Supporting Information

Water dispersed fluorescent organic aggregates for detection of picomolar ClO₄⁻ in water, soil and blood serum and atto g by contact mode

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1. Experimental Details

General Remarks:
All reagents were purchased from commercial suppliers (Aldrich, Across, SDFCL, Spectrochem etc.) and used without further purification. Solvents were purified and dried by standard methods prior to use. 1-(4-Biphenyl)benzimidazole (1) was synthesized by CuI, benzotriazole catalyzed N-arylation of benzimidazole with 4-bromobiphenyl as reported in literature. TLC analyses were performed on silica gel plates and column chromatography was carried out over silica gel (100 - 200 mesh).

¹H and ¹³C NMR spectra were recorded on BRUKER Bio spin AVANCE-III FT NMR HD-500 using CDCl₃ or DMSO-d₆ as solvent and tetramethylsilane (TMS) as internal standard. Data are reported as follows: chemical shifts in ppm relative to the tetramethylsilane (TMS) as an internal
standard, coupling constants J in Hz; multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet). HRMS spectra were recorded on Brucker MicroToff/QII. The time resolved fluorescence spectra were recorded with ISS Chronos-BH time-resolved fluorescence spectrophotometer. DLS experiments were performed on Malvern-Zetasizer.

For UV-Vis, fluorescence, DLS and FESEM studies, all the solutions were prepared in deionized water obtained from ULTRA UV/UF Rions Lab Water System Ultra 370 series.

**UV-Vis and fluorescence studies.** UV-Vis studies were carried out on Shimadzu UV-2450 machine using slit width of 1.0 nm and matched quartz cells. The cell holder was thermo stated at 25.0 ± 0.2°C. The fluorescence experiments were performed on Shimadzu 2450 and BH-CHRONOS fluorescence spectrophotometers with a quartz cuvette of path length 1 cm. The cell holder was thermo stated at 25.0 ± 0.2°C. All absorption and fluorescence scans were saved as ASCII files and were further processed in Microsoft Excel™ to produce all graphs shown. The stock solutions of CS-1 and CS-2 (1 mM) were prepared in DMSO. For experiments with CS-1, 3 ml of the solution containing CS-1 (60 µL, 0.1mM) and 2.94 mL HEPES buffer (0.05 M, pH 7.4) were taken in cuvette. Stock solutions (0.1 M) of sodium salts (Na⁺X⁻), where X = CN⁻, F⁻, Cl⁻, Br⁻, I⁻, ClO₄⁻, NO₃⁻, SO₄²⁻, HSO₄⁻, SCN⁻, AcO⁻ and H₂PO₄⁻ were prepared in deionized millipore water and were diluted as required. During titration experiments, after addition of each aliquot of ClO₄⁻ solution, the solution was allowed to remain stable for 2 minutes.

**DLS and SEM sample preparation.** The stock solutions of CS-1 (1 mM, DMSO) and water were filtered through 0.02 micron filter membrane to remove interfering impurities. Solutions of CS-1 (H₂O - 2% DMSO) and its mixtures with different concentrations of ClO₄⁻ were prepared. 2 ml of each of these solutions was taken in glass cuvette to record the DLS spectrum at 25 °C. The solutions prepared for DLS experiments were used for preparing thin films for SEM recording. 10 µL of each of the solution was added on the pre-cleaned surface of the separate glass slide and was allowed to dry in the incubator at 25 °C. The dried films were coated with Au before recording SEM images. The scanning electron microscope (SEM) images were obtained with a field emission scanning electron microscope SEM JEOL JSM-6610LV.

**Paper strips preparation.** Whatman filter paper strips (1 cm x 1 cm) were dipped into aqueous DMSO solution of CS-1 (0.1 mM) and were dried under vacuum at room temperature. These paper strips were used for naked eye (under 365 nm light) visualization of 10⁻¹⁵ to 10⁻⁹ M ClO₄⁻.
solution. The $10^{-15}$, $10^{-14}$, $10^{-13}$, $10^{-12}$, $10^{-11}$ M solutions of ClO$_4^-$ and $10^{-11}$ M, $10^{-9}$ M solution of all other anions were prepared in water and 6 μl aliquot of each of these solutions was added on separate paper strip. For control experiment, drop of water alone was added on the CS-1 coated paper strip. The fluorescence spectra of paper strips bearing ClO$_4^-$ ions were also recorded using front surface steady-state fluorescence on ISS Chronos BH fluorescence spectrophotometer.

**Blood Serum.** A real blood sample of a medically fit person was used for the experiments. The blood serum was isolated by centrifugation of the fresh blood sample after fasting at 4000 rpm for 30 min at 4 °C. The stock solution of the blood serum was prepared in 10 ml volumetric flask by diluting 1ml of serum with HEPES buffer (0.05 M) at pH 7.40.

**Quantum yield calculations**. Fluorescence quantum yields ($\Phi_s$) were determined by using an optically matching solution of QHS ($\Phi_r = 0.54$ in 0.1 M H$_2$SO$_4$) as standard at excitation wavelength of 290 nm and quantum yield is calculated using equation

$$\Phi_s = \Phi_r \times \frac{1}{10^{-\text{As}} - 1} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r}$$

$\Phi_s$ and $\Phi_r$ are the radiative quantum yields of sample and the reference, respectively. $D_s$ and $D_r$ are the respective areas of emission for the sample and reference, respectively. $A_s$ and $A_r$ are the absorbance; $L_s$ and $L_r$ are the lengths of the absorption cells; $N_s$ and $N_r$ are the refractive indices of the sample and reference solutions, respectively.

**Detection limit**. The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of CS-1 (2 μM) without perchlorate was measured by 10 times and the standard deviation of blank solution (without addition of perchlorate) measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the perchlorate concentration could be obtained in the $10^{-11} – 10^{-7}$ M ($R^2 = 0.9929$) for CS-1. The detection limit is then calculated with the equation:

Detection limit = $3\sigma_b / m$
Where, $\sigma_{bi}$ is the standard deviation of blank solution (without addition of perchlorate) measurements, $m$ is the slope between intensity versus sample concentration. The detection limit was measured to be 10 pM for CS-1 S/N = 3.
Figure S1. $^1$H NMR and $^{13}$C NMR of CS-1
Figure S2. $^1$H NMR and $^{13}$C NMR of CS-2
Figure S3. HRMS spectra of CS-1
Figure S4. HRMS spectra of CS-2
**Figure S5.** Change in absorption and emission spectrum of CS-1 with increasing volume fractions of water.

**Figure S6:** (X) DLS studies of CS-1 (HEPES- DMSO 98:2) in the presence of ClO$_4^-$: (a) only CS-1; (b) CS-1 + ClO$_4^-$ (10$^{-11}$ M); (c) CS-1 + ClO$_4^-$ (10$^{-9}$ M); (d) CS-1 + ClO$_4^-$ (10$^{-6}$ M); (Y) DLS of CS-1 (a) after 1h (b) after 24h; DLS of CS-1+10$^{-5}$ M ClO$_4^-$ in water (c) after 1h (d) after 24 h.
**Figure S7.** Effect of various anions (100 µM) on absorption spectrum of CS-1 (2 µM) in HEPES buffer – 2% DMSO (pH 7.4).

**Figure S8.** (A) Effect of various anions (100 µM) on emission spectrum (λex = 290 nm) of CS-1 (2 µM) in HEPES buffer – 2% DMSO (pH 7.4); (B) Effect of various aromatic hydrocarbons (50 µM) on the emission spectrum (λex = 290 nm) of CS-1 (2 µM) in HEPES buffer – 2% DMSO (pH 7.4).
**Figure S9.** Effect of different water - DMSO ratio (80 : 20 and 98 : 2) on the sensitivity of CS-1 for determination of ClO$_4^-$.

**Figure S10.** Job’s plot of chemosensor CS-1 (A) through absorption, (B) through emission in HEPES buffer – 2% DMSO solution (pH 7.4).
**Figure S11.** (A) Effect of different anions on the fluorescence quenching by ClO$_4^-$ (1) CS-1 only; (2) ClO$_4^-$ only; (3) F$^-$ + ClO$_4^-$; (4) Cl$^-$ + ClO$_4^-$; (5) Br$^-$ + ClO$_4^-$; (6) I$^-$ + ClO$_4^-$; (7) CN$^-$ + ClO$_4^-$; (8) OH$^-$ + ClO$_4^-$; (9) SO$_4^{2-}$ + ClO$_4^-$; (10) HSO$_4^-$ + ClO$_4^-$; (11) H$_2$PO$_4^-$ + ClO$_4^-$; (12) AcO$^-$ + ClO$_4^-$; (13) NO$_3^-$ + ClO$_4^-$; (14) SCN$^-$ + ClO$_4^-$. (B) Effect of various aromatic hydrocarbons (50 µM) on the emission spectrum ($\lambda_{ex} = 290$ nm) of CS-1 (2 µM) in the presence of ClO$_4^-$ ions (1000 nM) (1) ClO$_4^-$ only; (2) Benzene; (3) Toluene; (4) Phenol; (5) Benzoic acid; (6) Naphthalene; (7) Anthracene (error less than 3.1%).

**Figure S12:** Effect of pH on the fluorescence intensity of CS-1 (H$_2$O – DMSO, 98:2)
Figure S13. Job’s plot of CS-2 in HEPES buffer – 2% DMSO solution (pH 7.4).

Figure S14. $^1$H NMR titration of CS-1 with ClO$_4^-$ in DMSO - Water (7:3) ratio.
Figure S15. $^1$H NMR titration of CS-2 with ClO$_4^-$ in DMSO - Water (7:3) ratio.

Figure S16. Isosurface energy map of (A) CS-1 with ClO$_4^-$ and (B) CS-2 with ClO$_4^-$.
Figure S17. (A) HOMO of CS-1, (B) LUMO of CS-1, (C) HOMO of CS-1 + ClO$_4^-$ Complex, (D) LUMO of CS-1 + ClO$_4^-$.

Figure S18. Emission spectrum of CS-1, blood serum (BS) and CS-1 + blood serum in 98% aqueous buffered solution.