Electronic Supplementary Information

A Fluorescent "2 in 1" Proton Sensor and Polarity Probe based on Core Substituted Naphthalene Diimide.

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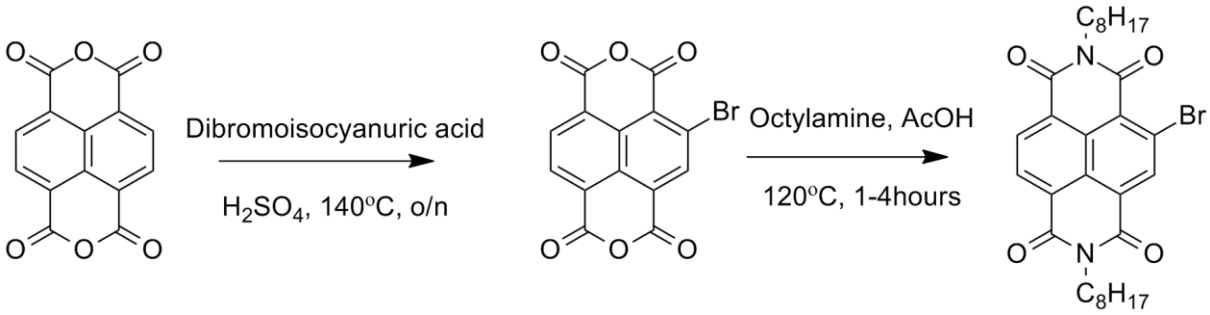
Supplementary synthesis data

1,4,5,8-naphthalenetetracarboxylic dianhydride, octylamine, 4-amino-1-benzylpiperidine, dichloromethane and methanol were obtained from Sigma-Aldrich reagents and used as received. Dibromoisocyanuric acid was prepared from isocyanuric acid following the method of Hammarstrom et al., (Chaignon, F.; Falkenstrom, M.; Karlsson, S.; Blart, E.; Odobel, F.; Hammarstrom, L., *Chem. Commun.* **2007**, 64-66). Analytical thin layer chromatography was performed on silica gel (Silica 60 F_{254}) coated aluminium plates. Column chromatography was performed using silica gel 60 (pore size 0.063 – 0.2 mm) purchased from Merck as the column stationary phase.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using either a Bruker DRX 300 or 400 MHz Spectrometer in the deuterated solvents stated. The chemical shifts (δ) were calibrated against the solvent peak in the spectra. In the ^{1}H NMR spectra each resonance was assigned according to the following convention: chemical shift (δ), measured in parts per million (ppm), multiplicity, coupling constant (*J*), measured in Hz, number of protons and assignment. Multiplicities are denoted as (s) singlet, (d) doublet, (t) triplet, (q) quartet, (p) pentet or (m) multiplet. The ^{13}C NMR spectra were assigned a chemical shift (δ) measured in parts per million.

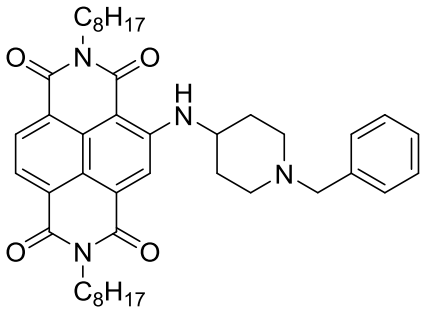
Mass spectrometry Low Resolution – Electrospray Ionisation Mass Spectrometry (LR-ESI) was performed on a Micromass Platform II API QMS – quadrupole electrospray mass spectrometer as the solutions specified. $[M]^+$ denotes the molecular ion. Mass spectrometry High Resolution – Electrospray Ionisation Mass Spectrometry (HR-ESI) was performed on an Agilent Technologies 6220 Accurate-Mass TOF – Time of Flight LC/MS as the solutions specified. $[M]^+$ denotes the molecular ion. All MS performed in positive ion mode (ESI^+) unless otherwise stated.

_N,N'_-bis(n-octyl)-2-bromo-1,4,5,8-naphthalenetetracarboxylic diimide (2)



Compound **2** was prepared from naphthalene dianhydride *via* literature procedures and obtained as a light pink solid in 30% yield. **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.94 (s, 1H, ArH), 8.83 (d, *J*=7.6 Hz, 1H, ArH), 8.78 (d, *J*=7.6 Hz, 1H, ArH), 4.22 (m, 4H, NCH_2), 1.79 (m, 4H, CH_2), 1.43 (m, 22H, CH_2), 0.88 (t, *J*=6.8 Hz, 6H, CH_3). **^{13}C NMR (100 MHz, $CDCl_3$):** 162.6, 162.0, 161.9, 161.2, 138.6, 131.8, 130.9, 128.8, 127.0, 126.2, 126.2, 125.9, 124.1, 41.7, 41.3, 31.9, 29.4, 29.4, 29.4, 29.3, 28.2, 28.1, 27.3, 27.2, 14.2. **Mass spectrum LR-MS (ESI, -ve):** 568.1 $[M-H]^-$

Sensor (1)



N,*N*'- bis(n-octyl)-2-bromonaphthalene-1,4,5,8-bis(dicarboximde) (100 mg, 176 µmol), and 4-amino-1-benzylpiperidine (100 mg, 526 µmol) was dissolved in *N*,*N*-dimethylformamide (3 ml) and heat at 80 °C for 24 hours. Most of the solvent was removed under reduced pressure, and the residue partitioned between dichloromethane and water. Organic layer was separated and washed with water (×2). The organic layer was concentrated to give a red solid residue and purified by flash chromatography on silica (0 – 2.5% dichloromethane/methanol) to afford a red solid (84 mg, 63%)

^{1}H NMR (300 MHz, $CDCl_3$, 30 °C): 10.22 (d, 6.3 Hz, 1H, NH), 8.60 (d, 5.85 Hz, 1H, ArH), 8.29 (d, 5.85 Hz, 1H, ArH), 8.16 (s, 1H, ArH), 7.2-7.4 (m, 5H, ArH), 4.14 (m, 4H, NCH_2), 3.87 (m, 1H, NHC<u>H</u>), 3.59 (s, 2H,CH_2Ar), 2.90 (m, 2H, N<u>C</u>H_2CH_2), 2.33 (t, 2H), 2.14 (m, 2H), 1.8 (m, 2H), 1.7 (m, 4H), 1.2-1.5 (m, 20H), 0.8 (m, 6H). **^{13}C NMR (300 MHz, $CDCl_3$, 30 °C):** 166.51, 163.64, 163.39, 163.27, 151.75, 131.57, 129.69, 129.41, 128.29, 127.51, 126.43, 124.77, 123.95, 120.22, 119.68, 100.31, 63.29, 52.02, 50.14, 41.26, 40.76, 32.75, 32.17, 32.14, 30.03, 29.69, 29.62, 29.58, 29.52, 28.41, 27.55, 27.44, 22.98, 22.96, 22.95, 22.94, 14.23, 14.40. **ESMS m/z** calculated: 679.42233 found: 679.4218.

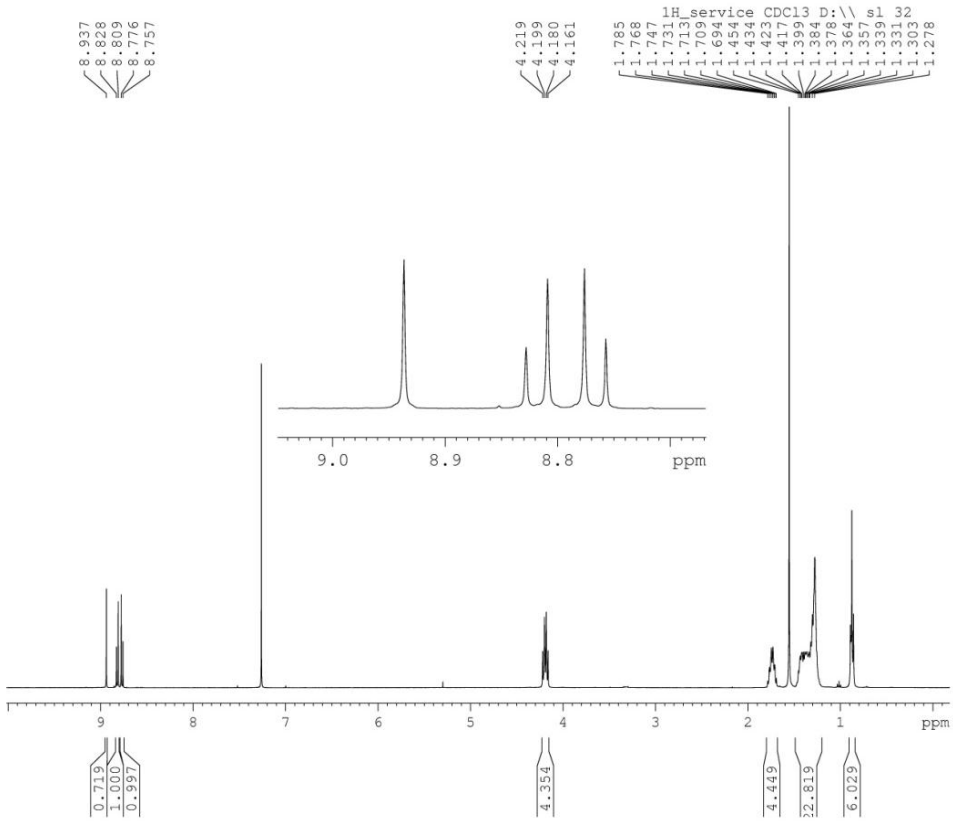


Fig. 1: ^{1}H NMR spectra of **2** (400 MHz, 300 K) in $CDCl_3$

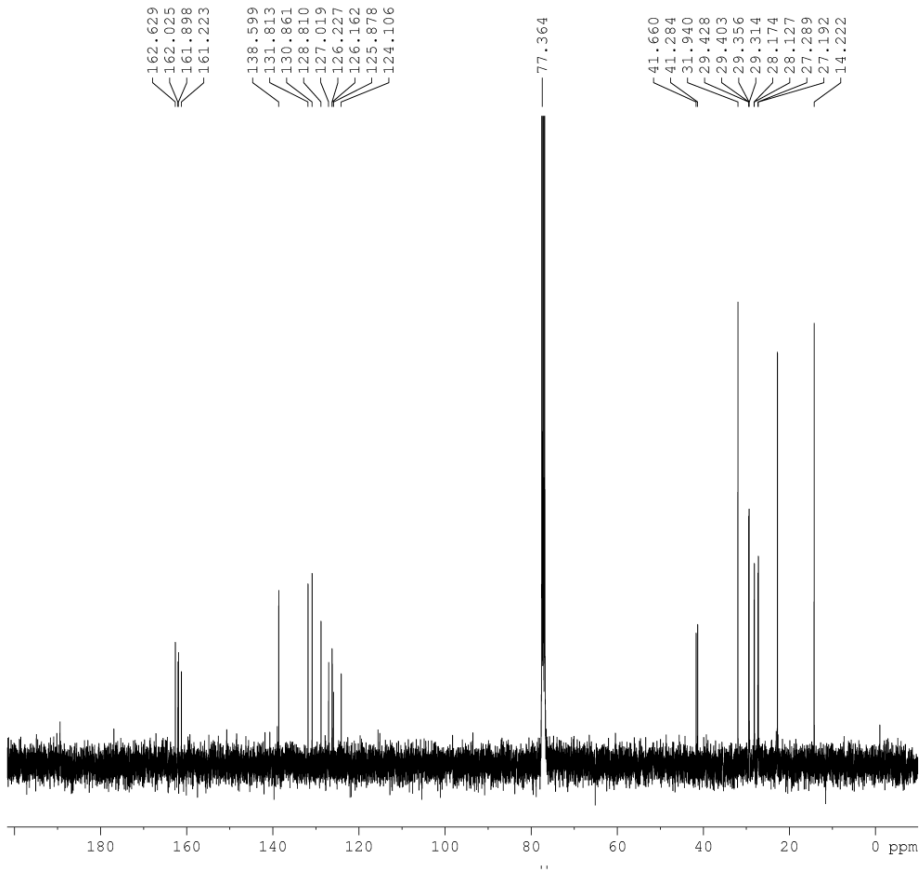


Fig. 2: ^{13}C NMR spectra of **2** (100 MHz, 300 K) in $CDCl_3$

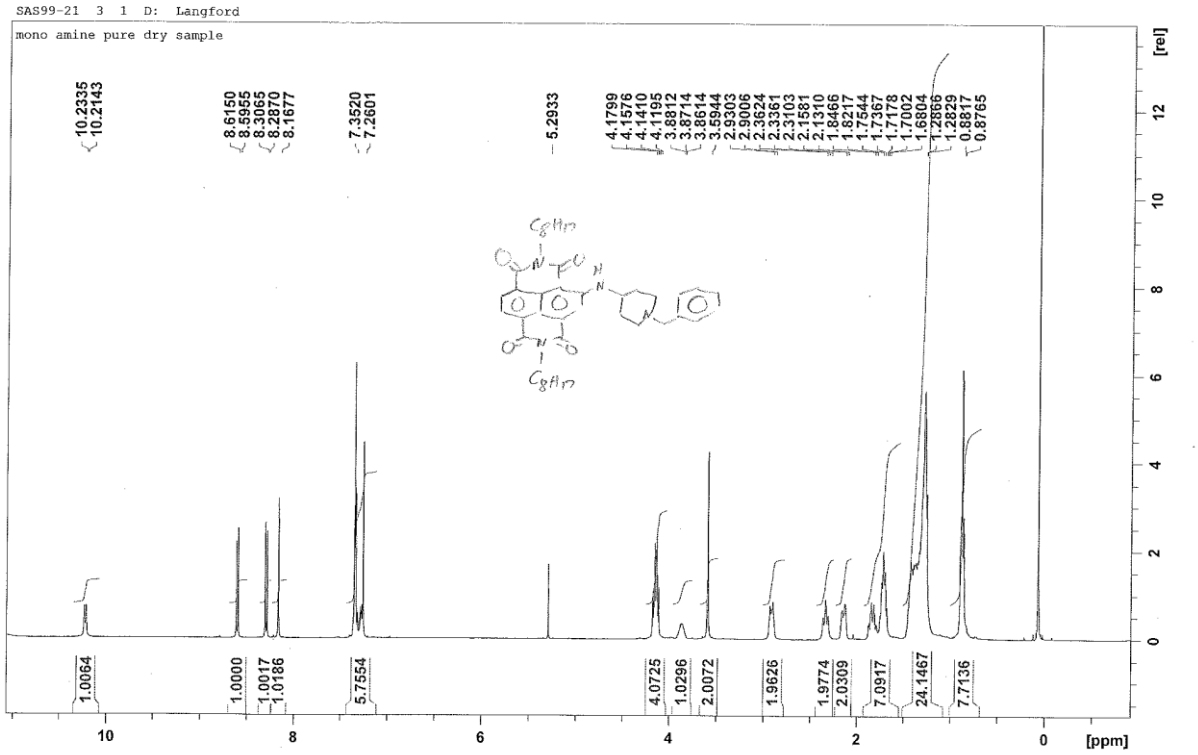


Fig. 3: ^{1}H NMR spectra of **1**(300 MHz, 300 K) in $CDCl_3$

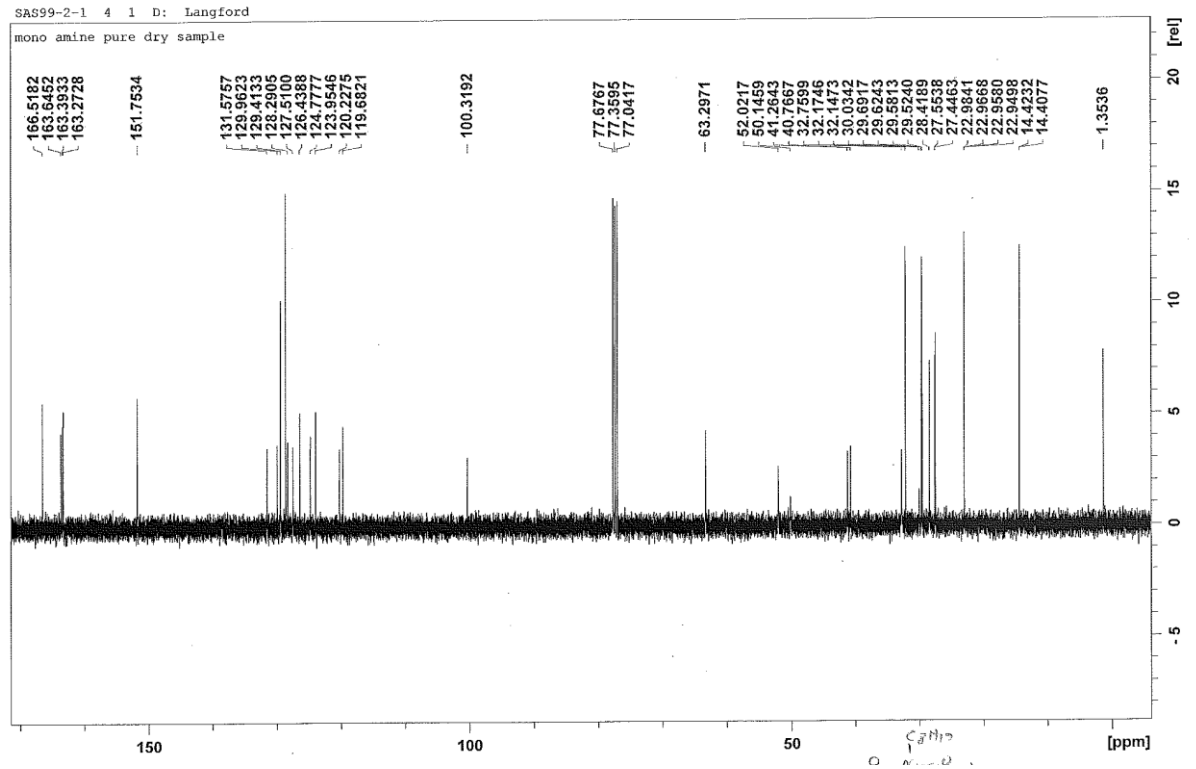


Fig. 4: ^{13}C NMR spectra of **1** (300 MHz, 300 K) in $CDCl_3$

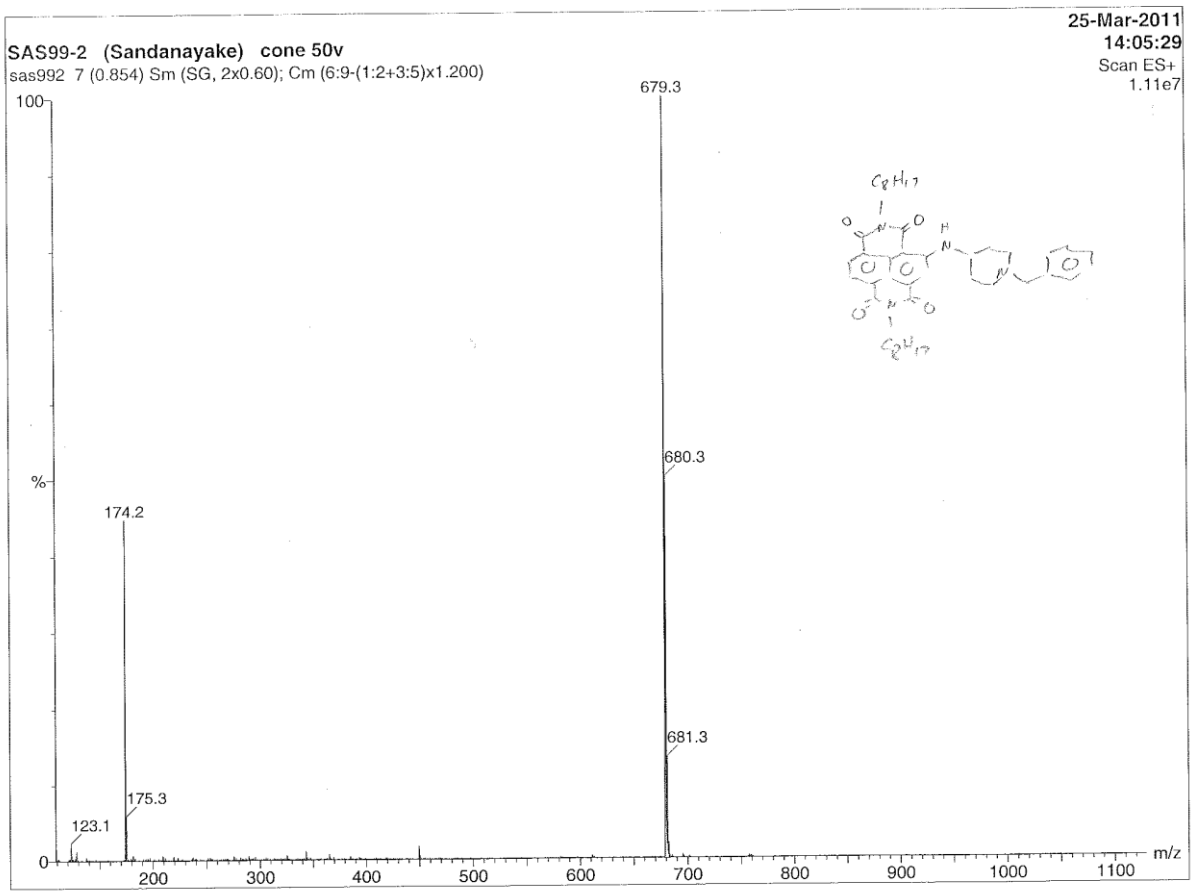


Fig. 5: Low resolution mass spectrum (ESI^+) of **1**

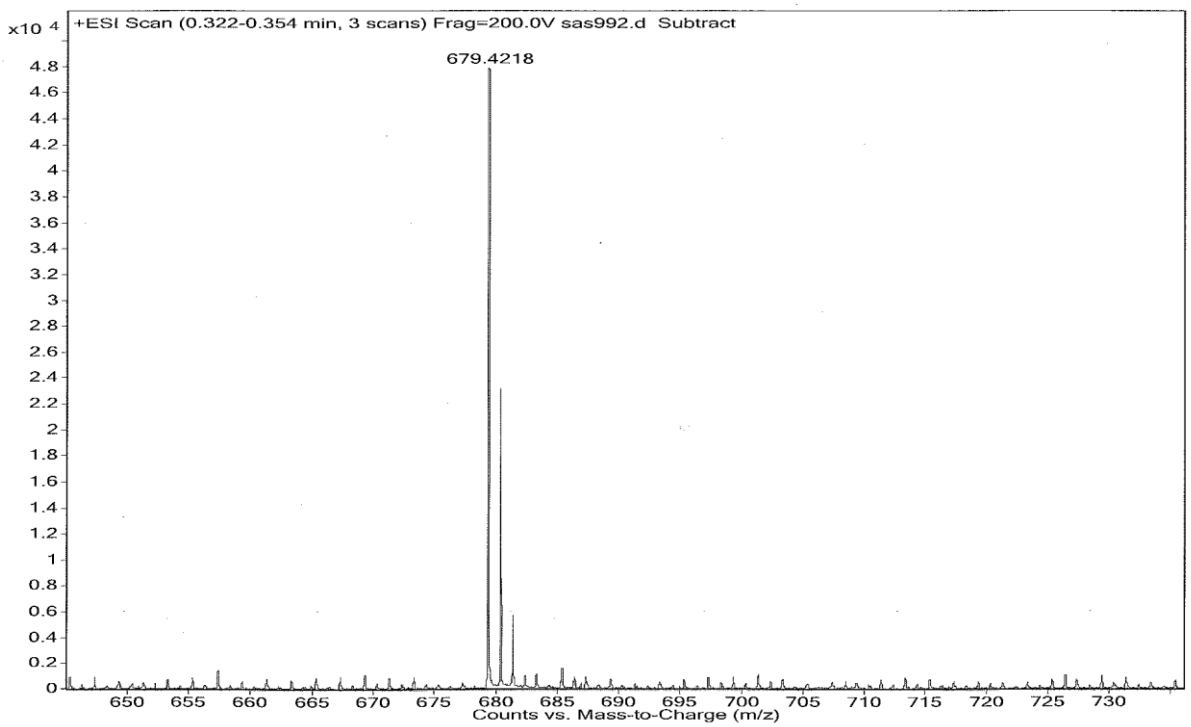


Fig. 6: High resolution mass spectrum (ESI^+) of **1**

Supplementary spectroscopic data

UV-visible spectra were recorded using a Varian model Cary 100 Bio UV-visible spectrophotometer and fluorescence excitation and emission spectra were performed on a Varian model Cary Eclipse fluorometer. Solvents for spectroscopic work of the highest available purity were used as received from Merck or Aldrich. Trifluoroacetic acid and triethylamine (Aldrich 99% grade) were used as received and diluted with the appropriate solvent. Samples for quantum yields and time-resolved fluorescence were degassed by bubbling with nitrogen for 20 minutes immediately prior to measurement.

For molar absorptivity coefficient determination, 10 ml standards of concentrations 0, 2, 5, 20 and 50 µmol L^{-1} of **1** were prepared in chloroform in 1 cm pathlength quartz cuvettes. The molar extinction coefficient was determined at the absorption maximum of 525 nm and determined to be 15,630 $M^{-1}cm^{-1}$. Samples for determination of the binding ratio *via* a Job's plot were prepared for concentrations of **1** from 0 to 10 µmol L^{-1} every 0.5 µM with a constant total molar concentration of **1** + TFA of 10 µmol L^{-1}.

For the fluorescence titration of **1** in $CHCl_3$, a 10 µmol L^{-1} solution (2.5 mL) of **1** was prepared and 10 µL aliquots (0.125 equivalents) of TFA were added until in excess.

Fluorescence quantum yields were determined by comparing areas under corrected emission spectra recorded under identical conditions with that of Rhodamine 101 which has a quantum yield of 1.0 in ethanol. (Karstens, T. and Kobs, K., *J. Phys. Chem.*, 1980, **84**, 1871).

Time-resolved fluorescence decays were measured on a previously described home built set-up. (Ref. 6, main text, Cox 2012) Briefly, excitation was provided by a super-continuum fibre laser (Fianium SC-400-4-pp), photons detected by an avalanche photodiode (Id-Quantique, Id-100) and photon emission times recorded and histogrammed by a photon counting module (Picoquant PicoHarp 300). Decay histograms were fit using a home written routine employing an iterative least-squares method based on the Marquardt algorithm. An instrument response function (IRF) and exponential decay components were included in the fitting function until a satisfactory fit was obtained as judged by the value of the χ^2 fitting parameter and the shape of the residuals to the data following fitting.

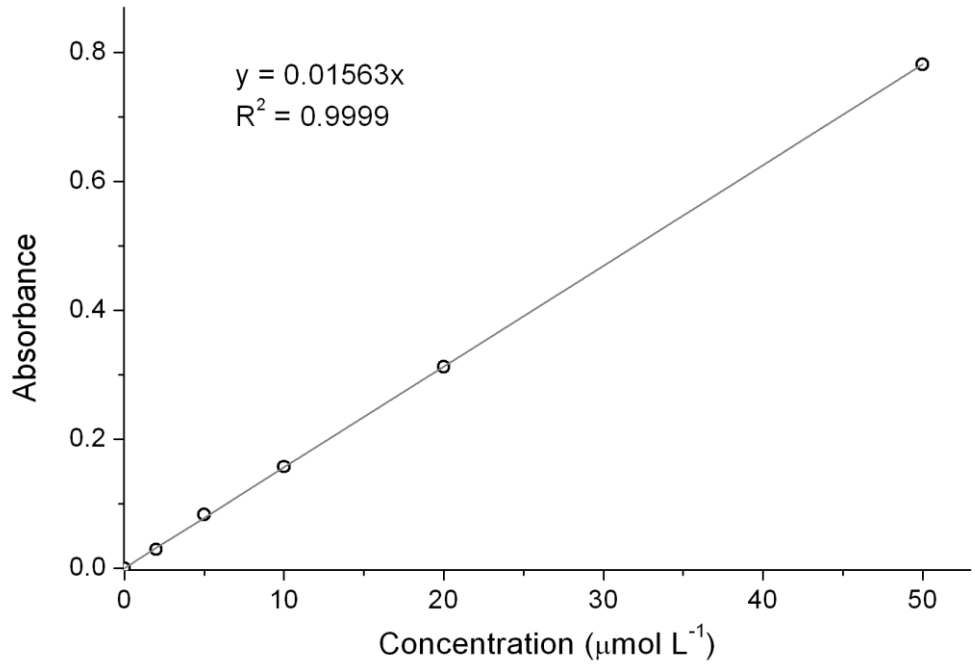


Fig. 7: Concentration versus absorbance of **1** in $CHCl_3$ at 525 nm. The data are fitted to a straight line passing through the origin. The gradient of this line is 15630 $M^{-1}cm^{-1}$.

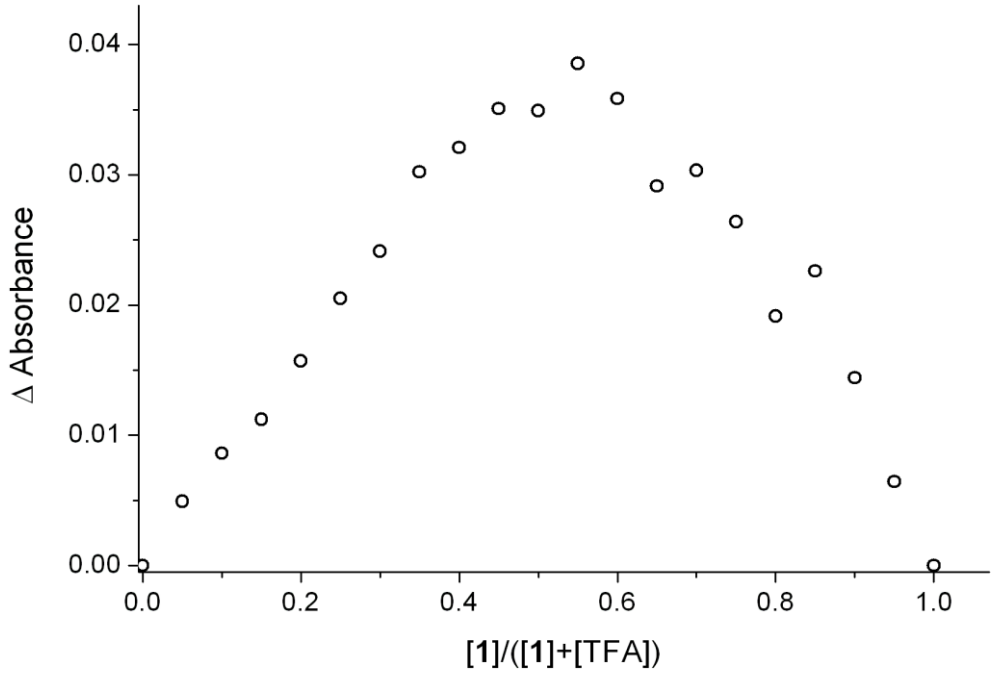


Fig. 8: Job's plot determined over range from 0 – 10 μmol L^{-1} **1** with a constant concentration of **1** + TFA = 10 μmol L^{-1}. Maximum ΔAbsorbance occurs at ~0.55 mole fraction of **1** indicating 1:1 binding between **1** and H^+. ΔAbsorbance = measured absorbance – calculated absorbance where the calculated absorbance is [**1**]/([**1**]+TFA) multiplied by the measured absorbance of the 10 μmol L^{-1} solution of **1**. The absorbance was taken over the range 510 – 530 nm. TFA does not absorb over this range.

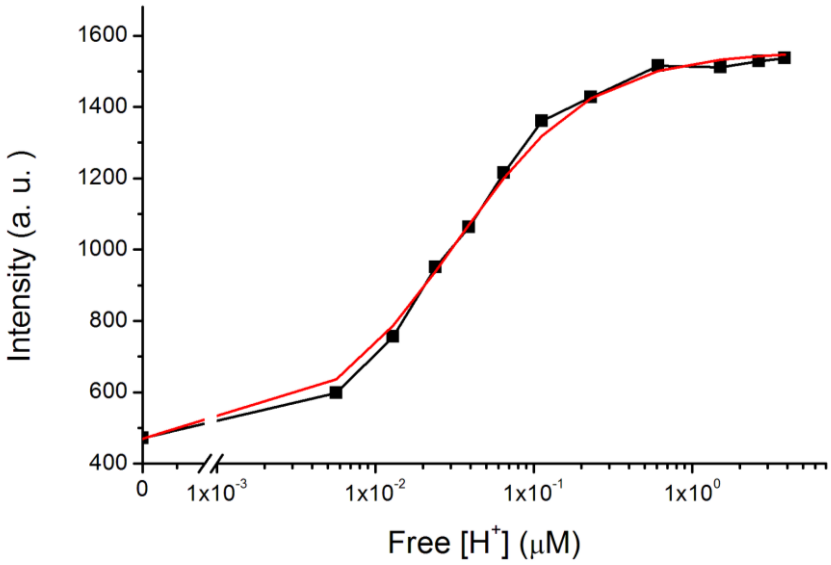


Fig. 9: Fluorescence titration of **1** in $CHCl_3$ with TFA. Each intensity data point was recorded following addition of 0.125 equivalents of H^+. >99% protonation was assumed to have been reached at 1.375 equivalents since no measurable increases in fluorescence intensity were observed on further addition of TFA and this intensity was taken as I_{max}. Intensity was fitted with a model assuming 1:1 binding between the host (**1**) and guest (H^+) following the method of Tsien et al. (Tsien, R.; Pozzan, T.; Rink, T. J., *J. Cell Biol.*, **1982**, 325-334): $I(H^+) = I_{min} + (I_{max} - I_{min})([H^+]^n / K_d)/(1+[H^+]^n / K_d)$, where *n* is the number of protons binding and K_d is the dissociation constant for the reaction $\mathbf{1.1H^+} \rightleftharpoons \mathbf{1} + H^+$. Intensities were calculated from areas under emission spectra and corrected for changes in absorbance with $[H^+]$ at the excitation wavelength (470 nm). The value of K_d returned by the fit is 0.031 μM when *n* is fixed to 1.0. (When both K_d and *n* are left free, values of 0.22 μM and 1.11 are returned with only marginal improvement to the fit. We assume therefore that the binding is 1:1 and K_d is given as that when *n* is fixed to 1.0.)

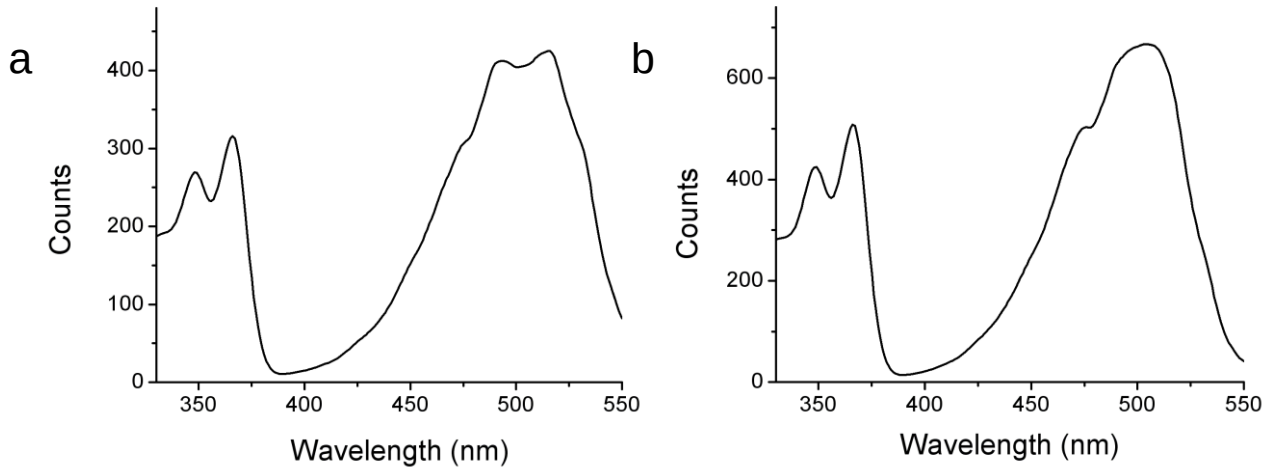
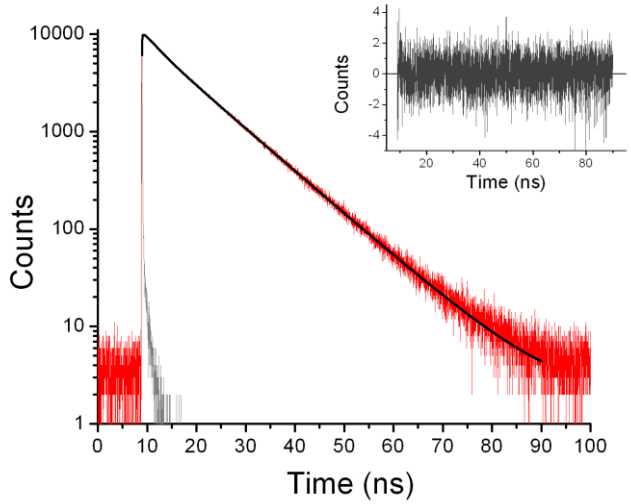
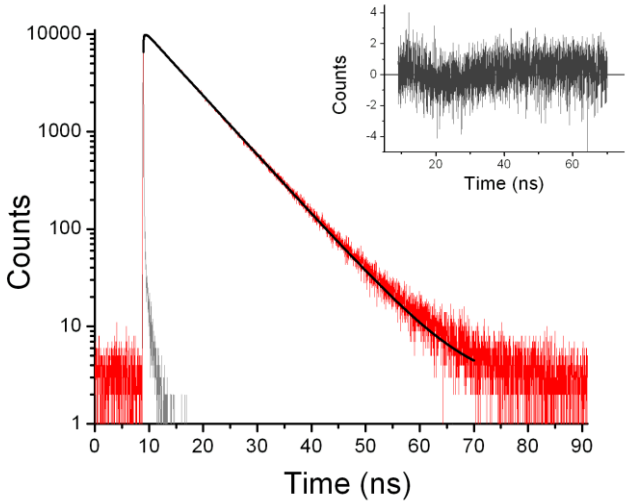


Fig. 10: Excitation spectra of **1** (a) and **1.1H**$^+$ (b) in $CHCl_3$. Emission monitored at 560 nm.

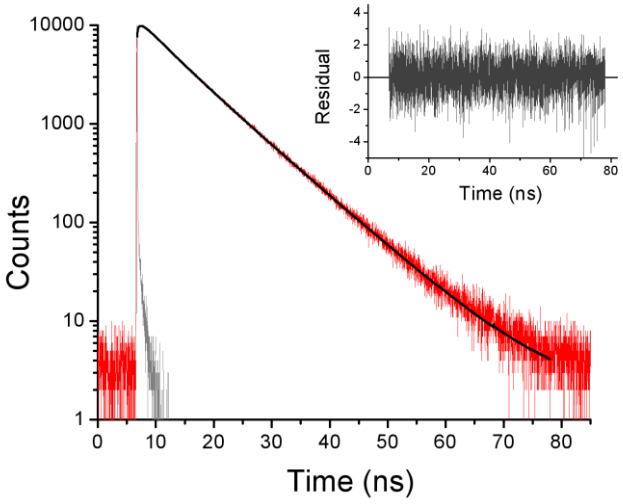
a



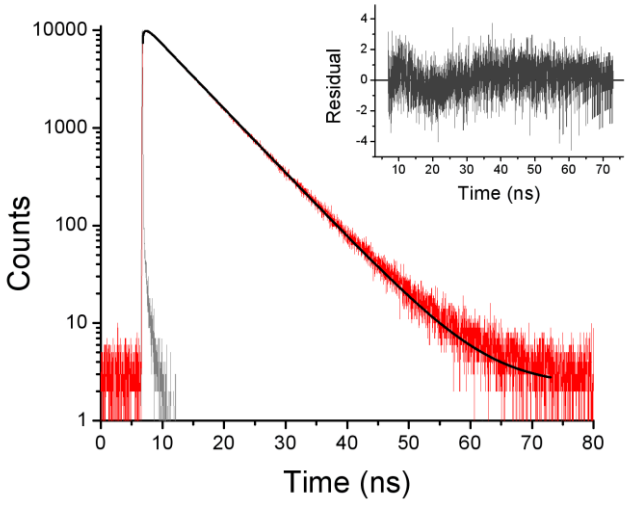
b



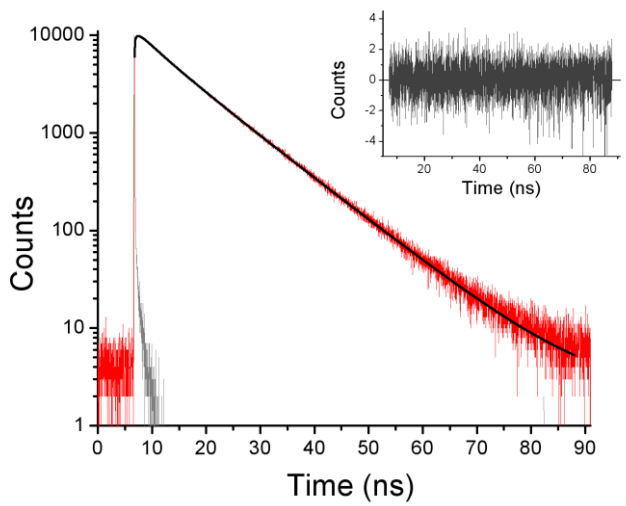
c



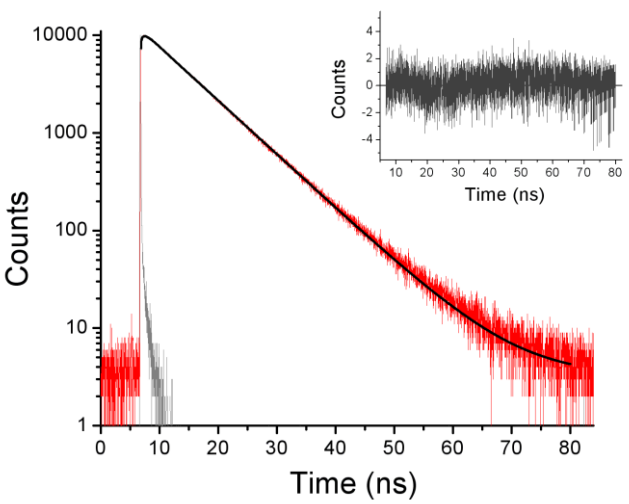
d



e



f



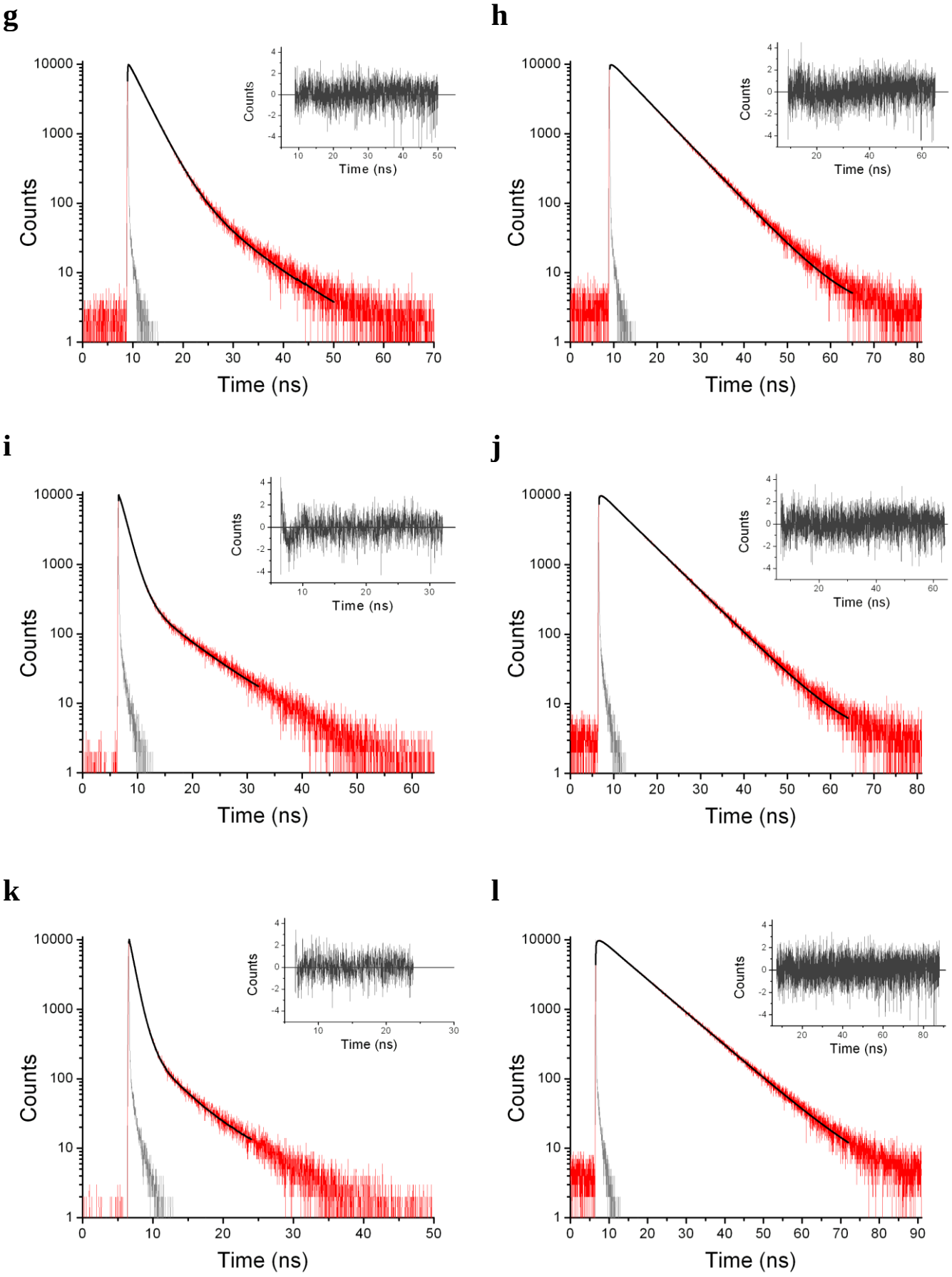


Fig. 11: Fluorescence decay histograms (red), fitted functions (black), instrument response functions (grey) and fit residuals (insets) of **1** in neutral solvents (left column) and solvents acidified with TFA (right column) for toluene (a,b; χ^2=1.11, 1.09) tetralin (c,d; χ^2=1.04, 1.07) *o*-xylene (e,f; χ^2=1.10, 1.12) $CHCl_3$ (g,h; χ^2=1.02, 1.15) CH_2Cl_2 (i,j; χ^2=1.14, 1.04) and benzonitrile (k,l; χ^2=1.00, 1.05).