hamal, K. B.; Pavlich, C. I.; Carlson, G. J.; Pinney, K. G.; Mason, R. P.; Lippert, A. R. Oxygen-Sensing Chemiluminescent Iridium(III) 1,2-Dioxetanes: Unusual Coordination and Activity. *Anal. Sens.* **2022**, e202200085.
(41) Haris, U.; Kagalwala, H. N.; Kim, Y. J.; Lippert, A. R. Seeking Illumination: The Path to Chemiluminescent 1,2-Dioxetanes for Quantitative Measurements and In Vivo Imaging. *Acc. Chem. Res.* **2021**, *54*, 2844−2857.
(42) Wang, Y.; Bian, Y.; Chen, X.; Su, D. Chemiluminescent Probes Based On 1,2-Dioxetane Structures For Bioimaging. *Chem.—Asian J.* **2022**, *17*, e202200018.
(43) Hananya, N.; Shabat, D. Recent Advances and Challenges in Luminescent Imaging: Bright Outlook for Chemiluminescence of Dioxetanes in Water. *ACS Cent. Sci.* **2019**, *5*, 949−959.
(44) Kagalwala, H. N.; Reeves, R. T.; Lippert, A. R. Chemiluminescent spiroadamantane-1,2-dioxetanes: Recent advances in molecular imaging and biomarker detection. *Curr. Opin. Chem. Biol.* **2022**, *68*, 102134.
(45) Kagalwala, H. N.; Lippert, A. R. Energy Transfer Chemiluminescent Spiroadamantane 1,2-Dioxetane Probes for Bioanalyte Detection and Imaging. *Angew. Chem., Int. Ed.* **2022**, *61*, e202210057.
(46) Schuster, G. B.; Turro, N. J.; Steinmetzer, H. C.; Schaap, A. P.; Faler, G.; Adam, W.; Liu, J. Adamantylideneadamantane-1,2-dioxetane. An Investigation of the Chemiluminescence and Decomposition Kinetics of an Unusually Stable 1,2-Dioxetane. *J. Am. Chem. Soc.* **1975**, *97*, 7110−7118.
(47) Trayhurn, P.; Thomas, M. E. A.; Duncan, J. S.; Black, D.; Beattie, J. H.; Rayner, D. V. Ultra-rapid detection of mRNAs on Northern blots with digoxigenin-labeled oligonucleotides and 'CDP-Star', a new chemiluminescence substrate. *Biochem. Soc. Trans.* **1995**, *23*, 494S.
(48) Matsumoto, M.; Watanabe, N.; Kasuga, N. C.; Hamada, F.; Tadokoro, K. Synthesis of 5-alkyl-1-aryl-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptanes as a Chemiluminescent Substrate with Remarkable Thermal Stability. *Tetrahedron Lett.* **1997**, *38*, 2863−2866.
(49) Matsumoto, M.; Watanabe, N.; Hoshiya, N.; Ijuin, H. K. Color modulation for intramolecular charge-transfer-induced chemiluminescence of 1,2-dioxetanes. *Chem. Record* **2008**, *8*, 213−228.
(50) Matsumoto, M. Advanced chemistry of dioxetane-based chemiluminescent substrates originating from bioluminescence. *J. Photochem. Photobiol. C* **2004**, *5*, 27−53.
(51) Adam, W.; Matsumoto, M.; Trofimov, A. V. Viscosity dependence of the chemically induced electron-exchange chemiluminescence triggered from a bicyclic dioxetane. *J. Am. Chem. Soc.* **2000**, *122*, 8631−8634.
(52) Matsumoto, M.; Watanabe, N.; Kobayashi, H.; Azami, M.; Ikawa, H. Synthesis and Chemiluminescence Of 3,3-Diisopropyl-4-Methoxy-4-(2-Naphthyl)-1,2-Dioxetanes. *Tetrahedron Lett.* **1997**, *38*, 411−414.
(53) Ciscato, L.; Bastos, E.; Bartoloni, F.; Günther, W.; Weiss, D.; Beckert, R.; Baader, W. Synthesis of Some Fenchyl-Substituted Alkenes and Enol-Ethers Containing 3-Oxyphenyl Substituents by The Barton-Kellogg Reaction. *J. Braz. Chem. Soc.* **2010**, *21*, 1896−1904.
(54) Ciscato, L.; Weiss, D.; Beckert, R.; Baader, W. Fenchyl Substituted 1,2-Dioxetanes as An Alternative to Adamantyl Derivatives for Bioanalytical Applications. *J. Photochem. Photobiol. A Chem.* **2011**, *218*, 41−47.
(55) Akhavan-Tafti, H.; Eickholt, R.; Arghavani, Z.; Schaap, A. A Novel Substitution Process for The Preparation of Alkoxy-, Aryloxy-, And Acyloxy-Substituted 1,2-Dioxetanes. *J. Am. Chem. Soc.* **1997**, *119*, 245−246.
(56) Matsumoto, M.; Suzuki, H.; Sano, Y.; Watanabe, N.; Ijuin, H. Rotamer-Dependent Chemiluminescence in The Intramolecular Charge-Transfer-Induced Decomposition of Bicyclic Dioxetanes Bearing A Hydroxyaryl Group. *Tetrahedron Lett.* **2008**, *49*, 5372−5375.
(57) Watanabe, N.; Kikuchi, M.; Maniwa, Y.; Ijuin, H.; Matsumoto, M. Synthesis of Sulfanyl-, Sulfinyl-, And Sulfonyl-Substituted Bicyclic Dioxetanes and Their Base-Induced Chemiluminescence. *J. Org. Chem.* **2010**, *75*, 879−884.
(58) Matsumoto, M.; Watanabe, N.; Shiono, T.; Suganuma, H.; Matsubara, J. Chemiluminescence Of Spiro[1,2-Dioxetane-3,1′-Dihydroisobenzofuran]S, Spiro[1,2-Dioxetane-3,1′-Isochroman]S And A Spiro[1,2-Dioxetane-3,1′-(2-Benzoxepane)]. *Tetrahedron Lett.* **1997**, *38*, 5825−5828.
(59) Sabelle, S.; Renard, P.-Y.; Pecorella, K.; de Suzzoni-Dezard, S.; Creminon, C.; Grassi, J.; Mioskowski, C. Design and Synthesis of Chemiluminescent Probes for the Detection of Cholinesterase Activity. *J. Am. Chem. Soc.* **2002**, *124*, 4874−4880.
(60) Maity, S.; Wang, X.; Das, S.; He, M.; Riley, L. W.; Murthy, N. A cephalosporin-chemiluminescent conjugate increases beta-lactamase detection sensitivity by four orders of magnitude. *Chem. Commun.* **2020**, *56*, 3516−3519.
(61) Watanabe, N.; Ichikawa, M.; Ono, A.; Murakami, H.; Matsumoto, M. New Triggering System for Dioxetane-based Chemiluminescence: Base-induced Decomposition of Bicyclic Dioxetanes Bearing a 3-Aminophenyl or 2-Phenylindol-6-yl Moiety. *Chem. Lett.* **2005**, *34*, 718.
(62) Matsumoto, M.; Mizuno, T.; Watanabe, N. Fluoride-induced chemiluminescent decomposition of 1,2-dioxetanes bearing a phenyl

moiety substituted with a methyl having an electron-withdrawing group. *Chem. Commun.* **2003**, 482−483.
(63) Matsumoto, M.; Mizuno, T.; Watanabe, N. Chemiluminescent decomposition of a dioxetane bearing a 3-(1-cyanoethenyl)phenol moiety induced by Michael addition of an anion of malonate. *Tetrahedron Lett.* **2004**, *45*, 3779−3782.
(64) Matsumoto, M.; Mizuno, T.; Watanabe, N. Intramolecular charge-transfer-induced chemiluminescent decomposition of 1,2-dioxetanes bearing a phenylmethanide. *Tetrahedron* **2005**, *61*, 9569−9585.
(65) Eilon-Shaffer, T.; Roth-Konforti, M.; Eldar-Boock, A.; Satchi-Fainaro, R.; Shabat, D. Ortho-Chlorination of Phenoxy 1,2-Dioxetane Yields Superior Chemiluminescent Probes For in Vitro and in Vivo imaging. *Org. Biomol. Chem.* **2018**, *16*, 1708−1712.
(66) Hisamatsu, Y.; Fukiage, T.; Honma, K.; Balia, A. G.; Umezawa, N.; Kato, N.; Higuchi, T. Effect of the o-Acetamido Group on pH-Dependent Light Emission of a 3-Hydroxyphenyl-Substituted Dioxetane Luminophore. *Org. Lett.* **2019**, *21*, 1258−1262.
(67) Huang, J.; Cheng, P.; Xu, C.; Liew, S.; He, S.; Zhang, Y.; Pu, K. Chemiluminescent Probes with Long-Lasting High Brightness For In Vivo Imaging of Neutrophils. *Angew. Chem., Int. Ed.* **2022**, *61*, e202203235.
(68) Hananya, N.; Reid, J.; Green, O.; Sigman, M.; Shabat, D. Rapid Chemiexcitation of Phenoxy-Dioxetane Luminophores Yields Ultrasensitive Chemiluminescence Assays. *Chem. Sci.* **2019**, *10*, 1380−1385.
(69) Ryan, L. S.; Gerberich, J. L.; Cao, J.; An, W.; Jenkins, B. A.; Mason, R. P.; Lippert, A. R. Kinetics-Based Measurement of Hypoxia in Living Cells and Animals Using an Acetoxymethyl Ester Chemiluminescent Probe. *ACS Sens.* **2019**, *4*, 1391−1398.
(70) Lin, V. S.; Lippert, A. R.; Chang, C. J. Cell-trappable fluorescent probes for endogenous hydrogen sulfide signaling: Imaging H2O2-dependent H2S production. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 7131−7135.
(71) Green, O.; Gnaim, S.; Blau, R.; Eldar-Boock, A.; Satchi-Fainaro, R.; Shabat, D. Near-Infrared Dioxetane Luminophores with Direct Chemiluminescence Emission Mode. *J. Am. Chem. Soc.* **2017**, *139*, 13243−13248.
(72) Yang, M.; Zhang, J.; Shabat, D.; Fan, J.; Peng, X. Near-Infrared Chemiluminescent Probe for Real-Time Monitoring Singlet Oxygen in Cells and Mice Model. *ACS Sens.* **2020**, *5*, 3158−3164.
(73) Edwards, B.; Sparks, A.; Voyta, J. C.; Bronstein, I. Unusual Luminescent Properties of Odd- and Even-Substituted Naphthyl-Derivatized Dioxetanes. *Journal of Bioluminescence and Chemiluminescence* **1990**, *5*, 1−4.
(74) Matsumoto, M.; Watanabe, N.; Kobayashi, H.; Azami, M.; Ikawa, H. Synthesis and Chemiluminescence of 3,3-Diisopropyl-4-mthoxy-4-(2-naphthyl)-1,2-dioxetanes. *Tetrahedron Lett.* **1997**, *38*, 411−414.
(75) Hoshiya, N.; Fukuda, N.; Maeda, H.; Watanabe, N.; Matsumoto, M. Synthesis and fluoride-induced chemiluminescent decomposition of bicyclic dioxetanes substituted with a 2-hydroxylnaphthyl group. *Tetrahedron* **2006**, *62*, 5808−5820.
(76) Matsumoto, M.; Mizoguchi, Y.; Motoyama, T.; Watanabe, N. Base-Induced Chemiluminescence Of 5- Tert -Butyl-1-(4-Hydroxybenz[D]Oxazol-6-Yl)-4,4-Dimethyl-2,6,7-Trioxabicyclo[3.2.0]Heptanes: Chemiluminescence−Chemiexcitation Profile In Aqueous Medium. *Tetrahedron Lett.* **2001**, *42*, 8869−8872.
(77) Watanabe, N.; Takatsuka, H.; Ijuin, H.; Matsumoto, M. Highly Effective and Rapid Emission of Light From Bicyclic Dioxetanes Bearing A 3-Hydroxyphenyl Substituted With A 4-P-Oligophenylene Moiety In An Aqueous System: Two Different Ways For The Enhancement Of Chemiluminescence Efficiency. *Tetrahedron* **2020**, *76*, 131203.
(78) Koči, J.; Grandclaude, V.; Massonneau, M.; Richard, J. A.; Romieu, A.; Renard, P. Y. A Novel and Unusually Long-Lived Chemiluminophore Based on the 7-Hydroxycoumarin Scaffold. *Chem. Commun.* **2011**, *47*, 6713−6715.
(79) Hananya, N.; Press, O.; Das, A.; Scomparin, A.; Satchi-Fainaro, R.; Sagi, I.; Shabat, D. Persistent Chemiluminescent Glow of Phenoxy-Dioxetane Luminophore Enables Unique CRET-Based Detection of Proteases. *Chem.—Eur. J.* **2019**, *25*, 14679−14687.
(80) Gnaim, S.; Gholap, S. P.; Ge, L.; Das, S.; Gutkin, S.; Green, O.; Shelef, O.; Hananya, N.; Baran, P. S.; Shabat, D. Modular Access to Diverse Chemiluminescent Dioxetane-Luminophores through Convergent Synthesis. *Angew. Chem., Int. Ed.* **2022**, *134*, e202202187.
(81) Ryan, L. S.; Nakatsuka, A.; Lippert, A. R. Photoactivatable 1,2-Dioxetane Chemiluminophores. *Results Chem.* **2021**, *3*, 100106.
(82) Patel, S. K.; Cao, J.; Lippert, A. R. A Volumetric Three-Dimensional Digital Light Photoactivatable Dye Display. *Nature Commun.* **2017**, *8*, 15239.
(83) Li, B.; Haris, U.; Aljowni, M.; Nakatsuka, A.; Patel, S. K.; Lippert, A. R. Tuning the Photophysical Properties of Spirolactam Rhodamine Photoswitches. *Isr. J. Chem.* **2021**, *61*, 244.
(84) Haris, U.; Plank, J. T.; Li, B.; Page, Z. A.; Lippert, A. R. Visible Light Chemical Micropatterning Using a Digital Light Processing Fluorescence Microscope. *ACS Cent. Sci.* **2022**, *8*, 67−76.
(85) Schaap, A. P.; Akhavan-Tafti, H. International Patent WO1990007511A1, 1990.
(86) Watanabe, N.; Kino, H.; Watanabe, S.; Ijuin, H. K.; Yamada, M.; Matsumoto, M. Synthesis of Bicyclic Dioxetanes Tethering a Fluororescer Through A ω-Carbamoyl-Substituted Linker and Their High-Performance Chemiluminescence In An Aqueous System. *Tetrahedron* **2012**, *68*, 6079−6087.
(87) Hananya, N.; Eldar Boock, A.; Bauer, C.; Satchi-Fainaro, R.; Shabat, D. Remarkable Enhancement of Chemiluminescent Signal by Dioxetane−Fluorophore Conjugates: Turn-ON Chemiluminescence Probes with Color Modulation For Sensing And Imaging. *J. Am. Chem. Soc.* **2016**, *138*, 13438−13446.
(88) Digby, E.; Tung, M.; Kagalwala, H. N.; Ryan, L. S.; Lippert, A. R.; Beharry, A. Dark Dynamic Therapy: Photosensitization Without Light Excitation Using Chemiluminescence Resonance Energy Transfer in A Dioxetane−Erythrosin B Conjugate. *ACS Chem. Biol.* **2022**, *17*, 1082−1091.
(89) Huang, J.; Li, J.; Lyu, Y.; Miao, Q.; Pu, K. Molecular optical imaging for early diagnosis of drug-induced acute kidney injury. *Nat. Mater.* **2019**, *18*, 1133−1143.