Support information for

A FRET-based ratiometric fluorescence probe for sensing trace water in organic solvents

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Computational section

All the calculations were implemented with the Gaussian09 program package. The structures of these molecules in transition state were optimized using the density functional theory (DFT), cam-b3lyp with the 6-31 g* basis set. Vibrational frequency analyses were carried out from the optimized transition state.

LOD and LOQ determination

The limit of detection and quantitation was determined from the linear regression of the fluorescence intensities' ratio at 615 nm and 455 nm versus the water content in the 0–1.8 % (v/v) range in MeCN upon excitation at 400 nm.

The limit of detection (LOD) and limit of quantitation (LOQ) are calculated according to the following equations, with σ being the standard deviation of the blank and S the slope of the calibration curve:

$LOD = 3.3\sigma/S$ $\quad$ $LOQ = 10\sigma/S$

The calibration equation was given by fitting the experimental data:

$\ln(I_{615}/I_{455}) = 1.52514[H_2O] - 1.77354$ $\quad$ ($R^2 = 0.99917$, $[H_2O]$ = (0-1.8 %)

S = 1.52514 (slope of calibration curve)

σ = 0.005131 (standard deviation of blank readings of ten replicate measurements at the zero level)

LOD = 3.3×0.005131/1.52514 = 0.01110

LOQ = 10×0.005131/1.52514 = 0.03364

Fluorescence quantum yield determination

Based on the above data, we can obtain the fluorescence quantum yield of RAP in different solvents. Because the fluorescence spectrum is affected by the size of the slit and many factors, we use the reference method to measure the relative value. The UV-Vis spectra and the fluorescence spectra of the corresponding slit were determined by using quinine sulfate as reference, and the quantum yield of the material in different solvents was calculated according to the following equation:

$$\Phi_{sample} = \Phi_{ref} \times \frac{F_{sample} \times A_{ref} \times {\eta_{sample}}^2}{F_{ref} \times A_{sample} \times {\eta_{ref}}^2}$$

Where F is the area under the fluorescence spectral curve and A is optical density of the compound at the excitation wavelength, η is the refractive index of the solvent used. The standard used for the measurement of fluorescence quantum yield was quinine sulphate (Φ = 0.547 in 0.05M H_2SO_4 aq.).

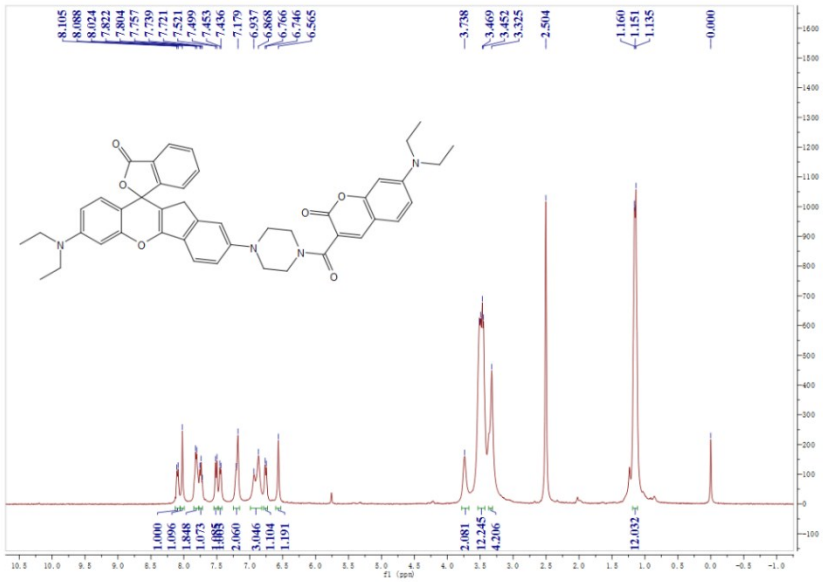


Fig. S1. ^{1}H NMR of RAP in DMSO-d_6

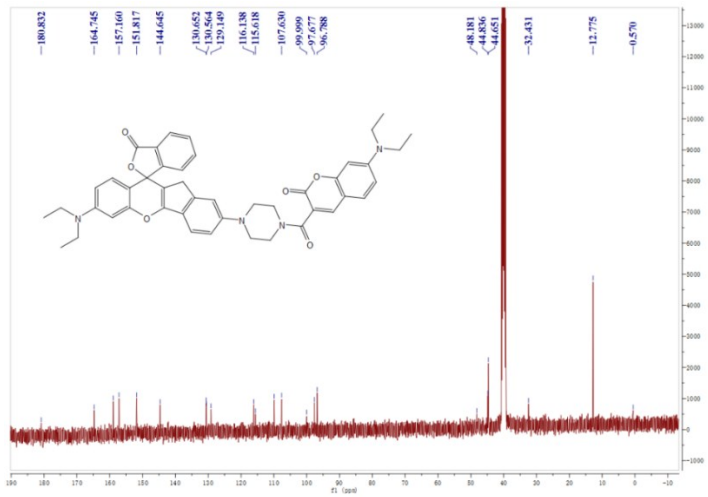


Fig. S2. ^{13}C NMR of RAP in DMSO-d_6

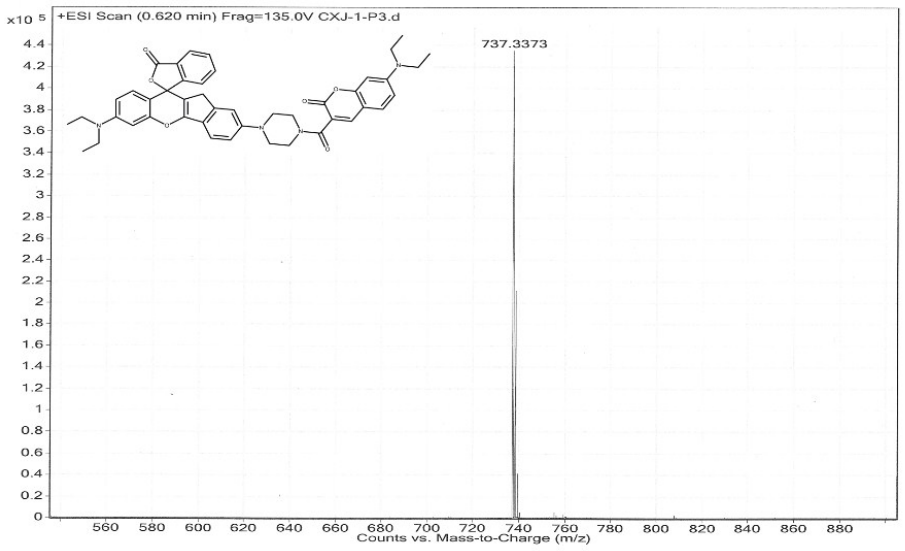


Fig. S3. HR-MS of RAP

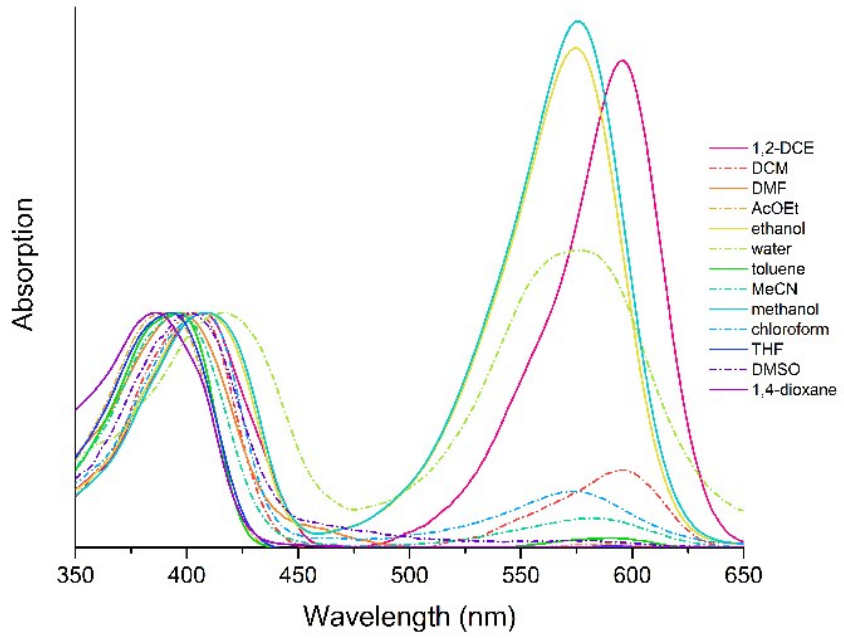


Fig. S4. Normalized UV-Vis spectra of RAP in different solvents

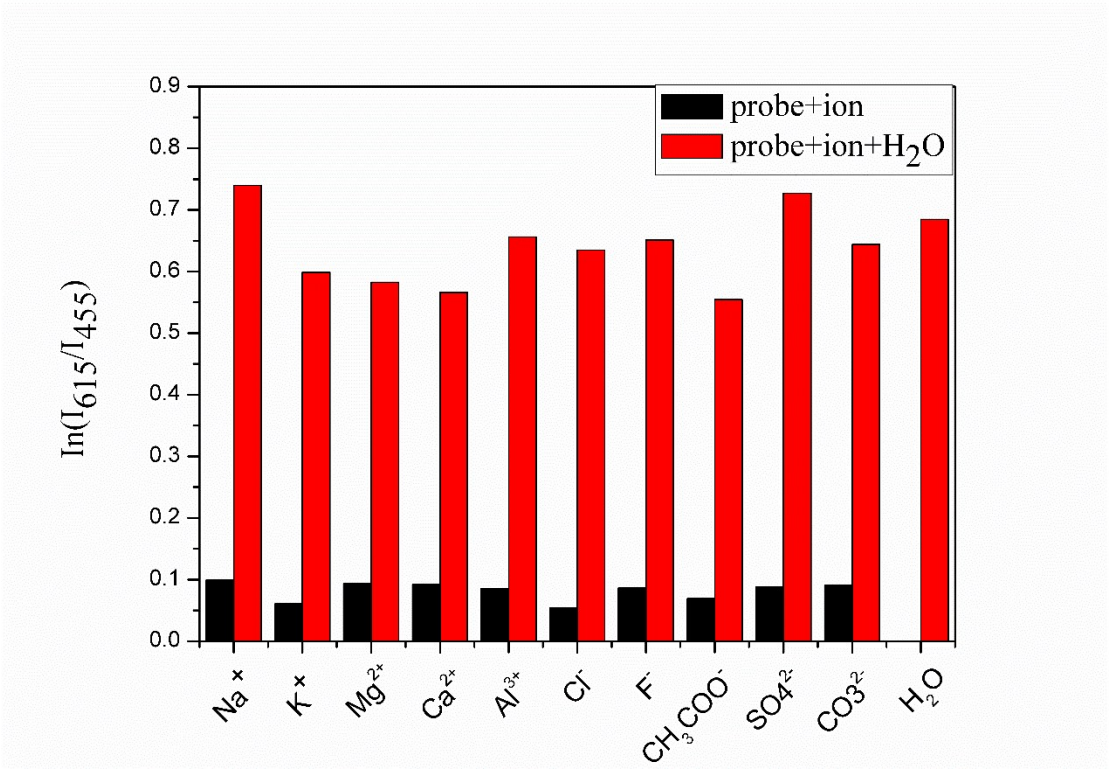


Fig. S5 The ratiometric responses $\ln(I_{615}/ I_{455})$ of RAW (c = 10^{-3} M) with or without water in the presence of various analytes (100 μM) in acetonitrile (λ_{ex} = 420 nm).

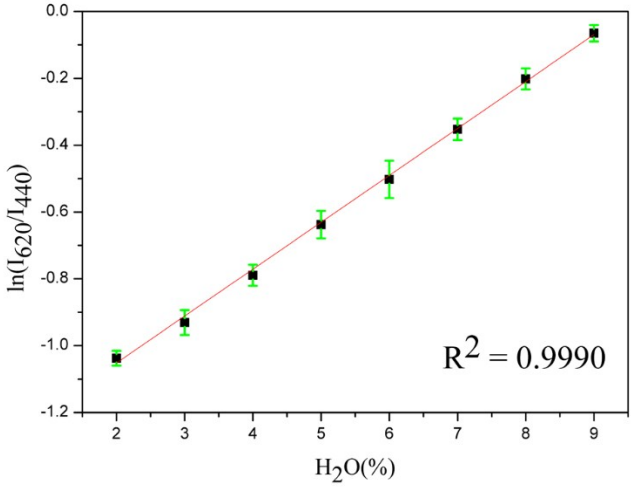


Fig. S6 The relationship of ratio of fluorescence intensity of RAW (c= 10^{-3} M) and water content in THF (λ_{ex} = 420 nm). Error bars (n = 3).

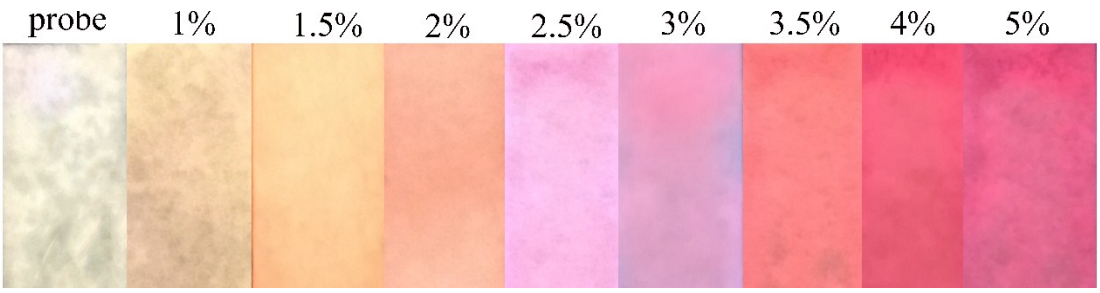


Fig. S7 Fluorescence changes of RAW (10^{-3} M)-coated test strips after soaked in THF with different water content (λex = 365 nm).

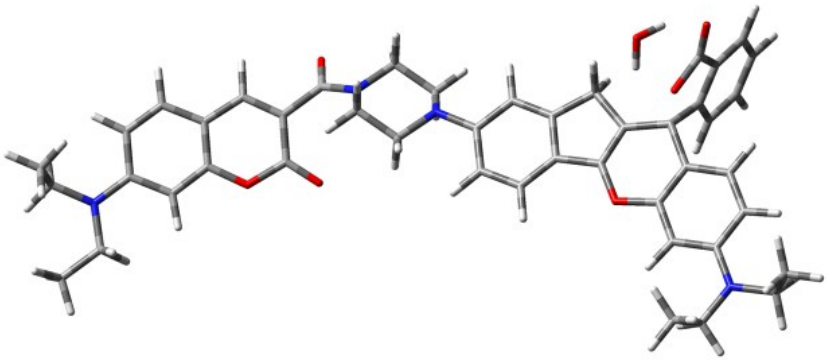


Fig. S8. The structure of RAP (optimized and calculated by theoretical calculation)

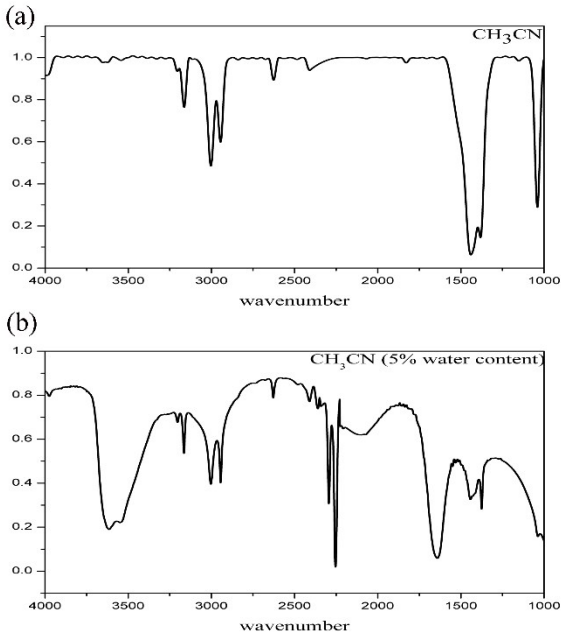
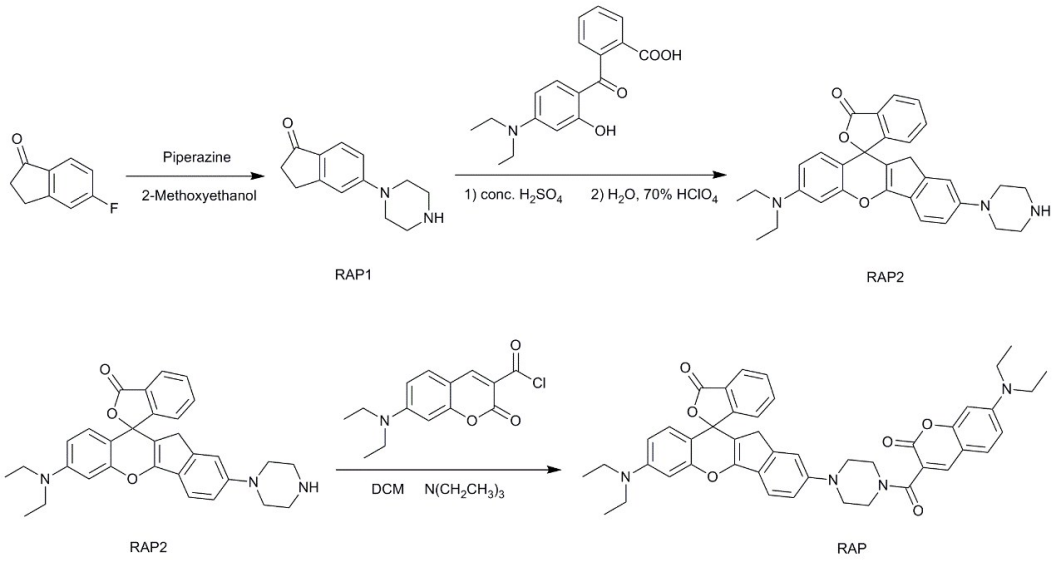


Fig. S9 IR for RAW in acetonitrile (a) and acetonitrile with 8 % water (b).



Scheme S1. Synthesis of probe RAP

Table S1. Optical properties of RAP[a]

Solvent	$E_T(30)$	$\lambda_{abs}{}^b$	$\lambda_{fl}{}^b$	$\Delta\upsilon^b$	$\lambda_{abs}{}^c$	$\lambda_{fl}{}^c$	$\Delta\upsilon^c$	Φ_F
toluene	33.9	396	434	2211.1	590	612	609.3	0.0376
1,4-dioxane	36	386	441	3231.0				0.0147
THF	37.4	393	443	2871.9				0.0859
AcOEt	38.1	390	443	3067.7	591	617	713.0	0.0738
chloroform	39.1	408	447	2138.4	574	602	810.3	0.1997
DCM	40.7	403	448	2492.5	596	621	675.5	0.1362
1,2-DCE	41.3	407	451	2397.1	596	629	880.3	0.2430
DMF	43.2	397	457	3307.1				0.0906
DMSO	45.1	402	461	3183.6				0.0552
MeCN	45.6	396	455	3274.5	582	616	948.4	0.0630
ethanol	51.9	407	458	2736.0	574	620	1292.6	0.2893
methanol	55.4	409	459	2663.4	576	622	1283.9	0.2096
water	63.1	417	467	2567.5	576	628	1437.5	0.0404

[a]The dye concentration was 100 μM, λ_{ex} = 400 nm, [b] coumarin derivatives fluorophores, [c] acceptor fluorophores.

$$\Delta\upsilon = \upsilon_{abs} - \upsilon_{fl} \quad (1)$$

$$\Phi_{sample} = \Phi_{ref} \times \frac{F_{sample} \times A_{ref} \times {\eta_{sample}}^2}{F_{ref} \times A_{sample} \times {\eta_{ref}}^2} \quad (2)$$

Table S2. Fitting results to the absorption maxima (υ_{abs}), fluorescence maxima (υ_{fl}) and Stokes shift ($\Delta\upsilon$) of RAP on the Catalan solvent parameter.

A	A_0	SP	SdP	SA	SB	R^2 [a]
υ_{abs} [b]	26748	-1709	-859	-1021	691	0.750
υ_{fl} [b]	24048	-900	1380	-636	-237	0.946
$\Delta\upsilon$ [b]	2700.3	-808	-520.9	-385.7	927.7	0.557
υ_{abs} [c]	18092	-1323	-154	378	-44	0.419
υ_{fl} [c]	18256	-2056	-597	-260	-481	0.385
$\Delta\upsilon$ [c]	-164.07	733.07	442.97	637.68	437.31	0.914

[a] $R^2 < 0.6$ no linearity, $0.6 < R^2 < 0.8$ poor linearity, $R^2 > 0.8$ good linearity, [b] represents coumarin derivatives fluorophores, [c] represents acceptor fluorophores.

Table S3. The G of different structures in different solvents (the data was calculated by DFT and TD-DFT)

G (Gibbs free energy)	RAP	RAPW	$\Delta G = G_{RAP} - G_{RAPW}$
G_{RAP} (in CH_3CN)	-2198.17488008	-2198.15495097	-0.01992911
G_{RAPW} (in water)	-2410.65213890	-2410.67390453	0.02176563