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A Photochemical Ligation System Enabling Solid-phase Chemiluminescence Read Out

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Abstract

The peroxyoxalate chemiluminescence (PO-CL) reaction is among the most powerful and versatile techniques for the detection of hydrogen peroxide (H_2O_2) and has been employed in various biological and chemical applications over the past 50 years. However, its two-component nature (peroxyoxalate and fluorophore) limits its use. This contribution introduces an innovative and versatile photochemical platform technology for the synthesis of inherently fluorescent PO probes by exploiting the nitrile imine-mediated tetrazole-ene cycloaddition (NITEC) reaction. In the presence of hydrogen peroxide, the pioneered "2-in-1" molecule emits either yellow or blue light – depending on tetrazole's (Tz) structure – visible to the naked eye. Even in the absence of base, the emitted light remains visible and H_2O_2 could be detected in the nanomolar range. Critically, the PO-Tz can be readily incorporated into polymeric materials. As a first application of this promising material, a tailor-made PO-Tz is grafted on poly(divinylbenzene) (PDVB) particles to enable solid-phase chemiluminescence on microspheres.

1. **Introduction**

Chemiluminescence (CL) reactions – the generation of light as a result of a chemical transformation – are powerful and versatile detection techniques that offer the unique read-out of emitting light in response to specific molecular events. In the realm of chemiluminescence, a plethora of applications ranging from analytical methods such as (immuno)essays,[1] pharmaceutical,[2] environmental[3] or food analysis[4-6] to biosensors[7, 8] have been developed over the last five decades exploiting its simplicity, low-cost, high sensitivity and fast dynamic response. Among the CL reactions, the peroxyoxalate (PO) reaction is of particular interest since it is possible to tune the color of light emitted as exemplified by the well-known glow sticks. The reaction can be classified as indirect or sensitized chemiluminescence as the excited species cannot emit light by itself but rather transfers its excess of energy to a fluorophore. Typically, hydrogen peroxide (H_2O_2) reacts with a peroxyoxalate to form an unstable energy-rich intermediate (1,2-dioxetanedione), which rapidly decomposes into carbon dioxide and releases energy. In the presence of an appropriate fluorescence dye, the chemical energy of the intermediate is converted to electronic excitation energy. The relaxation of the fluorophore from its excited state to its ground state releases photons and generates light. Even if the PO-CL mechanism has been studied in-detail, the chemiexcitation sequence is still not fully understood, yet it is assumed to proceed via the chemically induced electron exchange luminescence (CIEEL) mechanism.[9-11] The chemiluminescence quantum yield (*i.e.* the number of photons emitted per reacting molecule) mainly depends on the PO structure, its proximity with the fluorophore, the electronic nature of the fluorophore, the pH level and the oxalate ester leaving group.[12] Therefore, the parameters and the chemicals components can be advantageously adjusted to fulfil the requirements of the targeted applications. Since H_2O_2 plays a crucial role in regulating fundamental biological processes, CL sensors for H_2O_2 determination have found

interests in many applications. Indeed, H_2O_2 can be employed as an indicator for enzyme detection as its over expression is often a sign of serious disease (diabetes, cancers). Thus, the development of *in vivo* and *in vitro* H_2O_2 sensors hold great potential.[13] Critically, the PO reaction not only represents an efficient tool for imaging and monitoring enzymatic light emission in real time, yet also to evaluate the proliferation of tumor cells.[14] Lee et al. synthesized poly(ethylene glycol)-*b*-poly(ε-caprolactone) (PEG-PCL) micelles in the 50 nm range containing fluorescent dyes and oxalate esters in the hydrophobic core.[15, 16] Due to their nanomolar sensitivity and their small size, these advanced contrast agents found numerous applications for imaging H_2O_2. Employing nanoparticles as carriers further enables the loading of reactive molecules[17] or drugs.[18] However, all these processes are based on a three-component CL reaction, which requires a methodology for confining the peroxyoxalate and the fluorophore in close proximity *in vivo*. To overcome the limitation of a two-component system (*e.g.* leaching, distance) and elevate the PO reaction to an entirely new level, we herein introduce a "2-in-1" molecule. Surprisingly, since the first report of the peroxyoxalate reaction in the early nineteen sixties,[19, 20] the potential of combining the peroxyoxalate moiety and a fluorophore in the same molecule has barely been investigated. Motoyoshiya et al. observed that some non-fluorescent diaryl oxalates with electron-donating groups emitted weak light thanks to the emission from the excimer of the eliminating group.[21] The authors hypothesized that the emitting species would be formed by an intra- and intermolecular electronic interaction with a high-energy intermediate. To the extent of our knowledge, this contribution is the only report of a PO-CL reaction using a single molecule and the present work aims to introduce a new platform technology in which an inherently fluorescent PO molecule can emit strong light but also be readily grafted onto surfaces and incorporated in a wide variety of materials. This strategy relies on the nitrile imine-mediated tetrazole-ene cycloaddition (NITEC), which

produces a fluorescent five-membered pyrazoline ring.[22] Using either UV or visible light irradiation, tetrazoles (Tz) form highly reactive nitrile imines under release of nitrogen. The intermediate is capable of undergoing cycloaddition reactions with a vast array of dipolarophiles such as maleimides, acrylates and fumarates. Thus, the synthesis of an oxalate-based molecule bearing a maleimide functionality as dipolarophile will lead to a fluorescent moiety at the PO's vicinity **(Figure 1**). Our group has developed a wide library of UV and visible light sensitive tetrazoles bearing (meth)acrylate,[23] carboxylic acid or alcohol functionalities, enabling them to be incorporated into soft matter materials[24] and (bio)surfaces[25] of different nature. As one possible application of our new class of PO, we herein describe light emitting chemiluminescent polymeric microspheres (MS). Indeed, their large surface area as well as non-demanding handling, and long-term storage stability make microspheres highly attractive scaffolds. Solid-phase chemiluminescence presents a sensitive, fast and effective detection method and has shown promise for the detection of biological threat agents, water or food contaminants.[26] Current techniques rely on fluorogenic-chemiluminescence (FCL) and electro-chemiluminescence (ECL), which depend upon the isolation of the targeted analyte from the sample by magnetic separation employing magnetic microspheres as a support. Van Zoonen et al. have addressed the possibility of covalently immobilizing fluorophores onto controlled pore glass beads to enable solid-phase peroxyoxalate chemiluminescence in high-performance liquid chromatography (HPLC), resulting in a sensitive, cost-effective and simple H_2O_2 detection system in rain water.[27-29] Applying a similar approach, Pontén et al. compared six amino-based luminophores employing porous and non-porous methacrylate beads.[30-32] These authors concluded that porous materials were much more sensitive due to a higher surface area and degree of functionalization. To the best of our knowledge, this study from the mid-nineties is the only reported MS solid phase

luminophore. Since then, very little attention has been paid to solid-phase peroxyoxalate chemiluminescent polymeric microspheres, most likely because of the inherent difficulty of molecular anchoring the three reactive components in close proximity. Herein, we close this critical gap by grafting our new system onto the surface of hydrophilic microspheres.

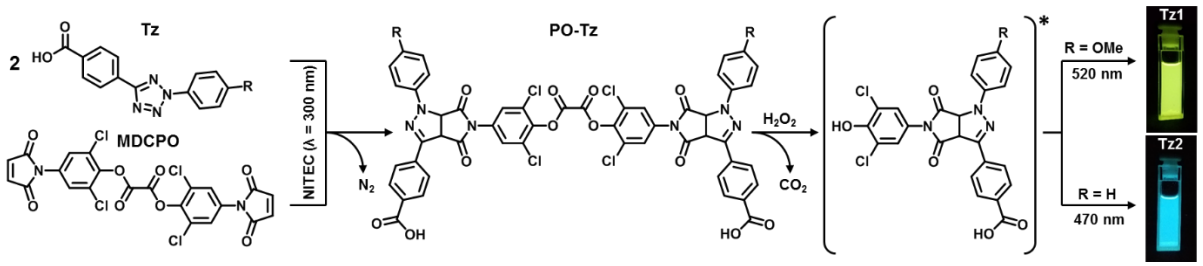

Figure 1. NITEC reaction of the MDCPO with tetrazole and simplified chemiluminescence activation pathway of the PO-Tz molecule. Pictures of the samples after addition of H_2O_2 are displayed next to the corresponding tetrazole.

2. Results and Discussion

2.1. Design of tetrazole-peroxyoxalate molecule

Initially, a suitable maleimide-PO was tailored. Peroxyoxalate esters are generally synthesized by reacting the respective functionalized phenol with oxalyl chloride under triethylamine (TEA) catalysis or with oxalic acid mediated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and a base. Due to the high reactivity of oxalyl chloride employed in the last step, the aromatic compound needs orthogonal functional groups to be able to incorporate maleimides as reactive double bond for the NITEC reaction. The most frequently used POs – bis(trichlorophenyl) oxalate (TCPO),[33] bis(2,4-nitrophenyl)oxalate (DNPO),[33] bis(pentafluorophenyl) oxalate (PFPO)[19] or divanillyl oxalate (DVO)[34] – do not present orthogonal pre or post-modifiable functionalities in their structure, requiring alternative synthetic pathways. Accordingly, a maleimide-PO compound (MPO) based on commercially

available 4-amino phenol was synthesized in a simple three-step procedure. ^{1}H and ^{13}C NMR spectroscopy confirmed the successful synthesis (for detailed analytical information, refer to Figures S2-S3) and its applicability in CL reactions was assessed in further experiments and compared to TCPO and DVO. Following a typical protocol, the MPO was dispersed in ethyl acetate (EA), together with a common fluorophore, here 9-10 diphenylanthracene (DPA). The light emitted was very weak, in contrast to the conventional PO which exhibited a very bright light, visible to the naked eye (Figure S16). We hypothesized that the maleimide deactivated the phenol as leaving group in subsequent CL reactions. Therefore, the second generation of maleimide-PO was tyramine-based (Figure S4-5) in order to introduce a small alkyl chain as spacer between the maleimide and the aromatic ring (MEPO). Unfortunately, the resulting CL intensity was even lower compared to the first generation MPO. Since aromatic substituents have a significant influence on the light emission intensity and electron-withdrawing groups such as halogens can decrease the pKa value of the respective phenol-group, their leaving group properties are enhanced.[12] As a consequence, the third generation was based on 4-amino-2,6-dichloro phenol, leading to bis(2,6-dichloro-4-N-maleimido)phenyl) oxalate (MDCPO) (Figure S6-9). The light intensity was strongly improved and a bright blue light was readily observable. Compared to TCPO, the light intensity was lower – TCPO contains 6 Cl whereas the MDCPO contains only 4 Cl – yet it was 3 times higher than DVO. The subsequent challenge was to assess the unconventional use of a NITEC adduct to act as a fluorophore in a PO-CL reaction employing a 2/1 molar ratio. As already noted, the PO-CL reaction requires a fluorophore, which is usually an organic dye such as 9,10- diphenylanthracene, rubrene, aminopyrene or Rhodamine B. Considering that the fluorescence quenching effects can be offset by too high concentration, fluorophores are generally employed in low concentrations, typically with a 0.05/1 molar ratio relative to the PO. In our strategy, the PO bears two

maleimide moieties, consequently leading to two fluorophores per PO after the reaction with the tetrazole. In a preliminary study, two UV-B reactive tetrazoles were synthesized, one bearing a methoxy phenyl substituent (Tz1) and one only bearing a phenyl group adjacent to the tetrazole (Tz2) (Figures S10-S14). Both tetrazoles were subsequently dissolved in 3 mL of THF or EA in the presence of MDCPO and irradiated with UV-B light (300 nm) for 30 min. ^{1}H NMR confirmed that the NITEC reaction proceeded with almost full conversion (94 %) after only 30 min (Figure S17) as resonances associated with the maleimide double bond protons (7.77 ppm and 7.04 ppm) disappeared while two doublets arose between 5.21 and 5.44 ppm. We further observed that aromatic proton resonances of the newly formed PO-Tz shifted upfield. Absorbance and fluorescence spectra (Figures S18-S19) additionally evidenced the successful formation of the highly fluorescent pyrazoline adduct with an emission wavelength of 525 nm for Tz1 and 480 nm for Tz2 (λ_{ex} = 390 nm). Chemiluminescence was subsequently triggered by adding 50 µL of a saturated urea hydrogen peroxide aqueous solution and 10 µL of a saturated sodium acetate solution. Indeed, CL reactions are usually conducted in the presence of a base which can catalyze nucleophilic H_2O_2 attack at the PO and increase the light intensity.[35] Both samples exhibited strong light emissions that were readily observable by the naked eye as shown on **Figure 2.** Tetrazole 1 emitted yellow light with a wavelength centered at 525 nm, while the second tetrazole emitted at 480 nm (blue light). In terms of intensity, tetrazole 1 exhibited a maximum irradiance at 0.024 uW cm^{-2} nm^{-1} whereas tetrazole 2 maximum irradiance was recorded at 0.014 uW cm^{-2} nm^{-1}. In the visible range, the total irradiance was 4.93 and 3.93 uW cm^{-2} nm^{-1} and the corresponding photosynthetically photon flux density (PPFD) was then calculated at 0.15 and 0.07 µmol m^{-2} s^{-1} for PO-Tz1 and PO-Tz2, respectively (**Figure 2A**). These experiments not only constitute the first report of a pyrazoline adduct being used as a fluorophore in a PO-CL reaction, but it is also the first functional PO-CL system that

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does not require additional fluorophores. Remarkably, the substituent near the tetrazole allows to access different emission wavelengths and colors as it is permitted with conventional dye. It is worth-noting that the PO-Tz was completely soluble in THF and only partially in EA. Nonetheless, the light intensity was higher in EA for both tetrazoles. Diethyl phthalate (DEP) – the solvent used in the glow sticks – was also investigated (Figures S20-S21 and **2B**). The MDCPO and Tz were partially soluble, but even if the emitted light intensity was slightly lower than in THF, it was sufficiently bright to be observed by the naked eye. Interestingly, due to the high viscosity of DEP, the emission lasted for 45 min instead of a few min when conducted in THF or EA (Figure S21). Taking advantage of the viscosity of DEP, the experiment performed in a 50/50 mixture DEP/EA lasted more than 10 min with a light intensity almost equivalent to neat EA. Changing the PO-Tz concentration led to higher light intensities with no linear correlation, most likely due to the moderate solubility of the PO-Tz in EA (Figure S22). The CL remained visible with a minimal concentration of 0.35 mmol L^{-1}.

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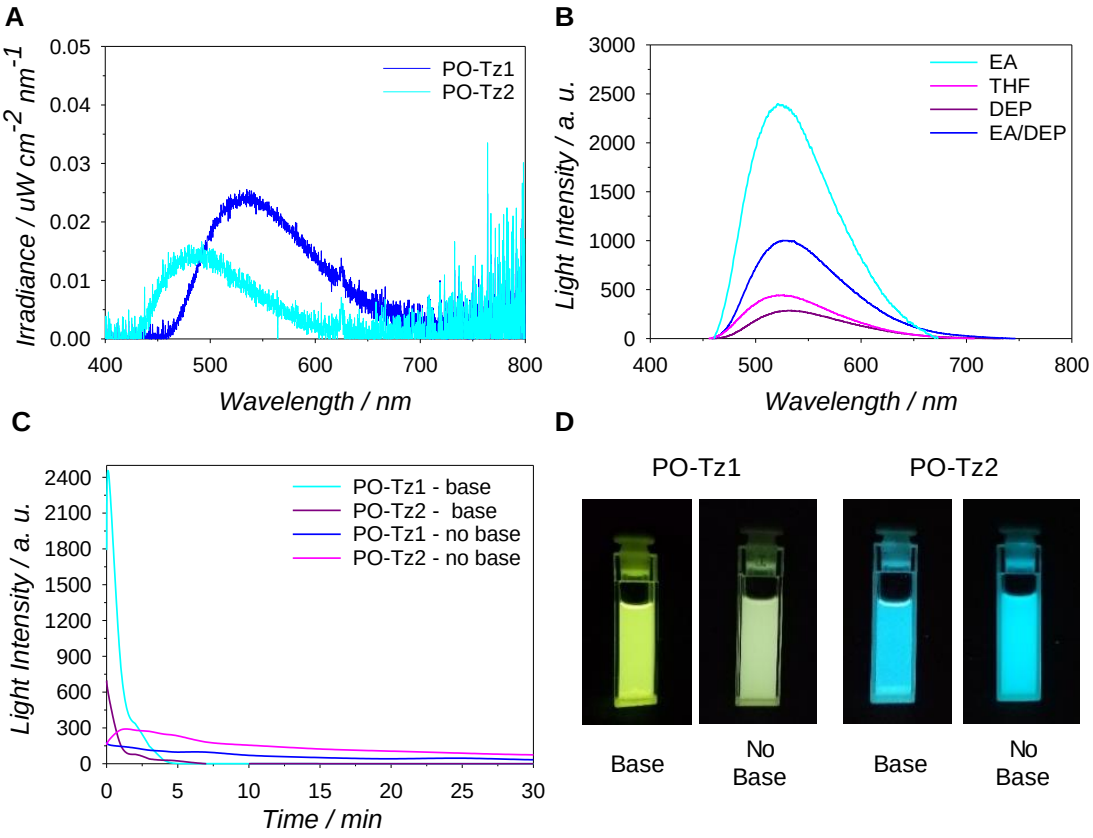

Figure 2. Chemiluminescence emission spectra of PO-Tz (Tz1, λ_{max} = 525 nm; Tz2, λ_{max} = 480 nm). Spectra were recorded in 3 mL of solvent in the presence of PO-Tz (4 mmol L^{-1}), 50 µL of urea hydrogen peroxide aqueous solution (6 mol L^{-1}) and 10 µL of sodium acetate (4 mmol L^{-1}). (A) Irradiance measurement (spectroradiometer, EA). (B) Influence of solvent (spectrophotometer set in medium voltage). (C) Kinetics of the light emitted in presence or absence of sodium acetate (medium voltage, EA). (D) Pictures of the PO-Tz after addition of H_2O_2.

We then moved to assess the peroxide detection limit of this new system by gradually decreasing the added amount of H_2O_2, whereby a weight detection limit (DL^W) of 10^{-6} mol was determined (Figure S23B). This threshold corresponds to the detection limit of the spectrophotometer and can still be observed by the naked eye. Classical PO-CL analytical methods state a DL^W between 10^{-11} and 10^{-14} mol[36, 37] with much more sensitive instruments. Therefore, an even lower detection limit could be measured, yet this is beyond the scope of the present work.

As previously established, employing different moiety adjacent to the tetrazole led to distinct colors and we were interested in investigating the effect of various functionalities on the CL. Hence, a library of tetrazoles bearing small (C2) or long alkyl chains (C11), hydroxyl or carboxyl groups as well as acrylates were assessed. Similarly to the initial Tz employed, all PO-Tz molecule exhibited a bright light, visible to the naked eye (Figure S24), demonstrating the versatility of our platform technology. In our aim to simplify the PO-CL reaction even further, we only employed the PO-Tz molecule with hydrogen peroxide without the use of any base (**Figures 2C-D**). Surprisingly, we observed that the light intensity slowly decayed for more than one hour where it lasted few minutes in the presence of a base. Several studies have stressed the importance of a catalyst in the CIEEL sequence and PO-CL reactions performed in the complete absence of base led to inconsistent results with a very weak light intensity.[38, 39] However, even if the light intensity was lower compared to the experiments conducted with sodium acetate, the emitted light remained very bright, which demonstrates that CL reactions can be triggered with a very simple two-component setup (PO-Tz and H_2O_2). Then, a plate reader was used for that experiment – employing acetonitrile as solvent – and hydrogen peroxide was detected down to 100 nmol L^{-1} (Figure S23C).

In summary, we introduce a 2-in-1 PO-Tz molecule which emits very bright light in the sole presence of H_2O_2. Further, various parameters such as solvent, employed Tz, or the concentration can be tuned depending on the final application. One key feature of our platform technology is its ability to use a tetrazole that can be readily incorporated into a polymer backbone due to its additional functional synthons.

2.2. All-in-one chemiluminescent microspheres

Driven by the potential of this new molecule, we functionalized the surface of polymeric microspheres (MS) to produce, for the first time, "all-in-one" CL MS (**Figure 3A**). Polymeric microspheres in the 0.1 to 2000 μm range can be synthesized by a variety of processes, yet are generally prepared by heterogeneous polymerization techniques such as (mini)emulsion, suspension, dispersion or precipitation polymerization.[40] Due to its simplicity we decided to employ the precipitation polymerization technique, which does not require any surfactant or stabilizers that can eventually hinder the surface's functionalization. The classical approach to synthesize microspheres by precipitation polymerization is to utilize a monomer and a crosslinker with a similar structure (*e.g.* styrene and divinylbenzene) in a near Θ-solvent (*e.g.* acetonitrile). During the initiation step, oligomers grow until they reach a critical length – after which they are no longer soluble in the solvent – precipitate and form spherical nuclei. The nuclei then grow by adsorbing further monomers and oligomers from solution to finally form narrow disperse polymeric particles. Thanks to the simplicity of this method, various morphologies (*e.g.* highly cross-linked, porous) can be achieved by tuning the reaction conditions respectively (solvent, monomer ratio, porogen...).[41] Based on well-established processes,[42] poly(divinylbenzene) (PDVB) MS were first synthesized as a highly crosslinked seed particle. However, this material is very hydrophobic and is not compatible with (aqueous) solutions of hydrogen peroxide. Therefore, in a second step, the residual double bonds of the seed particles were used to grow a shell of poly(2-hydroxyethyl methacrylate) (PHEMA) in order to make them suitable for polar systems. Using the residual hydroxyl group, the microspheres were functionalized with the tetrazole carboxylic acid under Steglich conditions.

The subsequent photoligation with the MDCPO was ultimately conducted with the aforementioned protocol to lead to so-called "all-in-one" chemiluminescent microspheres.

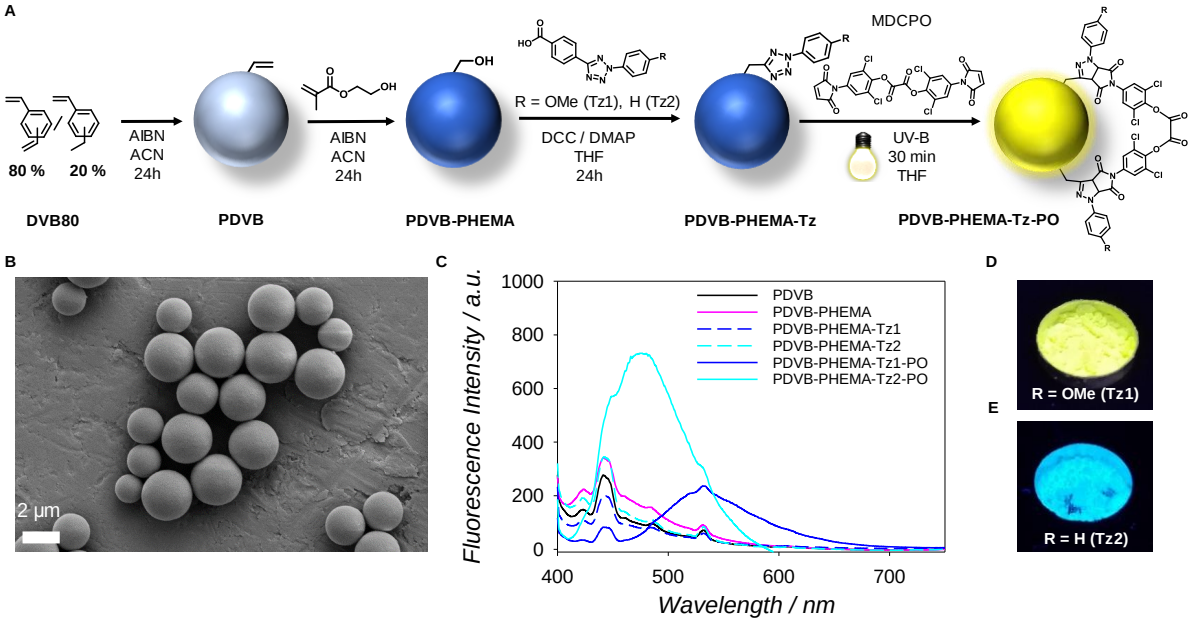

Figure 3. (A) Synthetic pathway for the formation of inherently fluorescent microspheres. (B) SEM pictures of the final PDVB-PHEMA-Tz2-PO. (C) Fluorescence spectra of the microspheres after each step (solid probe, λ_{ex} = 390 nm, medium voltage). (D-E) Pictures of the PDVB-PHEMA-Tz1-PO and PDVB-PHEMA-Tz2-PO particles under a TLC hand-held lamp (365 nm).

Since the tetrazole and MDCPO were used in excess to ensure full conversion during esterification and NITEC, the microspheres were thoroughly washed during each step to remove any unreacted species. Scanning electron microscopy (SEM) revealed spherical PDVB particles with an average diameter of 1.61 µm and a polydispersity (*U*) of 1.16 (**Table 1,** Figure S25). The diameter increased to 2.31 µm after the PHEMA shell grafting (Figure S26). As expected, the next steps only led to a minor increase in diameter since the grafting of small molecules (*i.e.* the tetrazole and the MDCPO) should not strongly affect the size (Figures S27-29, **Figure 3B**). The final particles were considered narrow disperse (*U* = 1.04)

and exhibited a diameter of 2.52 and 2.72 μm for MS-Tz1-PO and MS-Tz2-PO, respectively. The microsphere's surface composition was further analyzed by X-ray photoelectron spectroscopy (XPS) (Table 1) and revealed the presence of oxygen after the incorporation of the PHEMA chains and, most importantly, the presence of chlorine at 1.32 and 3.51 atom% only after the last step for MS-Tz1-PO and MS-Tz2-PO, respectively. Note the increase of the nitrogen content from 0.13 to 7.49 at% after the incorporation of tetrazole, followed by a slight decrease to 5.09 at% due to the release of N_2 during the NITEC reaction (MS-Tz2). Particles – after the final ligation step – were still fully dispersible in water which is critical for future applications in biological systems. Fluorescence was directly measured on the microsphere powder using a solid probe to confirm their intrinsic fluorescent nature (Figures 3C-E). The PDVB-PHEMA-Tz core-shell particles are slightly fluorescent and emitted at 443 nm, but a clear shift to higher wavelengths was observed after the NITEC reaction.

Table 1. SEM, XPS and Fluorescence analysis of the microspheres after each synthetic step.

	SEM[a]			XPS[b]				Fluorescence[c]	
Samples	*D*w [μm]	*D*n [μm]	U	C *1s* [at%]	N *1s* [at%]	O *1s* [at%]	Cl *2p* [at%]	λ_{em} [nm]	I [a.u]
PDVB	1.87	1.61	1.16	97.52 ± 0.09	0.17 ± 0.17	2.31 ± 0.08	0.00 ± 0.00	443	274
PDVB-PHEMA	2.52	2.31	1.09	74.39 ± 0.30	0.13 ± 0.13	25.49 ± 0.18	0.00 ± 0.00	443	339
PDVB-PHEMA-Tz1	2.51	2.60	1.04	75.94 ± 0.39	9.73 ± 0.12	14.34 ± 0.27	0.00 ± 0.00	443	201
PDVB-PHEMA-Tz1-PO	2.52	2.59	1.03	74.02 ± 0.44	9.43 ± 0.14	15.24 ± 0.21	1.32 ± 0.10	532	240
PDVB-PHEMA-Tz2	2.63	2.45	1.07	76.05 ± 0.25	7.49 ± 0.15	16.47 ± 0.11	0.00 ± 0.00	443	339
PDVB-PHEMA-Tz2-PO	2.72	2.61	1.04	73.90 ± 0.15	5.09 ± 0.21	17.51 ± 0.43	3.51 ± 0.07	475	730

[a]D_w represents the weight-average particle diameter, D_n represents the number-average particle diameter, and *U* represents the polydispersity index (D_w/D_n). [b]XPS measurements were performed on carbon tape and values were calculating using the average of two locations. [c]Fluorescence measurements were recorded using a solid probe with an excitation wavelength of 390 nm (medium voltage).

Consistent with the preliminary small molecule study, the MS-Tz1-PO emitted at 525 nm and the MS-Tz2-PO at 475 nm. Finally, chemiluminescence measurements were performed on the MS-Tz-PO. The light emission was recorded at 544 nm (I = 170 a.u.) for Tz1 (Figure S30). The light was not observable to the naked eye, therefore a photomultiplier suitable for solid samples (Lumipol 3) was employed to follow the kinetics. The material was loaded in an aluminium pan (10 mg) and 50 µL of a 2.10^{-2} mol L^{-1} solution of solid urea hydrogen peroxide in acetonitrile (equivalent to 1 µmol of hydrogen peroxide) was simply dropped on the powder. The light intensity of MS-Tz1-PO started at 1.5 10^7 counts then decreased to 4.10^4 counts within 30 min (**Figure 4A**). MS-Tz2-PO exhibited a similar behavior, with a light intensity of 2.5 10^7 counts – almost two times higher. The higher light intensity can be correlated to a higher chlorine content and, consequently, to more PO on the particle's surface. The loading content had a significant impact as weights of 2.0 and 5.0 mg exhibited lower intensities (Figure S31-32). The detection limit of hydrogen peroxide was assessed by successive dilution of the urea hydrogen peroxide solution (**Figure 4B** - Figure S33). A linear correlation between the quantity of H_2O_2 and the photon count was maintained to a concentration of 2.10^{-4} mol L^{-1}, although the light intensity was recorded until 2.10^{-6} mol L^{-1} (equivalent to 100 pmol of H_2O_2 for 5 mg of MS-PO).

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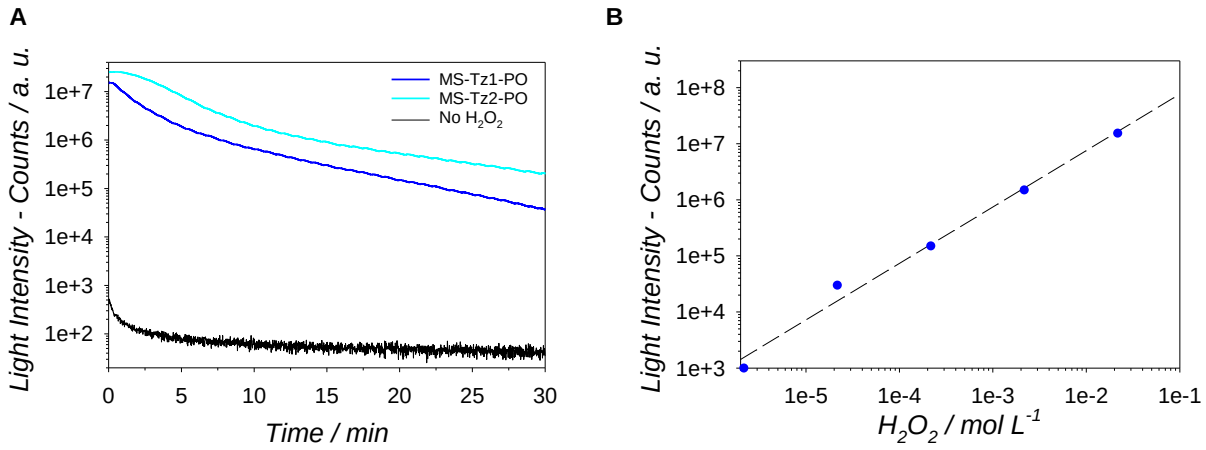

Figure 4. (A). Kinetics of the light emitted by MS-Tz1-PO and MS-Tz2-PO in presence of urea hydrogen peroxide: 10 mg MS-PO, 50 µL of a 2 10^{-2} mol L^{-1}solution of urea hydrogen peroxide in ACN. (B). Light intensity versus the hydrogen peroxide quantity (MS-Tz1-PO: 5 mg, 50 µL of a hydrogen peroxide solution in ACN varying from 2 10^{-2} to 2 10^{-6} mol L^{-1}, logarithm scale) (Lumipol). The dashed line is a guide to the eye.

3. Conclusion

We herein present a new class of molecule that combines a peroxyoxalate and a fluorophore. Undetermined by a robust chemiluminescent reaction, the effectiveness of this platform technology is based on the synthesis of a maleimide-PO that can subsequently react with a tetrazole under UV-B light to form a fluorescent pyrazoline adduct. By simply adding H_2O_2, the PO-Tz emits a very bright light visible by naked eyes. Furthermore, blue or yellow light are obtained by judiciously choosing the moiety adjacent to the tetrazole. Development of new tetrazole is attracting much attention and the colour of emitted light could be further tuned. Additionally, by using red-shifted tetrazole and thereby avoiding detrimental UV light, our platform technology could be employed in biological systems. Hence, this fluorescent peroxyoxalate molecule represents an innovative alternative to conventional multicomponent CL systems and has the potential to outperform current PO-CL reactions. Of fundamental

interest is the possibility of incorporating the molecule into various materials but also playing with its solubility in different media due to the end-group functionality of the tetrazole. In the present study, we employed an esterification, but a wide library of functional tetrazoles exists, enabling the PO-Tz to be readily tailored to the targeted application. Here, we grafted the PO-Tz onto the surface of polymeric microspheres resulting in solid-phase chemiluminescence. These "all-in-one" microspheres are highly fluorescent and easy to handle and store. The addition of hydrogen peroxide triggers CL and high photon counts are recorded even at low peroxide concentrations.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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A Photochemical Ligation System Enabling Solid-phase Chemiluminescence Read Out

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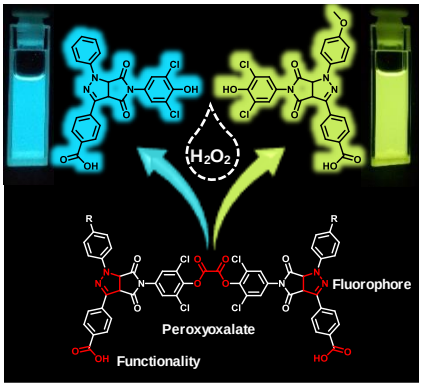
Keywords. Chemiluminescence, Peroxyoxalate, Tetrazole, Microsphere, Fluorescence.



A versatile platform technology combining a fluorescent pyrazoline-peroxyoxalate adduct is introduced. Upon addition of hydrogen peroxide, the 2-in-1 molecule emits a yellow or blue bright light visible to the naked eye. By judiciously selecting the tetrazole, this tailor-made molecule can be readily incorporated into polymeric materials, enabling solid-phase chemiluminescence read-out.