Highly sensitive and selective detection of the pyrophosphate anion biomarker under physiological conditions

Guzmán Sánchez,* David Curiel,* Witold Tatkiewicz,† Imma Ratera,† Alberto Tárraga,* Jaume Veciana,** and Pedro Molina*†

ELECTRONIC SUPPLEMENTARY INFORMATION

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NMR spectra of 1

Figure S1. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) spectra of 1.
Characterization of the monolayers

XPS data:

**Figure S2.** Deconvolution of the XPS peaks for each element detected.

<table>
<thead>
<tr>
<th>Element/orbital</th>
<th>Bond type</th>
<th>Calculated bond energy (eV)</th>
<th>Found bond energy (eV)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphur 2p</td>
<td>Bound thiol</td>
<td>162.1</td>
<td>161.93</td>
<td>2628.782</td>
</tr>
<tr>
<td></td>
<td>Bound thiol[a]</td>
<td>162.1</td>
<td>163.11</td>
<td>1419.567</td>
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<tr>
<td></td>
<td>Unbound thiol</td>
<td>164.7</td>
<td>164.21</td>
<td>627.583</td>
</tr>
<tr>
<td>Carbon 1s</td>
<td>C-C, C=C, C-H</td>
<td>284.6</td>
<td>284.32</td>
<td>14431.39</td>
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<tr>
<td></td>
<td>y C-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-O</td>
<td>286.1</td>
<td>285.03</td>
<td>14606.93</td>
</tr>
<tr>
<td></td>
<td>C=O</td>
<td>287.5</td>
<td>288.40</td>
<td>3054.977</td>
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<tr>
<td>Oxygen 1s</td>
<td>O=C</td>
<td>531.6</td>
<td>531.54</td>
<td>3256.887</td>
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<td></td>
<td>O=C</td>
<td>533.2</td>
<td>532.85</td>
<td>4811.747</td>
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<tr>
<td>Nitrogen 1s</td>
<td>N-C</td>
<td>399.5</td>
<td>399.53</td>
<td>1837.226</td>
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<tr>
<td></td>
<td>N⁺-H[b]</td>
<td>400.2</td>
<td>400.14</td>
<td>1881.968</td>
</tr>
</tbody>
</table>

[a] Although a bound thiol should exhibit only one band at 162.1 eV, the appearance of two different bands is not uncommon. [b] The protonation of NH groups is not infrequent during the XPS measurements.
TOF-SIMS data:

**Figure S3.** Partial TOF-SIMS spectrum with lateral resolution (positive ionization mode) for a gold substrate functionalized with 1 by means of μCP techniques.

PM-IRRAS data:

**Figure S4.** PM-IRRAS spectrum. The most relevant bands are shown as insets.
SEM and AFM data:

Figure S5. SEM and AFM images of printed 1·SAM. a) SEM image of 1·SAM; b) zoom in the border area of the SEM image, b) AFM image of 1·SAM and c) AFM profile of both regions found.
Surface Plasmon Resonance (SPR) titrations

1-Decanothiol SAM:

![Graph showing SPR sensogram of 1-decanothiol SAM upon addition of different aliquots of HP₂O₇³⁻ anion. An arrow denotes the injection of each concentration of guest.](image1)

**Figure S6.** SPR sensogram of 1-decanothiol SAM upon addition of different aliquots of HP₂O₇³⁻ anion. An arrow denotes the injection of each concentration of guest.

1·SAM in aqueous NaCl 0.1 M:

![Graph showing normalized sensogram obtained upon addition of different concentrations of the HP₂O₇³⁻ anion to 1·SAM in aqueous NaCl 0.1 M.](image2)

**Figure S7.** Normalized sensogram obtained upon addition of different concentrations of the HP₂O₇³⁻ anion to 1·SAM in aqueous NaCl 0.1 M. (a) baseline, (b) 10⁻¹⁰ M, (c) 10⁻⁹ M, (d) 10⁻⁸ M, (e) 10⁻⁷ M, (f) 10⁻⁶ M, (g) 10⁻⁵ M, and (h) 10⁻⁴ M. Inset: SPR response of a sensing chip functionalized with 1·SAM upon injection of different concentrations of HP₂O₇³⁻ anion; (I) denotes the injection of a 0.2 M NaCl aqueous solution.

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solution as a reference, (II) indicates the washing step with buffer solution (aqueous NaCl 0.1 M in this case) and (III) corresponds to the injection of HP$_2$O$_7^{3-}$ solutions of different concentrations.

**Figure S8.** Plots of apparent rate constants, $k_s$, vs [HP$_2$O$_7^{3-}$] at low concentrations (a) and at high concentrations (b).

**Figure S9.** Regeneration tests on 1·SAM with 10$^{-9}$ M solutions of HP$_2$O$_7^{3-}$. 
**1·SAM** in 20 mM HEPES-saline buffer at pH = 7.4:

![Normalized SPR sensogram](image1)

**Figure S10.** (a) Normalized SPR sensogram obtained upon addition of different concentrations of hydrogenpyrophosphate anion to 1·SAM in 20 mM HEPES-saline buffer (pH = 7.4). (a) baseline, (b) \([\text{HP}_2\text{O}_7^{3-}] = 10^{-10}\) M, (c) \([\text{HP}_2\text{O}_7^{3-}] = 10^{-8}\) M, (d) \([\text{HP}_2\text{O}_7^{3-}] = 10^{-7}\) M, (e) \([\text{HP}_2\text{O}_7^{3-}] = 10^{-6}\) M, (f) \([\text{HP}_2\text{O}_7^{3-}] = 10^{-5}\) M and (g) \([\text{HP}_2\text{O}_7^{3-}] = 10^{-4}\) M. Plots of apparent rate constants, \(k_s\), vs \([\text{HP}_2\text{O}_7^{3-}]\) at low concentrations (b) and at high concentrations (c).

![Bar chart](image2)

**Figure S11.** Selectivity of 1·SAM towards trivalent anions. The concentration used was \(10^{-9}\)M for HPPi and \(10^{-7}\)M for the rest of the anions (100-fold excess).
The interfering effects of trivalent citrate and trimesate anions, were tested in 100-fold excess. Surprisingly, the selectivity of the monolayer increased in such physiological media (see Figure S11). Since this environment is more competitive, we can assume that the affinity of $1 \cdot \text{SAM}$ is severely reduced. Nevertheless, while the signal towards hydrogen pyrophosphate is still good, the response showed for the possible competing anions decreased abruptly.

**Figure S12.** Selectivity of $1 \cdot \text{SAM}$ in 20 mM HEPES towards several phosphate anions. The concentration used for all anions were $10^{-7}$ M.

**Figure S13.** Regeneration tests of $1 \cdot \text{SAM}$ in 20 mM HEPES saline buffer using $[\text{HP}_2\text{O}_7^{3-}] = 10^{-9}$ M.
Scheme 1. Synthesis of compound 1.