

Supplementary

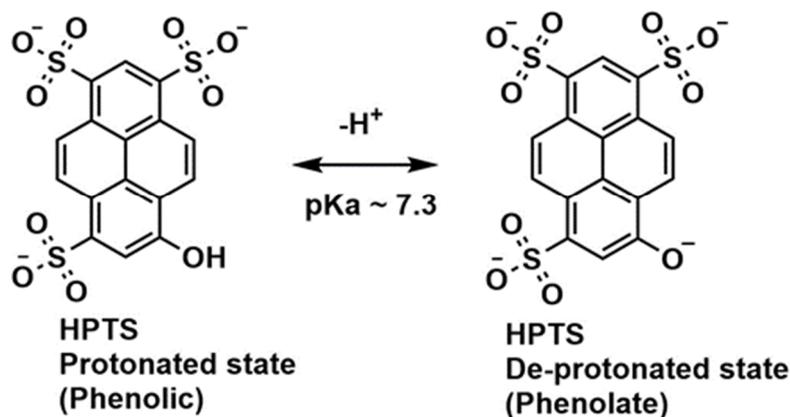
Development of Neutral Red as a pH/pCO₂ Luminescent Sensor for Biological Systems

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

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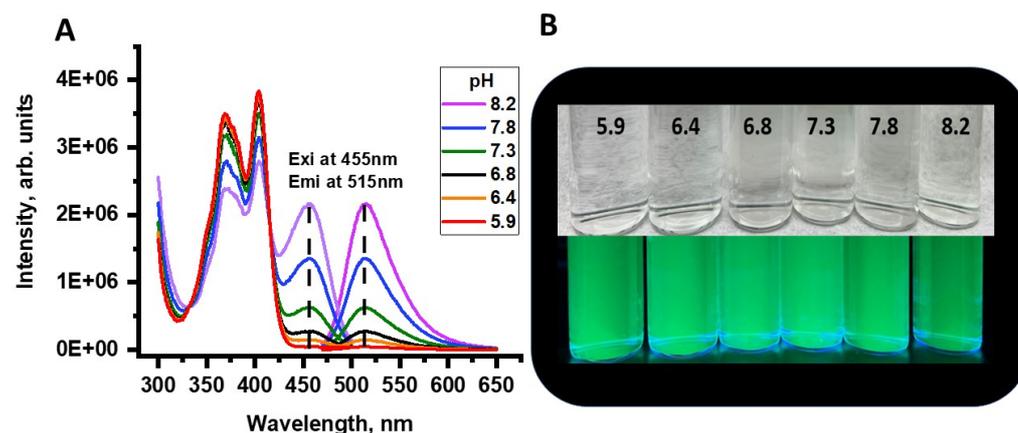
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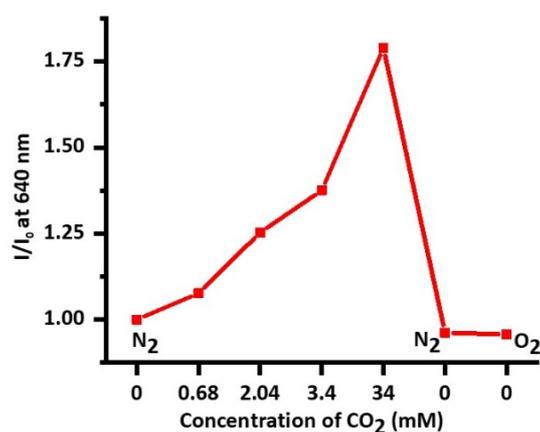
Scheme S2. (A) Excitation and emission studies of HPTS in different pH phosphate buffer media ranging from 5.9 to 8.2. (B) The color of the solutions depicted in the picture, TOP: Under ambient light and BOTTOM: Under UV irradiation.



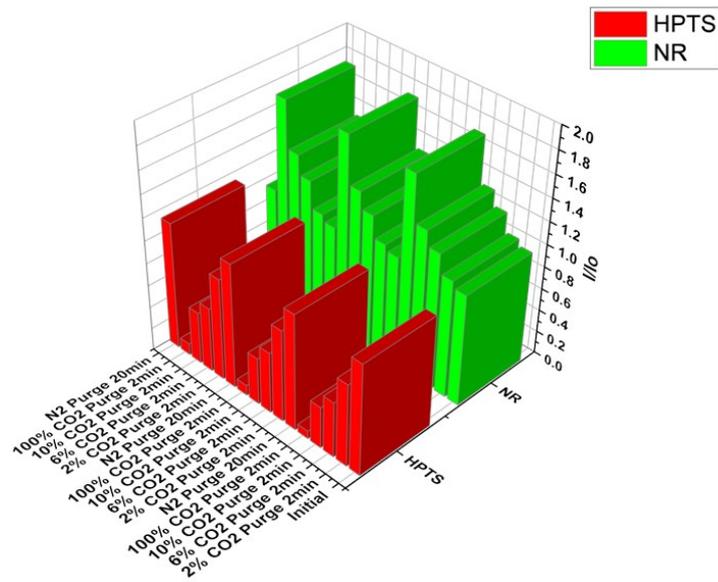
Scheme S3. Setup for the epifluorescence microscope. The components are labeled as 1. Zeiss Axio Observer (CAN-BUS) inverted microscope, 2. Power Supply, 3. Definite Focus module, 4. Temperature module, 5. CO₂ module, 6. O₂ module, 7. Heating device/humidity, 8. 100% CO₂ cylinder, 9. 100% N₂ cylinder, 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*).

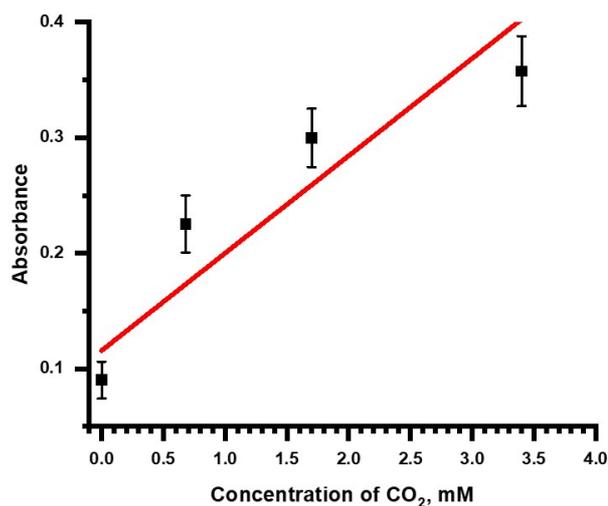
	Absorbance		Fluorescence		Fluorescence*	
	%Δ	Fold-Increase	%Δ	Fold-Increase	%Δ	Fold-Increase
0→0.68	149.500	2.495	88.298	1.883	33.806	1.338
0→1.7	232.100	3.321	128.323	2.283	N/A	N/A
0→2.04	N/A	N/A	N/A	N/A	62.468	1.625
0→3.4	296.000	3.960	161.100	2.611	N/A	N/A
0→8.5	365.200	4.652	176.205	2.762	N/A	N/A
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 O₂ 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 CO₂ 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