Electronic Supplementary Material (ESI) for New Journal of Chemistry. This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2019

# N-arylated bisferrocene pyrazole for dual-mode detection of

## hydrogen peroxide: AIE-active fluorescent "turn on/off" and

### electrochemical non-enzymatic sensor

Ezhumalai David,<sup>a</sup> Thamodharan Viswanathan,<sup>a1</sup> Selvam Prabu<sup>a1</sup> and Nallasamy Palanisami<sup>\*a</sup> <sup>a</sup>Department of Chemistry, School of Advanced Sciences, Vellore Institute of the Technology, *Vellore 632 014, Tamil Nadu, India.* Corresponding author: E-mail: <u>palanisami.n@qmail.com</u>; Tel: +91 9842639776. <sup>a1</sup>These authors contributed equally to this work

Supporting Information for New Journal of Chemistry

Table of Contents							
1.	Experimental	1					
2.	Reproducibility and stability	2					
3.	Solvatochromic data [ũmax (cm <sup>-1</sup> ) of the charge transfer band] for N–arylated bisferrocene pyrazole in different solvents with $\pi^*$ values by kamlet and Taft.						
4.	<sup>1</sup> H, <sup>13</sup> C, <sup>19</sup> F NMR and GC-Mass spectra of N-						
	arylated bisferrocene pyrazole 1-4						
5.	EI-Mass spectra of N-arylated bisferrocene pyrazole 1-4	S11-S14					
6.	FT-IR and absorbance, emission spectra						
	N-arylated bisferrocene pyrazole 1-4						
7.	UV-Vis absorption spectra of the N-arylated bisferrocene pyrazole						
	1-4 in different Polarity solvent (10 <sup>-4</sup> M)						
8.	Fluorescence emission spectra of N-arylated bisferrocene pyrazole						
	<b>1-4</b> AIE- fluorgens in CH <sub>3</sub> CN/water mixture (40:60) excited at 240 nm						
	with different concentrations of $H_2O_2$ (0-50µl)						
9.	Cyclic voltammetry of the N-arylated bisferrocene pyrazole 2 and 4						
	modified SPE electrode in 0.1 M PBS (pH 7.0) containing 10 $\mu M$ $H_2O_2$						
	at different scan rates. Inset is the relationship between the peak						
	current density and the $H_2O_2$ Concentration.						
10.	Electrochemical stability test in modified screen-printed electrode for						
	compound 1 and 3.						

#### Experimental

#### 1. Optimization of the experimental conditions

#### Optimized Electrochemical detection of H<sub>2</sub>O<sub>2</sub>

To optimize the working parameters involving the electroreduction of  $H_2O_2$  by the modified screen-printed electrode. Experiments were performed in  $1.0 \times 10^{-4}$  M in 0.1 M phosphate buffer during repetitive additions of  $H_2O_2$  and the influence of pH and applied potential are examined. Due to the mass-transport rate to a modified screen-printed electrode is very fast, stirring is required after injection of the  $H_2O_2$  solutions to a homogeneous mixture. The current responses obtained upon addition of  $H_2O_2$  over a pH range comprising 5 to 8, the potential being set at -0.25V to 0.0 V (1), 0.4V to 0.5V (3). A linear relationship between current and concentration was noticed at all pH values, with higher sensitivity at pH 7.0 and this value was selected for further sensing studies.

#### Fluorescent detection of H<sub>2</sub>O<sub>2</sub>.

The stock solution 100mM made freshly dissolving 11 mM of 30%  $H_2O_2$  in 989 mM deionized water. The growth solution consisted of  $1.0 \times 10^{-4}$  M in 0.1 M phosphate buffer solution (PBS, pH 7) and different concentrations of  $H_2O_2$  (10-50µM). The fluorescence intensities were recorded with excitation at 237nm for **1** and 242nm for **2** respectively.

#### 2. Reproducibility and stability

The electrode reproducibility was investigated for Bis-Fc-Pz (**1** and **3**) modified SPEs by preparing three electrodes under the same deposition conditions. Triplicate determination of  $10\mu$ M H<sub>2</sub>O<sub>2</sub> with each electrode was used in order to estimate the reproducibility. The reproducibility was expressed in relative standard deviation was found compound **1** for 2.88 % modified SPE. In the same manner, a relative standard deviation of compound **3** for 3.41 % was obtained for the modified SPE. The results showed good reproducibility of the electrodes.

Although the operational stability of Bis-Fc-Pz/SPE was verified by monitoring cyclic voltammetry response of the modified electrode in the potential range of -1 to +1 V for 20 continuous cycles (Fig. S26, S27), the electrode exhibits remarkable stability without any significant change in the peak current or peak potential. When not in use, the modified electrode was kept at room temperature. For the long-term stability of Bis-Fc-Pz/SPE was also investigated in the absence and presence of H<sub>2</sub>O<sub>2</sub> on every week over the period of 30 days. The electrode retained 90% of its initial current response for H<sub>2</sub>O<sub>2</sub> after two week of storage and could maintain 80% of current even after 30 days of storage. The observed results make Bis-Fc-Pz/SPE as a highly promising sensor towards the nonenzymatic determination of H<sub>2</sub>O<sub>2</sub>.

Solvants	a	ß	<del></del> *	Δῦmax			
Solvents	u	þ	Л	(1)	(2)	(3)	(4)
THF	0.00	0.55	0.58	33.89	34.48	33.67	34.12
DCM	0.13	0.10	0.82	34.36	34.60	34.60	34.72
CHCl₃	0.20	0.10	0.58	34.36	34.60	34.60	34.72
EtOAc	0.00	0.45	0.54	34.24	34.12	33.89	34.72
MeOH	1.00	0.66	0.69	34.12	34.48	34.24	34.60
EtOH	0.83	0.75	0.62	34.36	34.48	34.24	34.72
ACN	0.35	0.4	0.75	34.12	34.12	34.36	34.60
DMF	0.00	0.71	0.88	33.89	34.24	34.36	34.48
DMSO	0.00	0.76	1.00	34.12	34.48	34.36	34.36

**Table S1** Solvatochromic data [ $\tilde{u}$ max (cm<sup>-1</sup>) of the charge transfer band] for N-arylated bisferrocene pyrazole in different solvents with  $\pi^*$  values by kamlet and Taft.



Fig. S1 <sup>1</sup>H NMR spectrum of N-arylated bisferrocene pyrazole in CDCl<sub>3</sub>1



Fig. S2  $^{13}\text{C}$  NMR spectrum of N-arylated bisferrocene pyrazole in CDCl3 1



Fig. S3 <sup>1</sup>H NMR spectrum of N-arylated bisferrocene pyrazole in CDCl<sub>3</sub> 2



Fig. S4  $^{13}$ C NMR spectrum of N-arylated bisferrocene pyrazole in CDCl<sub>3</sub> 2



Fig. S5  $^{19}$ F NMR spectrum of N-arylated bisferrocene pyrazole in CDCl<sub>3</sub> 2



Fig. S6 <sup>1</sup>H NMR spectrum of N-arylated bisferrocene pyrazole in CDCl<sub>3</sub> 3



Fig. S7 <sup>19</sup>C NMR spectrum of N-arylated bisferrocene pyrazole in CDCl<sub>3</sub> 3



Fig. S8 <sup>1</sup>H NMR spectrum of N-arylated bisferrocene pyrazole in CDCl<sub>3</sub> 4



Fig. S9  $^{19}\text{C}$  NMR spectrum of N-arylated bisferrocene pyrazole in CDCl3 4



Fig. S10  $^{19}\text{F}$  NMR spectrum of N-arylated bisferrocene pyrazole in CDCl3 4



Fig. S11 El-Mass spectrum of N-arylated bisferrocene pyrazole 1



Fig. S12 EI-Mass spectrum of N-arylated bisferrocene pyrazole 2



Fig. S13 EI-Mass spectrum of N-arylated bisferrocene pyrazole 3



Fig. S14 EI-Mass spectrum of N-arylated bisferrocene pyrazole 4



Fig. S15 FT-IR spectrum of N-arylated bisferrocene pyrazole 1–4



Fig. S16 UV–Vis absorption spectrum of N-arylated bisferrocene pyrazole 1-4 in CH<sub>3</sub>CN solution ( $10^{-4}$ ) M



**Fig. S17** Fluorescence emission and excitation spectra of N-arylated bisferrocene pyrazole 1-4 in CH<sub>3</sub>CN solution  $10^{-4}$  M (excitation wavelength: 237(1 and 2), 242 (3 and 4))



**Fig. S18** UV-Vis absorption spectra of the N-arylated bisferrocene pyrazole **1** in different Polarity solvent  $(10^{-4} \text{ M})$ 



**Fig. S19** UV-Vis absorption spectra of the N-arylated bisferrocene pyrazole **2** in different Polarity solvent (10<sup>-4</sup> M)



**Fig. S20** UV-Vis absorption spectra of the N-arylated bisferrocene pyrazole **3** in different Polarity solvent (10<sup>-4</sup> M)



**Fig. S21** UV-Vis absorption spectra of the N-arylated bisferrocene pyrazole **4** in different Polarity solvent (10<sup>-4</sup> M)



**Fig. S22** Fluorescence emission spectra of N-arylated bisferrocene pyrazole **2** AIE-fluorgens in CH<sub>3</sub>CN/water mixture (40:60) excited at 240 nm with different concentrations of  $H_2O_2$  (0-50µl)



Fig. S23 Fluorescence emission spectra of N-arylated bisferrocene pyrazole 4 AIE-fluorgens in  $CH_3CN$ /water mixture (40:60) excited at 237 nm with different concentrations of  $H_2O_2$  (0-50µl)



**Fig. S24** Cyclic voltammetry of the N-arylated bisferrocene pyrazole (**2**) modified SPE electrode in 0.1 M PBS (pH 7.0) containing 5  $\mu$ M H<sub>2</sub>O<sub>2</sub> at different scan rates. Inset is the relationship between the peak current density and the H<sub>2</sub>O<sub>2</sub>Concentration.



**Fig. S25** Cyclic voltammetry of the N-arylated bisferrocene pyrazole (**2**) modified SPE electrode in 0.1 M PBS (pH 7.0) containing 5  $\mu$ M H<sub>2</sub>O<sub>2</sub> at different scan rates. Inset is the relationship between the peak current density and the H<sub>2</sub>O<sub>2</sub>Concentration.



Fig. S26 Electrochemical stability test in modified screen-printed electrode for compound 1.



Fig. S27 Electrochemical stability test in modified screen-printed electrode for compound 3.