Supplementary Information

Biocatalytic metal-organic framework nanomotors for active water decontamination

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Experimental section

1. Materials

Zinc nitrate hexahydrate, 2-methylimidazole, catalase from bovine liver (CAT) and fluorescein diacetate were purchased from Sigma Aldrich Australia. Isotope labelled ^{13}C8-PFOA was purchased from Wellington Laboratories (Guelph, Canada). UHPLC-MSMS used methanol, methyl-t-butyl ether (MTBE), glacial acetic acid and ammonium acetate were purchased from Merck (Australia) and of either LC-MS grade or > 99.9% purity. Solid-Phase Extraction cartridges (Oasis 500 mg hydrophilic/lipophilic balance) were purchased from Waters (Millford, USA). Sample extracts were evaporated using a TurboVap LV concentration workstation purchased from Caliper Life Sciences (Hopkinton, USA) and cylindered ultra-high purity nitrogen (BOC, Australia).

All other reagents were purchased from Sigma Aldrich (Australia) and used without further modification.

2. Synthesis and characterization

2.1 Fluorescent labeling of CAT

CAT was fluorescent labeled with fluorescein isothiocyanate (FITC). CAT (40 mg) and FITC (1 mg) was dissolved in phosphate buffer (2.5 mL, pH 7.4, 0.5 M) and kept stirring for 2 h at room temperature. The labeled enzymes were collected through NAP-25 column (GE Healthcare).

2.2 Synthesis of CAT-ZIF-8

The synthesis of CAT-ZIF-8 followed a previous report by our group with slight modification.[1] Briefly, CAT (0.6 mg) was dissolved in 800 μL of 2-methylimidazole solution (860 mM) and 200 μL of zinc nitrate solution (45 mM) was then added quickly followed by continuous stirring for 1 h. The resultant particles were collected by centrifugation (Eppendorf Centrifuge 5418) at 5000 rpm for 2 min. The particles were washed with Milli-Q water and centrifuged at 5000 rpm for three times and finally resuspended in 100 μL Milli-Q water.

2.3 Adsorbing efficiency for metal ions

The synthesized metal ions were pre-dissolved in 5mL Milli-Q water at an initial concentration of 5 ppm followed by the addition of hydrogen peroxide to 0.2% and gentle placement of 100μL CAT-ZIF-8 nanomotors at the bottom. The pH was adjusted to 7 by adding sodium hydroxide solution dropwise to rule out the fluctuation of pH resulted from the addition of metal salt and hydrogen peroxide. The mixed solution was left stationary for 20min and filtered with Millex-GP Syringe Filter (0.22 μm). The solution was sent to ICP-OES for accurate element concentration measurement.

The cycling performance was carried out with metal ions solution in the same concentration. 200 uL of nanomotors were placed at the bottom of the vial and the mixed solution was left stationary for 10 min, followed by centrifugation to recollect the nanomotors and two cycles of repeated absorbing experiment. After filtered with a Millex-GP syringe filter (0.22 μm), the solution was sent to ICP-OES for element concentration measurement.

2.4 Adsorbing efficiency for PFOA

The PFOA was pre-dissolved in 1mL Milli-Q water at an initial concentration of 20μM, followed by the addition of hydrogen peroxide to 0.2% and gentle placement of 20μL CAT-ZIF-8 nanomotors at the bottom. The mixed solution was left stationary for 2min and filtered with Millex-GP Syringe Filter (0.22 μm). 100μL was taken from each sample and diluted into 500mL Milli-Q water, spiked with isotope labelled internal standard (^{13}C8-PFOA, 5ng) and extracted with a solid phase extraction (SPE). The samples were rinsed with 2 x 5 mL Milli-Q water and dried with N_2 followed by separation by ultra-high-performance liquid chromatography and identification and quantification by tandem mass spectrometry (UHPLC-MSMS).

2.5 Characterizations

Fluorescent microscope images were taken with Olympus IX53 inverted microscope with sCMOS camera. Confocal laser scanning fluorescence microscopy images (CLSM) were taken under a Nikon A1 Intravital system. Scanning electron microscope (SEM) images of samples were taken on a FEI Nova Nano SEM 450 FE-SEM at an accelerating voltage of 5.0 kV. PXRD patterns were collected using Empyrean Thin-Film XRD Xpert Materials Research diffractometer (MRD). The particle movement was recorded as a video using a light microscope and the moving distance was pre-calibrated with a 4 mm stainless steel sphere. ImageJ software was used to extract the particle motion curve that enabled the calculation of the particle velocity change from the video. The adsorbing efficiency was carried out with ICP-OES (PerkinElmer Optima).

3. Calculation

3.1 Calculation of removal efficiency enhancement

The initial concentration of the metal ions was prepared to be 5 ppm in water as the standard, taking Cerium as an example:

$\eta_{with\ hydrogen\ peroxide} = 6.7\%$

$\eta_{with\ nanomotors} = 49.6\%$

$\eta_{with\ hydrogen\ peroxide\ and\ nanomotors} = 99.9\%$

$$\Delta_{with\ hydrogen\ peroxide} = \frac{\eta_{with\ hydrogen\ peroxide\ and\ nanomotors} - \eta_{with\ nanomotors}}{\eta_{with\ nanomotors}} \times 100\% = 101.4\%$$

The initial concentration of the metal ions was prepared to be 50 ppm in sea water as the standard, taking Cerium as an example:

$\eta_{with\ hydrogen\ peroxide} = 9.2\%$

$\eta_{with\ nanomotors} = 25.4\%$

$\eta_{with\ hydrogen\ peroxide\ and\ nanomotors} = 99.8\%$

$$\Delta_{with\ hydrogen\ peroxide} = \frac{\eta_{with\ hydrogen\ peroxide\ and\ nanomotors} - \eta_{with\ nanomotors}}{\eta_{with\ nanomotors}} \times 100\% = 292.2\%$$

3.2 Calculation of the zinc ions concentration:

The initial concentration of the zinc ions was prepared to be 5 ppm as the standard.

$C_{initial\ concentration} = 100\%$

$C_{with\ hydrogen\ peroxide} = 114.4\%$

$C_{with\ nanomotors} = 230.6\%$

$C_{with\ hydrogen\ peroxide\ and\ nanomotors} = 385.2\%$

$C_{HCl\ dissolved} = 7324.6\%$

The percentage of the increased zinc ions was calculated as followed.

$$\varphi_{with\ hydrogen\ peroxide} = \frac{C_{with\ hydrogen\ peroxide} - C_{initial\ concentration}}{C_{initial\ concentration}} \times 100\% = 14.4\%$$

$$\varphi_{with\ nanomotors} = \frac{C_{with\ nanomotors} - C_{initial\ concentration}}{C_{initial\ concentration}} \times 100\% = 130.6\%$$

$$\varphi_{with\ hydrogen\ peroxide\ and\ nanomotors} = \frac{C_{with\ hydrogen\ peroxide\ and\ nanomotors} - C_{initial\ concentration}}{C_{initial\ concentration}} \times 100\% = 285.2\%$$

$$\varphi_{HCl\ dissolved} = \frac{C_{HCl\ dissolved} - C_{initial\ concentration}}{C_{initial\ concentration}} \times 100\% = 7224.6\%$$

The percentage of the dissolved nanomotors was calculated as followed.

$$Dissolved\ nanomotor_{with\ hydrogen\ peroxide} = \frac{\varphi_{with\ hydrogen\ peroxide}}{\varphi_{HCl\ dissolved}} \times 100\% = 0.2\%$$

$$Dissolved\ nanomotor_{with\ nanomotors} = \frac{\varphi_{with\ nanomotors}}{\varphi_{HCl\ dissolved}} \times 100\% = 1.8\%$$

$$Dissolved\ nanomotor_{with\ hydrogen\ peroxide\ and\ nanomotors} = \frac{\varphi_{with\ hydrogen\ peroxide\ and\ nanomotors}}{\varphi_{HCl\ dissolved}} \times 100\% = 3.9\%$$

3.3 Calculation of buoyancy force:

The nanomotor was estimated to be a sphere with the radius of 250nm.

$$F_{buoyancy} = \rho_{water} gV = 10^3 kg/m^3 \times 9.8N/kg \times \frac{4}{3}\pi\left(2.5 \times 10^{-7} m\right)^3 = 6.41 \times 10^{-16} N$$

Table S1. Metal ions removal efficiency and increment in water

	Metal ions removal efficiency (%)			
	H_2O_2	Nanomotor	H_2O_2+Nanomotor	Increment
Ce	6.67	49.60	99.90	101.42
Cu	4.84	46.94	88.62	88.79
Co	1.11	11.61	47.19	306.43
Mn	1.35	2.70	32.90	1117.30
Ni	0.28	16.75	33.56	100.24

Table S2. Metal ions removal efficiency and increment in sea water

	Metal ions removal efficiency (%)			
	H_2O_2	Nanomotor	H_2O_2+Nanomotor	Increment
Ce	9.21	25.44	99.81	292.22
Cu	0.54	14.11	88.02	523.58
Co	1.25	4.96	13.91	179.99
Mn	0.67	5.69	14.67	157.52
Ni	1.02	4.90	10.45	113.01

Table S3. PFOA removal efficiency and increment in water and sea water

	PFOA removal efficiency (%)			
	H_2O_2	Nanomotor	H_2O_2+Nanomotor	Increment
water	0.50	69.29	84.53	21.99
sea water	3.98	72.00	82.24	14.2

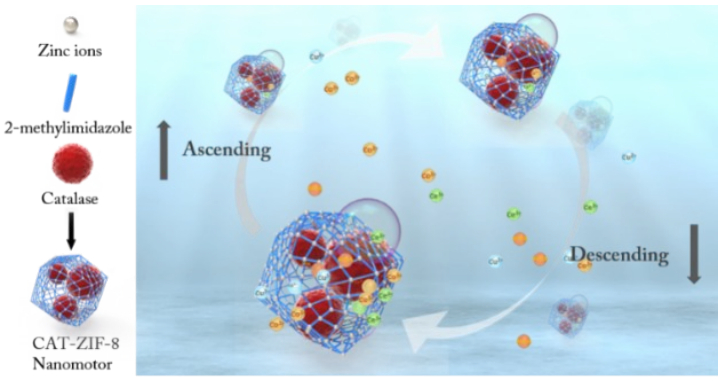

Figure S1. Schematic illustration of the biocatalytic CAT-ZIF-8 nano-cleaner in aqueous environments.

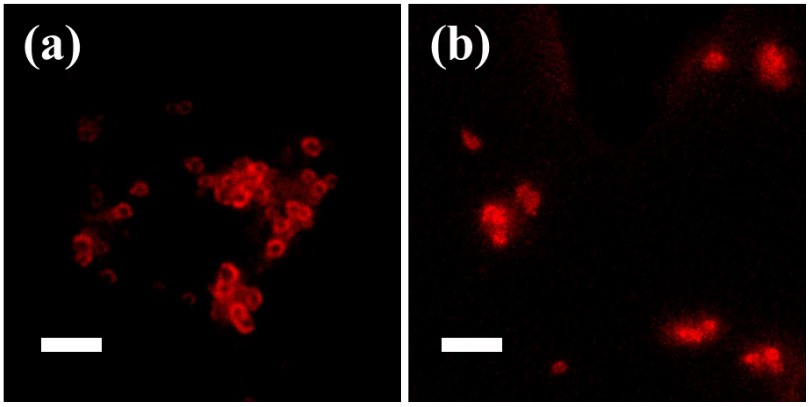

Figure S2. CLSM images of nanomotors with (a) post adsorption of catalase and (b) one-pot synthesis. Catalase was labelled with Alexa Fluor 647.

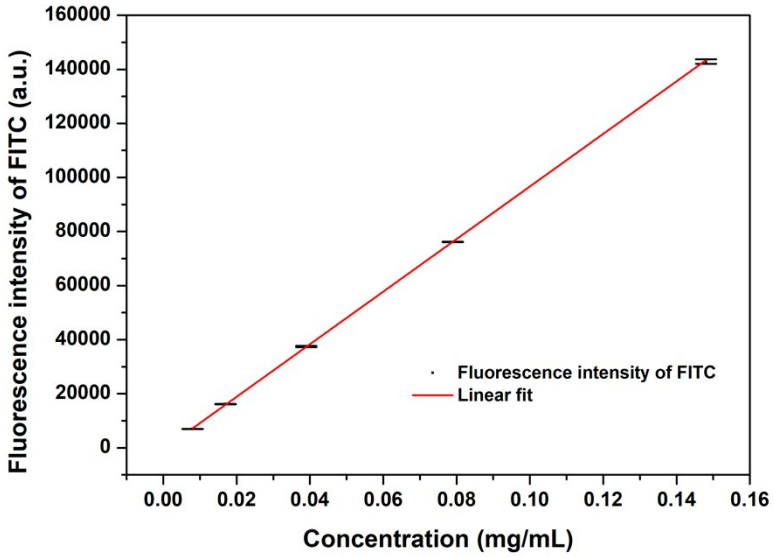

Figure S3. CAT concentration standard curve by measuring FITC-labeled CAT using a fluorescence spectrophotometer.

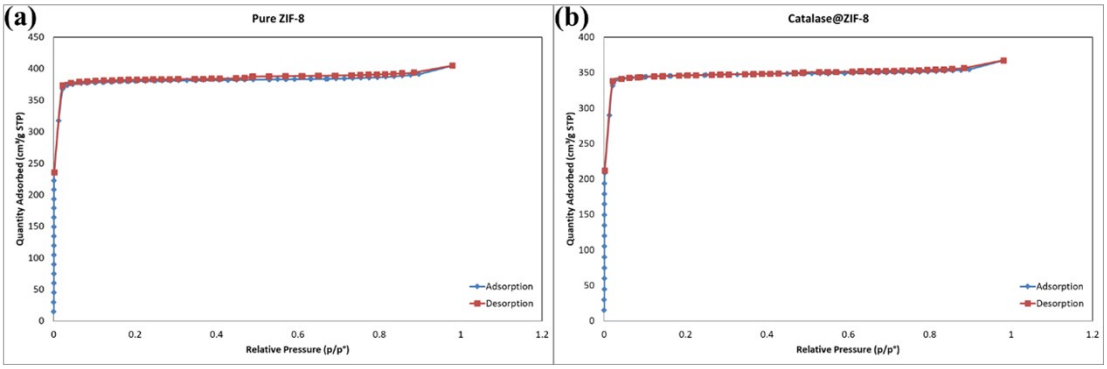

Figure S4. Nitrogen adsorption isotherms at 77 K for (a) pure ZIF-8 and (b) catalase@ZIF-8.

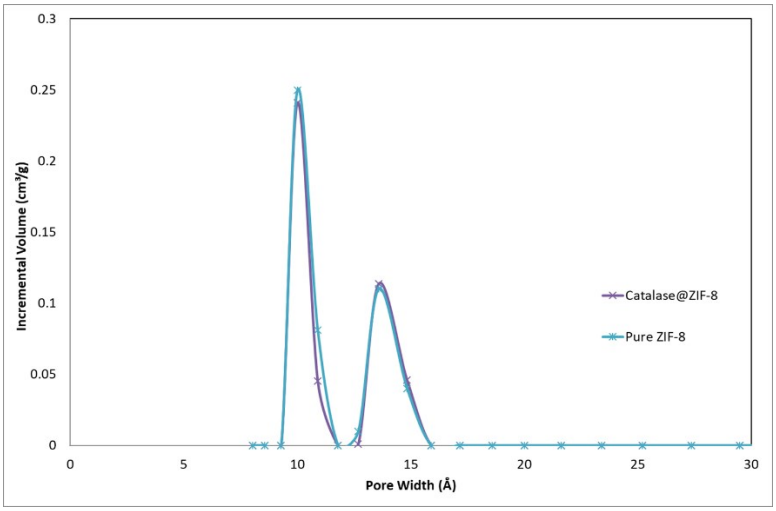

Figure S5. Pore size distribution of pure ZIF-8 and catalase@ZIF-8.

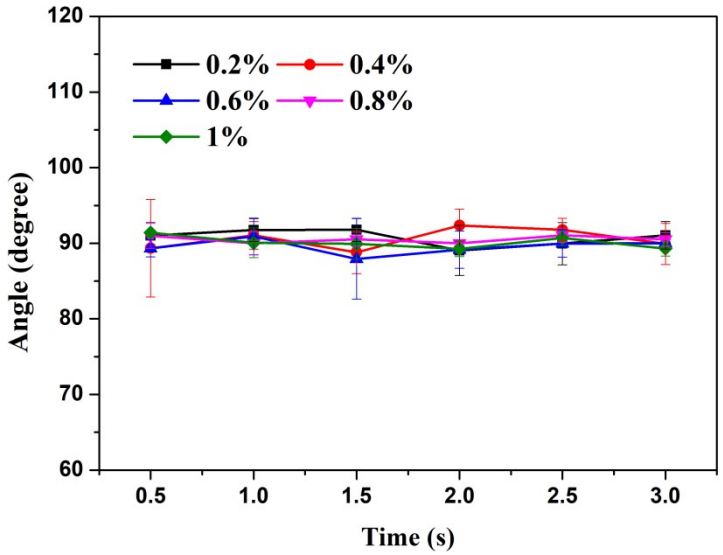

Figure S6. Angle variation between actual path and the horizontal line.

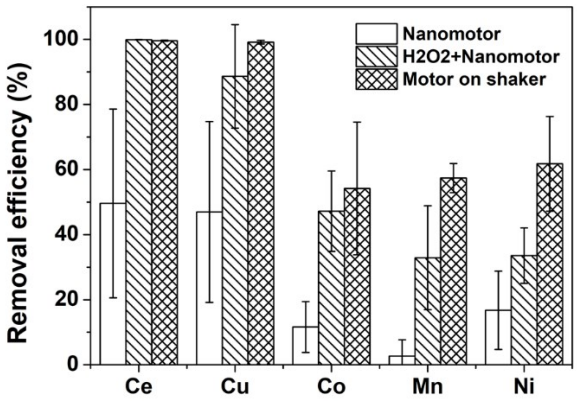

Figure S7. The removal efficiency of five metal ions with nanomotors without H_2O_2, with 0.2% H_2O_2 and nanomotors with mechanical stirring (no H_2O_2).

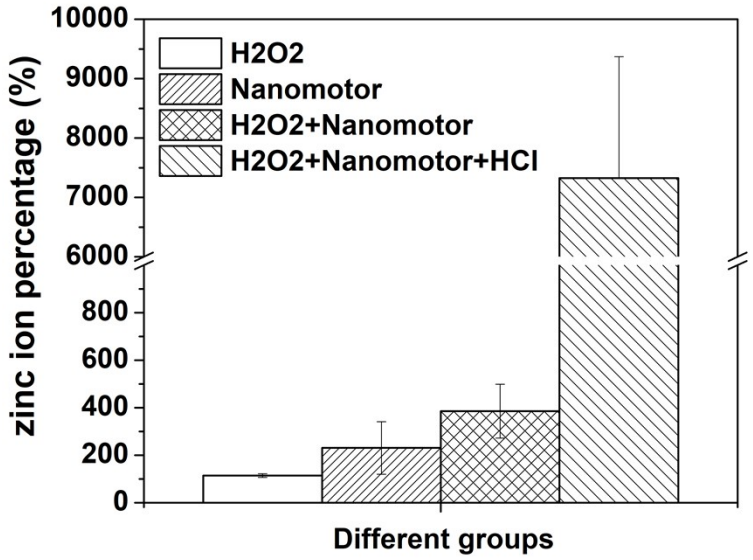

Figure S8. Zinc ions concentration variation in Milli-Q water: with the addition of hydrogen peroxide, CAT-ZIF-8 nanomotors, mobile CAT-ZIF-8 nanomotors with hydrogen peroxide, and mobile CAT-ZIF-8 nanomotors with hydrogen peroxide dissolved in hydrochloric acid.

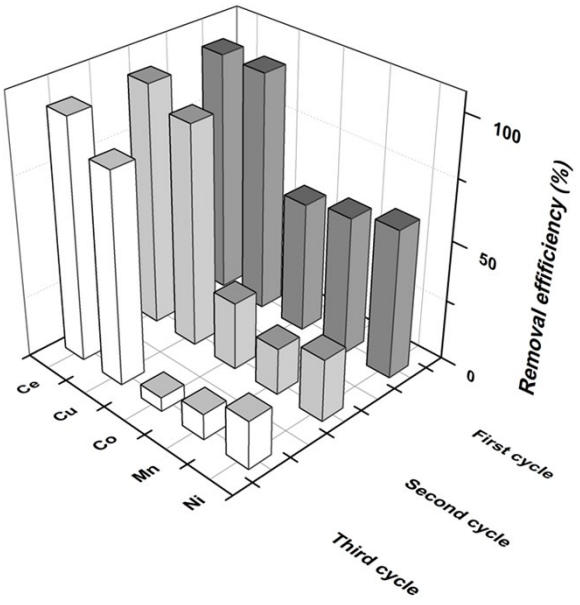

Figure S9. Cycling performance of CAT-ZIF-8 nanomotors on metal ions removal in the presence of hydrogen peroxide (0.2%) in water.

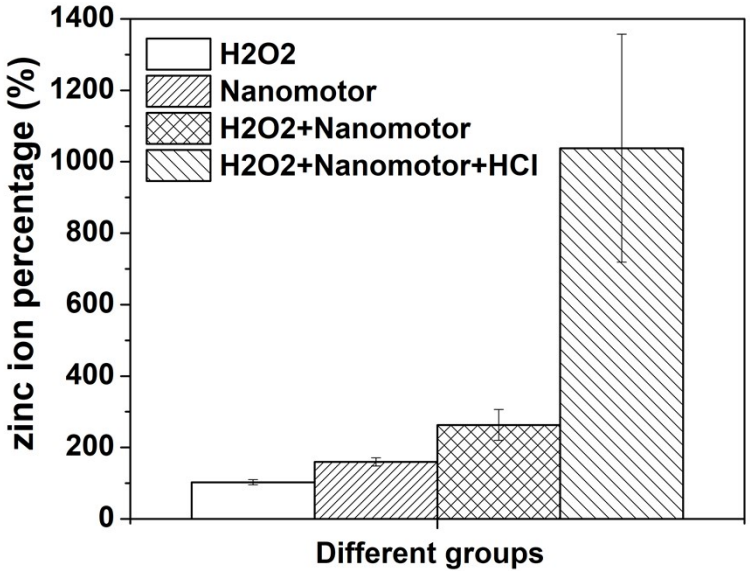

Figure S10. Zinc ions concentration variation in sea water: with the addition of hydrogen peroxide, CAT-ZIF-8 nanomotors, mobile CAT-ZIF-8 nanomotors with hydrogen peroxide, and mobile CAT-ZIF-8 nanomotors with hydrogen peroxide dissolved in hydrochloric acid.

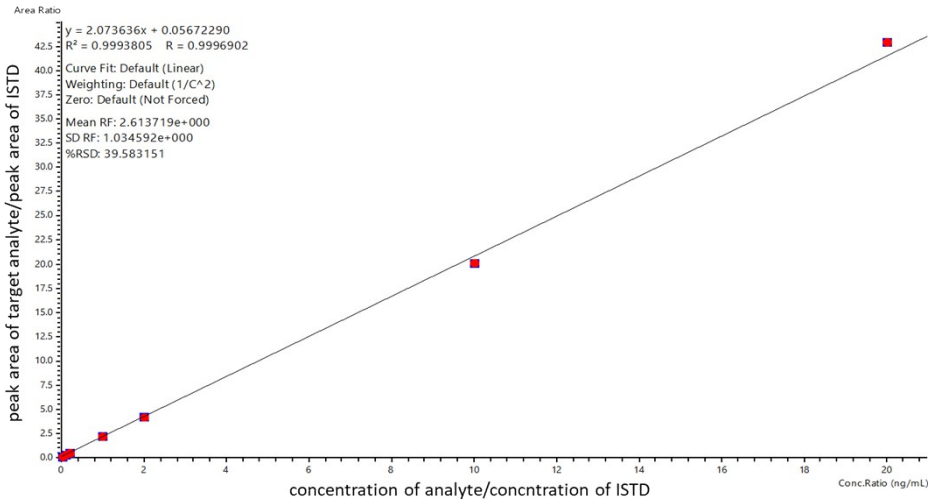

Figure S11. Calibration standard curve for PFOA concentration.

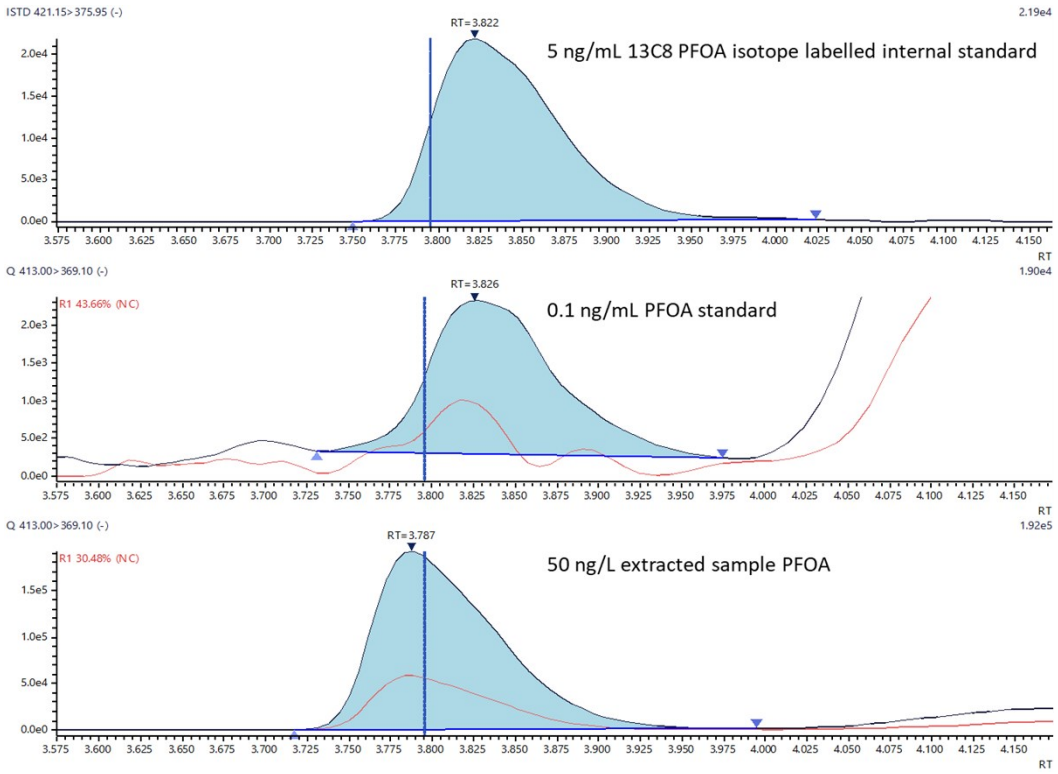

Figure S12. Chromatograms for 1 mL injection of isotope labelled internal standard, PFOA standard, and extracted sample PFOA.

Reference

[1]K. Liang, R. Ricco, C. M. Doherty, M. J. Styles, S. Bell, N. Kirby, S. Mudie, D. Haylock, A. J. Hill, C. J. Doonan and P. Falcaro, *Nat. Commun.*, 2015, **6**, 7240