

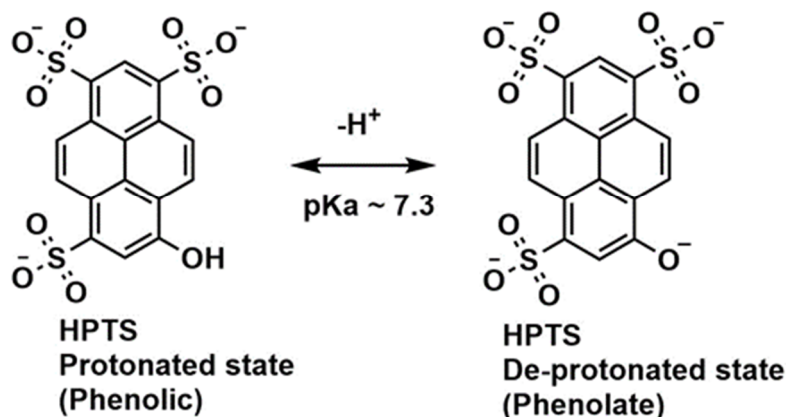
# Development of Neutral Red as a pH/pCO<sub>2</sub> Luminescent Sensor for Biological Systems

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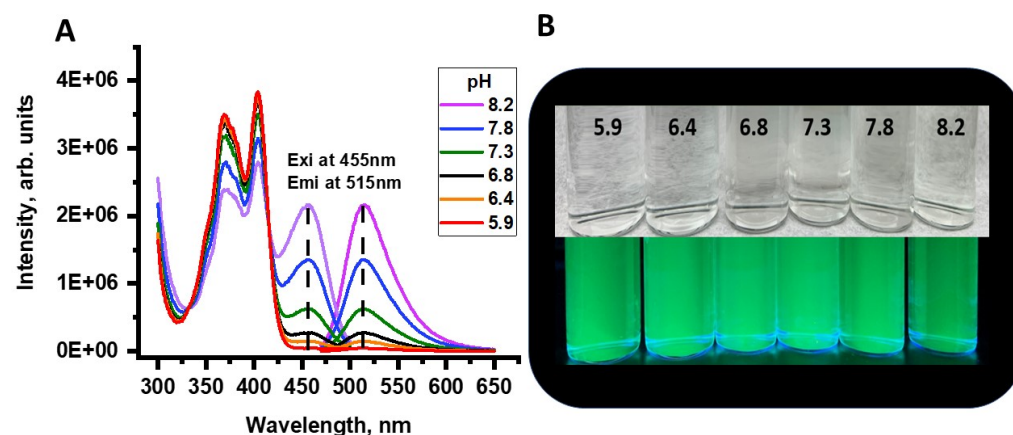
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**Scheme S1.** Chemical structure of HPTS molecule showing pKa structural variations at equilibrium.



**Scheme S2.** (A) Excitation and emission studies of HPTS in different pH phosphate buffer media ranging from 5.9 to 8.2. (B) The color of the solutions depicted in the picture, TOP: Under ambient light and BOTTOM: Under UV irradiation.

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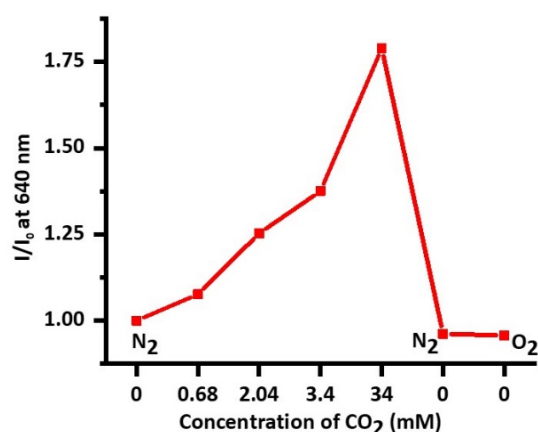
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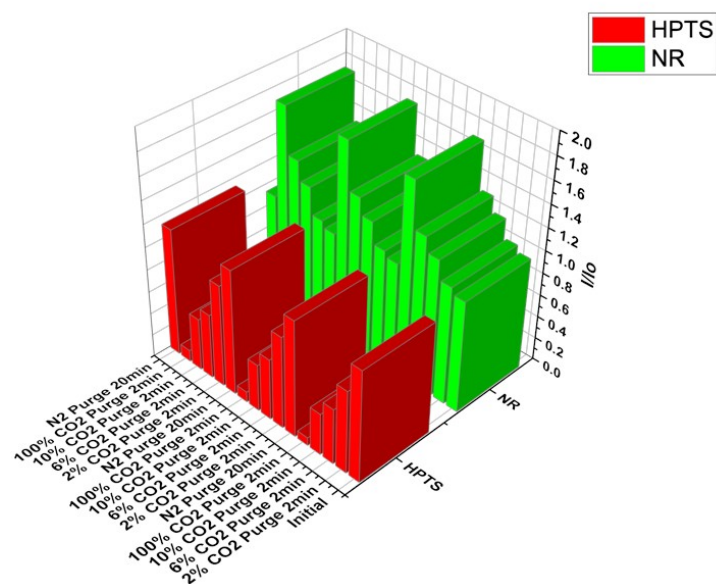
**Scheme S3.** Setup for the epifluorescence microscope. The components are labeled as 1. Zeiss Axio Observer (CAN-BUS) inverted microscope, 2. Power Supply, 3. Definite Focus module, 4. Temperature module, 5. CO<sub>2</sub> module, 6. O<sub>2</sub> module, 7. Heating device/humidity, 8. 100% CO<sub>2</sub> cylinder, 9. 100% N<sub>2</sub> cylinder, 10. Incubator chamber.

**Table S1.** Carbon dioxide sensitivity numbers obtained from absorption and fluorescence measurements. Fluorescence measurements are collected using spectrophotometer (fluorescence), and epifluorescent microscope (fluorescence\*).

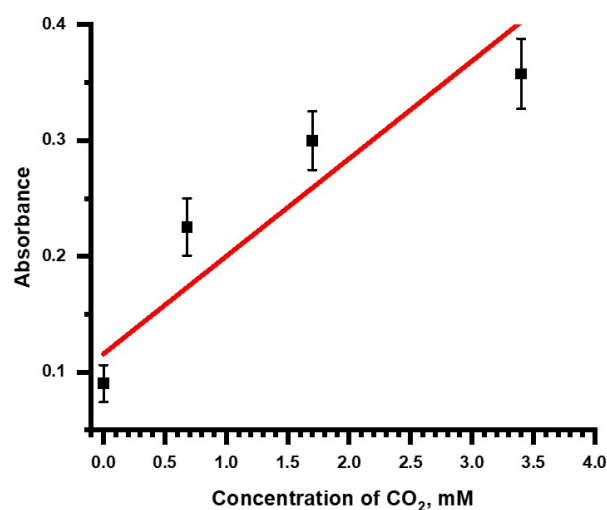
	Absorbance		Fluorescence		Fluorescence*	
	%Δ	Fold-Increase	%Δ	Fold-Increase	%Δ	Fold-Increase
0→0.68	149.500	2.495	88.298	1.883	33.806	1.338
0→1.7	232.100	3.321	128.323	2.283	N/A	N/A
0→2.04	N/A	N/A	N/A	N/A	62.468	1.625
0→3.4	296.000	3.960	161.100	2.611	N/A	N/A
0→8.5	365.200	4.652	176.205	2.762	N/A	N/A
0→17	385.8	4.858	176.354	2.764	N/A	N/A
0→34	433.6	5.336	199.335	2.993	115.265	2.153
0.68→1.7	33.106	1.331	21.256	1.213	N/A	N/A
0.68→2.04	N/A	N/A	N/A	N/A	21.4206	1.214
0.68→34	111.225	2.139	58.969	1.590	60.8784	1.609
2.04→34	N/A	N/A	N/A	N/A	32.4969	1.325
1.7→3.4	19.240	1.192	14.355	1.144	N/A	N/A
3.4→8.5	17.475	1.175	5.786	1.058	N/A	N/A
8.5→17	4.428	1.044	0.054	1.000	N/A	N/A
17→34	9.839	1.098	8.316	1.083	N/A	N/A



**Scheme S4.** Effect of O<sub>2</sub> gas purging on the emission intensity of NR buffer solution. Refer to last two points in the graph.



**Scheme S5.** Comparison of emission intensity changes at different CO<sub>2</sub> levels for HPTS and NR in phosphate buffer media.



**Scheme S6.** Linear fit analysis for the calculation of residual standard deviation and slope.

**Table S2.** Linear fit analysis for the calculation of residual standard deviation and slope.

Slope	0.08429±0.02309
Residual Standard Deviation	0.0593