## **Supporting Information**

## A smart material for the in situ detection of mercury in fish

José García-Calvo, Saúl Vallejos, Félix C. García, Josefa Rojo, José M. García,\* and Tomás Torroba\*

Department of Chemistry, Faculty of Science, University of Burgos, 09001 Burgos, Spain.

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#### **Experimental Part:**

Materials and methods: The reactions performed with air sensitive reagents were conducted under dry nitrogen. The solvents were previously distilled under nitrogen over calcium hydride or sodium filaments. Melting points were determined in a Gallenkamp apparatus and are not corrected. FT-IR spectra were recorded on potassium bromide pellet with a JASCO FT/IR-4200. NMR spectra were recorded in Varian Mercury-300 and Varian Unity Inova-400 machines, in DMSO-*d*<sub>6</sub>, CDCI<sub>3</sub>, CD<sub>3</sub>CN, CD<sub>3</sub>OD. Chemical shifts are reported in ppm with respect to residual solvent protons, coupling constants  $(J_{X-X'})$  are reported in Hz. Elemental analyses of C, H and N were taken in a Leco CHNS-932. El mass spectra were taken in a Micromass AutoSpec machine, by electronic impact at 70 eV. MALDI-TOF mass spectra were measured on a MALDI-TOF Bruker Autoflex Mass Spectrometry instrument using DCTB and DIT matrixes. Quantitative UV-visible measures were performed with a Hitachi U-3900, in 1 cm UV cells at 25°C. Fluorescence spectra were recorded in a Varian Cary Eclipse or a Hitachi F-7000 FL spectrofluorometers. in 1 cm guarz cells at 25°C. The pH values were measured at room temperature using a Metrohm 16 DMS Titrino pH meter fitted out with a combined glass electrode and a 3 M KCl solution as a liquid junction, which was calibrated with Radiometer Analytical SAS buffer solutions.

#### SYNTHESIS:

Synthesis of tert-butyl 4-(5-(1-oxoindan-5-yl)pyrimidin-2-yl)piperazine-1-carboxylate:



5-Bromoindanone (500 mg, 2.38 mmol), 2-[4-(N-Boc-piperazin-1-yl]pyrimidine-5-boronic acid pinacol ester (1003 mg, 2.57 mmol) and Na<sub>2</sub>CO<sub>3</sub> (1500 mg, 14.2 mmol) were dissolved in a mixture of THF:H<sub>2</sub>O (10:1, 33 mL) under stirring in a nitrogen atmosphere, then Pd(PPh<sub>3</sub>)<sub>4</sub> (5 mg) was added and the resulting mixture was heated under reflux for 24 hours. Then the mixture was added to water (100 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 50 mL). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (silica, 3 x 30 cm), from CH<sub>2</sub>Cl<sub>2</sub> to CH<sub>2</sub>Cl<sub>2</sub>:AcOEt (6:1), to get tert-butyl 4-(5-(1-oxoindan-5-yl)pyrimidin-2vl)piperazine-1-carboxylate (930 mg, 95%) as a white solid, mp: 198-199 °C (decomp.). IR (KBr, cm<sup>-1</sup>): 2975, 2927, 2860, 1717 (C=O), 1599, 1525, 1421, 1362, 1310, 1277, 1251, 1181, 1025, 996, 944, 840. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ: 8.52 (s, 2H, H-C=N), 7.72 (d, J = 8.0 Hz, 1H, ArH), 7.49 (s, 1H, ArH), 7.42 (d, J = 8.0 Hz, 1H, ArH), 3.81 (t, J = 6.0 Hz, 4H,  $2 \times CH_2$  3.46 (t, J = 6.0 Hz, 4H,  $2 \times CH_2$ ), 3.11 (t, J = 5.8 Hz, 2H, CH<sub>2</sub>), 2.65 (t, J = 5.80 Hz, 2H, CH<sub>2</sub>), 1.43 (s, 9H, 3×CH<sub>3</sub>). <sup>13</sup>C NMR & DEPT (CDCl<sub>3</sub>, 75 MHz) δ: 206.4 (C=O), 161.3, 156.4 (CH<sub>Ar</sub>), 156.3, 154.9, 142.1, 136.0, 125.2 (CH<sub>Ar</sub>), 124.7 (CH<sub>Ar</sub>), 123.5 (CH<sub>Ar</sub>), 122.2, 80.2, 43.9 (CH<sub>2</sub>), 36.6 (CH<sub>2</sub>), 28.6 (CH<sub>3</sub>), 26.0 (CH<sub>2</sub>). MS (EI) m/z (%): 394 (M<sup>+</sup>, 39), 338 (24), 264 (22), 252 (34), 238 (100), 226 (32). HRMS (EI): calcd. for C<sub>22</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub>: 394.2005 (M<sup>+</sup>); found: 394.1989.



Figure S1: <sup>1</sup>H NMR (CDCI<sub>3</sub>, 300 MHz) of tert-butyl 4-(5-(1-oxoindan-5-yl)pyrimidin-2yl)piperazine-1-carboxylate

![](_page_3_Figure_2.jpeg)

Figure S2: <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) of tert-butyl 4-(5-(1-oxoindan-5-yl)pyrimidin-2yl)piperazine-1-carboxylate

![](_page_4_Figure_0.jpeg)

Figure S3: DEPT (CDCI<sub>3</sub>, 75 MHz) of tert-butyl 4-(5-(1-oxoindan-5-yl)pyrimidin-2yl)piperazine-1-carboxylate

Synthesis of 5-(2-(piperazin-1-yl)pyrimidin-5-yl)indan-1-one:

![](_page_5_Figure_1.jpeg)

Trifluoroacetic acid (10 mL, p= 1.489 g/mL, 130.59 mmol) was added dropwise with stirring to a solution of tert-butyl 4-(5-(1-oxoindan-5-yl)pyrimidin-2-yl)piperazine-1carboxylate (874 mg, 2.22 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and the resulting mixture was stirred at room temperature for 15 minutes. Then the mixture was added to water (50 mL), basified to pH= 10 with 5% NaOH and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 x 50 mL). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), the solvent was evaporated under reduced pressure and the residue was recrystallized from CH<sub>2</sub>Cl<sub>2</sub>-MeOH (5:1) to get 5-(2-(piperazin-1yl)pyrimidin-5-yl)indan -1-one (617 mg, 95%) as a white solid, mp: 189-190 °C (decomp.). IR (KBr, cm<sup>-1</sup>): 3221 (N-H), 2940, 2843, 1706 (C=O), 1603, 1502, 1455, 1363, 1307, 1260, 1117, 940. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ: 8.55 (s, 2H, H-C=N), 7.76 (d, J = 8.0 Hz, 1H, ArH), 7.52 (s, 1H, ArH), 7.44 (d, J = 8.0 Hz, 1H, ArH), 3.84 (d, J = 6.0 Hz, 4H, 2×CH<sub>2</sub>), 3.15 (d, J = 6.0 Hz, 2H, CH<sub>2</sub>), 2.92 (d, J = 6.0 Hz, 4H, 2×CH<sub>2</sub>), 2.69 (d, J = 6.0 Hz, 2H, CH<sub>2</sub>), 1.84 (s, 1H, NH). <sup>13</sup>C NMR & DEPT (CDCl<sub>3</sub>, 75 MHz) δ: 206.3 (C=O), 161.3, 156.2 (CH<sub>Ar</sub>), 156.2, 142.2, 135.8, 125.0 (CH<sub>Ar</sub>), 124.5 (CH<sub>Ar</sub>), 123.2 (CH<sub>Ar</sub>), 121.5, 46.1 (CH<sub>2</sub>), 45.1 (CH<sub>2</sub>), 36.5 (CH<sub>2</sub>), 25.9 (CH<sub>2</sub>). MS (EI) m/z (%): 294 (M<sup>+</sup>, 22), 252 (100), 238 (29), 226 (59). HRMS (EI): calcd. for C<sub>17</sub>H<sub>18</sub>N<sub>4</sub>O: 294.1481 (M<sup>+</sup>); found: 294.1482.

![](_page_6_Figure_0.jpeg)

Figure S4: <sup>1</sup>H NMR (CDCI<sub>3</sub>, 300 MHz) of 5-(2-(piperazin-1-yl)pyrimidin-5-yl)indan-1-

![](_page_6_Figure_2.jpeg)

Figure S5: <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) of 5-(2-(piperazin-1-yl)pyrimidin-5-yl)indan-1-

one

![](_page_7_Figure_0.jpeg)

Figure S6: DEPT (CDCI<sub>3</sub>, 75 MHz) of 5-(2-(piperazin-1-yl)pyrimidin-5-yl)-indan-1-one

Synthesis of *N*-(4-azidophenyl)-4-(5-(1-oxo-indan-5-yl)pyrimidin-2-yl)piperazin-1-carbothioamide JG10:

![](_page_8_Figure_1.jpeg)

A 50 ml Schlenk, with a magnetic stirrer, was charged with 50 mg of 5-[4-(piperazine-1yl)pyrimidin]indanone (0.17 mmol) and 20 ml of CHCl<sub>3</sub>. The resulting solution stirred under nitrogen in an ice bath for five minutes and then 40.66 mg of 1-azide-4isothiocyanatebenzene (0.23 mmol) were added to the solution. The solution was stirred for 20 minutes at 0 °C, then for 21 hours at room temperature and then refluxed for 30 minutes at 35 °C. The presence of the initial reagents was followed by thin layer chromatography. TLC was eluted with dichloromethane:methanol (50:1). The solvent was evaporated and the product was purified under flash column chromatography from dichloromethane to dichloromethane: methanol 50:1 v/v to obtain 55.4 mg of N-(4azidophenyl)-4-(5-(1-oxoindan-5-yl)pyrimidin-2-yl)piperazin-1-carbothioamide (56% yield) as a white solid, mp: 188-190 °C (decomp.). IR (KBr, cm<sup>-1</sup>): 3359 (N-H), 3022, 2921, 2851, 2110 (N<sup>-</sup>=N<sup>+</sup>=N-), 1697 (C=O), 1597, 1503, 1453, 1420, 1355, 1307, 1259, 1224. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ: 9.43 (s, 1H, N-H), 8.86 (s, 2H, H-C=N), 7.89 (s, ArH), 7.73 (d, J = 8.7 Hz, 1H, ArH), 7.68 (d, J = 8.1 Hz, 1H, ArH), 7.36 (d, J = 8.7 Hz, 2H, ArH), 7.06 (d, J = 8.7 Hz, 2H, ArH), 4.06 (m, 4H, 2×CH<sub>2</sub>) 3.93 (m, 4H, 2×CH<sub>2</sub>), 3.14 (t, J = 5.8 Hz, 2H, CH<sub>2</sub>), 2.66 (t, J = 5.8 Hz, 2H, CH<sub>2</sub>). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 100 MHz) δ: 205.9 (C=O), 181.4 (C=S), 160.5, 156.4 (2×CH<sub>Ar</sub>), 156.2, 141.1, 138.1, 135.3, 135.2, 127.0 (2×CH<sub>Ar</sub>), 124.7 (CH<sub>Ar</sub>), 123.6 (CH<sub>Ar</sub>), 123.3 (CH<sub>Ar</sub>), 121.2, 118.7 (2×CH<sub>Ar</sub>), 47.5 (CH<sub>2</sub>), 43.0 (CH<sub>2</sub>), 36.1 (CH<sub>2</sub>), 25.5 (CH<sub>2</sub>). MS (MALDI) m/z (%): 471 (M<sup>+</sup>+1, 21), 443 (100), 411 (45), 393 (19), 337 (95), 295 (43). HRMS (MALDI): calcd. for C<sub>24</sub>H<sub>23</sub>N<sub>8</sub>OS: 471.1710 (M<sup>+</sup>+1); found: 471.1803 (M<sup>+</sup>+1).

![](_page_9_Figure_0.jpeg)

Figure S7: <sup>1</sup>H NMR (CDCI<sub>3</sub>, 300 MHz) of *N*-(4-azidophenyl)-4-(5-(1-oxo-indan-5yl)pyrimidin-2-yl)piperazin-1-carbothioamide JG10

![](_page_9_Figure_2.jpeg)

Figure S8: <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) of *N*-(4-azidophenyl)-4-(5-(1-oxo-indan-5yl)pyrimidin-2-yl)piperazin-1-carbothioamide JG10

#### Preparation of the functional polymers

![](_page_10_Figure_1.jpeg)

![](_page_10_Figure_2.jpeg)

Figure S9: Synthesis of a fluorescent crosslinked polymer of 2-hydroxyethyl acrylate

The film-shaped functional membrane (Mem) was prepared by the photochemically initiated radical polymerization of the hydrophilic monomer 2-hydroxyethylacrylate, 2HEA, and propargylmetacrylate, PGM. Ethylenglycolmetacrylate, EGDMA, was used as crosslinking agent. The co-monomer molar ratio **2HEA/PGM//EGDMA** was 95/5//5, respectively. 2,2-Dimethoxy-2-phenylacetophenone (DMPA. 1.5% wt) was emploved as а photochemical initiator. The photoinitiated bulk polymerization was performed in a 100 µm thick silanized glass hermetic mould upon irradiation with a UV mercury lamp (250w, Philips HPL-N, emission band in the UV region at 304, 314, 335, and 366 nm, with maximum emission at 366 nm), at 20 °C, for 4 h. The water-swelling percentage (WSP) of the membrane was 60% and the DMF swelling 300 %. Then, N-(4-azidophenyl)-4-(5-(1oxo-indan-5-yl)pyrimidin-2-yl)piperazin-1-carbothioamide (JG10) (80 mg, 0.17 mmol) and DMF (30 ml) as solvent were added to a glass crystallizer and then nitrogen was sparged. Finally, about 0.400 g of the polymer membrane **Mem** and a spatula tip of the Cu(I) catalyst were added. The reaction was stirred in an orbital shaker for 72 hours. After that, the reaction was finished and the polymer had a light orange-yellow colour. The polymer was washed by adding distilled water dropwise and removing the mixture water-DMF until the percentage of water was near to 100 %, then the polymer was laid in a surface until it was dried, taking care not to bending or breaking it. Finally, the polymer was cleaned again for several times with distilled water.

![](_page_10_Picture_5.jpeg)

Figure S10. Picture of membranes **JG25** once the reaction has finished.

**Characterization:** The characterization was carried out by SEM analysis, with gold and carbon recap. The atomic proportion was indicated by X-ray analysis on different areas of the polymer. The proportion between oxygen or carbon and sulfur atoms was very similar to the theoretical results associated to a 100 % stoichiometric reaction (60-100 % depending on the area).

![](_page_11_Picture_1.jpeg)

[A]

Coating	%S <sub>real</sub> /%S <sub>teor</sub>
Gold	79,4
Gold	100,7
Gold	83,5
Carbon	84,9
Carbon	68,5
Carbon	93,7

![](_page_11_Figure_4.jpeg)

Figure S11. [A] SEM image of JG25 [B] X-ray fluorescence analysis from JG25.

![](_page_11_Figure_6.jpeg)

Figure S12. IR (ATR, cm-1): 3529-3356 (O-H), 2968-2859 (C-H), 1708 (C=O), 1514 (Car-Car), 1441, 1393, 1264, 1167, 1077, 1043, 893, 839 (fingerprint zone). The signal at 1514 and 1598 (very low) cm<sup>-1</sup> are the only signals associated to the presence of the probe, because there is no presence of these signals on the initial IR spectrum, and their intensity has to be very low due to the low percentage (5%) (Inset in the Figure S12).

#### TGA study:

![](_page_12_Figure_2.jpeg)

![](_page_12_Figure_3.jpeg)

# Synthesis of a mercury sensitive polymer derivative from 2-hydroxyethyl acrylate soluble in water: (JG32)

![](_page_13_Figure_1.jpeg)

Figure S14: Synthesis of a mercury sensitive polymer derivative from 2-hydroxyethyl acrylate soluble in water (JG32)

The intial polymer reagent was synthesized from a mixture of 99% molar of 2-hydroxyethyl acrylate and 1% molar of propargyl methacrylate, and was obtained as an uncoloured slime. The initial polymer reagent (0.682 g, 5.87 mmol) was dissolved in 30 ml of DMF in a round bottom flask and stirred until all the polymer was dissolved, then N-(4-azidophenyl)-4-(5-(1-oxo-indan-5-yl)pyrimidin-2-yl)piperazin-1-carbothioamide (JG10) (25 mg, 0.05 mmol) was added and then a spatula tip of the Cu(I) catalyst was added. The reaction was left under stirring at 30 °C for 24 hours. Then 150 ml of diethylether were added dropwise to the solution under stirring and the solution was left stirred for 24 hours until the solid polymer precipitated. The polymer was separated and the remainder of the mixture DMF-diethylether was subjected to centrifugation to separate the rest of the polymer. The process allowed obtaining 0.561 g of orange-yellow polymer slime, 80% yield.

![](_page_13_Figure_4.jpeg)

Figure S15: IR (ATR, cm-1): 3506-3330 (O-H), 2953-2878 (C-H), 1720 (C=O), 1444, 1392, 1167, 1076, 888, 839 (fingerprint zone).

In this compound, the signals at 1600-1500 cm<sup>-1</sup> can be barely distinguished from the noise. It should be because, for this polymer, the percentage of the fluorogenic probe is too low (1%) which agrees with the previous results.

### QUALITATIVE TESTS

General conditions:

The fluorescence tests were done with some cations and anions. The solutions of the probe were tested by increasing the concentration of cations and anions in solution and checking the variation in colour and/or fluorescence under visible and UV light (366 nm).

The cations and anions solutions were prepared in deionized water and the counterions were selected as non-coordinant counterions; tetrabutylamonium for anions and perchlorate or triflate for cations, except Au<sup>3+</sup> which is a HAuCl<sub>4</sub> solution in water.

The results of these tests are shown when there is a change that can be appreciated, the anions tests aren't usually shown because there is no change in presence of anions.

#### Tests with JG10, azide probe:

In order to check the fluorescence of the azide probe before reaction with the polymer, a qualitative test was done from  $10^{-5}$  M solutions in MeOH:H<sub>2</sub>O 60:40, due to its low solubility in water.

![](_page_15_Picture_7.jpeg)

Figure S16. Fluorescence in the presence of cations excess after 24 hours

The fluorescence increases very little in presence of  $Ag^+$  and increases strongly in the presence of  $Hg^{2+}$  and  $Au^{3+}$ .

#### Tests with JG25:

Pieces of polymer were added to different vials and 60  $\mu$ l of the cations solutions (5·10<sup>-3</sup> M) were added, enough to cover all the surface of the polymer. Then, the polymers were left to dry.

![](_page_15_Figure_12.jpeg)

Figure S17. Fluorescence changes in the presence of 60 µl of cations (UV light)

![](_page_16_Figure_0.jpeg)

Figure S18. Fluorescence changes in the presence of 60  $\mu$ l of cations,  $\lambda_{exc}$  = 364 nm.  $\lambda_{em}$  = 445 nm

About the changes of the polymer in presence of cations, it can be seen an increase in fluorescence for Ag<sup>+</sup>, although Hg<sup>2+</sup> and MeHg<sup>+</sup> produce a higher increase in fluorescence. This is easily quantified by measuring with a fluorometer, albeit it is hard to distinguish by the naked eye.

## Tests with the soluble modified polymer (1 % sensor) (JG32):

The solution of the polymer was prepared and the concentration is  $1.2 \times 10^{-2}$  g/L of the polymer (1% of the probe,  $10^{-6}$  equivalents/L). Then the cations were added:

![](_page_16_Figure_5.jpeg)

Figure S19. Fluorescence cations excess after (upper) 2 minutes and (lower) 30 minutes

The polymer was dissolved in water  $(1.2 \times 10^{-2} \text{ g/L})$ , the relative concentration of the probe is 1% in mass, so the final concentration of the probe would be  $10^{-6}$  equivalents/L

supposing that the reaction had 100 % yield. 60 µl of the cations were added (around 300 theoretical equivalents), and there weren't any changes under visible light. The fluorescence under UV light (366 nm) increased for Hg<sup>2+</sup> and decreased for Au<sup>3+</sup>. The fluorescence increased very slightly for Fe<sup>3+</sup> and Sn<sup>2+</sup>, an increase associated to their acid behaviour and it is lower and need more time to take effect.

The fluorescence was checked with other cations. Sequence: Water, Hg<sup>2+</sup>, MeHg<sup>+</sup>, Au<sup>3+</sup>, Pd(0), Rh<sup>3+</sup>, Ir<sup>3+</sup>, Pt<sup>2+</sup>, Co<sup>2+</sup>, Pd<sup>2+</sup>. (As chlorides, except Pd(0) that was Pd(dba)<sub>2</sub>).

![](_page_17_Figure_2.jpeg)

Figure S20. Fluorescence cations excess after (upper) 2 minutes and (lower) 70 hours

Two processes were observed, an increase of the fluorescence under UV light for  $Hg^{2+}$  and MeHg<sup>+</sup> and a decrease for  $Au^{3+}$  and  $Pd^{2+}$ .

![](_page_17_Figure_5.jpeg)

Figure S21. Variation of fluorescence with cations after 60 minutes,  $\lambda_{exc}$  = 320 nm,  $\lambda_{em}$  = 455 nm

![](_page_18_Figure_0.jpeg)

Figure S22. Variation of fluorescence with 60  $\mu$ L of cations + 60  $\mu$ L of Hg<sup>2+</sup>,  $\lambda_{exc}$  = 364 nm.  $\lambda_{em}$  = 445 nm, mother solutions 5 x 10<sup>-3</sup> M, 0.5 mL solution

![](_page_18_Figure_2.jpeg)

Figure S23. Variation of fluorescence with 60  $\mu$ L of cations + 60  $\mu$ L of MeHg<sup>+</sup>,  $\lambda_{exc}$  = 364 nm.  $\lambda_{em}$  = 445 nm, mother solutions 5 x 10<sup>-3</sup> M, 0.5 mL solution

A major increase in fluorescence occurs in presence of  $Hg^{2+}$  and  $MeHg^+$ , moreover there was minor increases in presence of  $Sn^{2+}$  and  $Fe^{3+}$ , associated to the acidity of these cations. It can be seen that the effect of the presence of the different cations is by increasing the final fluorescence if it is  $Sn^{2+}$  and  $Fe^{3+}$  (the same effect of increasing the acidity of the solution), and total inhibition of the signal for  $Au^{3+}$  or partial inhibition of the signal for  $Cu^{2+}$ .

#### QUANTITATIVE TESTS:

General conditions: Tests were carried out for the two synthesized polymers, the soluble in water (JG32) and the film with water affinity (JG25). Because of that, there are two procedures to measure in the fluorometer:

In the case of solid polymers the sample was put between two magnetic sheets with • a hole in the middle and placed in an angle of 45 degrees between the lamp and the detector (See the Figure S24). The concentration of analyte in the solution (in contact with the polymer) is successively increased in the cuvette and the changes in the fluorescence of the polymer are then measured.

For the soluble polymer, a linear regression was necessary in order to • find a concentration in which little variations change linearly the emission intensity and the absorbance.

Then the limit of detection of Hg<sup>2+</sup> and MeHg<sup>+</sup> was calculated, using the program "R" by following the next procedure:

- Adjusting to a mean square linear regression.
- Removing the "outliers".
- Adjusting to a linear regression.
- Calculation of the LOD when the probability of false positive

and false negative is equal or inferior to 5 %.

180

160

140

120

100

80

60

ΰ

1<u>0</u>0

#### Measurements with the solid crosslinked polymer JG25:

Kinetic response to Hg<sup>2+</sup>:

Emission intensity (au)

![](_page_19_Figure_13.jpeg)

Time (min)

3<u>0</u>0

200

– 10<sup>-4</sup> M Hg<sup>2+</sup>

4<u>0</u>0

500

![](_page_19_Figure_14.jpeg)

S24: Experimental procedure

The signal increases in intensity very quickly during the first five minutes, then increases linearly for 70 minutes. The process is very slow and supposes a 54 % increase in the global fluorescence; reaching 6 % in the first 5 minutes and 33 % in the first 90 minutes. Moreover, this process is dependent on the concentration of Hg<sup>2+</sup> added, but the results are very similar when the time is very short, less than twenty minutes.

![](_page_20_Figure_1.jpeg)

Figure S26. Kinetic response of **JG25** to an aqueous solution of Hg(ClO<sub>4</sub>)<sub>2</sub> 10<sup>-4</sup> M.  $\lambda_{exc}$  = 369 nm.  $\lambda_{em}$  = 445 nm, 100 minutes

#### • Titration experiments by adding Hg<sup>2+</sup>:

After the kinetic results, a deep study on the equilibrium was too difficult and less interesting, because of the long waiting time and the relation between concentration and this time. Therefore, there are some alternatives, but because the changes at the beginning of the additions are linear, by adding quantities of Hg<sup>2+</sup> every 5 minutes the resulting graphic plot is very similar to the typical plot for species studied in equilibrium.

![](_page_20_Figure_5.jpeg)

Figure S27. Left: Fluorescence curves by addition of increasing concentrations of Hg(ClO<sub>4</sub>)<sub>2</sub>,  $\lambda_{exc}$  = 369 nm. Right: Titration plot by using integral surfaces of the fluorescence curves between 379-650 nm in response to increasing concentrations of Hg(ClO<sub>4</sub>)<sub>2</sub>  $\lambda_{exc}$  = 369 nm.

In this way, the limit of detection obtained will be a little higher than taking measurements when the interaction  $Hg^{2+}$ -probe reaches the equilibrium after a long time. But the value is perfectly valid for an assigned concentration and the time passed when that concentration was added. The saturation of the signal was reached when the concentration of  $Hg^{2+}$  was near 10<sup>-3</sup> M (two hours and a half after the first addition) and, in a concentration below 10<sup>-5</sup> M, the variation of intensity between additions was linear.

**Results of the LOD calculation:** By using the program "R" and the data from the previous titration, a limit of detection was calculated:

![](_page_21_Figure_2.jpeg)

![](_page_21_Figure_3.jpeg)

Therefore, the LOD is  $6.6 \times 10^{-6}$  M or 1.3 ppm of Hg<sup>2+</sup> in water. Value reached 15 minutes after the first measure, so this is the average time that is necessary to detect a noticeable increase of the fluorescence of the solution.

#### • Kinetic response to MeHg<sup>+</sup>:

The behaviour of the probe in the presence of MeHg<sup>+</sup> resulted to be different to the previous behaviour in the presence of Hg<sup>2+</sup>. The following graph in Figure S21 shows the changes in fluorescent emission on time by normalizing the spectra:

![](_page_22_Figure_0.jpeg)

Figure S29. Normalized kinetic response to MeHgCl aqueous solution.  $\lambda_{exc}$  = 364 nm.  $\lambda_{em}$  = 445 nm

- The increase on the emission is more or less the same independently of the MeHg<sup>+</sup> concentration.

- When the concentration is high, the maximum of emission is reached in 90 minutes, after that the concentration decreases.

- When the concentration is low, the emission increase is slower, but the decrease starts after 8 hours when the concentration is  $5 \times 10^{-6}$  M.

The presence of two processes is a possible explanation for these facts. The first one is the complexation of the cation, which is very fast and needs a very low amount of MeHg<sup>+</sup> to reach the saturation. Then, there is another process in which the MeHg<sup>+</sup> modifies the structure of the complex between the polymer and the MeHg<sup>+</sup> cation (reaction) which is slower and depends on the concentration.

## • Titration experiments by adding MeHg<sup>+</sup>:

By the same way than in the case of  $Hg^{2+}$ , the titration experiments with the polymer where done by adding the solution of the cation every 5 minutes (Figure S20).

![](_page_23_Figure_0.jpeg)

Figure S30. Left: Fluorescence curves by addition of increasing concentrations of MeHgCl,  $\lambda_{exc}$  = 364 nm. Right: Titration plot by using integral surfaces of the fluorescence curves between 380-650 nm in response to increasing concentrations of MeHgCl,  $\lambda_{exc}$  = 364 nm.

During the measurements the emission intensity increases faster than in the case of Hg<sup>2+</sup>.

#### **Results of the LOD calculation:**

![](_page_23_Figure_4.jpeg)

Figure S31. Regression plot by using integral surfaces of the fluorescence curves between 380-650 nm in response to increasing concentrations of MeHgCl,  $\lambda_{exc}$  = 364 nm. LOD calculation.

Therefore the LOD is  $1.5 \times 10^{-6}$  M or 0.3 ppm. This value is reached in less than 20 minutes.

### • Effect of pH on JG25:

Although the quantitative measures were done in deionized water (pH = 8 approximately), it is important to study the pH effect on the samples in order to do further studies such as the measurements on fish samples, which are the final objective of the work.

A cuvette buffered at pH = 7.8 (HEPES buffer, 5 mM) was acidified by adding 5  $\mu$ L of HCl 1M and measuring the changes in pH and fluorescence. The results indicate that an increase of fluorescence is measured at lower pH. Then, by using the same process, the fluorescence increase was measured in a sample that contains 10<sup>-5</sup> M of Hg<sup>2+</sup>.

pН	Emission intensity (au)	Emission intensity + Hg <sup>2+</sup> (au)
7.8	39	89.2
7.5	43.5	100.6
7.1	49.4	116.7
6.6	60.9	148.4
5.4	69.7	173.0
4.6	96.4	243.0
4.0	110.1	282.7
3.5	121.9	318.7

Table S1: Emission intensity, with and without  $Hg(CIO_4)_2$ , at different pH, of **JG25** 

![](_page_25_Figure_0.jpeg)

Figure S32. Emission intensity, with and without Hg(ClO<sub>4</sub>)<sub>2</sub>, at different pH, of JG 25;  $\lambda_{exc}$  = 365 nm,  $\lambda_{em}$  = 455 nm

The proportional increase in fluorescence with  $Hg^{2+}$  is higher in acidic pH, being lower if the buffer is basic. The best pH is a controlled acidic or neutral pH.

#### Measurements with the soluble polymer JG32:

#### • Work concentration calculation:

In order to check that the fluorescence and absorbance change linearly with concentration the absorbance at 320 nm and emission at 453 nm ( $\lambda_{exc}$  = 320 nm) was measured for different concentrations.

![](_page_26_Figure_3.jpeg)

Figure S33. Absorbance and emission intensity ( $\lambda_{exc}$  = 320 nm) for different solutions.

The absorbance and fluorescence change is linear from  $2 \times 10^{-7}$  to  $2 \times 10^{-6}$  eq/L of probe. Due to the low quantity of the sensor (1%) a good work concentration is 0.012 g/L of polymer or what is the same,  $2 \times 10^{-6}$  eq/L of probe; so the rest of the tests were performed in that concentration.

• Kinetic response to Hg<sup>2+</sup>:

![](_page_26_Figure_7.jpeg)

Figure S34. Kinetic response of JG32 to aqueous solution of Hg(ClO<sub>4</sub>)<sub>2</sub> 10<sup>-4</sup> M,  $\lambda_{exc}$  = 320 nm,  $\lambda_{em}$  = 445 nm. Measures were taken every minute for 100 minutes

The increase in fluorescence is instantaneous but the equilibrium is reached after 50 minutes. These values depend on the polymer concentration and the Hg<sup>2+</sup> concentration, because of that the measurements of the detection limit with successive additions of Hg<sup>2+</sup> are done by waiting a constant time between additions (3 minutes).

#### Titration experiments by adding Hg<sup>2+</sup>:

![](_page_27_Figure_1.jpeg)

Figure S35. Left: Fluorescence curves by addition of increasing concentrations of Hg(ClO<sub>4</sub>)<sub>2</sub>,  $\lambda_{exc}$  = 320 nm. Right: Titration plot by using integral surfaces of the fluorescence curves between 380-650 nm in response to increasing concentrations of Hg(ClO<sub>4</sub>)<sub>2</sub>,  $\lambda_{exc}$  = 320 nm.

From 0 to  $2 \times 10^{-6}$  M the fluorescence decreases linearly, then it remains constant until  $3 \times 10^{-6}$  M and finally it increases.

#### Results of the LOD calculation:

![](_page_27_Figure_5.jpeg)

Figure S36. Regression plot by using integral surfaces of the fluorescence curves between 369-700 nm in response to increasing concentrations of Hg(ClO<sub>4</sub>)<sub>2</sub>,  $\lambda_{exc}$  = 320 nm. LOD calculation.

The LOD is  $7.6 \times 10^{-6}$  M or 1.5 ppm. Calculated from the point in which the fluorescence starts to increase. This limit is reached in 20 minutes. To use this limit with real samples, the fluorescence should be superior to the initial value to be quantified, therefore there are two possible results: If the fluorescence is lower than the initial value, the quantity of Hg<sup>2+</sup> stays between 0 to  $2.2 \times 10^{-5}$  M. If the fluorescence is higher than the initial value, the quantity of Hg<sup>2+</sup> is higher to  $2.2 \times 10^{-5}$  M and can be quantified.

For this polymer there is a clear second effect of decreasing the fluorescence when the concentration is very low. Without more data this effect could be associated to a kinetic effect. To check that effect, some solutions near the LOD were prepared and the fluorescence was measured at different times.

![](_page_28_Figure_1.jpeg)

Figure S37. Emission intensity difference at low concentrations of Hg(ClO<sub>4</sub>)<sub>2</sub> at different times,  $\lambda_{exc}$  = 320 nm.

The analysis of the graph leads to the conclusion that the results under low concentrations barely change. This behaviour was checked three times giving similar results.

• Kinetic response to MeHg<sup>+</sup>:

![](_page_28_Figure_5.jpeg)

Figure S38. Kinetic response of JG32 to aqueous solutions of Hg(ClO<sub>4</sub>)<sub>2</sub> and MeHgCl at different concentrations,  $\lambda_{exc}$  = 320 nm,  $\lambda_{em}$  = 456 nm. Measures were taken for 55 minutes.

The increase in fluorescence is faster with MeHg<sup>+</sup> at the beginning, the first 5 minutes, but the maximum is reached more slowly and the final fluorescence is lower at the same concentration of each species.

#### • Titration experiments by adding MeHg<sup>+</sup>:

The emission maximum was 458 nm.

![](_page_29_Figure_3.jpeg)

Figure S39. Left: Fluorescence curves by addition of increasing concentrations of MeHgCl,  $\lambda_{exc}$  = 320 nm,  $\lambda_{em}$  = 458 nm. Right: Titration plot by using the maximum of the fluorescence curves in response to increasing concentrations of MeHgCl,  $\lambda_{exc}$  = 320 nm,  $\lambda_{em}$  = 458 nm.

The titration profile is very similar to the one obtained for experiments with  $Hg^{2+}$ . For MeHg<sup>+</sup> the emission decreases until a concentration of  $5 \times 10^{-6}$  M of  $Hg^{2+}$  is reached and then it starts to increase when a concentration of  $1.5 \times 10^{-5}$  M of  $Hg^{2+}$  is reached.

**Results of the LOD calculation:** 

![](_page_29_Figure_7.jpeg)

Figure S40. Regression plot by using the maximum of the fluorescence curves between 380-650 nm in response to increasing concentrations of MeHgCl,  $\lambda_{exc}$  = 320 nm,  $\lambda_{exc}$  = 458 nm. LOD calculation.

The LOD is  $2.8 \times 10^{-5}$  M or 6.5 ppm. Calculated after the concentration in which the fluorescence starts to increase. The limit is reached in 40 minutes. To use this limit with real samples, the fluorescence should be higher to the initial value to be quantified, therefore there are two possible results: If the fluorescence is lower than the initial value, the quantity of MeHg<sup>+</sup> stays between 0 to  $3.5 \times 10^{-5}$  M. If the fluorescence is higher than the initial value, the quantity of MeHg<sup>+</sup> is higher to  $3.5 \times 10^{-5}$  M and can be quantified.

## • Quantum Yield:

Quantum yield was determined by its general equation:

$$\Phi = \Phi_R \frac{n^2 A_R F}{n_R^2 A F_R}$$

Where

- Φ is the quantum yield.
- n represents the refractive index of the solvent.
- A is the absorbance.
- F is the fluorescence.
- R is associated to the patron samples.

The quantum yield was calculated by using quinine sulfate in  $H_2SO_4$  0.1 M as reference, and 320 nm as excitation wavelength. The results are the average of 3 measurements:

 $\Phi$  (JG32) = 0.10 ± 0.01

 $\Phi$  (JG32 + Hg<sup>2+</sup>) = 0.17 ± 0.01

## **RESULTS SUMMARY**

## JG25 (Solid polymer with water affinity) (5 %probe)

## Hg(ClO<sub>4</sub>)<sub>2</sub>

Maximum increase in fluorescence is 380 %. LOD Hg<sup>2+</sup> = 6.6×10<sup>-6</sup> M or 1.3 ppm

Time dependent results:

- $\circ\,$  The samples reach a saturation point, and don't decrease (at least in several hours).
- $\circ$  10<sup>-4</sup> M implies 8 hours until saturation
- $\circ$  15 minutes are the time necessary to have this LOD or better.

## • MeHgCl

Maximum increase in fluorescence is 180 %. LOD MeHg<sup>+</sup> =  $1.5 \times 10^{-6}$  M or 0.3 ppm

Time dependent results:

- The fluorescence increases and when the maximum is reached it starts to decrease.
- Higher concentrations imply faster increase of the fluorescence, but once reached the maximum it decreases faster.
- $\circ~$  The maximum of fluorescence is not higher when the concentration is higher than 5×10^-6 M.
- $_{\odot}$  The maximum of fluorescence is reached faster than with Hg^{2+} 90 minutes at high concentrations and less than 3 hours with 5×10<sup>-6</sup> M.
- $\circ~$  The increase in fluorescence is lower than with Hg^2+.
- 20 minutes is enough to have a good reproducibility of this LOD.

• The pH changes the response to Hg<sup>2+</sup> by increasing the emission intensity when the pH is lower, but the initial emission of the polymer is also higher.

## JG32 (polymer soluble in water) (1 % probe) 0.012 g/L

Φ (JG32) = 0.10 ± 0.01

 $\Phi$  (JG32 + Hg<sup>2+</sup>) = 0.17 ± 0.01

• Hg(ClO<sub>4</sub>)<sub>2</sub>

Maximum increase in fluorescence is 177 %. LOD  $Hg^{2+} = 2.2 \cdot 10^{-5} M$  or 4.4 ppm.

Time dependent results:

- The samples reach a maximum fluorescence value that then does not decrease (at least within 100 minutes).
- $\circ~$  A concentration 10  $^4$  M needs 50 minutes until maximum value of fluorescence is reached.
- To have a reliable LOD the time necessary for measurements is 20 minutes.

The fluorescence starts to increase when the concentration is higher than  $2 \times 10^{-6}$  M, and this process is independent of time.

#### MeHgCl

Maximum increase in fluorescence 160 % from the minimum, 140 % from the initial fluorescence. LOD MeHg<sup>+</sup> =  $3.9 \times 10^{-5}$  M or 7.5 ppm

Time dependent results:

- $\circ$  The fluorescence increases faster than with Hg<sup>2+</sup> within the first 5 minutes.
- The final increase in fluorescence is lower than with Hg<sup>2+</sup>.
- 40 minutes is enough to have a good reproducibility of the LOD.

The fluorescence starts to increase when the concentration is higher than 5×10<sup>-6</sup> M, and this process is not time-dependent.

#### **ANALYSIS OF FISH SAMPLES**

![](_page_32_Figure_1.jpeg)

Figure S41: A scheme of treatment for analysis of fish samples.

In order to find the best conditions to have reproducible and reliable measures of the presence of mercury in fish samples, some preliminary tests are necessary:

- To measure the mercury extracted from the fish samples by ICP-mass analysis.
- Qualitative measures of the fluorescent changes of the polymer in the presence of the mercury extracts.
- Measurements of some different fish samples.

#### Analysis of mercury extracted from fish samples:

#### a) Extraction of mercury species from fish

Two methods were tested: acidic extraction and silica extraction. The basic extraction was discarded because of the high temperatures, the extracts are colored and the probe has no good results at pH superior than 8 so it had interferences in fluorescence measures. All the measurements were done with lyophilized fish because the quantity of water is vital in the concentration calculation.

**Acid extraction:** 5 ml of HCl 5 M in water and 5 ml of NaCl 0.25 M in water were added to 0.5 g of lyophilized fish in a microwave Owen vial. The mixture was sonicated for 10 minutes and heated for 10 minutes at 60 °C in the microwave Owen. Then the mixture was centrifuged at 4000 rpm for 10 minutes and at 7000 rpm for additional 10 minutes. The liquid phase was filtered in a glass fibre filter of 0.22  $\mu$ m pore. Finally, the samples to be measured by fluorescence were concentrated to 1 ml.

**Silica extraction:** 0.5 g of fish and 2 ml of water were mixed in a mortar and the mixture was grinded, 1g of silica was then added and mixed. The mixture was then treated with HCI 5 M in water following the same procedure used for the acid extraction, but without using the microwave Owen. Finally, the samples to be measured by fluorescence were concentrated to 1 ml.

### b) Determination of mercury by ICP-Mass analysis

The samples were lyophilized, in some cases it is expected a loss in weigh of 70-80 %.

Table S2: Amount of mercury detected by ICP-Mass analysis on fish samples, left, lyophilized fish, right, the corresponding amount to fresh fish.

	Conc. Hg (pp	m) lyophilized		Conc. Hg (ppm) fresh	
Sample	Acid extraction	Silica extraction	% (water)	Acid extraction	Silica extraction
Swordfish	5.1 ± 0.1	6.0 ± 0.6	73	1.4 ± 0.1	1.6 ± 0.6
Tuna	3.1 ± 0.1	5.1 ± 1.4	71	0.9 ± 0.1	1.5 ± 1.4
Panga	1.1 ± 0.4	1.3 ± 0.6	78	0.2 ± 0.1	0.3 ± 0.1
Salmon	0.015 ± 0.016	0.07 ± 0.01	72	0	0
Conger eel	2.4 ± 0.1	2.2 ± 0.2	80	0.5 ± 0.1	0.4 ± 0.1
Dogfish	6.8 ± 0.3	3.9 ± 0.1	75	1.7 ± 0.1	1.0 ± 0.1

![](_page_33_Figure_4.jpeg)

Figure S42. Graphical representation of the amounts of mercury detected by ICP-Mass analysis on fish samples, (left) lyophilized, (right) the corresponding amount to fresh fish.

The data show that the concentration is higher when the fish is bigger. Another important result is that the concentration of mercury in salmon is 0, because salmons are obtained

from a fish farm, not from the sea, so the concentration of mercury is 0. The concentrations of mercury in swordfish, tuna, conger eel and dogfish are high enough to be measured with the solid polymer and, in case of swordfish, around the maximum amount per week recommended by the FDA, (1.3 ppm). The LOD of the polymer JG25 is 1.3 ppm for Hg<sup>2+</sup> and 0.3 ppm for MeHg<sup>+</sup>, therefore a direct measure is possible, depending on the possible interferents. In any case, we started by making an extraction and checking the results.

#### c) Measures of mercury extracts with JG25:

#### • Quantitative measures:

In a cuvette, 1 ml of the extract was added to a vial with a portion of JG25. Then the fluorescence was checked. The variation of intensity was measured with water (blank) and then by adding the extract, the difference is the value to be measured. Elimination of interferences from the position of the polymer is very important in the case of solid materials.

	$\Delta$ Emission intensity, $\lambda_{exc}$ = 365 nm (au)					
Extraction Method	Swordfish	Tuna	Panga	Salmon	Conger eel	Dogfish
Acid	301.6	229.6	0.91	5.5	152.1	377.3
Silica	354.2	307.2	27.7	6.4	162.5	171.7

Table S3: Emission intensity variation of JG25 in contact to fish samples.

![](_page_34_Figure_6.jpeg)

Figure S43. Emission intensity variation of JG25 in contact to fish samples,  $\lambda_{exc}$  = 365 nm,  $\lambda_{em}$  = 455 nm.

From the experiments it is clear that the fluorescence is related to the percentage of mercury in the fish samples.

### Comparative ICP – JG25 study:

To compare the results, the process followed was:

• Normalizing the graphs, taking as reference maximum the ICP-Mass result from the acid extraction of dogfish.

• Considering the results of the ICP-Mass in ppm and converting the fluorescence values to ppm relative to ICP-Mass values.

![](_page_35_Figure_4.jpeg)

Figure S44. A comparison between the values of mercury in fish samples obtained by ICP-Mass analysis and fluorescent measurements.

The different results are a consequence of multiple factors such as the treatment of the samples or the matrix of the fish, but there are very close results between all methods. The increase of fluorescence in solution is enough to see the signal of fluorescence for the samples with higher concentrations of mercury, namely swordfish, tuna and dogfish. None of the samples of salmon shows an increase in fluorescence. For catfish the values are very close to the LOD therefore it is expected that there is no signal in certain cases in the fluorescence measurements. As a result, the method might be useful in order to measure quantitatively the mercury concentration. The method is reliable as an indicator of the presence of the metal in quantities higher to the LOD, so it could be a very easy and cheap method to detect the presence of mercury in fish.

## 4.5.1.4.4.2. Direct analysis of mercury on fish samples with JG25:

3 tests were performed:

- Preliminary tests with fish samples that contain high quantities of mercury such as tuna and swordfish.

- Tests with several samples of fish, tuna, swordfish, conger and panga.
- Systematic analysis of some of the samples.

### • Test 1:

Three pieces of polymer (JG25) were placed in contact to three new fish samples containing fresh tuna, fresh swordfish, and a mixture of swordfish+Hg<sup>2+</sup>. Every fish sample, 2 g, was grinded and mixed with 5 ml of water and then left in contact with a polymer fragment. After a period of time the polymer fragment was taken from the solution and dried in order to compare the fluorescence of every polymer fragment. Previously, by ICP-Mass, the samples of fresh fish showed that tuna had a concentration of 0.7  $\pm$  0.1 ppm and swordfish had a concentration of 3.7  $\pm$  0.8 ppm. After one hour the difference between the fish samples and the blank is clear and correlates quite well with the real amounts of mercury present in every sample. But after one day in contact the increase is even higher. This analysis was a preliminary test in order to check the possibility of these kinds of measures, because of that it was repeated with more polymer fragments and more fish samples in order to compare the results of fish samples with high and low amounts of mercury.

#### • Test 2:

Four pieces of polymer (JG25) were put in presence of four new fish samples containing: tuna – swordfish – conger – panga. Qualitatively, the only clear difference is noticed between the blank and the panga with no fluorescence and low fluorescence respectively. The polymer fragment with added Hg<sup>2+</sup> is the most fluorescent and the rest of the samples give variations in fluorescence that cannot be evaluated quantitatively by the naked eye, but may give a clear qualitative evaluation of the presence or absence of mercury in the fish samples.

### • Test 3:

In this case, we used the same samples originally used for extractions, in order to compare the results with fish samples and extracts, taking in account the large variability of mercury contamination between different fish samples from different specimens, from them, 0.5 g of lyophilized fish samples were mixed with 2 ml of water. Then a piece of the polymeric sensor was added. To check the difference in fluorescence every polymer fragment in contact with fish samples was measured at different waiting times in the fluorometer, obtaining the results in Table S4 and Figure S26. The results can be compared with the corresponding results from the extraction by normalizing to one of them (dogfish in this case) (Figure S27).

Table S4: The relation between the concentration of mercury and the obtained values of fluorescence for fish samples and the polymeric sensor.

	$\Delta$ Emission intensity 365 nm (au)				
Sample/ time (h)	Swordfish	Tuna	Panga	Conger eel	Dogfish
0.5	220.5	212.2	21.3	32.0	230.93
1	260.8	237.7	21.97	65.79	272.2
24	301.6	280.0	30.68	136.3	318.4

![](_page_37_Figure_0.jpeg)

Figure S45. Emission intensity variation with fresh fish samples ( $\lambda_{exc}$  = 365 nm,  $\lambda_{em}$  = 455 nm) at different waiting times.

![](_page_37_Figure_2.jpeg)

Figure S46. Emission intensity variation in experiments with fresh fish samples ( $\lambda_{exc}$  = 365 nm,  $\lambda_{em}$  = 455 nm) compared with the results from the extracts.

Therefore, a relation between the concentration of mercury and the obtained values of fluorescence is confirmed.