Microenvironment-Mediated Oxidative Enhancement of Conjugated Polyelectrolytes for the Detection of Hydrogen Peroxide

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Fluorescence Spectra of MPS-PPV upon Addition of Hydrogen Peroxide in the Absence of Polyvinyl Pyrrolidone

The fluorescence spectra of 12.5 μg/mL MPS-PPV show a clear hydrogen peroxide–dependent turn-on response in DI, 10 mM HEPES buffer (pH = 7), and 10 mM HEPES 150 mM NaCl buffer (pH = 7). At low H_2O_2 concentrations, the fluorescence intensity increases gradually, indicating the onset of oxidation-induced emission enhancement. This is followed by a more pronounced increase at intermediate concentrations. At higher H_2O_2 levels, the fluorescence intensity approaches a plateau and begins to stabilize, suggesting saturation of the oxidative process responsible for fluorescence enhancement.
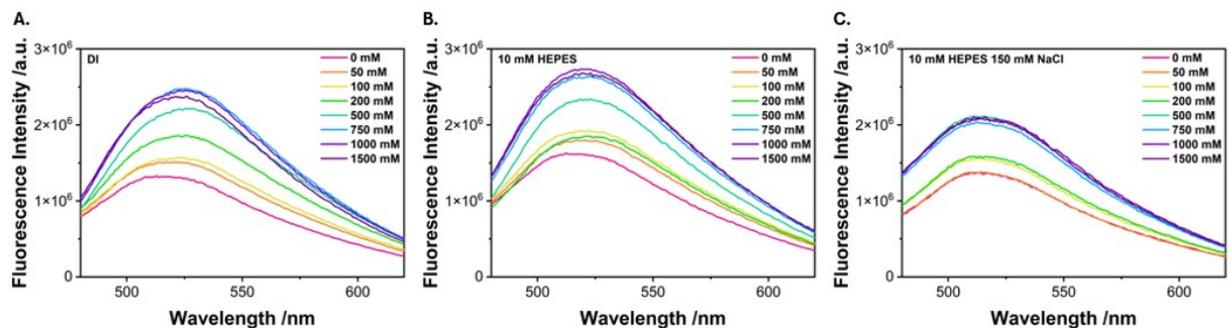

Figure S1: Fluorescence spectra of 12.5 μg/mL MPS-PPV upon increasing the concentration of hydrogen peroxide from 0 mM to 1500 mM in (A) (DI), (B) 10 mM HEPES (pH = 7), and (C) 10 mM HEPES and 150 mM NaCl (pH = 7). Samples were excited at 450 nm and the emission spectra were collected at 20°C.

Blue Shift in the Normalized Fluorescent Signal of MPS-PPV upon its Incubation with Hydrogen Peroxide for 24 Hours.

The normalized fluorescent intensity of MPS-PPV shifts from 513 nm, in the absence of H_2O_2, to 475 nm, after its incubation with 1.5 M of hydrogen peroxide for 24 hours. The samples were tested under the same experimental conditions in the same medium (pH = 7 buffer solution of 10 mM HEPES). The blue shift highlights the shortening of the conjugated length of MPS-PPV.
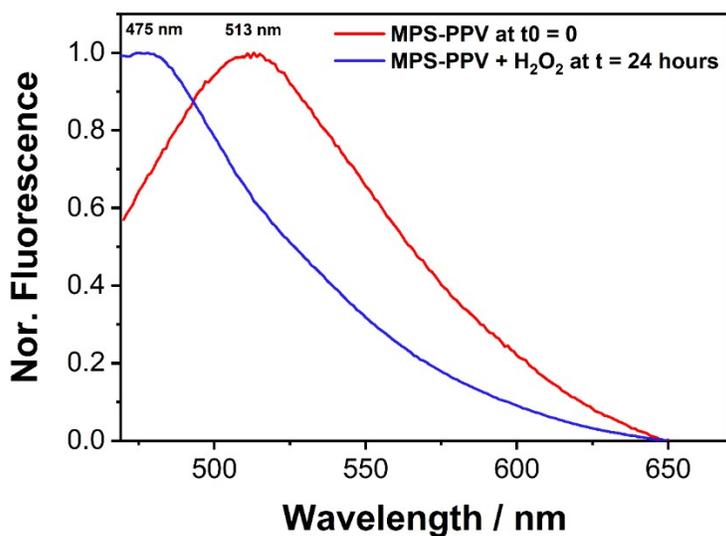
Figure S2: Blue shift of the normalized fluorescent signal of MPS-PPV upon its incubation with hydrogen peroxide for 24 hours. The measurements were done in a pH = 7 buffer solution of 10 mM HEPES. The emission spectra were collected at 20°C.

Effect of Varying the Concentration of PVP on the Sensitivity of Hydrogen Peroxide Detection.

Different PVP concentrations of the same molecular weight (3500 g/mol) were tested under the same experimental conditions in the same medium (pH = 7 buffer solution of 10 mM HEPES and 150 mM NaCl). Even at low PVP concentrations, as low as 0.0050 mg/mL, the fluorescent intensity increases as the concentration of hydrogen peroxide increases. As the molecular weight of PVP was kept constant, the sensor showed the highest sensitivity with the lowest concentration of PVP (0.0050 mg/mL).
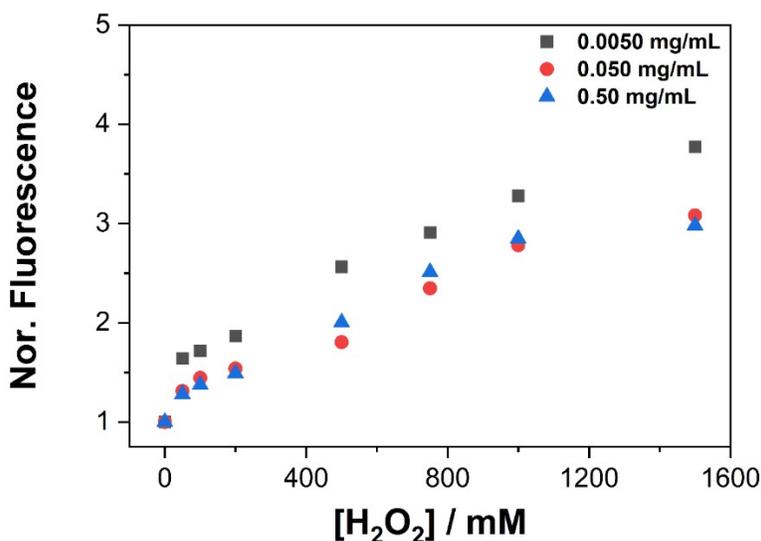
Figure S3: Fluorescence intensity changes of 12.5 µg/ml MPS-PPV are shown upon incremental additions of hydrogen peroxide (0-1500 mM) in the presence of 0.0050 mg/mL PVP 3500 g/mol, 0.050 mg/mL PVP 3500 g/mol, and 0.50 mg/mL PVP 3500 g/mol. The measurements were done in a pH = 7 buffer solution of 10 mM HEPES and 150 mM NaCl. Samples were excited at 450 nm and the emission spectra were collected at 520 nm at 20°C.

Effect of Varying the Molecular Weight of PVP on the Sensitivity of Hydrogen Peroxide Detection.

Same concentrations of different molecular weights of PVP (3500 g/mol, 8K g/mol, 55K g/mol) were tested under the same experimental conditions in the same medium (pH = 7 buffer solution of 10 mM HEPES and 150 mM NaCl). The fluorescent intensity increases as the concentration of hydrogen peroxide increases. As the concentration of PVP was kept constant, the sensor showed the highest sensitivity with PVP of the lowest molecular weight (3500 g/mol).
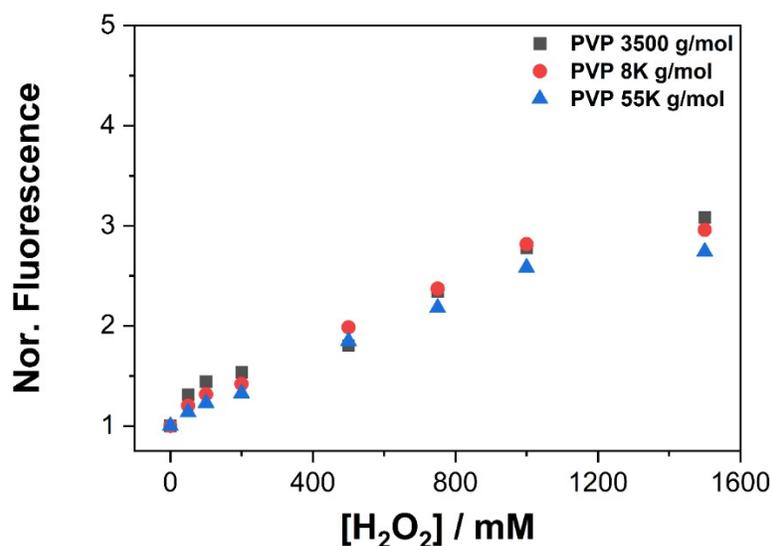
Figure S4: Fluorescence intensity changes of 12.5 µg/ml MPS-PPV are shown upon incremental additions of hydrogen peroxide (0-1500 mM) in the presence of 0.050 mg/mL PVP 3500 g/mol, 0.050 mg/mL PVP 8K g/mol, and 0.050 mg/mL PVP 55K g/mol. The measurements were done in a pH = 7 buffer solution of 10 mM HEPES and 150 mM NaCl. Samples were excited at 450 nm and the emission spectra were collected at 520 nm at 20°C.

Fluorescence Spectra of MPS-PPV upon Addition of Hydrogen Peroxide in the Presence of Polyvinyl Pyrrolidone.

The fluorescence spectra of 12.5 µg/mL MPS-PPV in the presence of 0.0050 mg/mL PVP (3500 g/mol) exhibit a consistent hydrogen peroxide–dependent turn-on response across all tested media. In DI water and 10 mM HEPES, increasing H_2O_2 concentration from 0 to 1500 mM leads to a progressive enhancement of the emission intensity, while the overall spectral shape and emission maximum remain largely unchanged. Notably, the magnitude of fluorescence enhancement depends on the solution composition. While a clear concentration-dependent increase is observed in DI water and HEPES buffer, the presence of electrolytes further influences the response. In particular, NaCl and $CaCl_2$ containing buffers show a stronger fluorescence enhancement at higher H_2O_2 concentrations, with Ca^{2+} producing the most pronounced effect. Overall, these results demonstrate that the MPS-PPV/PVP system maintains a robust oxidative fluorescence response to H_2O_2 across different ionic environments, with the trend approaching saturation at elevated oxidant concentrations.
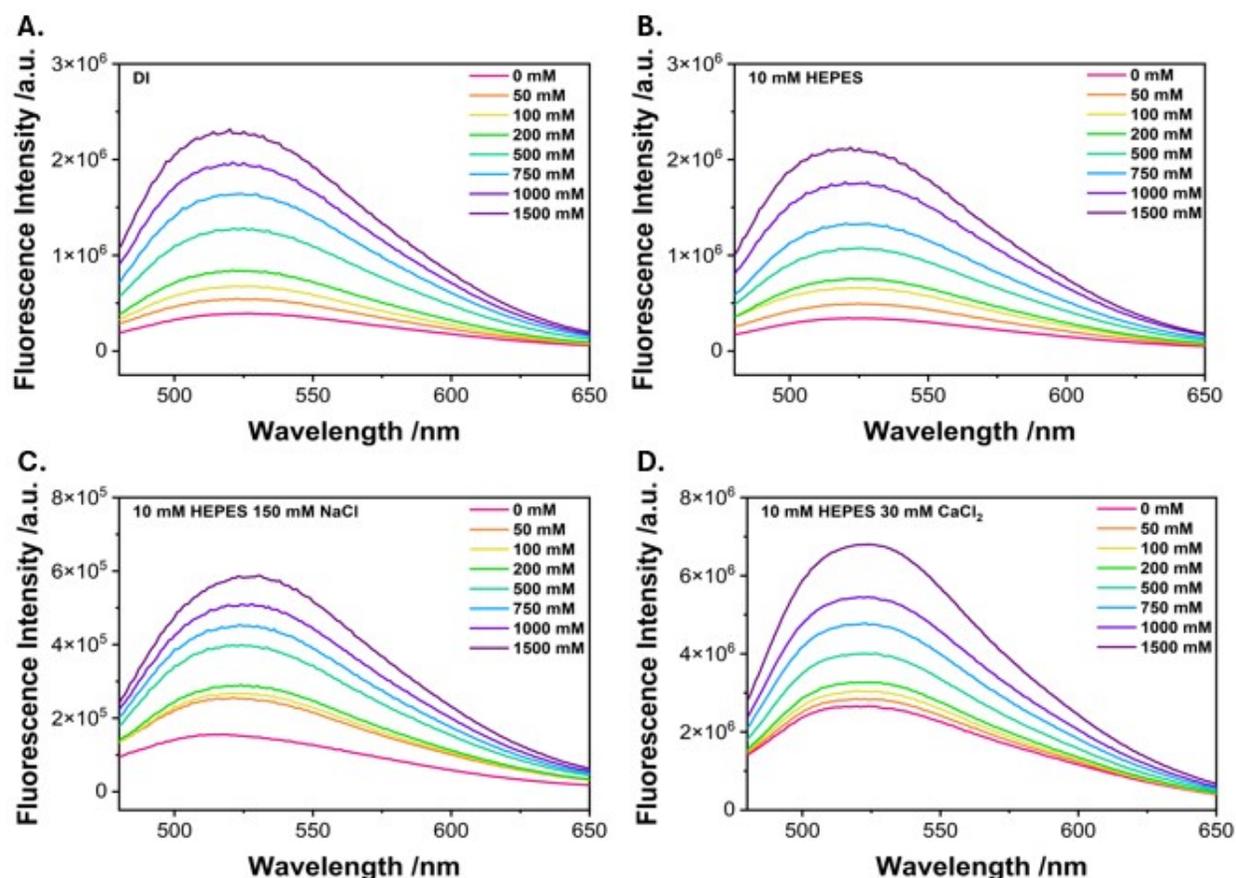
Figure S5: Fluorescence spectra of 12.5 μg/mL MPS-PPV, in the presence of 0.0050 mg/mL of PVP 3500 g/mol, upon increasing the concentration of hydrogen peroxide from 0 mM to 1500 mM in (A) DI, (B) 10 mM HEPES (pH = 7), (C) 10 mM HEPES 150 mM NaCl (pH = 7), and (D) 10 mM HEPES 30 mM CaCl$_2$ (pH = 7). Samples were excited at 450 nm and the emission spectra were collected at 20°C.

Table S1: Comparing the performance of our sensor against recently reported H$_2$O$_2$ detection methods.

Materials Used	Detection mechanism / Signal Mode	Linear range	LOD	References
PFP-NMe$_3^+$ + peroxyfluor-1	Boronate deprotection + FRET : Turn-off ratiometric	15 - 600 nM	15 nM	[1]
PF-FB	Intramolecular FRET : Ratiometric	4.4 - 530 μM	4.4 μM	[2]
PPESO$_3$ + HRP + H$_2$Q	Enzyme-generated quinone quenching : Turn-off Photoluminescence	6 μM - 2 mM	1 μM	[3]
Fluorescent polymer + Fe^{2+}	Fenton reaction : Turn-off	1 to 10 μM	0.6 μM	[4]
MPS-PPV + PVP	Fluorescent Turn-on	0 – 1500 mM	26 μM	This work
N-doped Reduced Graphene Oxide	Non-enzymatic electrochemical detection via chronoamperometry	0.1 mM to 10.7 mM	26.0 μM	[5]

Dual-emission carbon nanodots synthesized from citric acid and urea	Fluorescent/Colorimetric (Dual-mode) sensing where H_2O_2 causes aggregation and surface property changes	50 mM to 500 mM	14 mM	6
Metal-free brominated graphene (GBR) coated on a glassy carbon electrode	Electrochemical (Electro-oxidation) mimicking catalase activity	0.1 mM to 10 mM	48 µM (via Cyclic Voltammetry) or 63 µM (via DPV)	7
Horseradish Peroxidase (HRP) covalently immobilized on a conducting polymer film	Amperometric (Electrochemical) biosensing	0.1 mM to 30 mM	30 µM	8

Effect of PVP on the Fluorescence Intensity of MPS-PPV Following the Addition of Hydrogen Peroxide

The flureoscence intensity of MPS-PPV showed a two-fold increase upon the addition of 1.5 M of hydrogen peroxide in a pH = 7 buffer solution of 10 mM HEPES. After introducing polyvinylpyrrolidone, the intensity of MPS-PPV increases three-fold further, reaching a 6.5-fold fluroerscent enhancement of the initial signal, which highlights the crucial role of PVP in our developed sensor.
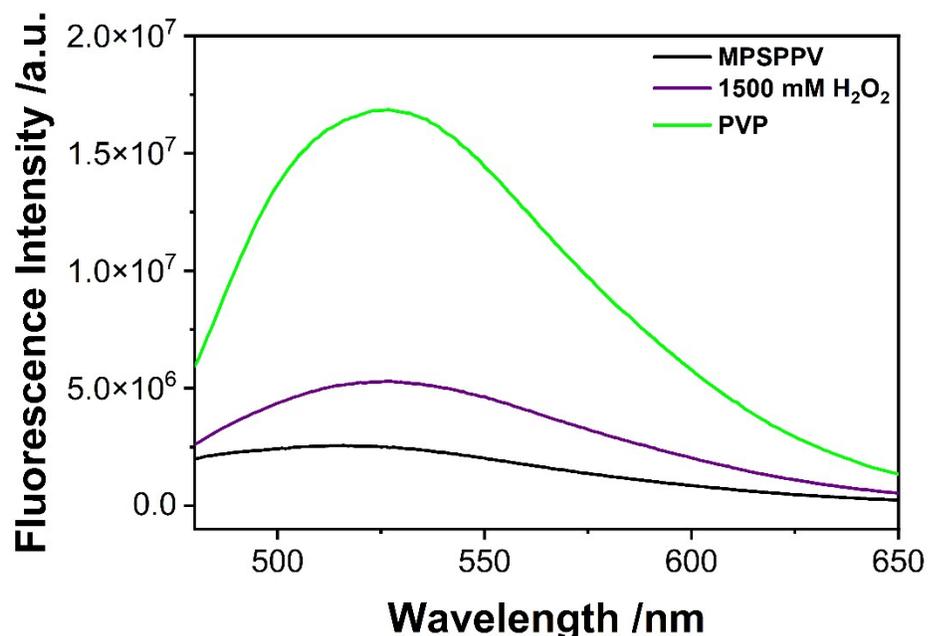
Figure S6: Fluorescence enhancement of MPS-PPV upon adding 1500 mM of hydrogen peroxide followed by the addition of 0.0050 mg/mL of PVP 3500 g/mol. The measurements were done in a pH = 7 buffer solution of 10 mM HEPES. Samples were excited at 450 nm and the emission spectra were collected at 20°C.

Control Experiments with Non-Complexing Polymer (Polyethylene Glycol).

To evaluate whether the observed fluorescence enhancement originates from the distinctive dual functionality of PVP rather than from a generic polymeric effect, control experiments were performed using polyethylene glycol (PEG, MW = 8,000 g/mol). PEG was chosen as a water-soluble reference polymer because it possesses comparable solubility to PVP but lacks carbonyl groups capable of interacting with H_2O_2 and does not form complexes with the oxidant. Under otherwise identical experimental conditions, including polymer concentration, H_2O_2 concentration, buffer composition, and pH, the MPS-PPV/PEG system displayed only a two-fold increase in fluorescence intensity upon H_2O_2 addition. This response is markedly weaker than the six-fold enhancement observed in the presence of PVP (Figure 5). The substantial difference between the two systems demonstrates that the enhanced fluorescence cannot be explained by nonspecific polymer effects such as increased viscosity, molecular crowding, or general stabilization. Instead, these findings hilight the critical role of PVP's moieties in H_2O_2 complexation and stabilization of oxidized polymer fragments, which collectively enable the pronounced turn-on fluorescence response.
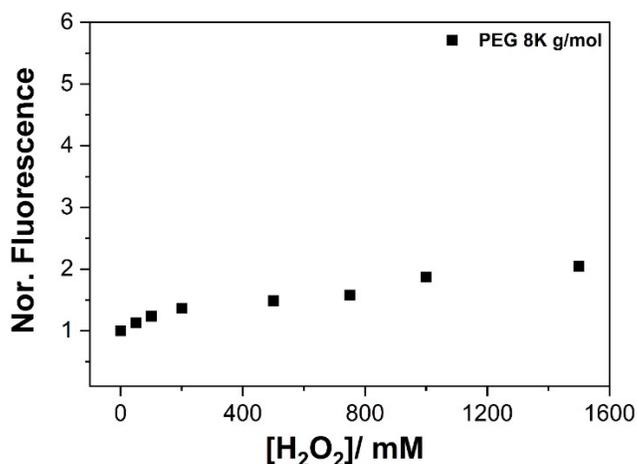

Figure S7: Fluorescence intensity changes of 12.5 µg/ml MPS-PPV are shown upon incremental additions of hydrogen peroxide (0-1500 mM) in the presence of 0.0050 mg/mL of PEG 8K g/mol. The measurements were done in a pH = 7 buffer solution of 10 mM HEPES. Samples were excited at 450 nm and the emission spectra were collected at 480 nm at 20°C. Symbols are added for visual aid.

Selectivity Studies: Response to Alternative Oxidants

To assess the selectivity of the MPS-PPV/PVP sensor for H_2O_2 over other oxidizing species that may be present in biological or environmental samples, we tested the fluorescence response to hypochlorous acid (HOCl) and tert-butyl hydroperoxide (TBHP). These oxidants were selected because HOCl is a biologically relevant oxidant produced by myeloperoxidase, while TBHP is a commonly used organic peroxide model compound. Solutions containing MPS-PPV/PVP were treated with either HOCl or TBHP under identical conditions to those used for H_2O_2 sensing. As shown in Figure S8 neither HOCl nor TBHP produced significant fluorescence enhancement, in stark contrast to the pronounced response observed for H_2O_2 at equivalent concentrations. This high selectivity for H_2O_2 is consistent with our mechanistic proposal that hydroxyl radicals attack the conjugated polymer backbone reducing the non-emissive sites, thus increasing the fluorescent intensity.
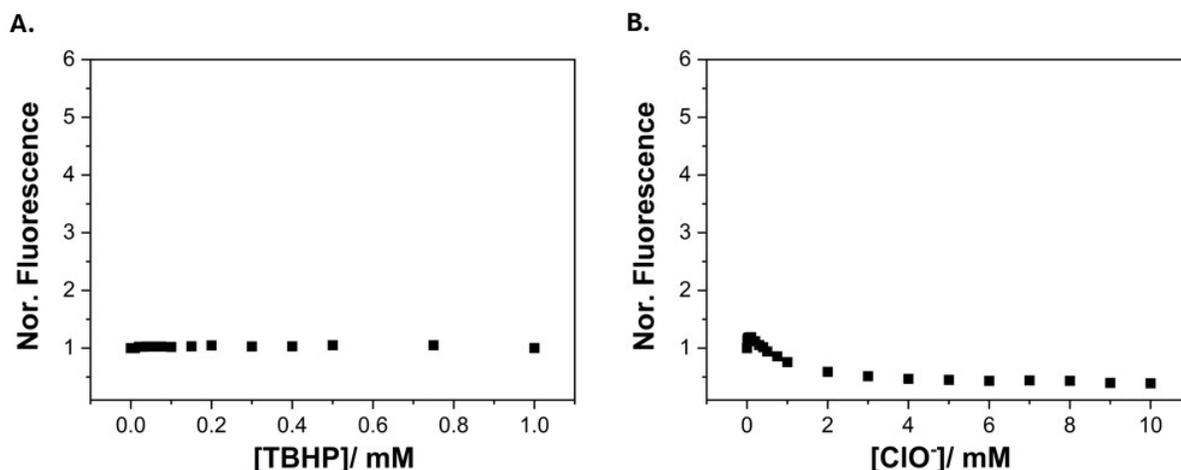

Figure S8: Fluorescence intensity changes of 12.5 µg/ml MPS-PPV are shown upon incremental addition of (A) TBHP (0-1 mM) and (B) HClO (0-10 mM) in the presence of 0.0050 mg/mL PVP 3500 g/mol. The measurements were done in a pH = 7 buffer solution of 10 mM HEPES. Samples were excited at 450 nm and the emission spectra were collected at 520 nm at 20°C.

Changes in the Fluorescent Signal upon the Addition of Hydrogen Peroxide.

The fluorescent intensity of PVP/MPS-PPV shifts to the blue upon the addition of hydrogen peroxide, with the disappearance of the red species. The samples were tested under the same experimental conditions in the same medium (pH = 7 buffer solution of 10 mM HEPES). The blue shift showcases the reduction of the conjugated length of MPS-PPV upon its oxidation by hydrogen peroxide. Changes in the absorbance was also observed with the increase in the hydrogen peroxide concentrations: a decrease in the measured intensity and a shift in the max absorbance.
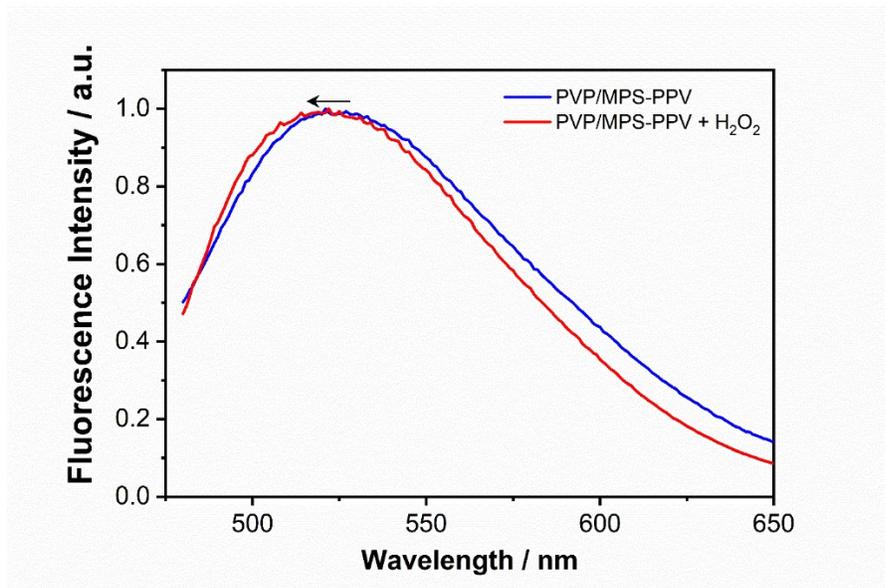
Figure S9: Emission spectra of PVP/MPS-PPV before and after the addition of hydrogen peroxide. The measurements were done in a pH = 7 buffer solution of 10 mM HEPES. Samples were excited at 450 nm at 20°C.
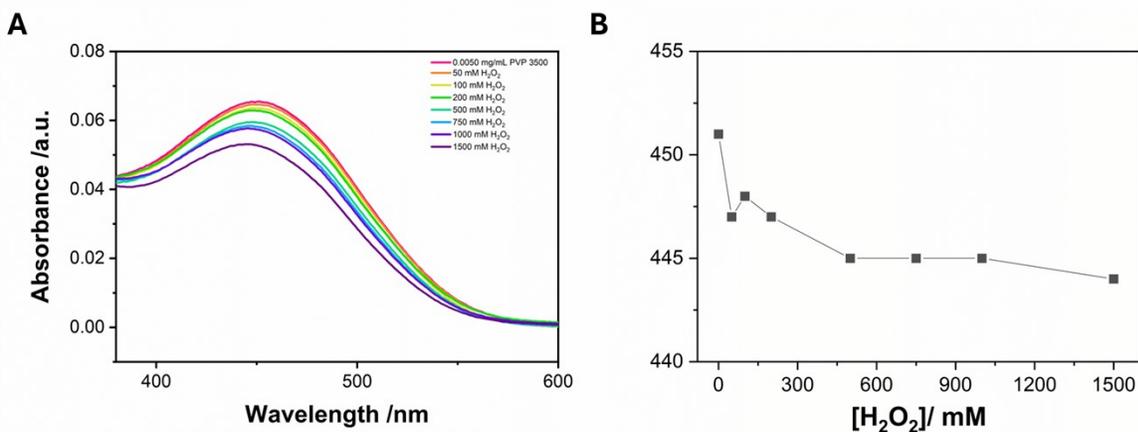

Figure S10: Changes in the absorbance upon incremental addition of hydrogen peroxide. The measurements were done in a pH = 7 buffer solution of 10 mM HEPES.

Dynamic Light Scattering Analysis of Polymer Fragmentation

To investigate structural changes in the MPS-PPV/PVP complex upon H_2O_2 exposure and verify our hypothesis of oxidative backbone fragmentation, we performed dynamic light scattering (DLS) measurements before and after prolonged H_2O_2 treatment. The rational for this experiment was to determine whether oxidation causes polymer chain cleavage (expected to decrease particle size). DLS analysis revealed that the average hydrodynamic diameter of the MPS-PPV/PVP complex decreased from approximately **3237** nm before H_2O_2 addition to

approximately **2120** nm after extended exposure to H_2O_2 (Table S2). This substantial reduction in particle size provides direct evidence of oxidative polymer backbone fragmentation, consistent with hydroxyl radical-mediated chain scission. This observation supports our central mechanistic hypothesis: PVP stabilizes the oxidized polymer fragments in solution, preventing the aggregation-induced fluorescence quenching that typically plagues CPE-based oxidant sensors and instead enabling the observed turn-on fluorescence response.

Table S2: Changes in the hydrodynamic diameter of MPS-PPV/PVP in the presence of 1500 mM hydrogen peroxide (A) at t0 = 0 and (B) at t = 24 hours.

Solution	Hydrodynamic Diameter (nm)
MPS-PPV/PVP + H_2O_2 at $t_0 = 0$	3237
MPS-PPV/PVP + H_2O_2 at t = 24 hours	2120

Effect of Divalent Cations: Magnesium Ion Studies

To further investigate the role of electrostatic interactions in sensor performance, we examined the effect of magnesium ions (Mg^{2+}), another physiologically important divalent cation, on the H_2O_2 sensing response. As shown in Figure S11, Mg^{2+} produced a dose-dependent reduction in sensor response similar to that observed with Ca^{2+}.
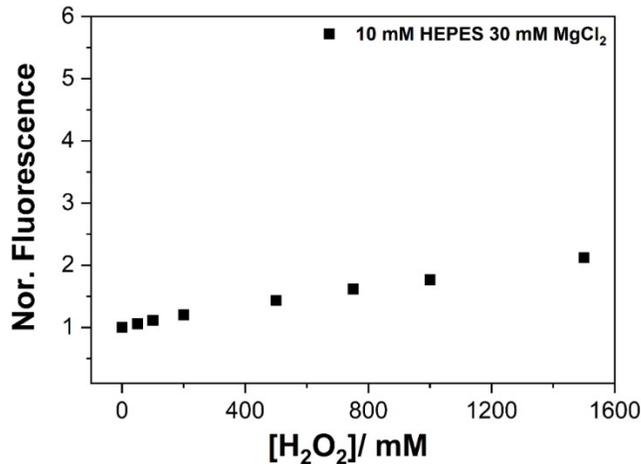

Figure S11: Fluorescence intensity changes of 12.5 µg/ml MPS-PPV are shown upon incremental additions of hydrogen peroxide (0-1500 mM) in the presence of 0.0050 mg/mL of PVP 3500 g/mol. The measurements were done in a pH = 7 buffer solution of 10 mM HEPES and 30 mM $MgCl_2$. Samples were excited at 450 nm and the emission spectra were collected at 480 nm at 20°C.

Hydroxyl Radical Quenching Mechanism of Ascorbic Acid:

Ascorbic acid scavenges hydroxyl radicals via a sequential two-step process. In the initial step, interaction with a hydroxyl radical produces an ascorbyl radical intermediate, which subsequently reacts with a second hydroxyl radical to yield dehydroascorbate. As the result, the fluorescence intensity decreases in the presence of ascorbic acid, which supports the suggested mechanism.
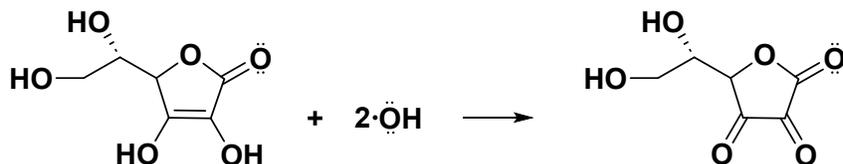

Figure S12: The chemical reaction for the quenching of free radicals by ascorbic acid.

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