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Supporting Information

Fluorescent polymeric aggregates for selective response to Sarin surrogates

Anca D. Rusu[a,c], Ioana Moleavin[a,b], Nicolae Hurduc[c], Matthieu Hamel[a], Licinio Rocha[a]

[a]*CEA, LIST, Laboratoire Capteurs et Architectures Electroniques, 91191, Gif-sur-Yvette, Cedex, France*

[b]*Petru Poni Institute of Macromolecular Chemistry, Ghica Voda Alley 41A, 700487, Iasi, Romania*

[c]*Department of Natural and Synthetic Polymers, ''Gheorghe Asachi'' Technical University of Iasi, Prof. D. Mangeron Street 73, 700050 Iasi, Romania*

Email of corresponding author: rusuancadaniela@yahoo.com

Experimental

General Experimental.

Diethylchlorophosphate (DCP) was purchased from Aldrich and distilled under vacuum before use.

Dichlorvos (DDVP) (Sigma Aldrich), dimethyl methylphosphonate (DMMP) (Fluka) and Fluorescein were used without any purification. All other reagents were obtained from commercial suppliers and used as received.

NMR spectra were recorded on a Bruker 400 MHz spectrometer.

The aggregates morphology was studied by Dynamic Light Scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments, Southborough, MA) equipped with a He–Ne laser source (λ= 633 nm), the autocorrelation function being automatically calculated.

The aggregates aqueous solutions were prepared by *precipitation method*: 5 mL of 6.6×10^{-5} M solution of polymer in THF were added dropwise under magnetic stirring to 10 mL of deionized water. The obtained solution was kept under stirring 2-3 hours at room temperature until all the THF was evaporated. If necessary, after the solvent evaporation, the volume of water was completed to 10 mL in order to obtain a 3.3×10^{-5} M solution.
All the solutions were left 24 h after preparation for stabilization. A Zeta potential around -50 mV revealed the formation of some stable polymeric aggregates in water.

Fluorescence measurements were performed with a Fluoromax-4P spectrofluorimeter (Horiba Jobin Yvon) and the FluoroEssence software for data recording.

Absorption measurements were performed with a 6715 UV/Vis JENWAY spectrophotometer.

Synthesis

Fluorescein sodium salt preparation

In a typical synthesis 0.5 g of Fluorescein was dissolved in 20 mL THF in a 50 mL round flask. After the Fluorescein solubilisation, 0.06 g NaOH was added (molar ratio 1:1). The mixture was heated 5 hours at 60-70°C under reflux and under stirring. The Fluorescein sodium salt precipitated from the solution during the reaction. The final product was filtered, washed 5 times with THF (in order to remove potentially unreacted Fluorescein) and dried under vacuum.

Fluorescent polymer synthesis (PSI-Fl)

0.5 g of Polysiloxane was dissolved in 6 mL of DMSO in a 50 mL round flask. A given amount of Fluorescein salt was added in function of the desired substitution degree. For a 56% substitution degree, the molar ratio between Fluorescein sodium salt and Polysiloxane was 21:1. The synthesis was catalysed by 0.1 g Bu_4NBr. The reaction mixture was heated at 80°C for 7 h, with stirring. The obtained polymer was precipitated twice in methanol and washed with methanol until the solvent remained clear and all the unreacted salt was removed.

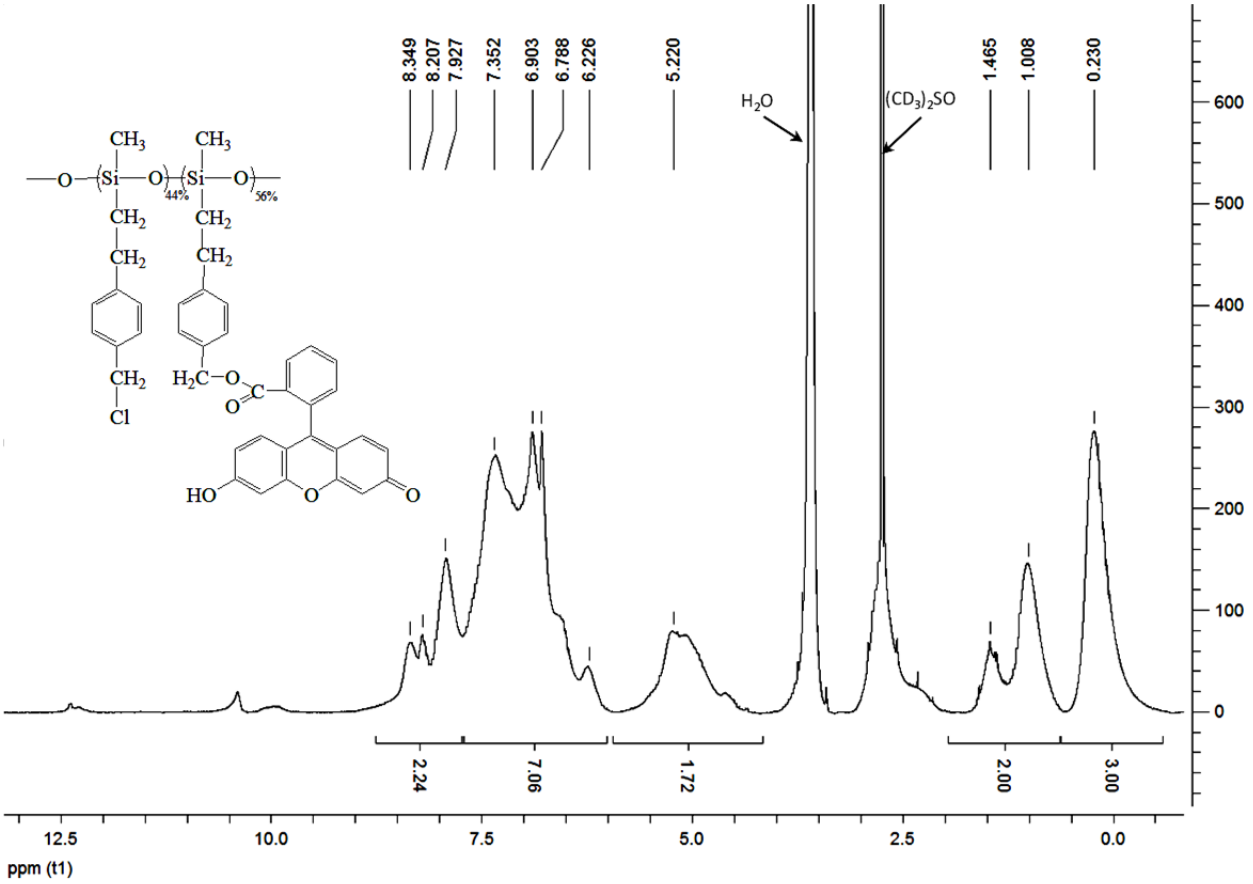

Fig. S1 ^{1}H-NMR spectrum of Polysiloxane substituted with Fluorescein moieties (56%) (PSI-FI)

The chemical structure was confirmed by ^{1}H-NMR ($(CD_3)_2SO$, 25 °C, 400 MHz), δ (ppm): 0.23 (**CH_3**-Si-); 0.98-1 (-Si-**CH_2**-CH_2-, β-isomer); 1.46 [-Si-CH-(**CH_3**), α-isomer]; 4.57 (-**CH_2**Cl); 4.9 (-**CH_2**-O-Fluorescein); 7-7.7 (-CH_2-O-**C_6H_4**-); 6-7 and 7.8-8.4 ppm (Fluorescein protons). The signals from 2.21 ppm [-Si-**CH-**(CH_3), α-isomer] and 2.74 ppm (-Si-CH_2-**CH_2**-, β-isomer) are superposing with the solvent signal. Details concerning the presence of α and β isomers in polysiloxane were previously explained.[1]

The substitution degree was calculated by comparing the signal of (**CH_3**-Si-) from 0.23 ppm with the signal of the 4 Fluorescein protons from 7.8-8.4 ppm.

The chemical reaction between Fluorescein and DCP

Fluorescein (0.55 g; 1.65 mmol) was dissolved in 20 mL THF to form an orange solution and then 240 µL (1.65 mmol) of freshly distilled DCP were added. The reaction mixture was stirred at 75°C for 22 hours in a dry environment formed by a 50 mL reaction flask connected to a condenser containing $CaCl_2$. A yellow powder is separating during the reaction. Nevertheless, the reaction product is found to be in the soluble part. The product was separated by decantation, concentrated and precipitated in water (to remove the traces of DCP) and dried under vacuum. The obtained product was chromatographed on Silica gel with CH_2Cl_2: Methanol / 90:10

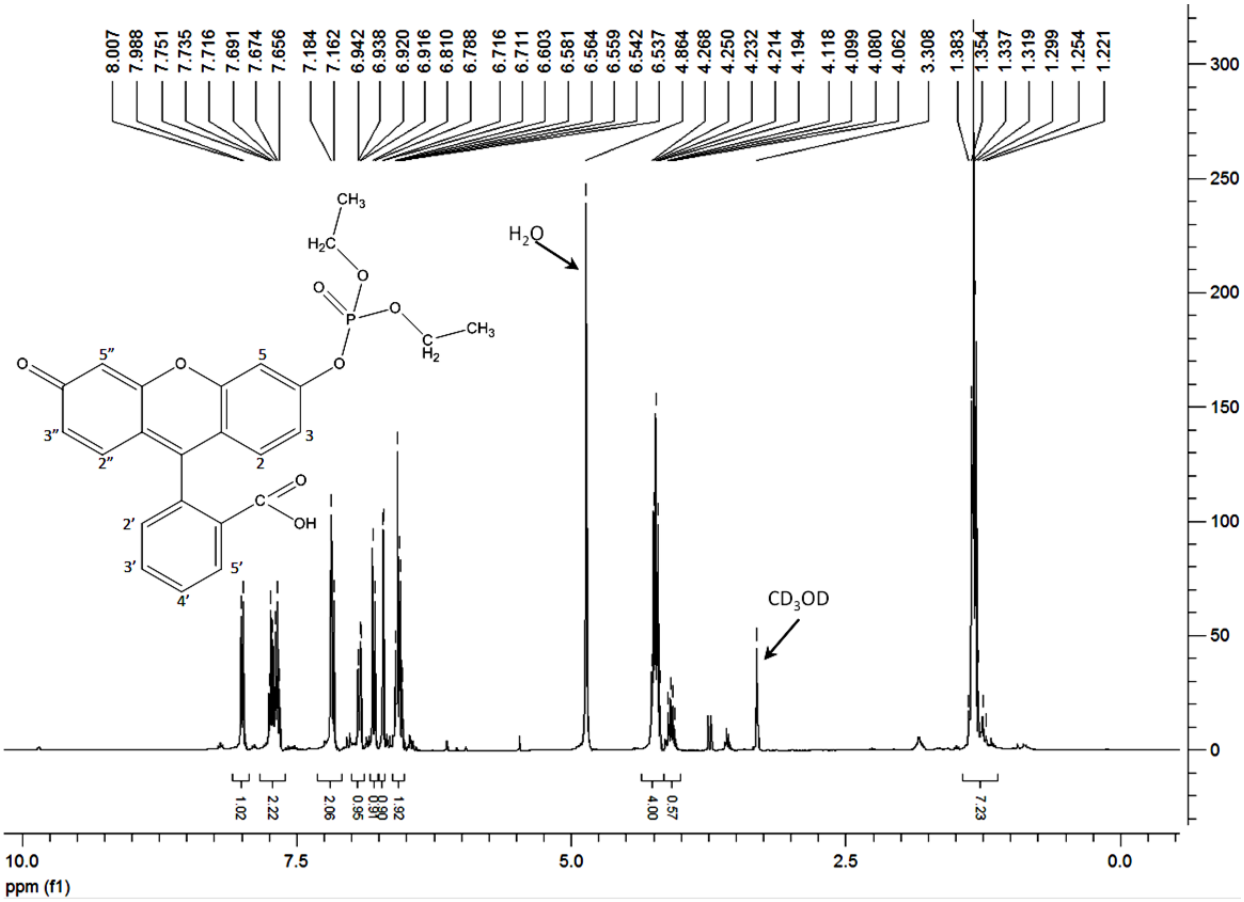

Fig. S2 ^{1}H-NMR spectrum of Fluorescein bonded with diethyl phosphoryl moiety

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 1.33 (t, 6H, $^{4}J_{HP}$=1.0 Hz, $^{3}J_{HH}$=5.1 Hz CH_3); 4.19-4.27 (m, 4H, OCH_2); 6.54-6.62 (m, 2H, H_3", H_5"); 6.71- (d, 1H, J=6.7 Hz, H_2"); 6.78 (d, 1H, J=6.8 Hz, H_5'); 6.94 (dd, 1H, J=1.1Hz, J=2.6, H_3'); 7.16-7.81 (m, 2H, H_2', H_4'); 7.65-7.75 (m, 2H, H_3,H_5); 7.98 (d, 1H, J=8.0 Hz, H_2). The signal from 4.06-4.11 ppm (m, 4H, OCH_2) represents traces of unbounded DCP.

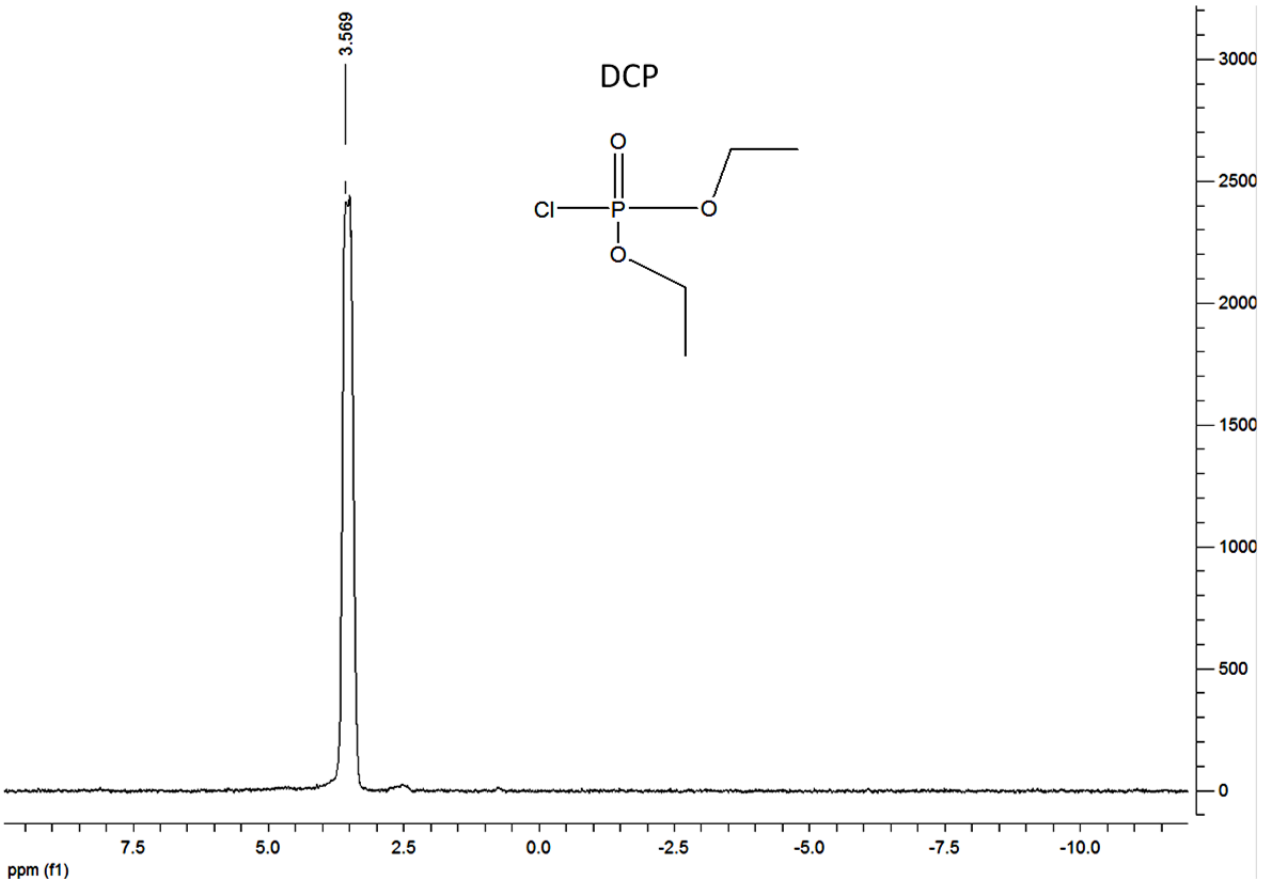

Fig. S3 ^{31}P-NMR spectrum DCP (161.9 MHz, CD_3OD)

δ_P = 3.5 ppm [2]

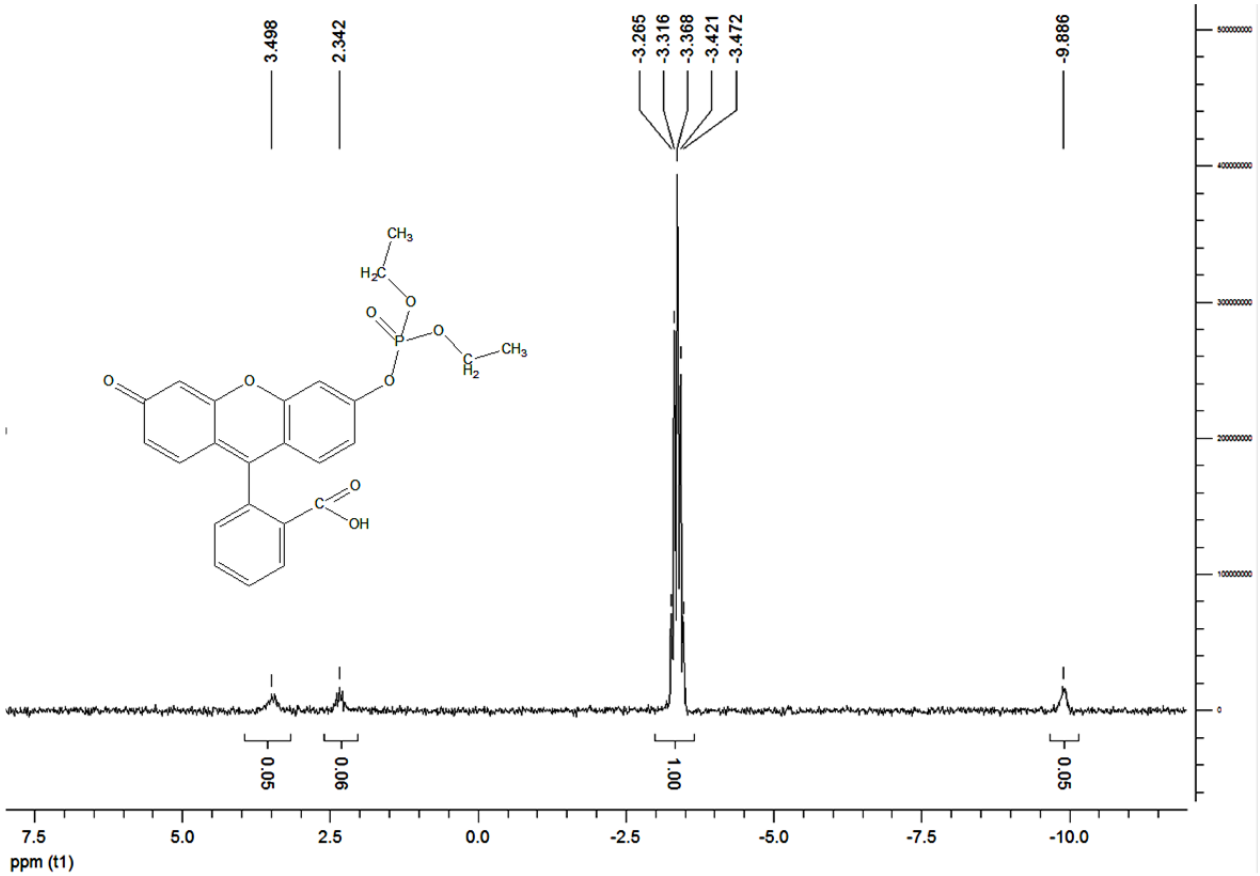

Fig. S4 ^{31}P-NMR spectrum of Fluorescein bonded with diethyl phosphoryl moiety

^{31}P NMR (161.9 MHz, CD_3OD) δ: -3.4 ÷ -3.2 ppm Fl-O-(PO)-$(OC_2H_5)_2$; 3.498 ppm traces of unreacted DCP; 2.34 ppm PO_4^{-3}, -9.886 ppm traces of side reaction product

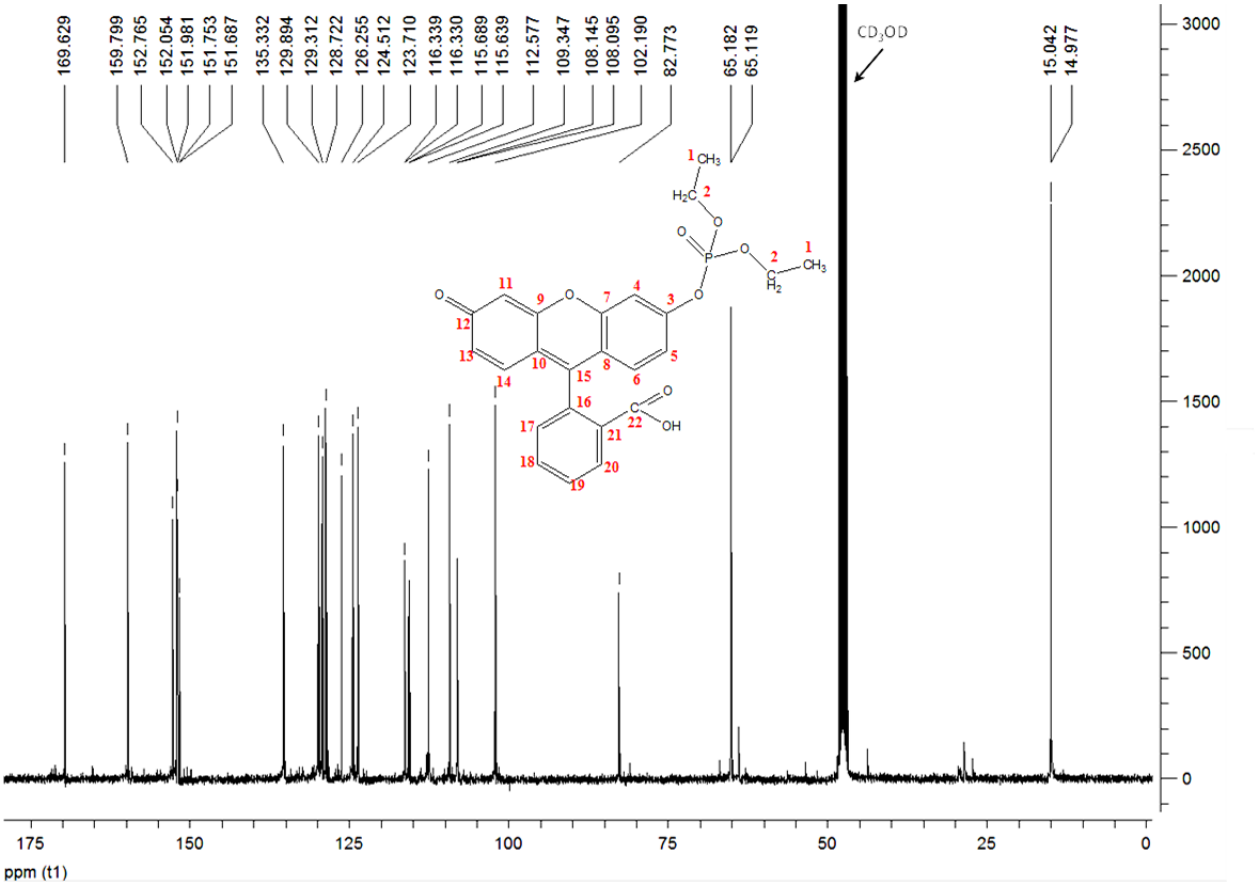

Fig. S5. ^{13}C-NMR spectrum of Fluorescein bonded with diethyl phosphoryl moiety (100.6 MHz, CD_3OD) δ (ppm): 14.9 (d, J = 6.5, C1), 65.1 (d, J = 6.3, C2), 82.7 (C15), 102.1 (C11), 108.0 (d, J = 5.0, C4), 109.3 (C13),112.5 (C10), 115.6 (d, J = 5.0, C5),116.3 (C8), 123.7/124.5 (C17/C20), 126.2 (C21), 128.7/129.3 (C18/C19), 129.8 (C14), 135.3 (C6), 151.6 (d, J = 6.6, C3), 151.9 (C7), 152.0 (C9), 152.7 (C16), 159.7 (C12) and 169.6 (C22).

Supplementary fluorescence spectra

All the spectra are for aqueous polymeric aggregates solutions 3.3×10^{-5} M with an aggregates average size of 50 nm.

Experiments were made in $1 \times 1 \times 3$ cm^3 quartz cells using a 2 mL sample.

Each pollutant addition was made under stirring using a 10 µL syringe and the spectrum was recorded immediately after the addition. For small pollutants volume, a dilution in acetonitrile was necessary.

All the results are given in pollutant concentrations in part per million (ppm).

Excitation spectra

Excitation wavelengths: 300-520 nm; emission wavelength: 540 nm

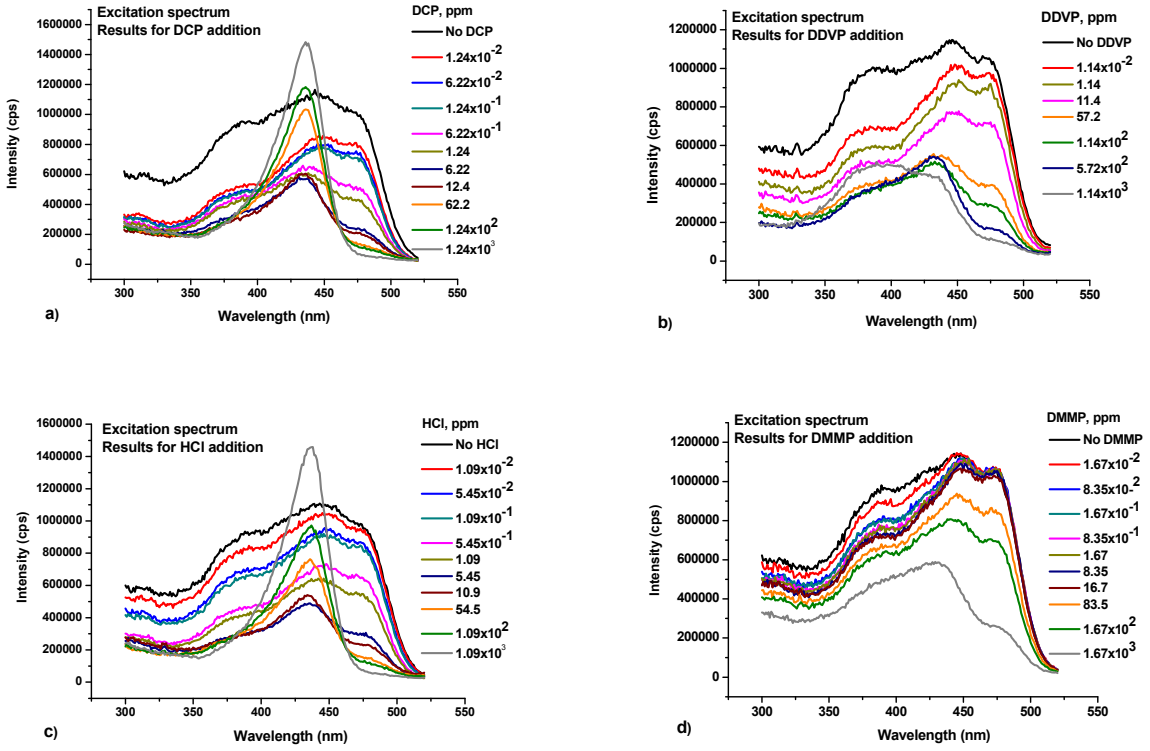

Fig. S6 Excitation spectra changes of PSI-FI aqueous solution before and after addition of various volumes of pollutants.

The fastest sample response is to DCP (a) where the ratio I/I_0 is decreasing with 26% after 12.4 ppb added into the solution compared with smaller variation in case of DDVP (b) (12%) and HCl (c) (4%), and no changes in case of DMMP (d), for similar concentration additions. After a certain volume of DCP or HCl the shape of the spectrum is changing revealing a modification of the Fluorescein molecule through the hydroxyl function[3,4], which in the case of HCl can be attributed to the presence of Fluorescein in its cationic form . The maximum of intensity is shifted from 442 nm to 436 nm. For the DDVP and DMMP addition, the modification of the Fluorescein molecule is less obvious.

Emission spectra

Excitation wavelength: **416 nm**; emission wavelengths: 436-650 nm

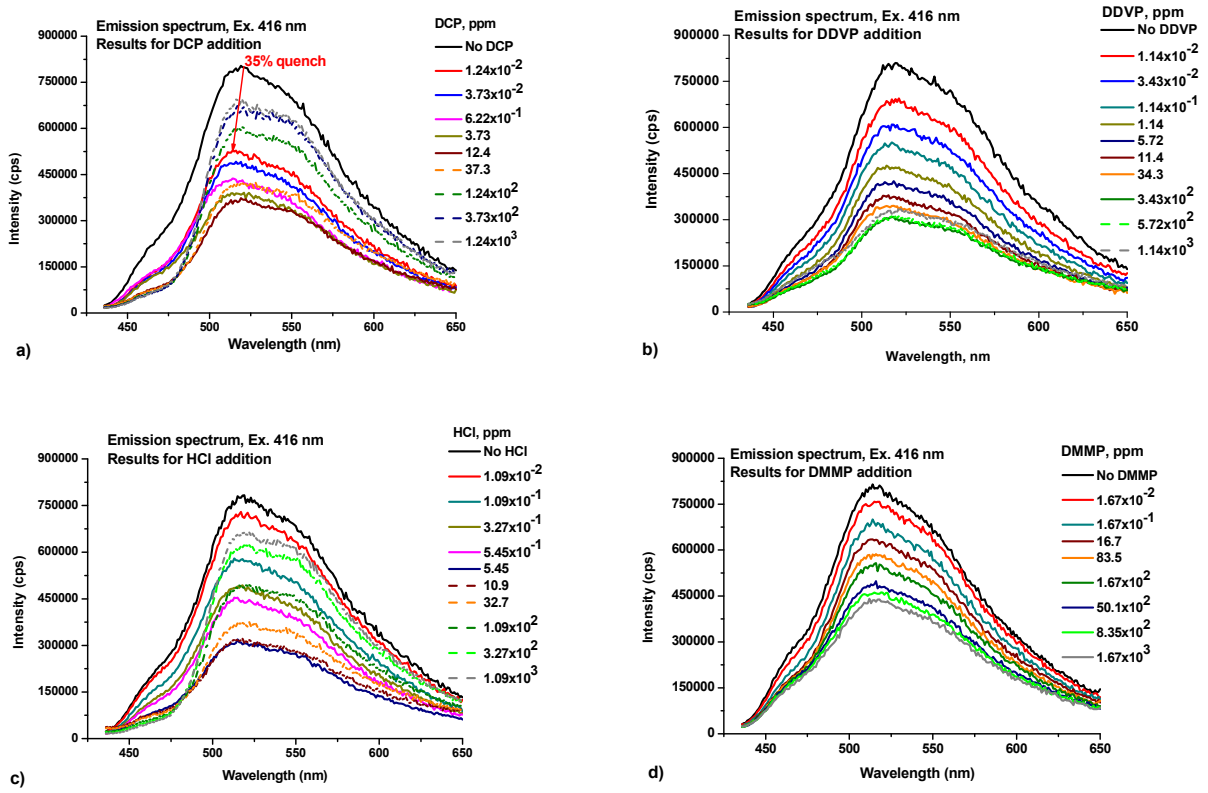

Fig. S7 Emission spectra changes of PSI-FI aqueous solution before and after addition of various volumes of pollutants for a $\lambda_{Ex.}$= 416 nm. Solid line - Fluorescence is decreasing with pollutant addition for small pollutant concentrations; **Dotted line** - Fluorescence is (starts to) increasing with pollutant addition. An increase of fluorescence intensity can be observed after the shifting of the excitation spectrum when DCP and HCl are added.

Excitation wavelength: **470 nm**; emission wavelengths: 490-650 nm

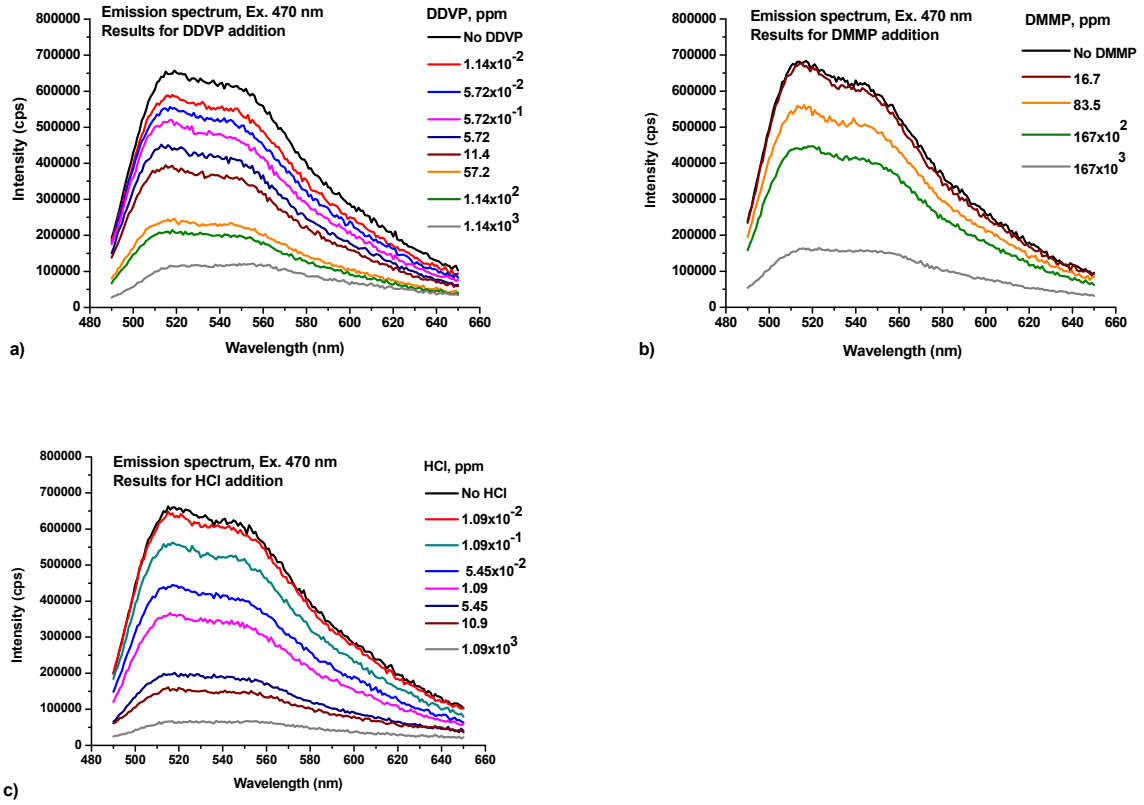

Fig. S8 Emission spectra changes of PSI-FI aqueous solution before and after addition of various volumes of pollutants for a $\lambda_{Ex.}$= 470 nm

Dynamic Light Scattering

DLS experiments were performed at 25°C in 1 × 1 × 3 cm^3 quartz cells using a 1 mL sample volume. The pollutants were added under stirring. The equilibration time was set to 2 minutes and three records were made for each measurement.

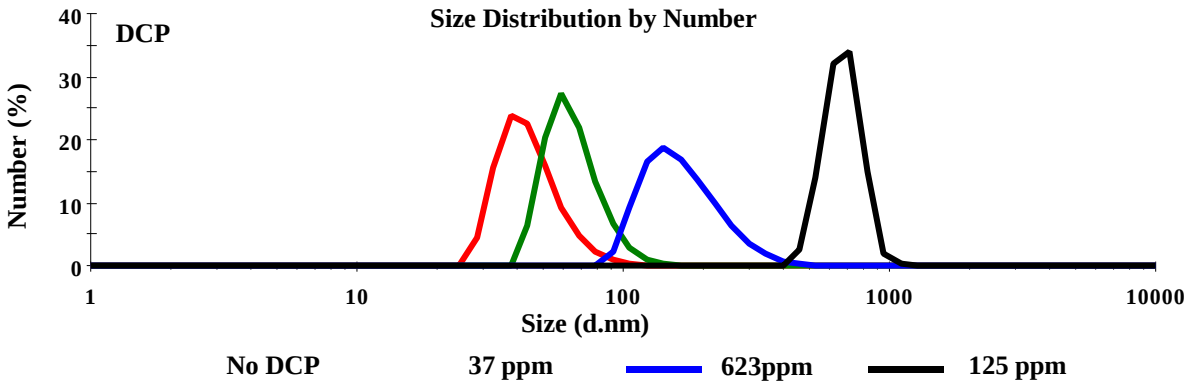

Fig. S9 Size distribution variation by number for DCP in aqueous polymeric solution 3.3×10^{-5} M

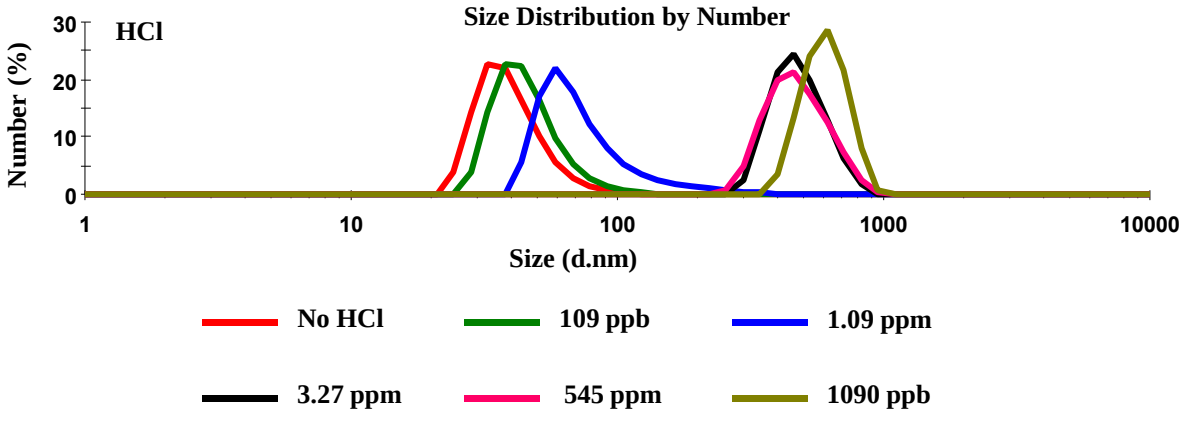

Fig. S10 Size distribution variation by number for HCl in aqueous polymeric solution 3.3×10^{-5} M

Real time measurements

The system depicted in **Fig. S11** has been employed to measure in real time the variation of the polymeric aggregates solutions fluorescence and light diffusion after the pollutant addition. The solutions fluorescence were excited in the absorption band of the aggregates Fluorescein groupments using a 488 nm solid state laser. A laser source emitting at 632 nm has been used to measure the diffusion properties (related to the aggregates structural properties). For each measurement 2 ml of aqueous polymer solution were used. The solution fluorescence and optical diffusion were collected simultaneously by means of a spherical lens focusing the generated light into an optical fiber coupled to a spectrometer.

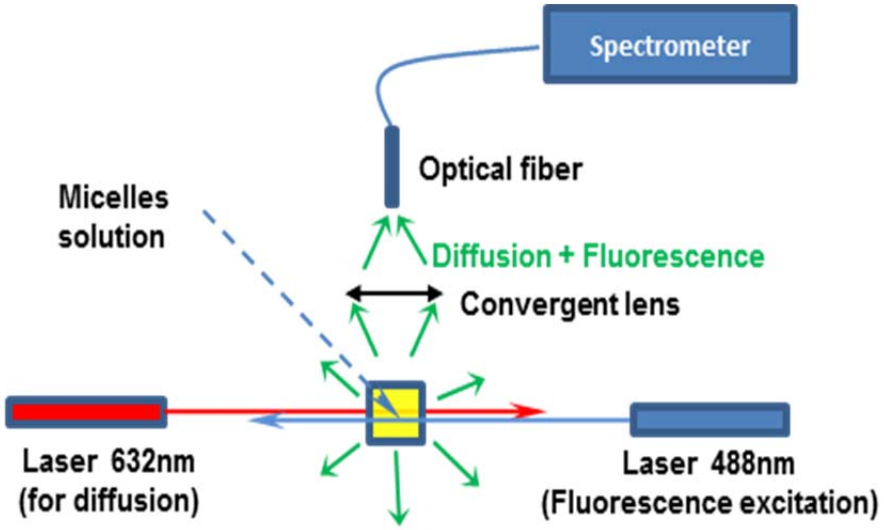

Fig. S11 The experimental configuration used for real time measurements. The experiments were performed at 25°C under stirring in 1 × 1 × 3 cm^3 quartz cells using a 2 mL sample volume.

In the next figure is represented the evolution of the fluorescence and diffusion signals as a function of time for a 3.3×10^{-5} M fluorescent polymer water solution (56% Fluorescein).

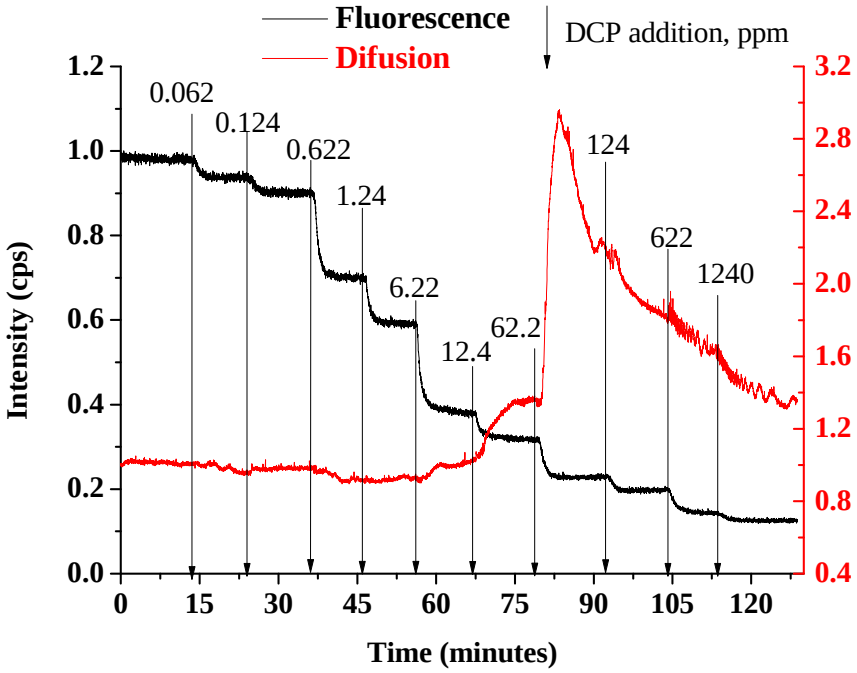

Fig. S12 Time variation of fluorescence and diffusion with DCP addition.

The experiment was started after both signals (fluorescence and diffusion) were stable. The intensity variation for fluorescence was recorded at 536.54 nm and for diffusion at 632 nm. After the first DCP addition (62 ppb) a small decrease in fluorescence signal was noticed (5%) and the signal becomes stable after approximatively 3 minutes. The fluorescence intensity is decreasing with each addition, and after 1240 ppm of DCP the sample is presenting just 12% from its initial intensity. Also, no influence of the long exposure time could be noticed on the fluorescence signal variation, the response to DCP is instantaneous and the signal is stabilised after around 3 minutes.

For higher amounts of DCP added (12.4 ppm), the decrease of the fluorescence is followed by an increase of the diffusion resulting from the interaction between the pollutant and the fluorescent moieties with a strong impact on the nanoparticles reorganisation. After 62.2 ppm a sharp increase in diffusion could be noticed followed by a decrease after just 4 minutes when the polymers starts to separate from the solution reducing the optical diffusion of the solution. By

correlating the resulting with the DLS ones we noticed that the aggregation/separation process during real time measurements takes place for a little lower DCP addition. The explanation is the fact that the steering process is facilitating the interaction between the aggregates.

The aqueous aggregates polymeric system can be used for real time measurements.

References

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