

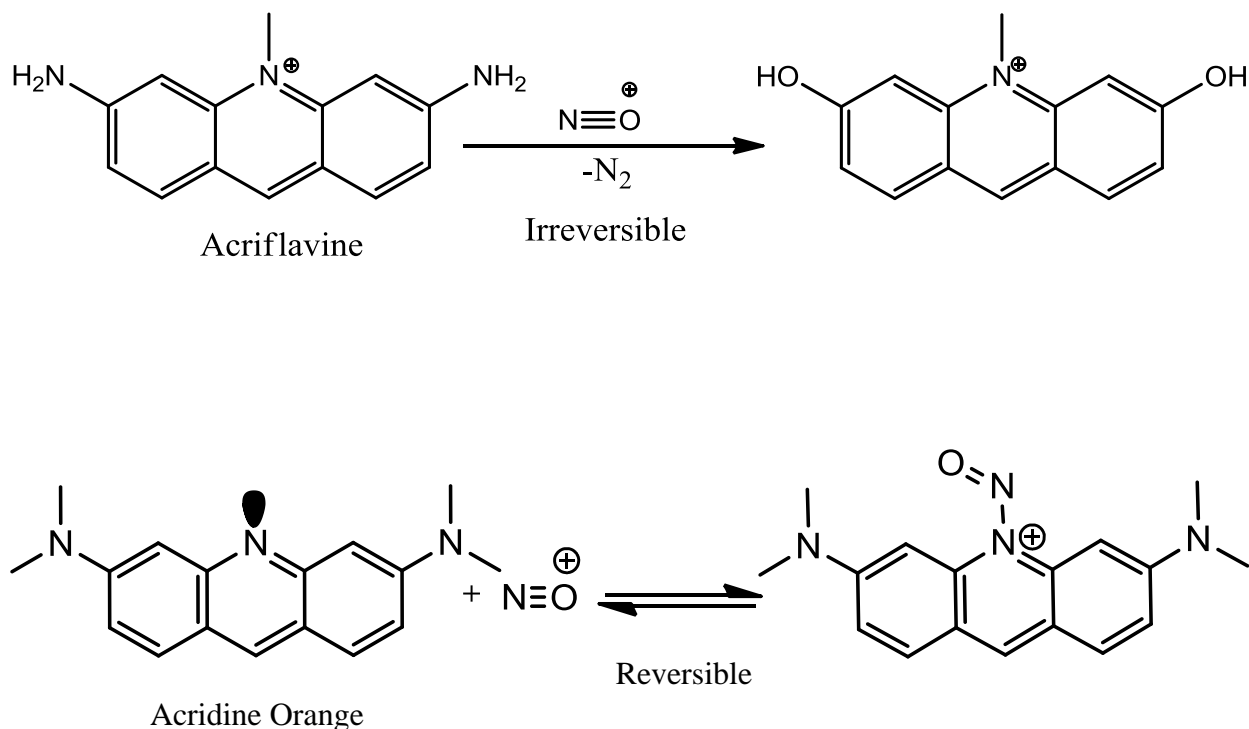
Supporting Information:

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**Photophysical Effects of Nitric Oxide and S-Nitrosocysteine on Acridine Orange: Use as Sequential Sensing Platform for NO, Cysteine, Cysteine-NO and Hg<sup>2+</sup> under Physiological Condition**

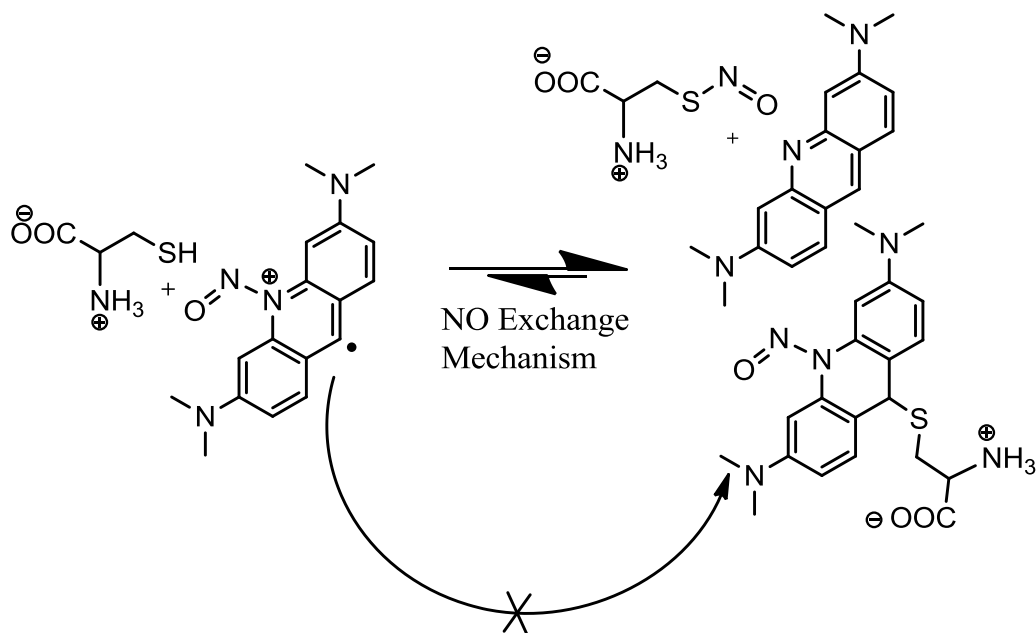
Kallol Bera, Barun Kumar Maity, Moupriya Nag, Manjur Oyasim Akram and Soumen Basak\*

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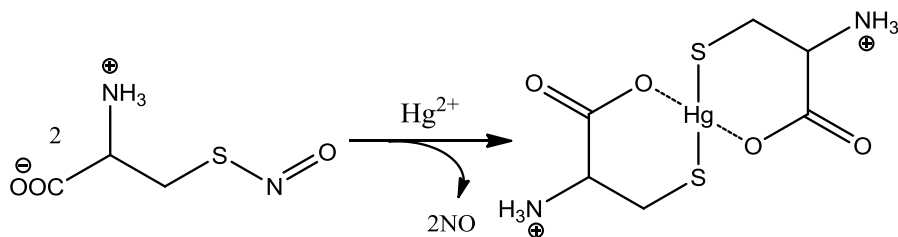


**Scheme S1:** Mode of Reaction of NO with Acriflavine and Acridine Orange

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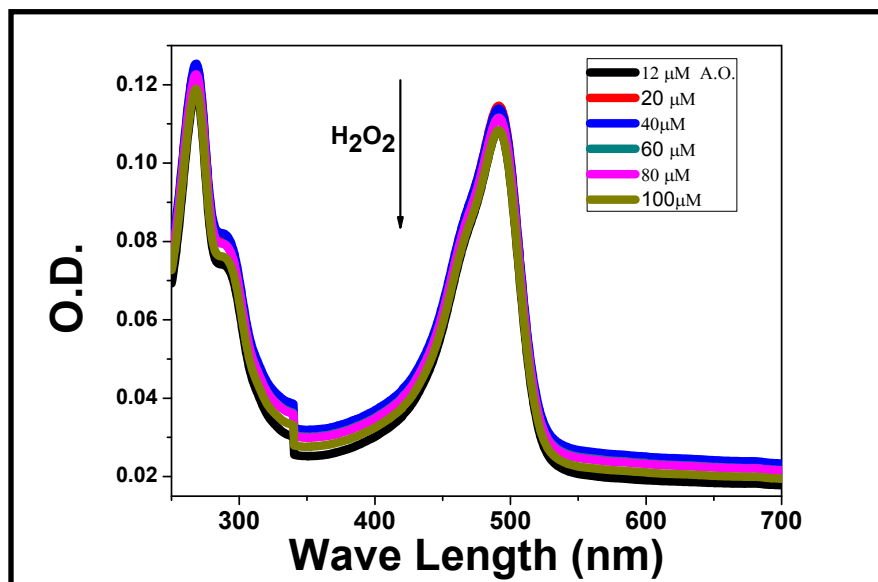


**Scheme S2:** A plausible NO exchange mechanism.

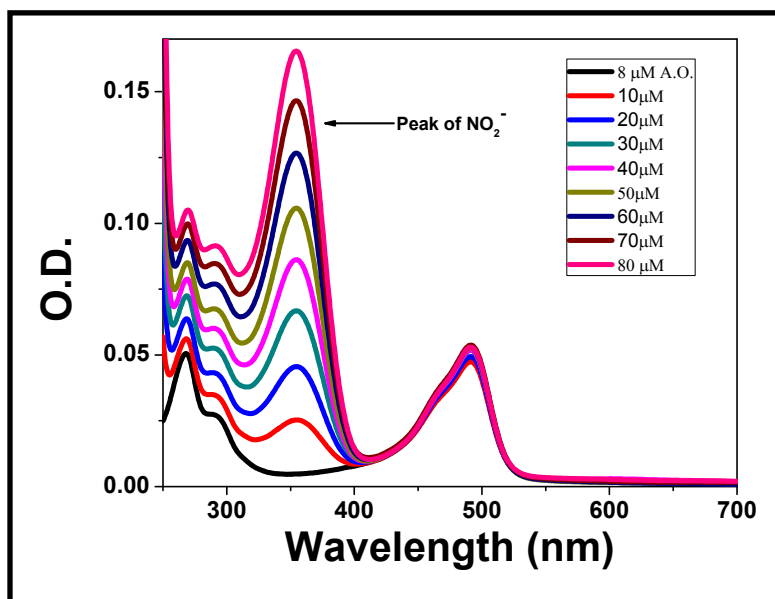


**Scheme S3:** Formation of  $\text{Hg}^{2+}$ -Cysteine complex

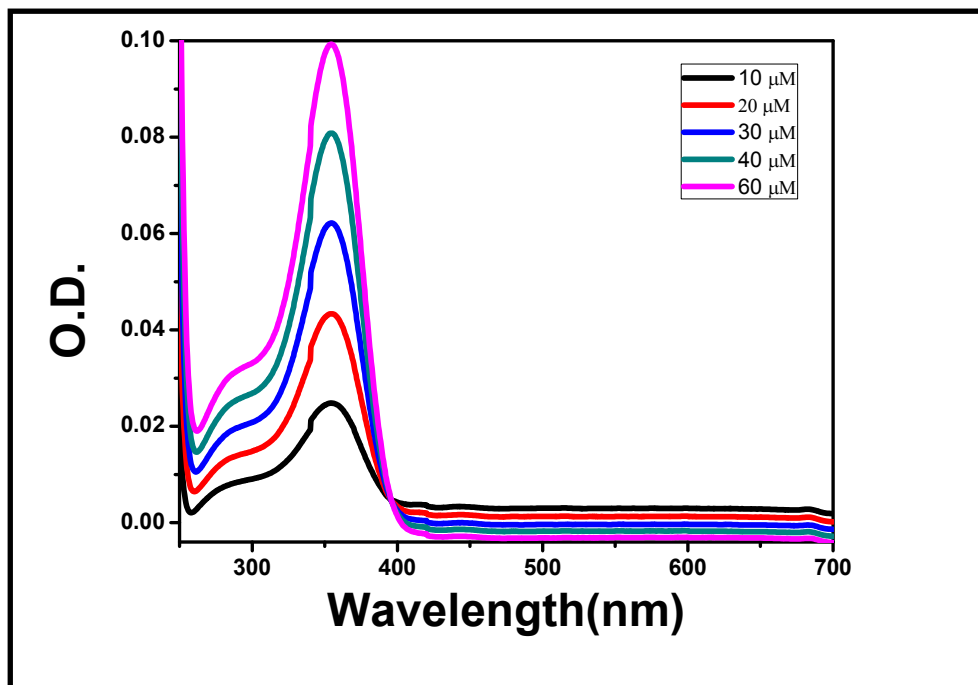
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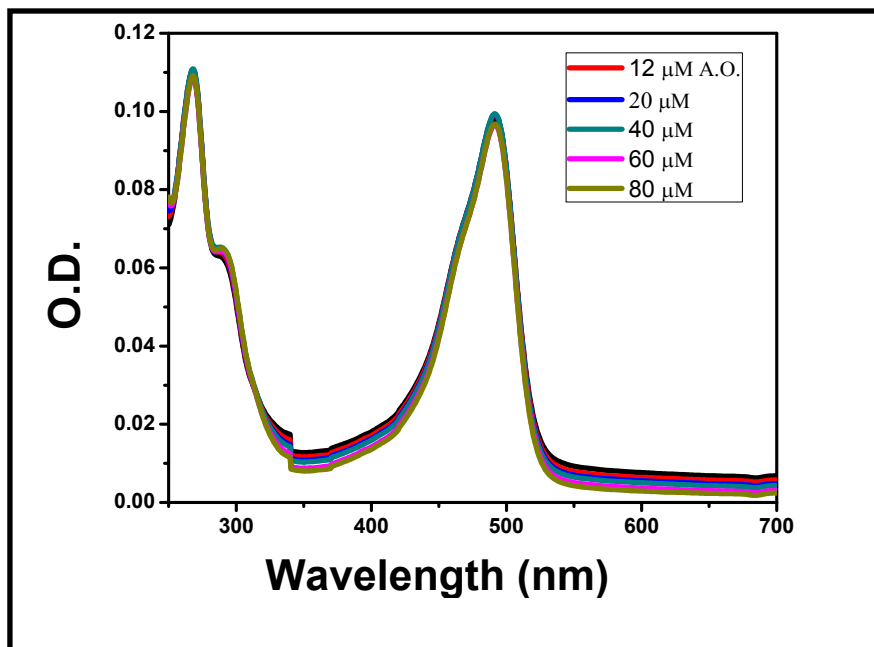
**Figure S1:** Titration of AO (12 μM) with increasing concentration of H<sub>2</sub>O<sub>2</sub> (20-100 μM).



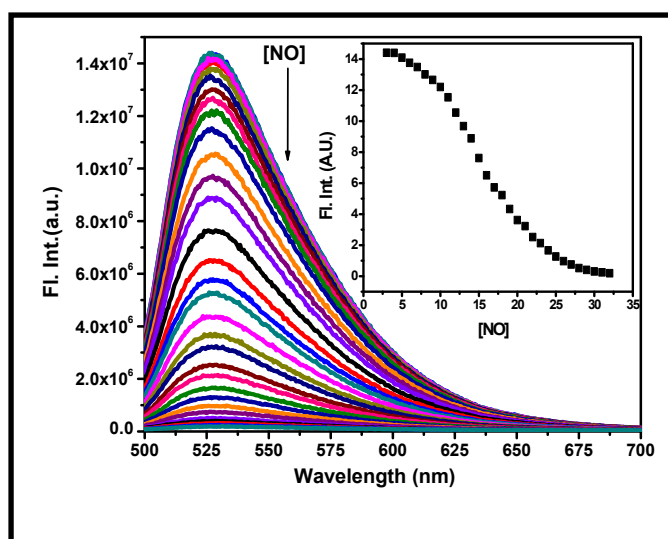
**Figure S2:** Titration of AO (8 μM) with increasing concentration NO<sub>2</sub><sup>-</sup> (10-80 μM).



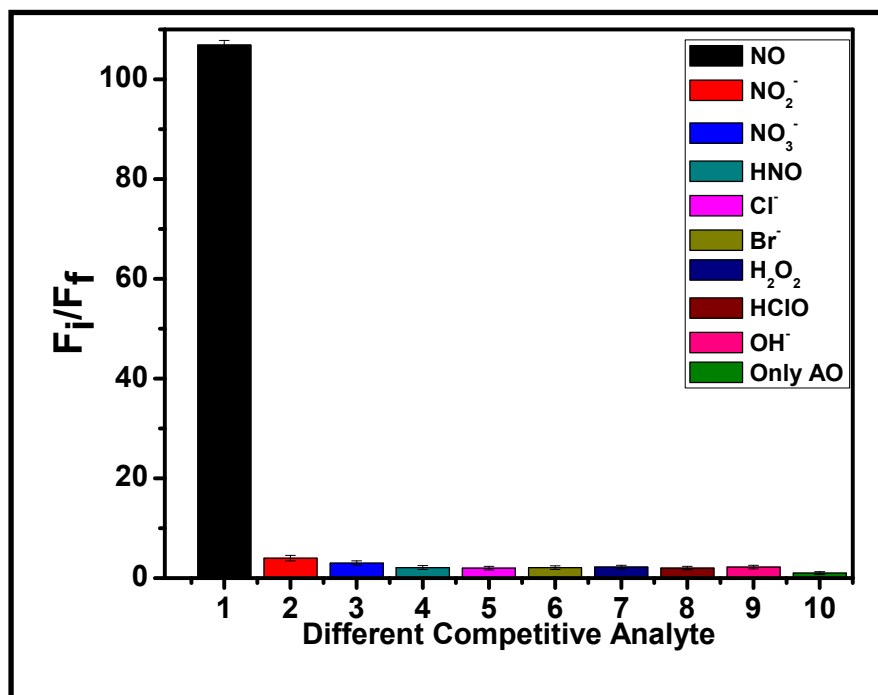
**Figure S3:** Absorption spectra of only  $\text{NO}_2^-$  (10-60  $\mu\text{M}$ ) in distilled water.



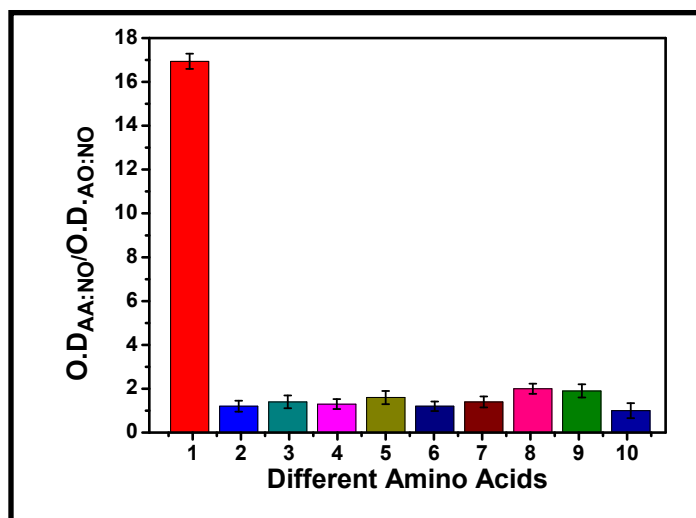
**Figure S4:** Titration of AO (12  $\mu\text{M}$ ) with increasing concentration  $\text{NO}_3^-$  (20-80  $\mu\text{M}$ ).



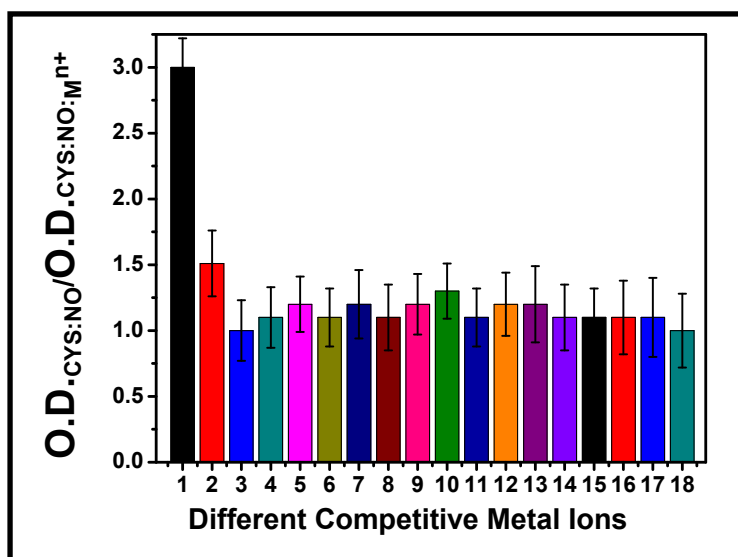
**Figure S5.** Fluorescence spectra of AO (10 μM) in presence of increasing concentration of NO (0-32 μM) in PBS at pH 7.2 at 25<sup>0</sup>C. Inset: Plot of fluorescence intensity at 527 nm vs number of equivalents of NO.



**Figure S6:** Relative decrease in fluorescence response at 527 nm (excitation at 491 nm) of AO (10 μM) in water in presence of 40 μM each of NO (1), NO<sub>2</sub><sup>-</sup> (2), NO<sub>3</sub><sup>-</sup> (3), HNO (4), OH<sup>-</sup> (5), Cl<sup>-</sup> (6), Br<sup>-</sup> (7), H<sub>2</sub>O<sub>2</sub> (8), ClO<sup>-</sup> (9), AO only (10).



**Figure S7 :** (b) Relative change in O.D. (at 336 nm) of AO:NO (10:32  $\mu\text{M}$ ) in pure water in presence of 40  $\mu\text{M}$  each of cysteine (1), glycine (2), aspartic acid (3), lysine (4), histamine (5), alanine (6), phenyle alanine (7), tyrosine (8), tryptophan (9) and 10:24  $\mu\text{M}$  AO:NO (10).



**Figure S8:** Relative decrease in O.D. (at 335 nm) of AO:Cys:NO (10:40:32  $\mu\text{M}$ ) in water in presence of 16  $\mu\text{M}$  of  $\text{Hg}^{2+}$  (1),  $\text{Cu}^{2+}$  (2),  $\text{Zn}^{2+}$  (3),  $\text{Ag}^+$  (4),  $\text{Pb}^{2+}$  (5),  $\text{Cd}^{2+}$  (6),  $\text{Ni}^{2+}$  (7),  $\text{Co}^{2+}$  (8),  $\text{Fe}^{2+}$  (9),  $\text{Mn}^{2+}$  (10),  $\text{Mg}^{2+}$  (11),  $\text{Ca}^{2+}$  (12),  $\text{Ba}^{2+}$  (13),  $\text{Li}^+$  (14),  $\text{K}^+$  (15),  $\text{Na}^+$  (16),  $\text{Cr}^{2+}$  (17) and Cys-NO (18).