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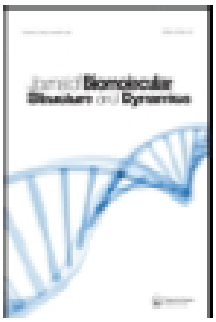

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# Elucidating the chemical and biochemical applications of *Citrus sinensis* mediated silver nanocrystal

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# A benzothiazole-conjugated hemicyanine dye as a ratiometric NIR fluorescent probe for the detection and imaging of peroxynitrite in living cells†

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Herein, we report a new red-emitting ratiometric fluorescent probe containing a benzothiazole-hemicyanine scaffold (BPVI) linked with a C=C double bond, which is highly selective and sensitive for the detection of peroxynitrite ($ONOO^-$) in biological systems. The dual colorimetric and ratiometric fluorescent response of the probe BPVI was due to the peroxynitrite-induced oxidation of the central double bond, which inhibited the intramolecular charge-transfer (ICT) process. The probe BPVI showed a rapid response to $ONOO^-$ over other reactive ROS/RNS at room temperature, and an attractive ratiometric fluorescence change from contessa (red region) to sapphire blue was observed in the presence of $ONOO^-$, which was well justified in terms of ESIPT and ICT. Moreover, in the presence of competitive ROS species, our probe did not show any significant changes in both color and emission intensity ratio ($I_{630}/I_{700}$); this proved the high selectivity and sensitivity of the probe BPVI towards $ONOO^-$, with a very low limit of detection (LOD, 37.0 nM). To verify the electronic properties of the BPVI probe, DFT and TDDFT calculations were performed. Moreover, it was found that the auto-fluorescence of biomolecules in living systems and aqueous solution did not significantly affect the detection procedure; the utility of the BPVI probe in the detection of $ONOO^-$ in live cells was also indicated using adenocarcinomic human alveolar basal epithelial cells.

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## Introduction

Peroxynitrite ($ONOO^-$) is a highly reactive powerful oxidant, which is endogenously produced by the diffusion-controlled coupling reaction of superoxide radical anions ($O^{2\cdot-}$) and nitric oxide ($NO$)[1] at the rate of ~1010 $M^{-1}$ $s^{-1}$ without any requirement of enzymatic catalysis.[2–4] Among the strong abilities of peroxynitrite are its oxidizing and nitrating capabilities, by which it can cause the oxidation of multiple molecules *via* direct or highly reactive second-generation radicals. This process can help in the development of a structural correction and modifications of dysfunctions in nucleic acids, proteins and lipids with significant cytotoxic importance.[5] *Via* an extreme control over the level of $ONOO^-$, it can exert a positive influence on cellular functions such as signal transduction, redox homeostasis and the immune response.[7–9] Peroxynitrite at high levels can cause cardiovascular disease, inflammation, Alzheimer's disease, multiple sclerosis, Parkinson's disease, traumatic brain injury, Huntington's disease,[10–12] and neurodegenerative disorders.[13] Considering the urgent demand and significant interest in the development of $ONOO^-$-specific sensing systems, it is highly desirable to produce meaningful quantification tools. Consequently, the exploitation of ratiometric fluorescent techniques for the selective and sensitive detection of peroxynitrite is the prime focus of this article.

In the last few years, a number of fluorescent probes have been reported for the selective detection of $ONOO^-$. These reported probes contain various oxidisable functional groups such as boronates,[14–23] active ketones,[24] hydrazides,[25–28] 4-amino-and 4-hydroxybenzenes,[27–33] selenium[33–36] and tellurium species[37,38] and others.[39–52] To detect the fluorescence of peroxynitrite, boronate-based fluorescent probes have been fabricated by some groups, namely Kim and James, for peroxynitrite sensing.[20,24] Many chemosensors in which $ONOO^-$ reacts with activated ketones to form dioxiranes have been developed by Yang *et al.*[24,47] A three-channel fluorescent probe capable of differentiating $ONOO^-$ from hypochlorite was formulated by

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