Supplementary information for

A fluorescent three-sensor array for heavy metals in environmental water sources

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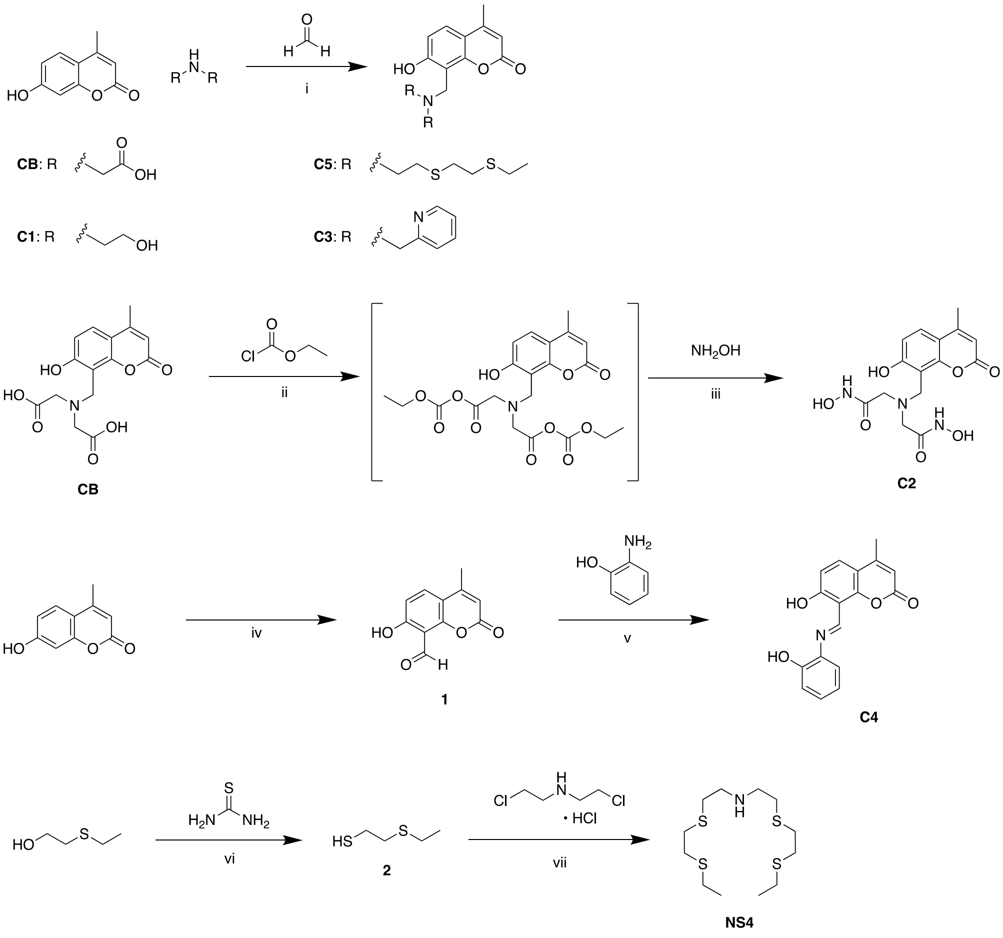
Contents

Experimental methods

Chemicals and characterisation

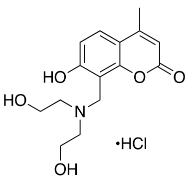
All chemical reagents and solvents used were laboratory or analytical grade purchased from Sigma-Aldrich, Merck or Combi-blocks. Metal salts used were lead(II) nitrate, iron(III) nitrate nonahydrate, copper(II) nitrate trihydrate, zinc(II) nitrate hexahydrate, cobalt(II) nitrate hexahydrate, nickel(II) nitrate hexahydrate, cadmium(II) nitrate tetrahydrate, mercury(II) nitrate monohydrate silver(I) nitrate, chromium(III) chloride hexahydrate and potassium dichromate. Flash column chromatography was performed on silica (Merck Silica gel 60 0.040–0.063 mm, 230–400 mesh). ^{1}H NMR spectroscopy was performed on the Bruker AVANCE300 NMR Spectrometer at a frequency of 300 MHz. Spectra are reported as chemical shifts (δ) in ppm, multiplicity, coupling constant (J) in Hz and relative integration. ^{13}C NMR spectroscopy was performed on the same instrument as ^{1}H NMR spectroscopy at a frequency of 75 MHz. Melting points for solid compounds were acquired on the MPA160 (Stanford Research Systems). Low resolution mass spectrometry was performed using a Bruker amaZon SL mass spectrometer operating in electrospray ionisation (ESI) mode. High-resolution mass spectrometry was performed on a Bruker Apex-Ultra spectrometer operating on ESI mode using an Apollo II ESI/MALDI dual source.

Synthesis of sensors C1-C5



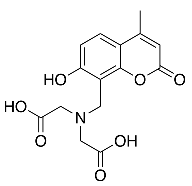
Scheme S1: Synthetic routes and conditions to achieve fluorescent sensors **C1** to **C5**. i (ethanol/MeCN, reflux), ii (Et$_2$O, N-methylmorpholine, 0˚C), iii (MeOH, KOH, 0 ˚C), iv (AcOH, hexamethylenetetramine, 75 ˚C), v (THF, N$_2$, 30 ˚C), vi (HBr (48%), reflux), vii (EtOH, Na, reflux).

C1



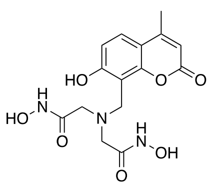
Method was adapted from Elderfield and Mehta.[1] A stock solution was prepared by dissolving paraformaldehyde (2.7 g, 91 mmol) and diethanolamine (7.7 mL, 79 mmol) in absolute ethanol (100 mL). 7-hydroxy-4-methylcoumarin (0.65 g, 3.3 mmol) was dissolved in absolute ethanol (6 mL) The prepared stock solution (5 mL) was added and the resultant solution was heated and stirred at 60 ˚C for 7 h. The solvent was removed under reduced pressure to give a light brown oil. The oil was dissolved in MeOH (10 mL) and the pH adjusted to 1 with hydrochloric acid (10 M) to effect precipitation of the desired product as a pale yellow solid (0.50 g, 62%). m.p. decomposition without melting at 205℃ (observed) 210℃ (reported).[1] ^{1}H NMR (300 MHz, D$_2$O) δ: 7.71 (d, J = 8.9, 1H), 6.95 (d, J = 8.9, 1H), 6.16 (s, 1H), 4.60 (s, 2H), 3.97 (t, *J* = 5.1, 4H), 3.44 (t, *J* = 5.1, 4H), 2.42 (s, 3H). ^{13}C NMR (75 MHz, D2O) δ: 163.6, 160.2, 157.1, 153.3, 129.3, 113.7, 113.3, 110.7, 103.9, 56.3, 55.7, 47.9, 18.7. LRMS (ESI) *m/z* calculated for $C_{15}H_{20}NO_5^+$ 294.13; found 294.15. $C_{15}H_{20}NO_5^+$

CB



Method was adapted from Huitink, Poe and Diehl.[2] 7-hydroxy-4-methylcoumarin (1.76 g, 10 mmol) was added to iminodiacetic acid (1.62, 12.2 mmol) and formaldehyde (37% aqueous solution, 1.2 mL, 12 mmol) in glacial acetic acid (35 mL) and the resultant suspension was heated to reflux for 15 h. The resultant white precipitate was collected by vacuum filtration and washed with deionised water (20 mL). The solid was re-dissolved in water adjusted to pH 7 with sodium hydroxide (10 M). The pH was adjusted to pH 4 with hydrochloric acid (10 M) to effect precipitation. The precipitate was collected by vacuum filtration, washed with deionised water (20 mL), and re-dissolved in water at pH 7 adjusted with sodium hydroxide (10 M). The pH was adjusted to pH 4, and the fine white precipitate was collected by centrifugation and decantation. The solid was suspended in absolute ethanol (30 mL) and removing the solvent under reduced pressure gave the desired product as a white solid (0.99 g, 31%). m.p. > 250˚C (observed), > 300˚C (reported).[2] ^{1}H NMR (300 MHz, DMSO-d$_6$) δ: 11.88 (b, 2H), 7.58 (d, *J* = 8.7, 1H), 6.82 (d, *J* = 8.7, 1H), 6.13 (s, 1H), 4.13 (s, 2H), 3.51 (s, 4H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ: 172.3, 160.8, 159.9, 153.8, 152.8, 125.5, 112.8, 111.8, 110.0, 109.2, 53.7, 46.8, 18.2. LRMS (ESI) *m/z* calculated for $C_{15}H_{14}NO_7^-$ 320.08; found 320.01.

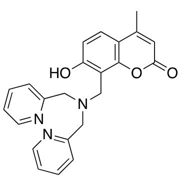
C2



CB (0.67 g, 2.1 mmol) was dissolved in diethyl ether (10 mL). Ethylchloroformate (0.44 mL, 4.6 mmol) and N-methylmorpholine (0.57 mL, 5.2 mmol) were added and the resultant solution was stirred at 0 ˚C for 15 min. The precipitate was removed by vacuum filtration, washing with cold diethyl ether (10 mL). A hydroxylamine solution was prepared by adding a solution of hydroxylammonium chloride (1.0 g, 14 mmol) in MeOH (5 mL) to a solution of potassium hydroxide (0.84 g, 15.0 mmol) in MeOH (5 mL) and stirring for 15 min at 0 ˚C. The precipitate was removed by vacuum filtration, washing with cold MeOH (10 mL). The filtrates of the two reaction mixtures were combined and stirred for 15 min at 0 ˚C. The solvent was removed under reduced pressure to obtain a yellow oil. Triturating the oil in diethyl ether (20 mL) and MeOH (10 mL) gave the desired product as a pale solid (24 mg, 30%) m.p. decomposition without melting at 122 ˚C (no reported value). ^{1}H NMR (300 MHz, DMSO-d$_6$)

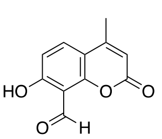
δ: 8.93 (b, 1H), 7.57 (d, *J* = 8.7, 1H), 6.82 (d, *J* = 8.7, 1H), 6.13 (s, 1H), 4.03 (s, 2H), 3.37 (s, 4H), 2.37 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d$_6$) δ: 172.13, 166.95, 159.95, 153.82, 152.91, 125.48, 112.93, 111.81, 110.00, 109.56, 53.50, 46.56, 18.26. LRMS (ESI) *m/z* calculated for $C_{15}H_{20}N_3NaO_7^+$ 374.10; found 374.16.

C3



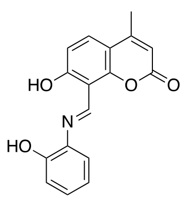
2,2'-Dipicolylamine (0.53 mL, 3.1 mmol) was dissolved in MeCN (30 mL). Formaldehyde (37% aqueous solution, 0.32 mL, 3.2 mmol) was added and the resultant solution was heated to 60 ˚C and stirred for 40 min. 7-hydroxy-4-methylcoumarin (0.50 g, 3.1 mmol) was added, and the reaction mixture was heated to reflux and stirred for 24 h. The solvent was removed under reduced pressure to give a yellow oil. The oil was dissolved in $CHCl_3$ (50 mL), washed with deionised water (50 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by flash chromatography (silica gel, CH_2Cl_2:MeOH:AcOH (95:5:3)) gave the desired product as a light brown oil (0.82 g, 67%). $\boldsymbol{\nu}_{max}$/cm^{-1} 3058, 2926, 2548, 1908, 1704, 1625, 1593, 1501, 1478, 1432, 1369, 1272, 1217, 1171, 1151, 1121, 1069, 1049, 1006. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.47 (b, 1H), 8.55 (d, *J* = 8.6, 2H), 7.64 (td, *J* = 7.7, 1.6, 2H), 7.41–7.34 (m, 3H), 7.17 (t, *J* = 6.2, 2H), 6.85 (d, *J* = 6.9, 1H), 6.06 (s, 1H), 4.09 (s, 2H), 3.90 (s, 4H), 2.35 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.7, 161.3, 157.2, 153.4, 152.9, 148.0, 137.4, 124.7, 123.4, 122.5, 113.7, 112.0, 110.2, 110.0, 58.3, 47.7, 18.6. HRMS (ESI) *m/z* calculated for $C_{23}H_{21}N_3O_3^+$ 388.16557; found 388.16502.

1



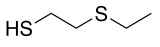
Method was adapted from Huang *et.al.*[3] 7-hydroxy-4-methylcoumarin (4.9 g, 28 mmol) and hexamethylenetetramine (10 g, 71 mmol) were stirred in glacial acetic acid (50 mL) at 75 ˚C for 24 h. Hydrochloric acid (90 mL, 20% (v/v)) was added and stirred for 1 h at 75 ˚C. The reaction mixture was extracted with diethyl ether (2 ×100 mL), washed with brine (100 mL) and concentrated *in vacuo* to give a pale yellow solid. Recrystallisation from hot ethanol gave the desired product as pale yellow needles (0.49 g, 8.6%). m.p. 178–180 ˚C (observed) 176–177 ˚C (reported).[4] ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.17(s, 1H), 10.58, (s, 1H), 7.70 (d, *J* = 8.89 1H), 6.87 (d, *J* = 8.90, 1H), 6.17 (s, 1H), 2.40 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 193.4, 165.3, 159.2 156.2, 152.8, 133.0, 114.4, 112.1, 112.0, 108.7, 19.0. LRMS (ESI) *m/z* calculated for $C_{11}H_8NaO_4^+$ 227.03; found 227.08.

C4



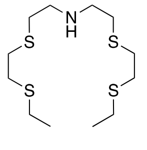
Method adapted from Dong *et al.*[5] A solution of **1** (0.11 g, 0.50 mmol) in anhydrous THF (8 mL) was stirred under a nitrogen atmosphere at room temperature for 15 min. 2-aminophenol (65 mg, 0.60 mmol) in absolute ethanol (5 mL) was added and the resultant solution was stirred at 30 ˚C for 24 h. The resultant orange precipitate was collected by centrifugation and recrystallisation from hot ethanol gave the desired product as orange needles (98 mg, 65%). m.p. > 250 ˚C (observed) 246 ˚C (reported).[6] ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.27 (b, 1H), 9.34 (s, 1H), 7.74 (d, *J* = 9.12, 1H), 7.60 (dd, *J* = 8.03, *J* = 1.37, 1H), 7.19 (td, *J* = 7.63, *J* = 1.45, 1H), 7.01 (dd, *J* = 8.26, *J* = 1.08, 1H), 6.92 (td, *J* = 7.41, J = 1.18, 1H), 6.80 (d, *J* = 9.05, 1H), 6.18 (s, 1H), 2.40 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.9, 159.3, 154.6, 154.2, 153.6, 150.4, 130.8, 130.5, 128.8, 119.9, 119.6, 116.5, 116.2, 109.2, 109.0, 105.7, 18.4. LRMS (ESI) *m/z* calculated for $C_{17}H_{13}NNaO_4^+$ 318.07; found 318.16.

2



Method was adapted from Zeng *et al.*[7] Formation of 2-thiapentan-1-thiol was achieved by adding ethyl 2-hydroxyethylsulfide (4.25 g, 40 mmol) to a solution of thiourea (3.05 g, 40 mmol) in 48% hydrobromic acid (8.5 mL). The mixture was heated to reflux for 16 h under a nitrogen atmosphere then cooled to room temperature. A solution of sodium hydroxide (3.2 g) in water (20 mL) was added slowly to the reaction mixture and then heated to reflux for 12 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and extracted with CH_2Cl_2 (100 mL). The organic phase was separated and washed sequentially with water and brine, then dried (Na_2SO_4) and filtered. The extract was concentrated *in vacuo* to yield the thiol as a colourless oil that was used in the next step without further purification (3.9 g, 79%).

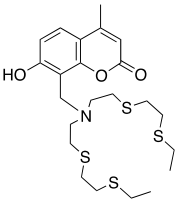
NS4



Method was adapted from Zeng *et al.*[7] Under a nitrogen atmosphere, sodium (1.29 g, 56 mmol) was dissolved slowly in ethanol (60 mL). **2** was added and the resultant solution was heated to reflux. A solution of bis(2-chloroethyl)amine hydrochloride (1.59 g, 11 mmol) in ethanol (35 mL) was added dropwise over 30 min and the resultant reaction mixture was heated to reflux for a further 4 h, then concentrated *in vacuo*. The residue was dissolved in chloroform (200 mL) and washed sequentially with water, saturated sodium bicarbonate and brine, then dried (Na_2SO_4), filtered and concentrated *in vacuo.* Purification by flash column chromatography (silica gel, 4% methanol/CH_2Cl_2), gave the desired compound as a pale orange oil

(2.35 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) 2.77 (t, J=6.0 Hz, 4H), 2.67 (m, 12H), 2.50 (q, J = 8.0 Hz, 4H), 1.84 (br, 1H), 1.97 (t, J = 8 Hz, 6H) ppm. ^{13}C NMR (100 MHz; $CDCl_3$) 53.4, 48.2, 32.1, 31.8, 26.1, 14.8 ppm. LRMS (ESI) *m/z* calculated for $C_{12}H_{27}NS_4^+$ 313.10; found 313.8.

C5



Method was adapted from Sakamoto *et al.*[8] A solution of **NS4** (314 mg, 1.0 mmol) was dissolved in ethanol (6 mL). Formaldehyde (150 uL of a 37% solution) was added and the resultant mixture was stirred at 60 °C for 30 min. 4-methyl-7-hydroxycoumarin (176 mg, 1.0 mmol) in ethanol (6 mL) was added and the resultant solution was heated to reflux for 12 h. The solvent was removed under reduced pressure. Purification by flash chromatography (silica gel, EtOAc:hexane (1:1)) gave the desired product as a pale yellow oil (270 mg, 54%). ^{1}H NMR (300 MHz; $CDCl_3$) 7.43 (d, J = 9.0 Hz, 1H), 6.80 (d, J = 9.0 Hz, 1H), 6.09 (s, 1H), 4.14 (s, 2H), 2.84-2.69 (m, 16H), 2.54 (q, J = 7.5 Hz, 4H), 2.39 (s, 3H), 1.24 (t, J = 7.5 Hz, 6H). ^{13}C NMR (101 MHz; $CDCl_3$) 161.9, 161.1, 153.4, 152.4, 124.9, 113.5, 112.5, 110.8, 108.1, 53.2, 50.1, 32.3, 31.6, 28.9, 26.1, 18.8, 14.8 ppm. HRMS (ESI) *m/z* calculated for $C_{23}H_{35}NO_3S_4H^+$ 502.1572; found 502.1563.

Supplementary tables and figures

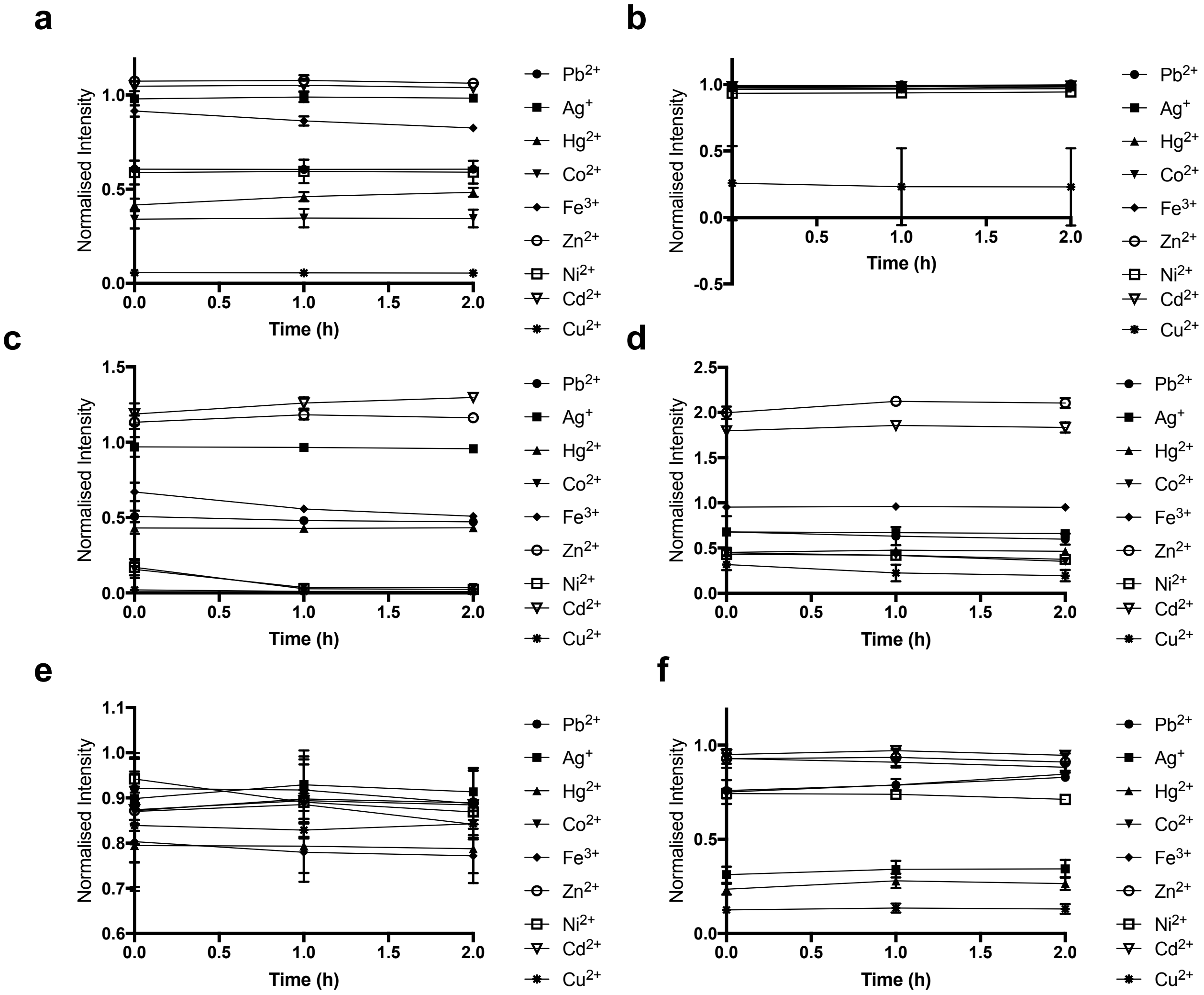


Figure S1: Normalised fluorescence responses of metals (10 µM) to sensors **CB (a)** and **C1–C5 (b-f)** (10 µM) in HEPES (20 mM, pH 7.4) and DMF (1% (v.v)) collected after incubation for 0, 1 and 2 h. Error bars represent standard deviation of replicate measurements, *n* = 5. No error bars are shown where the error bars would be shorter than the height of the symbol.

Table S1: ICP-MS instrument specifications for pond water metal analysis.

Specification	Details
Nebuliser	Meinhard glass
Spray Chamber	Cyclonic, glass
RF generator	1500 W
Plasma gas flow rate	16 L/min, Ar
Nebuliser gas flow rate	1-1.1 L/min, Ar
Auxiliary gas flow rate	1.2 L/min, Ar
Kinetic energy discrimination (KED) gas flow rate	5 L/min, He
Sample uptake rate	300-500 µL/min (9 rpm peristaltic pump with Perkin Elmer PVC MP2 Peripump flared tubing 0.38 mm)
Number of replicates	3
Integration time (ms)	1000 for all except V (5000) and Se (3000)
Internal standards	193-Ir, 103-Rh delivered through a T-piece.
Isotopes	27-Al, 75-As, 138-Ba (with Ce and La corrections) 111-Cd, 43-Ca, 52-Cr, 59-Co, 63-Cu, 57-Fe, 24-Mg, 55-Mn, 60-Ni, 30-P, 39-K, 82-Se (with Kr correction), 28-Si, 107-Ag, 23-Na, 88-Sr, 47-Ti, 51-V, 66-Zn, 206-Pb, 207-Pb, 208-Pb
Autosampler	CETAC ASX-520

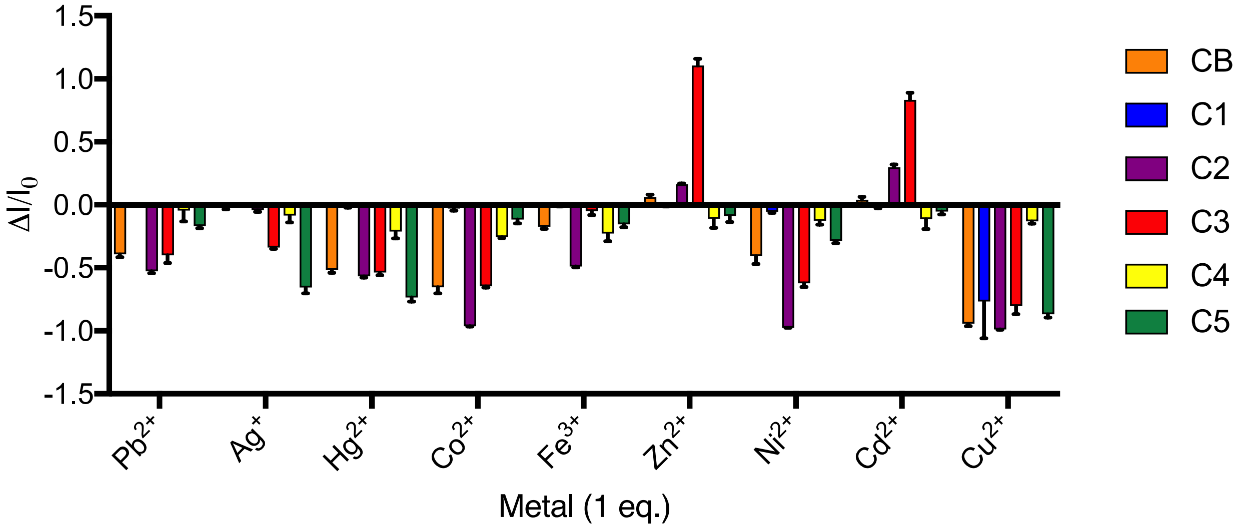


Figure S2: Fluorescence responses of metals (10 µM) to sensors **CB** and **C1–C5** (10 µM) in HEPES (20 mM, pH 7.4) and DMF (1% (v.v)). Error bars represent standard deviation of replicate measurements, *n* = 5.

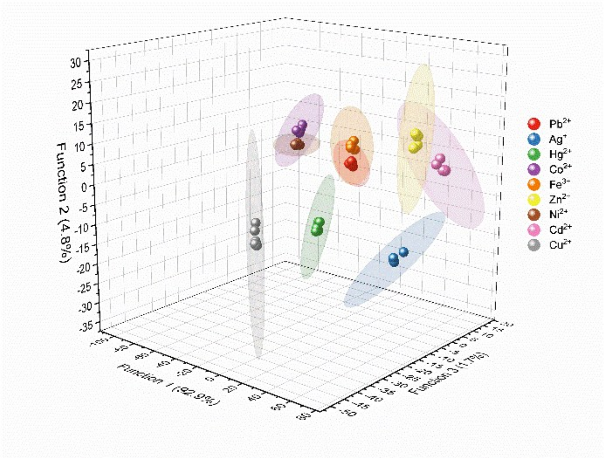


Figure S3: 6-sensor array LDA score plot for the analysis of 9 metal ions performed in quintuplicate (10 µM metal ion, 10 µM sensors in HEPES (20 mM, pH 7.4), and DMF (1% v/v)) with 95% confidence ellipsoids.

Table S2: LDA classification of 6-sensor array (10 µM metal, 10 µM sensor in HEPES (10 mM, pH 7.4) and DMF (1% v/v)). Red cells indicate false classifications.

		Pb(II)	Ag(I)	Hg(II)	Co(II)	Fe(III)	Zn(II)	Ni(II)	Cd(II)	Cu(II)
Original	Pb(II)	5	0	0	0	0	0	0	0	0
	Ag(I)	0	5	0	0	0	0	0	0	0
	Hg(II)	0	0	5	0	0	0	0	0	0
	Co(II)	0	0	0	5	0	0	0	0	0
	Fe(III)	0	0	0	0	5	0	0	0	0
	Zn(II)	0	0	0	0	0	5	0	0	0
	Ni(II)	0	0	0	0	0	0	5	0	0
	Cd(II)	0	0	0	0	0	0	0	5	0
	Cu(II)	0	0	0	0	0	0	0	0	5
Cross-validated	Pb(II)	5	0	0	0	0	0	0	0	0
	Ag(I)	0	5	0	0	0	0	0	0	0
	Hg(II)	0	0	5	0	0	0	0	0	0
	Co(II)	0	0	0	5	0	0	0	0	0
	Fe(III)	0	0	0	0	5	0	0	0	0
	Zn(II)	0	0	0	0	0	5	0	0	0
	Ni(II)	0	0	0	0	0	0	5	0	0
	Cd(II)	0	0	0	0	0	0	0	5	0
	Cu(II)	0	0	0	0	0	0	1	0	4

Table S3: Anti-image correlations with measurements of sampling adequacies (MSAs)[a] from PCA of 6-sensor array.

	CB	**C1**	**C2**	**C3**	**C4**	**C5**
CB	.781[a]	-.484	-.570	-.022	-.146	-.166
C1	-.484	.556[a]	-.165	.487	.295	-.511
C2	-.570	-.165	.591[a]	-.739	-.205	.627
C3	-.022	.487	-.739	.579[a]	.227	-.739
C4	-.146	.295	-.205	.227	.491[a]	-.116
C5	-.166	-.511	.627	-.739	-.116	.454[a]

Table S4: Sensor communality scores from PCA extraction of 6-sensor array.

	Initial	**Extraction**
CB	1.000	.907
C1	1.000	.694
C2	1.000	.880
C3	1.000	.814
C4	1.000	.721
C5	1.000	.678

Table S5: Sensor correlation matrix from PCA extraction of 6-sensor array. *P<0.05, **P<0.005 from 1-tailed T-test.

	CB	**C1**	**C2**	**C3**	**C4**	**C5**
CB	1.000	.647**	.886**	.801**	.259*	.510**
C1	.647**	1.000	.421**	.357*	-.053	.577**
C2	.886**	.421**	1.000	.871**	.315*	.325*
C3	.801**	.357*	.871**	1.000	.194	.577**
C4	.259*	-.053	.315*	.194	1.000	-.027
C5	.510**	.577**	.325*	.577**	-.027	1.000

Table S6: LDA classification of 3-sensor array (10 µM metal, 10 µM sensor in HEPES (20 mM, pH 7.4) and DMF (1% v/v)). Red cells indicate false classifications.

		Pb(II)	Ag(I)	Hg(II)	Co(II)	Fe(III)	Zn(II)	Ni(II)	Cd(II)	Cu(II)
Original	Pb(II)	5	0	0	0	0	0	0	0	0
	Ag(I)	0	5	0	0	0	0	0	0	0
	Hg(II)	0	0	5	0	0	0	0	0	0
	Co(II)	0	0	0	5	0	0	0	0	0
	Fe(III)	0	0	0	0	5	0	0	0	0
	Zn(II)	0	0	0	0	0	5	0	0	0
	Ni(II)	0	0	0	0	0	0	5	0	0
	Cd(II)	0	0	0	0	0	0	0	5	0
	Cu(II)	0	0	0	0	0	0	0	0	5
Cross-validated	Pb(II)	5	0	0	0	0	0	0	0	0
	Ag(I)	0	5	0	0	0	0	0	0	0
	Hg(II)	0	0	5	0	0	0	0	0	0
	Co(II)	0	0	0	5	0	0	0	0	0
	Fe(III)	0	0	0	0	5	0	0	0	0
	Zn(II)	0	0	0	0	0	5	0	0	0
	Ni(II)	0	0	0	0	0	0	5	0	0
	Cd(II)	0	0	0	0	0	0	0	5	0
	Cu(II)	0	0	1	0	0	0	0	0	4

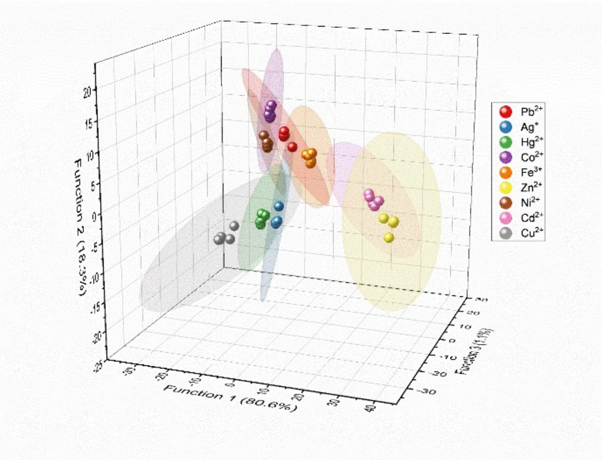


Figure S4: 3-sensor array LDA score plot for the analysis of 9 metal ions performed in quintuplicate (10 µM metal ion, 10 µM sensors in HEPES (20 mM, pH 7.4), and DMF (1% v/v)) with 95% confidence ellipsoids.

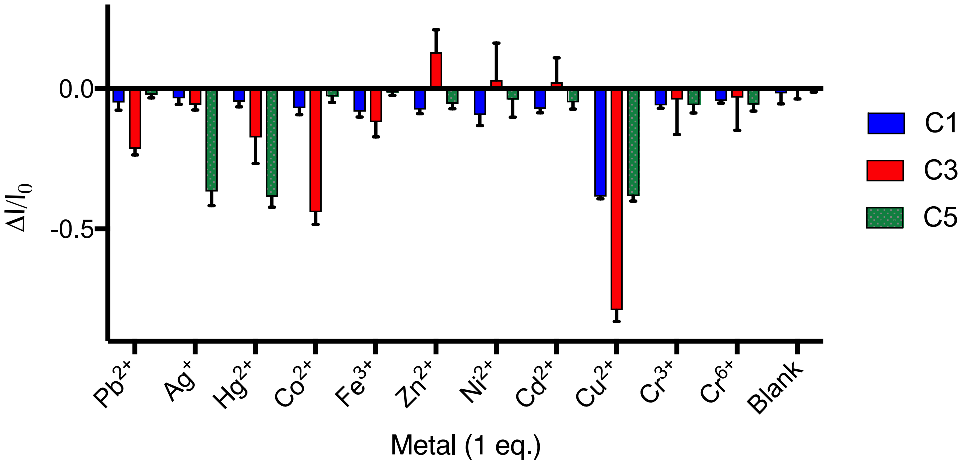


Figure S5: Fluorescence responses of metals (1 µM) to sensors **C1**, **C3** and **C5** (1 µM) in HEPES (10 mM, pH 7.4) and DMF (1% (v.v)). Error bars represent standard deviation of replicate measurements, *n* = 5.

Table S7: LDA classification of 3-sensor array (1 µM metal, 1 µM sensor in HEPES (10 mM, pH 7.4) and DMF (1% v/v)). Red cells indicate false classifications.

		Pb(II)	Ag(I)	Hg(II)	Co(II)	Fe(III)	Zn(II)	Ni(II)	Cd(II)	Cu(II)	Cr(III)	Cr(VI)	Blank
Original	Pb(II)	5	0	0	0	0	0	0	0	0	0	0	0
	Ag(I)	0	5	0	0	0	0	0	0	0	0	0	0
	Hg(II)	0	1	4	0	0	0	0	0	0	0	0	0
	Co(II)	0	0	0	5	0	0	0	0	0	0	0	0
	Fe(III)	1	0	0	0	4	0	0	0	0	0	0	0
	Zn(II)	0	0	0	0	0	3	0	2	0	0	0	0
	Ni(II)	0	0	0	0	0	2	2	0	0	1	0	0
	Cd(II)	0	0	0	0	0	1	0	3	0	1	0	0
	Cu(II)	0	0	0	0	0	0	0	0	5	0	0	0
	Cr(III)	0	0	0	0	1	1	0	0	0	3	0	0
	Cr(VI)	0	0	0	0	0	1	0	0	0	0	4	0
	Blank	0	0	0	0	0	0	0	0	0	0	0	5
Cross-validated	Pb(II)	4	0	0	0	0	0	0	0	0	0	0	1
	Ag(I)	0	3	2	0	0	0	0	0	0	0	0	0
	Hg(II)	0	1	4	0	0	0	0	0	0	0	0	0
	Co(II)	0	0	0	5	0	0	0	0	0	0	0	0
	Fe(III)	1	0	0	0	4	0	0	0	0	0	0	0
	Zn(II)	0	0	0	0	0	3	0	2	0	0	0	0
	Ni(II)	0	0	0	0	1	2	1	0	0	1	0	0
	Cd(II)	0	0	0	0	1	1	1	1	0	1	0	0
	Cu(II)	0	0	0	0	0	0	0	0	5	0	0	0

	Cr(III)	0	0	0	0	1	1	0	0	0	1	2	0
	Cr(VI)	0	0	0	0	0	1	0	0	0	2	2	0
	Blank	0	0	0	0	0	0	0	0	0	0	1	4

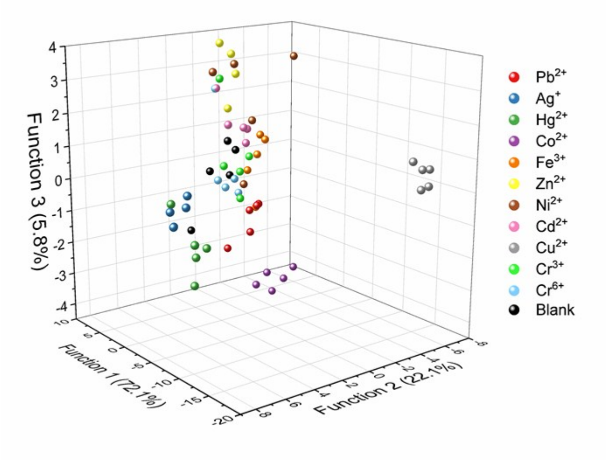


Figure S6: 3-sensor array LDA score plot for the analysis of 9 metal ions performed in quintuplicate (1 µM metal ion, 1 µM sensors in HEPES (10 mM, pH 7.4), and DMF (1% v/v)).

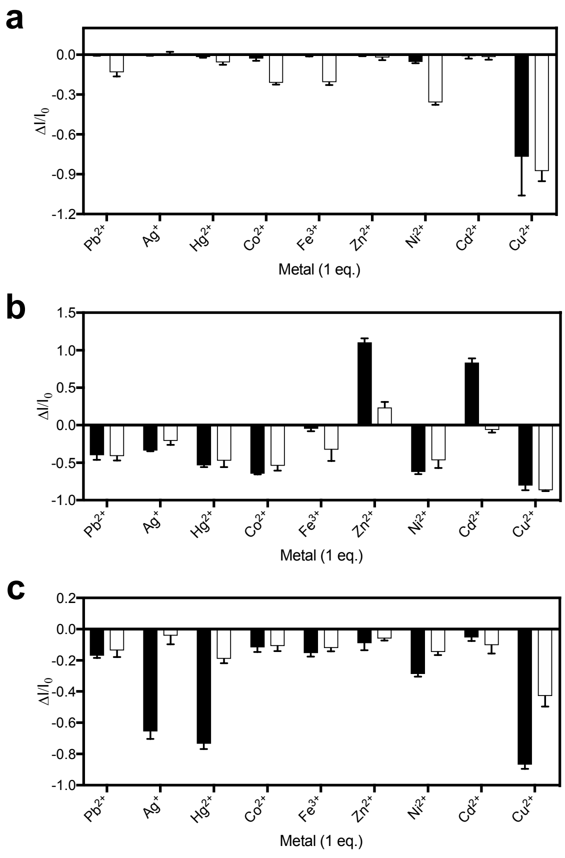


Figure S7: Fluorescence responses of **C1** (a) **C3** (b) and **C5** (c) (10 µM) of metal ions (10 µM) in HEPES (20 mM, pH 7.4) (black) and pond water (white). Error bars represent standard deviation of replicate measurements, *n* = 5.

Table S8: Metal concentrations in pond water measured by ICP-MS. Concentrations below the lower limit of quantification (LLOQ) are indicated and determined based on their readings being lower than the background equivalent concentration (BEC) of the instrument. Values given to 3 significant figures, considering relative standard deviation (RSD).

Element	Isotope	Concentration (ppb)	RSD (%)	Concentration (µM)	BEC (ppb)
Na	23	29400	2%	1280	2.17
Mg	24	4340	1%	181	0.257
Al	27	6.75	12%	0.250	0.181
K	39	6350	2%	163	30.5
Ca	43	8860	6%	206	2.42
Cr	52	0.647	4%	0.0124	0.0094
Mn	55	0.142	25%	0.00258	0.0619
Fe	57	2.29	16%	0.0402	0.415
Co	59	0.0491	11%	0.000832	0.000739
Ni	60	9.21	7%	0.154	0.0115
Cu	63	12.2	5%	0.194	0.0918
Zn	66	16.6	8%	0.252	0.669
Sr	88	103	2%	1.170	0.0324
Ag	107	0.044	12%	0.000411	0.00275
Cd	111	LLOQ	20%	LLOQ	0.778
Ba	138	34.7	2%	0.251	0.00838
Pb	206	LLOQ	23%	LLOQ	0.0128
Pb	207	LLOQ	186%	LLOQ	0.014
Pb	208	LLOQ	56%	LLOQ	0.0124

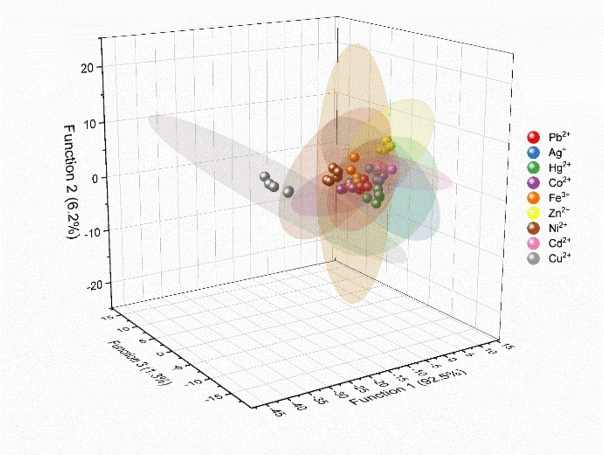


Figure S8: 3-sensor array LDA score plot for the analysis of 9 metal ions performed in quintuplicate (10 µM metal ion, 10 µM sensors in pond water and DMF (1% v/v)) with 95% confidence ellipsoids.

Table S9: LDA classification of 3-sensor array (10 µM metal, 10 µM sensor in pond water. Red cells indicate false classifications.

		Pb(II)	Ag(I)	Hg(II)	Co(II)	Fe(III)	Zn(II)	Ni(II)	Cd(II)	Cu(II)
Original	Pb(II)	5	0	0	0	0	0	0	0	0
	Ag(I)	0	5	0	0	0	0	0	0	0
	Hg(II)	0	0	5	0	0	0	0	0	0
	Co(II)	0	0	0	5	0	0	0	0	0
	Fe(III)	0	0	0	1	4	0	0	0	0
	Zn(II)	0	0	0	0	0	5	0	0	0
	Ni(II)	0	0	0	0	0	0	5	0	0
	Cd(II)	0	0	0	0	0	0	0	5	0
	Cu(II)	0	0	0	0	0	0	0	0	5
Cross-validated	Pb(II)	5	0	0	0	0	0	0	0	0
	Ag(I)	0	5	0	0	0	0	0	0	0
	Hg(II)	0	0	5	0	0	0	0	0	0
	Co(II)	0	0	0	5	0	0	0	0	0
	Fe(III)	1	0	0	1	3	0	0	0	0
	Zn(II)	0	0	0	0	0	5	0	0	0
	Ni(II)	0	0	0	0	0	0	5	0	0
	Cd(II)	0	0	0	0	0	0	0	5	0
	Cu(II)	0	0	0	0	0	0	0	0	5

Table S10: LDA classification of 3-sensor array for Pb2+ quantification (10 µM sensor in pond water). Red cells indicate false classifications.

	Concentration (µM)	0	5	10	50	100
Original	0	5	0	0	0	0
	5	0	5	0	0	0
	10	0	0	5	0	0
	50	0	0	0	5	0
	100	0	0	0	0	5
	Unknown (20 µM)	0	0	1	4	0
Cross-validated	0	5	0	0	0	0
	5	0	5	0	0	0
	10	0	1	4	0	0
	50	0	0	1	4	0
	100	0	0	0	0	5

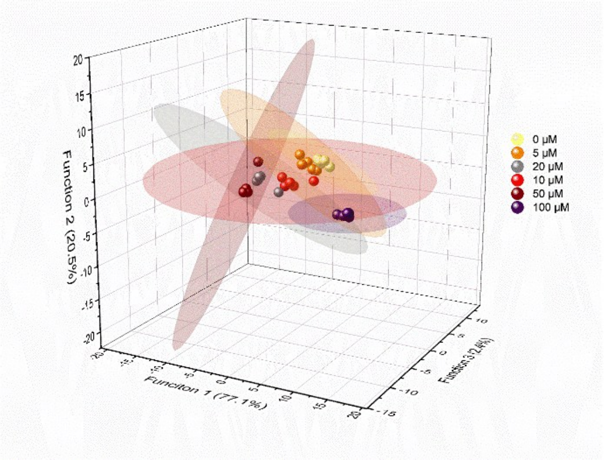


Figure S9: 3-sensor array LDA score plot for the classification of 5 concentrations of Pb^{2+} (0, 5, 10, 50, 100 µM) and prediction of unknown Pb^{2+} concentration to the LDA analysis (20 µM) performed in quintuplicate (10 µM sensors in pond water and DMF (1% v/v)) with 95% confidence ellipsoids.

Supplementary references

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