Electronic Supplementary Material (ESI) for Analyst

Supporting Information

Fluorogenic and Chromogenic Probe for Detection of a nerve agent simulant DCP

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Electronic Supplementary Material (ESI) for Analyst

General methods

Rhodamine B and 2-aminoethanol was purchased from Alfa Aesar.

Column chromatography was performed on silica gel (300-400 mesh).

NMR spectra (^{1}H at 400 MHz and ^{13}C at 100 MHz) were recorded on a JNM-ECA-400 instrument using tetramethyl silane as the internal reference.

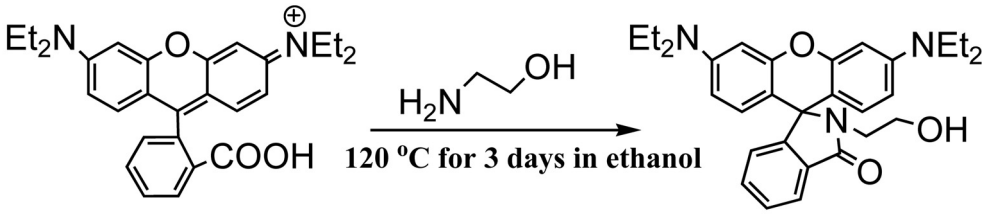
The samples were analyzed on an Applied Biosystems 4700 Proteomics Analyzer (USA) (MALDI-TOF/TOF operated in reflection mode) or a Thermo Advantage Max mass spectrometer (USA) (ESI-MS).

Fluorescence measurements were recorded in a Hitachi FP-4500 fluorescence spectrometer at RT using a 1-cm pathlength quartz cell.

The excitation wavelength was set to 520 nm (slit width = 5nm), and emission was monitored from 530-600nm (slit width = 5nm).

UV absorption spectra were obtained using a Tian-mei 2300 UV/Vis spectrophotometer.

Synthesis of RB-AE probe



RB-AE was prepared according to previous reports.[1, 2] Briefly Aminoethanol (500 µL, 8.32 mM) was added to a solution of Rhodamine B (1.00g, 2.08 mmol) in ethanol (100 mL), and the resulting mixture was heated at 120 ºC for 3 days. The reaction mixture was cooled to room temperature and the solvent was evaporated off. The residue was then dissolved in EtOAc (100 mL) and successively washed with water (20 ML) and brine (20 mL). The resultant solution was dried with Na_2SO_4 overnight. The solvent was further evaporated under vacuo. The obtained solid was purified by silica gel chromatography (10% MeOH in CH_2Cl_2) to give RB-AE as an off-white solid.

Yield: 0.72 g (71%).

1H NMR ($CDCl_3$, 400 MHz): δ 1.17 (12H, t, J = 7.0 Hz), 3.28-3.36 (10H, m), 3.46 (2H, broad), 6.29 (2H, d, J = 8.8 Hz), 6.38 (2H, s), 6.49 (2H, dd, J = 8.8 Hz), 7.07 (1H, m), 7.44-7.46 (2H, m), 7.89-7.91 (1H, m); 13C NMR ($CDCl_3$, 100 MHz): δ:170.11, 153.87, 153.20, 148.81, 132.72, 130.33, 128.49, 128.14, 123.76, 122.88, 108.14, 104.63, 97.65, 65.85, 62.70, 44.61, 44.33, 12.56 ppm; ESI-MS ($C_{30}H_{35}N_3O_3$): calculated ($M+H^+$): 486.28, found: 486.28.

The inner filter effect (IFE)

IFE is a photophysical phenomenon where a donor's fluorescence emission is absorbed by an acceptor's. RB-oxazoline molecule in our study can act as both a donor and an acceptor. IFE is especially obvious in situations where there is significant wavelength overlap between the sample's emission and absorption spectra. It is very important to subtract such an effect from the raw quenching data. The extent of this effect can be approximately estimated with the following formula[3, 4]:

$$F_{corr}=F_{obs}\ \text{antilog}(OD_{ex}/2+OD_{em}/2)$$

where F_{corr} is the corrected fluorescence value, Fobs is the measured fluorescence value, OD_{ex} is the absorption value at the excitation wavelength, and Od_{em} is the absorption value at the emission wavelength.

The fluorescence spectra of Figure 3 and FigureS2 show significant IFEs in this paper. The inset of Figure 3 shows the corrected result after compensating IFEs. The corrected fluorescence intensity (red triangle) in absence of IFE has a stronger linear correlation with DCP concentration than the observed intensity does (black rectangle). The bottom graph of Figure S2 also showed the corrected data by subtracting IFEs. As expected, there is a clear linear relationship between DCP concentration and the fluorescence intensity without IFE.

RB-AE probe detects DCP using DCM as medium.

In our study, addition of DCP to RB-AE in DCM or DMF solvents quickly leads to the development of a pink color. DMF was the preferred assay medium when compared to DCM. The reason is that the blank of RB-AE in DCM has a little pink color and fluorescence (Figure S1), affecting the detection limit of DCP with RB-AE probe.

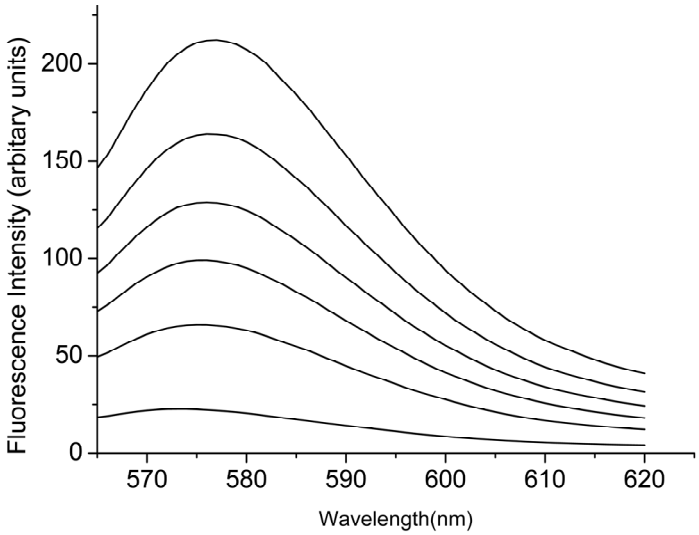


Figure S1 Fluorescence spectra of RB-AE probe (2 mg/ml) with DCP at different concentrations (from top to bottom: 250, 150, 100, 50, 25, and 0 ppm) in DCM media.

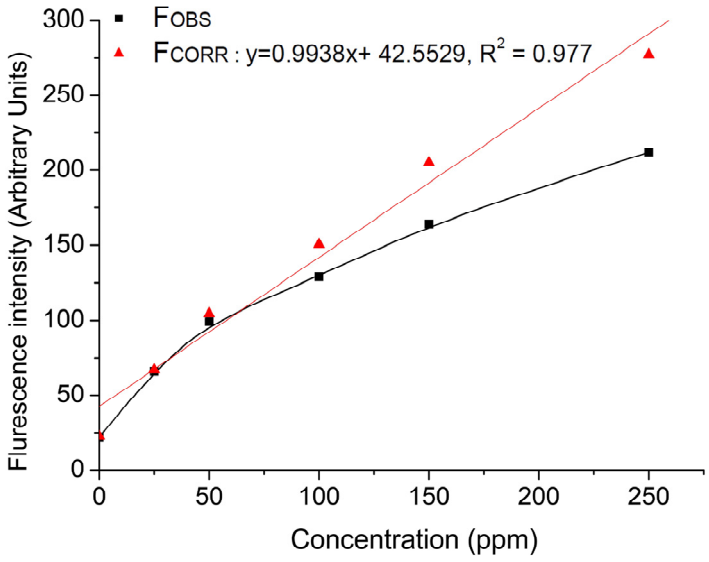


Figure S2 The fluorescence intensity profile of RB-AE probe upon addition of DCP (25-250 ppm) (the intensity was taken at the peak height). The data were obtained in DCM at room temperature. The black rectangles show the measured intensities and the red triangles indicate the corrected intensities after compensating the IFEs.

The RB-AE probe failed to detect DCP in basic DMF solutions.

We tested the efficacy of RB-AE probe to detect DCP in DMF containing TEA (3%, v/v). The analysis result showed no fluorescent (Figure S3) and colored species were generated after DCP (Figure S4) was added to the basic solutions of RB-AE, which indicated that the intramolecular spirolactam of RB-AE remains closed under the basic conditions. For the low amounts of DCP, the bases in DMF solvent obviously prevent the formation of intermolecular oxazoline and thus affect the DCP detection limit.

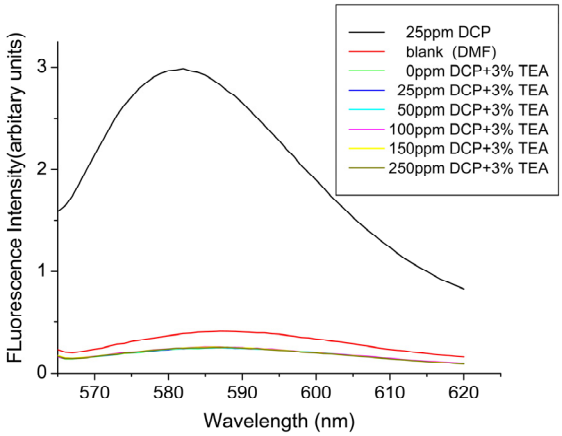


Figure S3 RB-AE probe failed to detect DCP in basic DMF solutions (with 3% TEA).

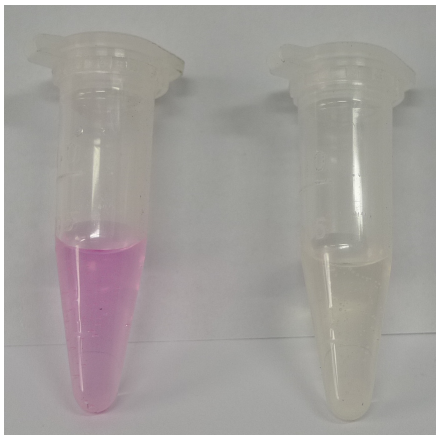
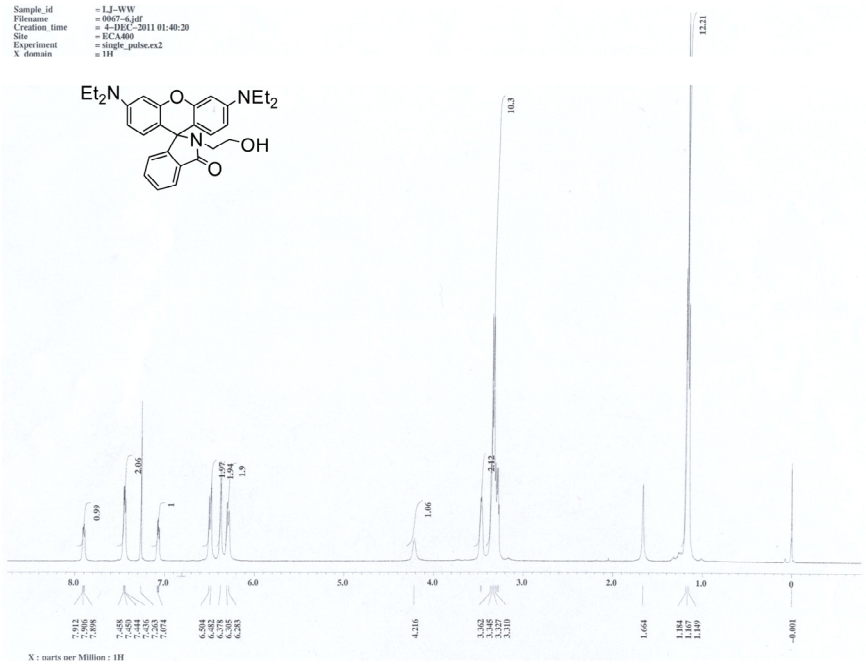
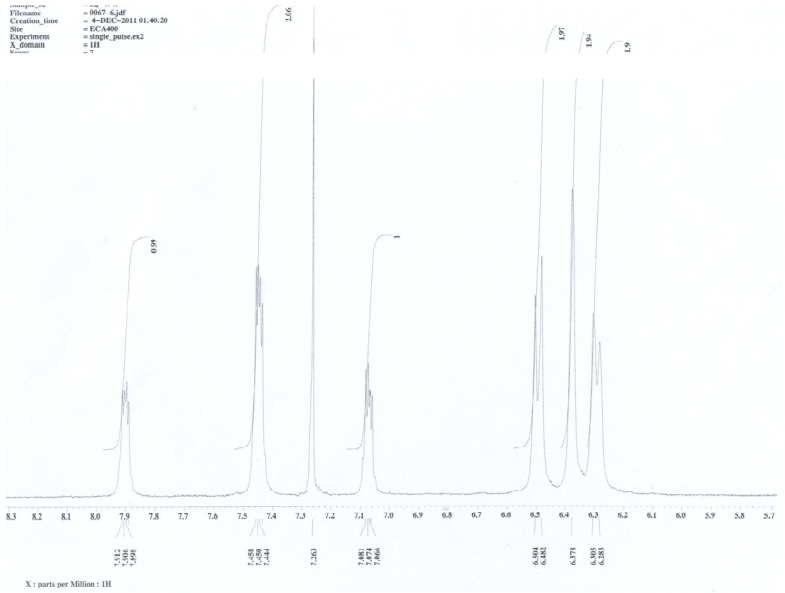


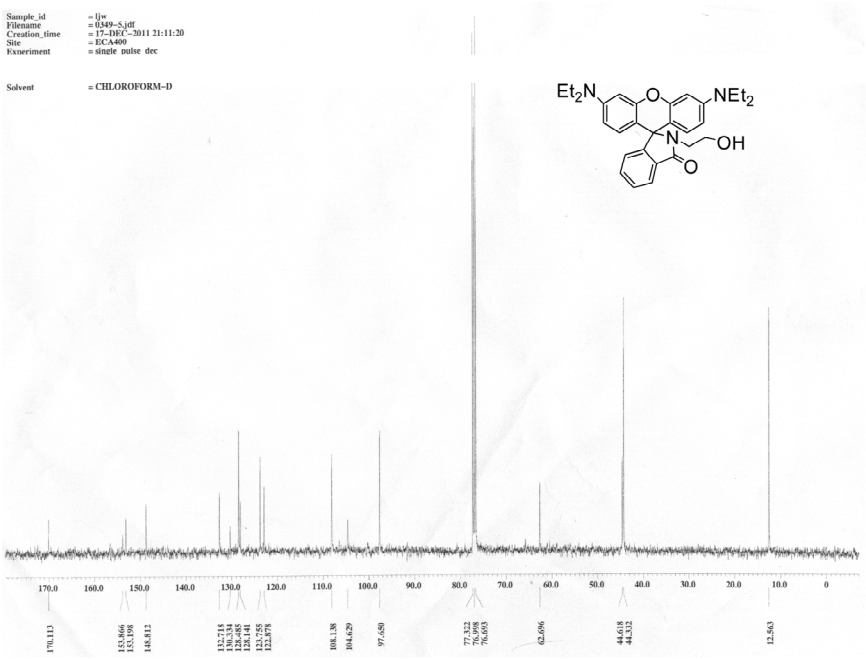
Figure S4 Visual color comparison between the RB-AE probe (2 mg/ml) in DMF (left) and DMF (3% TEA) (right) upon addition of the nerve agent simulant DCP (50 ppm), after 10 s at room temperature.

1H-NMR spectrum of RB-AE in $CDCl_3$.

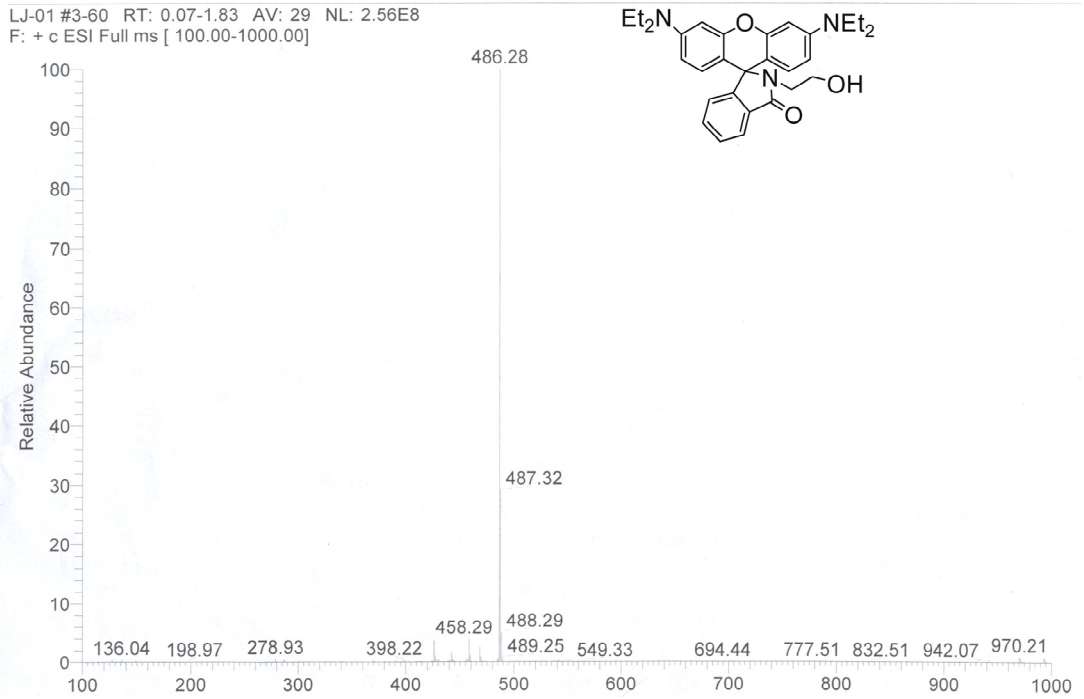




13C-NMR spectrum of RB-AE in $CDCl_3$.



ESI-MS spectrum of RB-AE in $CDCl_3$.



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2. A. Chatterjee, M. Santra, N. Won, S. Kim, J. K. Kim, S. B. Kim and K. H. Ahn, *Journal of the American Chemical Society*, 2009, **131**, 2040-2041.
3. M. Kubista, R. Sjoback, S. Eriksson and B. Albinsson, *Analyst*, 1994, **119**, 417-419.
4. J. R. Lakowicz, *Principles of fluorescence spectroscopy*, 2006, 3rd edn., Springer Science+Business Media, New York.