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

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ELECTRONIC SUPPLEMENTARY INFORMATION

NMR spectra of 1

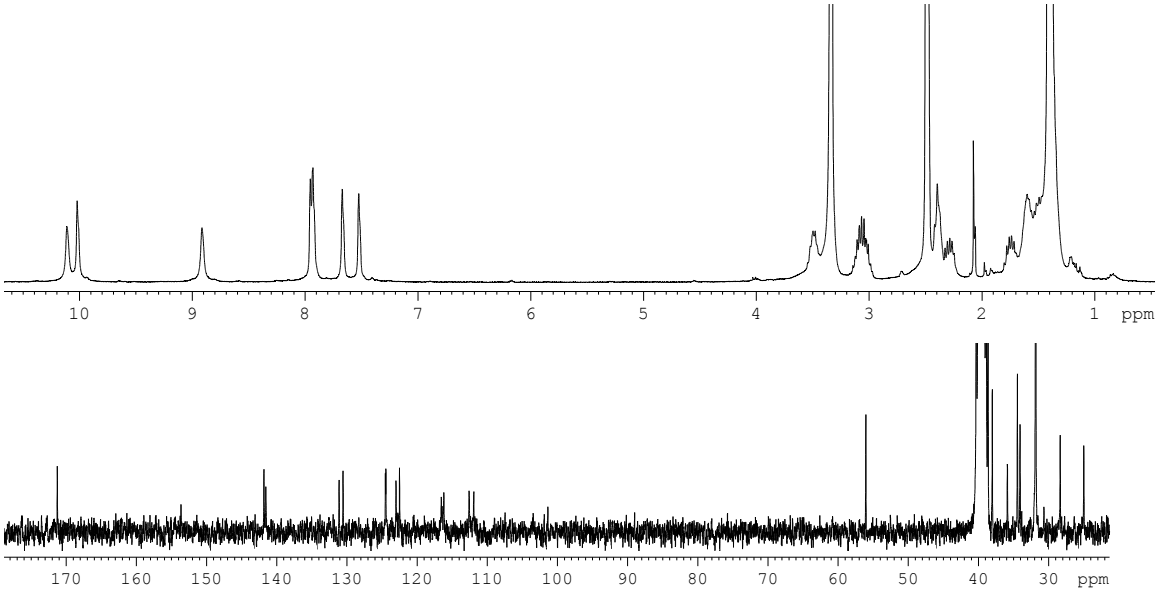

Figure S1. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **1**.

Characterization of the monolayers

XPS data:

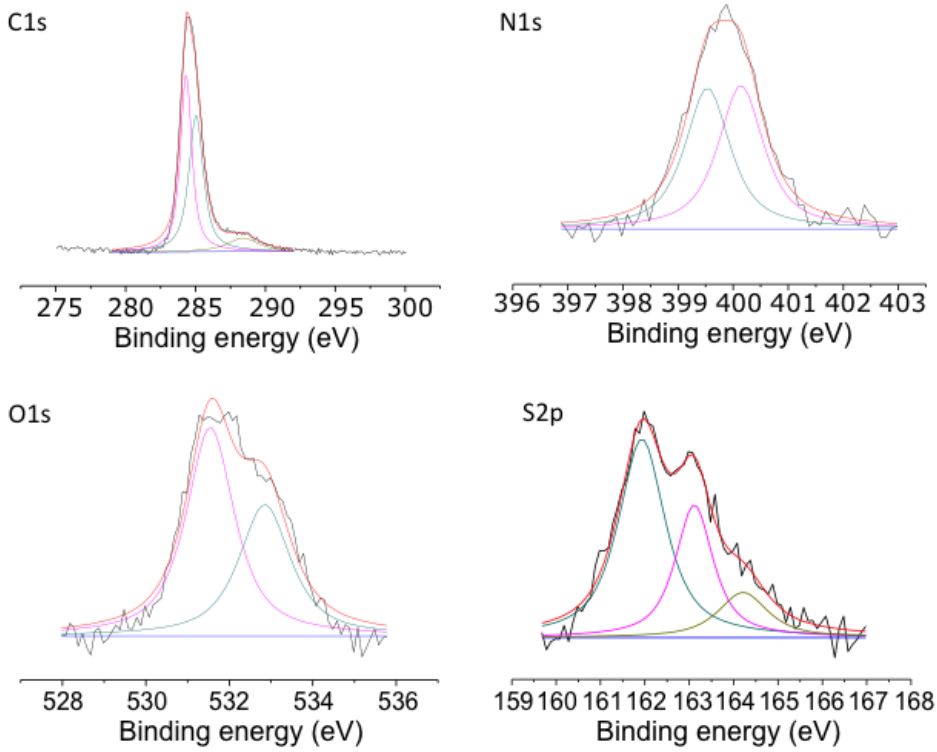

Figure S2. Deconvolution of the XPS peaks for each element detected.

Table S1. Parameters rendered by the XPS analysis.

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

[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:

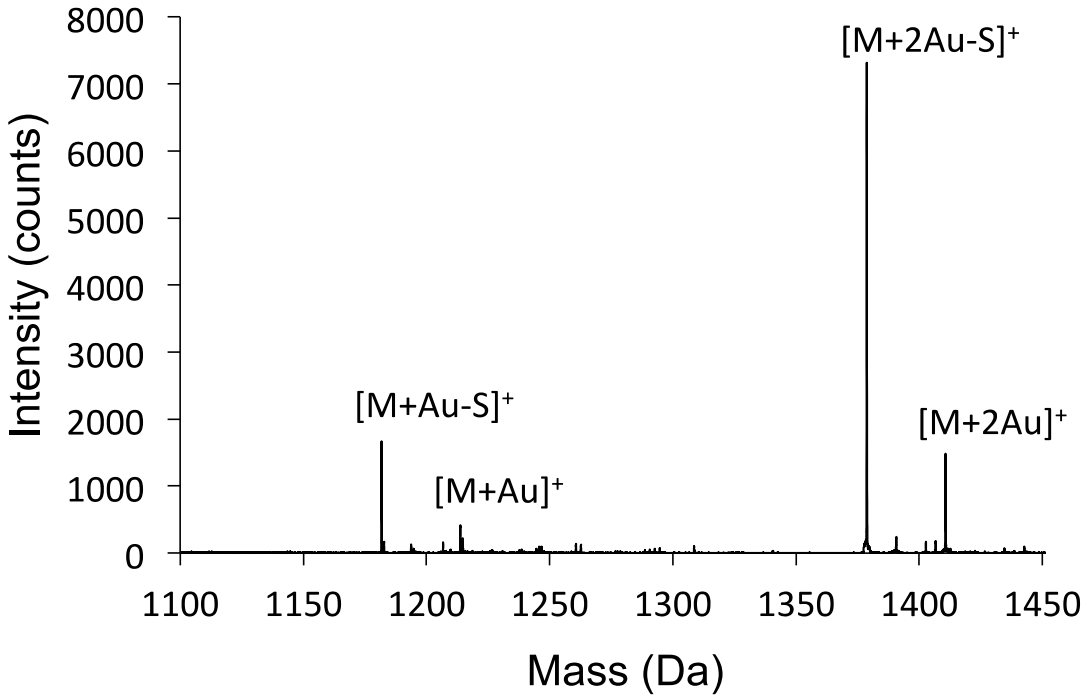

Figure S3. Partial TOF-SIMS spectrum with lateral resolution (positive ionization mode) for a gold substrate functionalized with **1** by means of μCP techniques.

PM-IRRAS data:

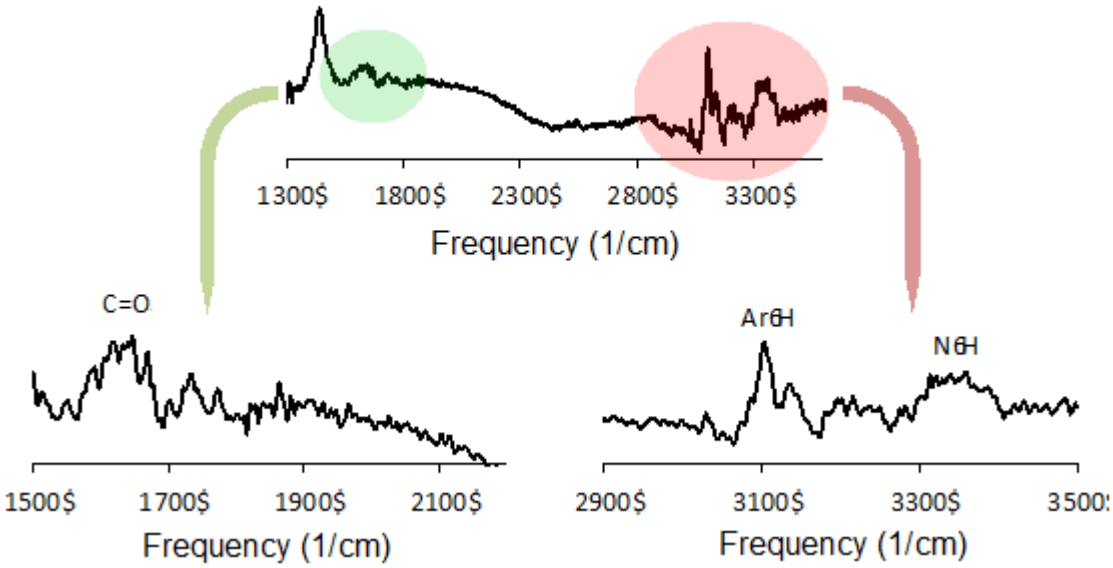

Figure S4. PM-IRRAS spectrum. The most relevant bands are shown as insets.

SEM and AFM data:

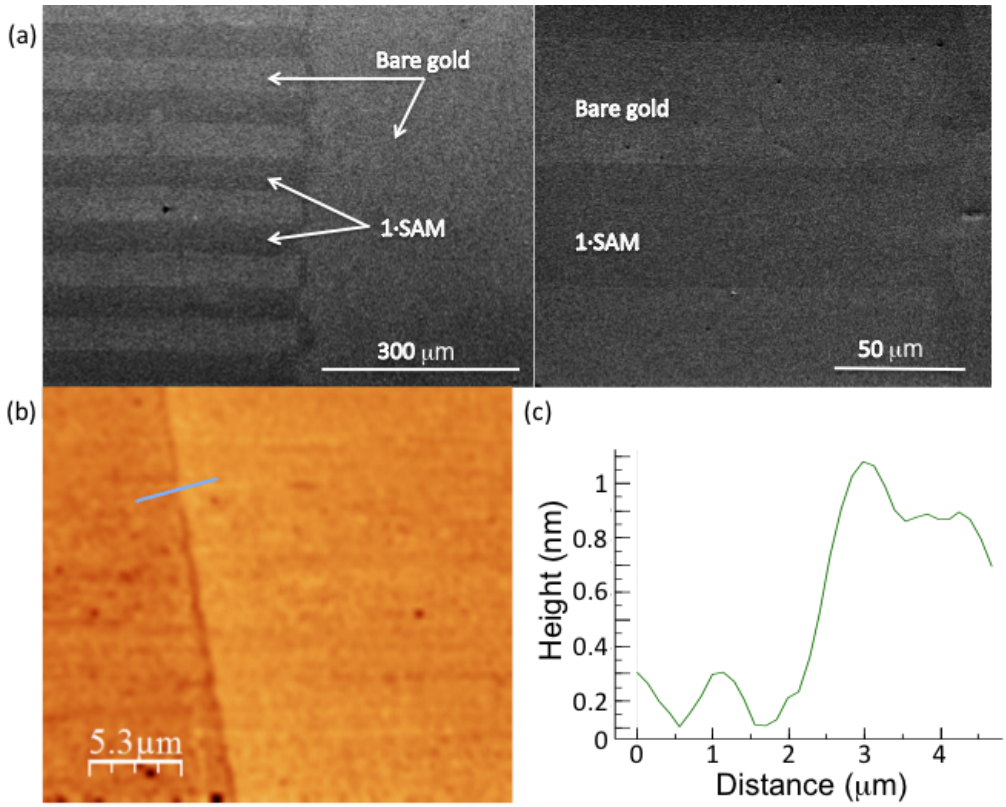

Figure S5. SEM and AFM images of printed **1·SAM**. a) SEM image of **1**·SAM; b) zoom in the border area of the SEM image, b) AFM image of **1·SAM** and c) AFM profile of both regions found.

Surface Plasmon Resonance (SPR) titrations

1-Decanothiol SAM:

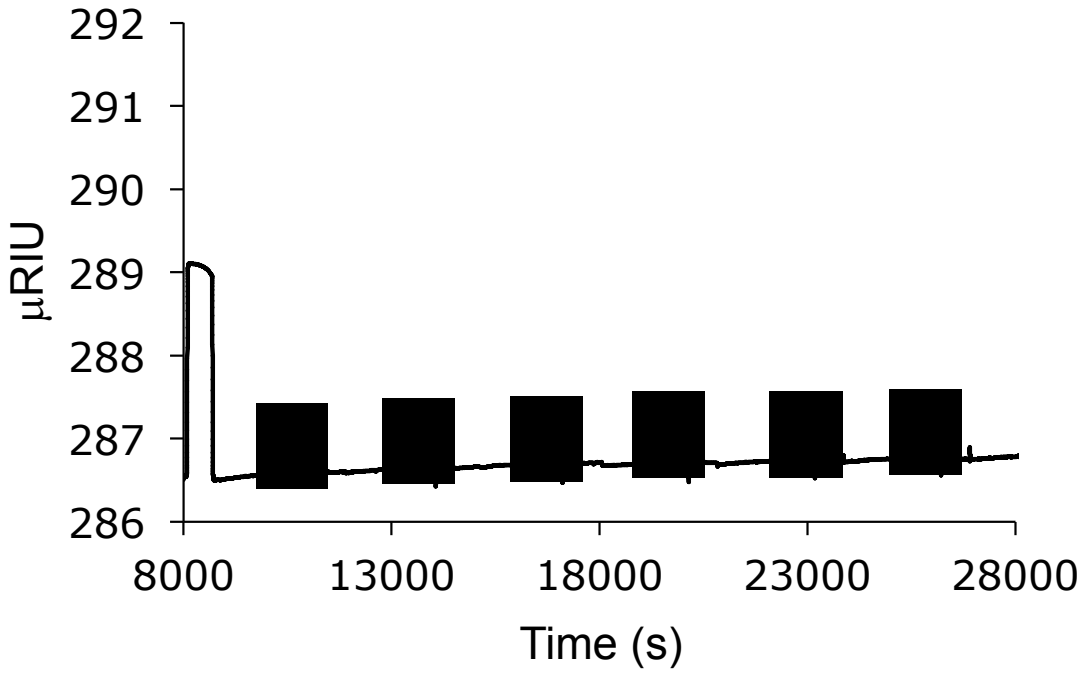

Figure S6. SPR sensogram of 1-decanothiol SAM upon addition of different aliquots of $HP_2O_2^{3-}$ anion. An arrow denotes the injection of each concentration of guest.

1·SAM in aqueous NaCl 0.1 M:

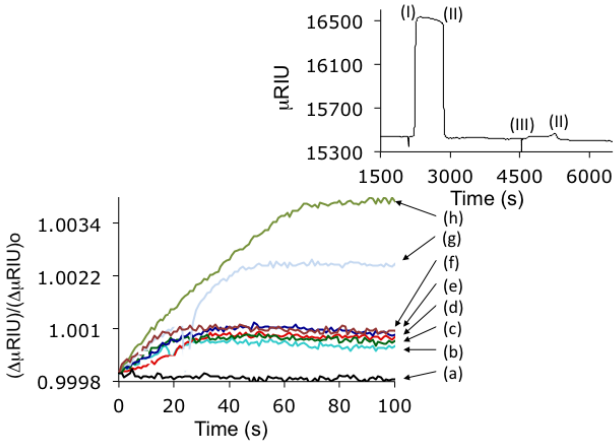

Figure S7. Normalized sensogram obtained upon addition of different concentrations of the $HP_2O_7^{3-}$ anion to **1·SAM** in aqueous NaCl 0.1 M. (a) baseline, (b) 10^{-10} M, (c) 10^{-9} M, (d) 10^{-8} M, (e) 10^{-7} M, (f) 10^{-6} M, (g) 10^{-5} M, and (h) 10^{-4} M. Inset: SPR response of a sensing chip functionalized with **1·SAM** upon injection of different concentrations of $HP_2O_7^{3-}$ anion; (I) denotes the injection of a 0.2 M NaCl aqueous

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 $HP_2O_7^{3-}$ solutions of different concentrations

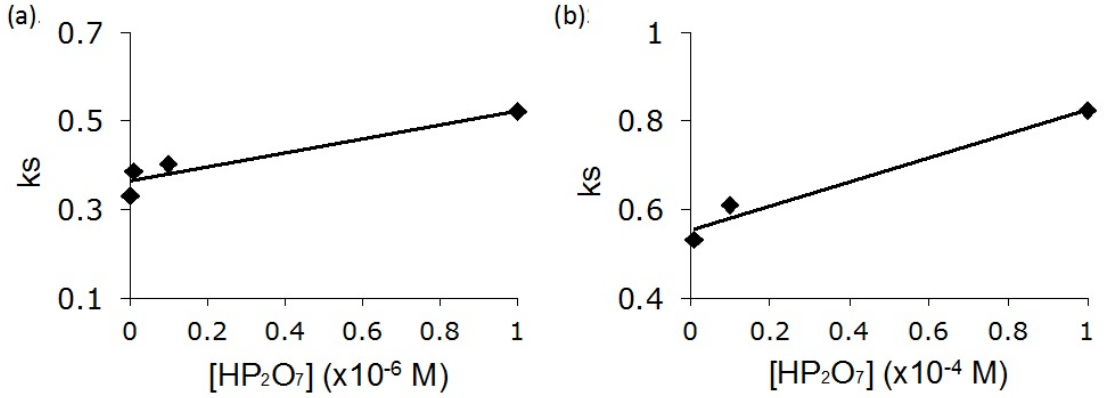

Figure S8. Plots of apparent rate constants, k_s, *vs* $[HP_2O_7^{3-}]$ at low concentrations (a) and at high concentrations (b).

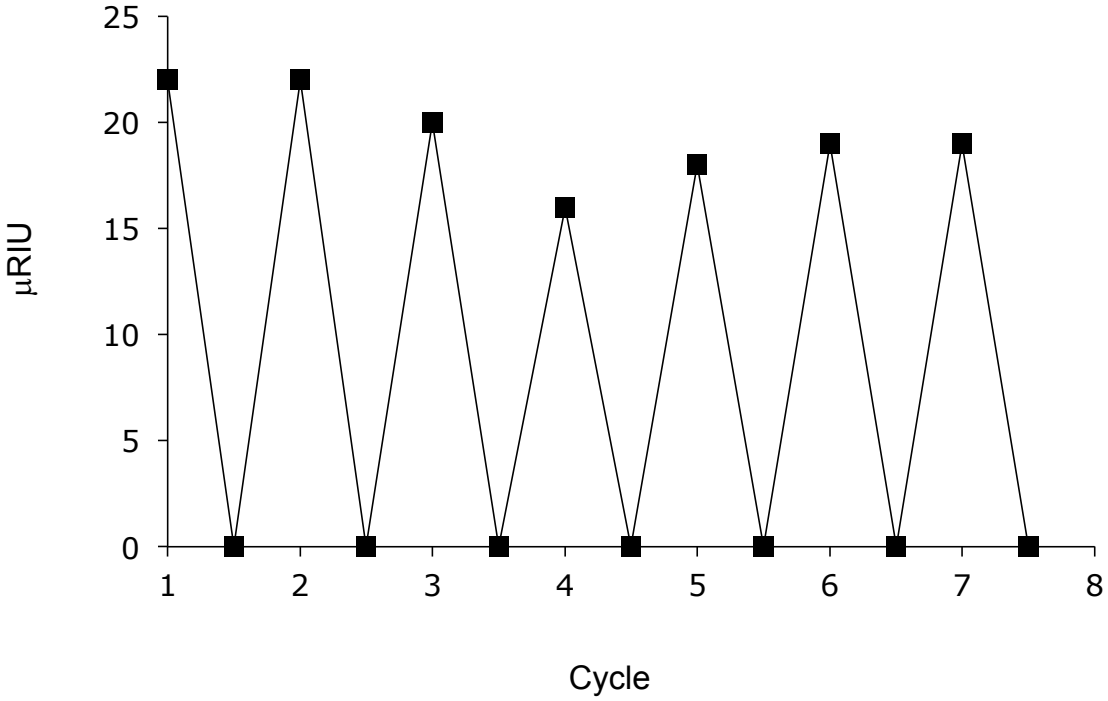

Figure S9. Regeneration tests on **1·SAM** with 10^{-9} M solutions of $HP_2O_7^{3-}$.

1·SAM in 20 mM HEPES-saline buffer at pH = 7.4:

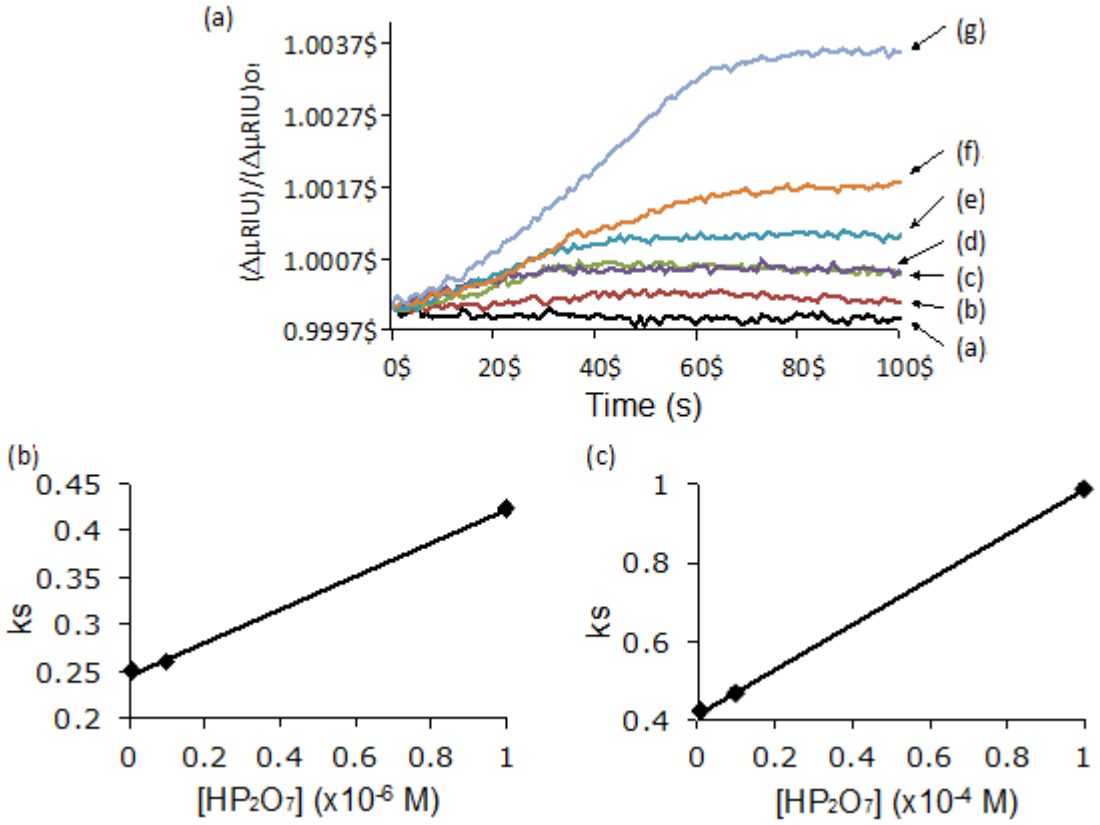

Figure S10. (a) Normalized SPR sensogram obtained upon addition of different concentrations of hydrogenpyrophosphate anion to **1·SAM** in 20 mM HEPES-saline buffer (pH = 7.4). (a) baseline, (b) $[HP_2O_7^{3-}] = 10^{-10}$ M, (c) $[HP_2O_7^{3-}] = 10^{-8}$ M, (d) $[HP_2O_7^{3-}] = 10^{-7}$ M, (e) $[HP_2O_7^{3-}] = 10^{-6}$ M, (f) $[HP_2O_7^{3-}] = 10^{-5}$ M and (g) $[HP_2O_7^{3-}] = 10^{-4}$ M. Plots of apparent rate constants, k_s, *vs* $[HP_2O_7^{3-}]$ at low concentrations (b) and at high concentrations (c).

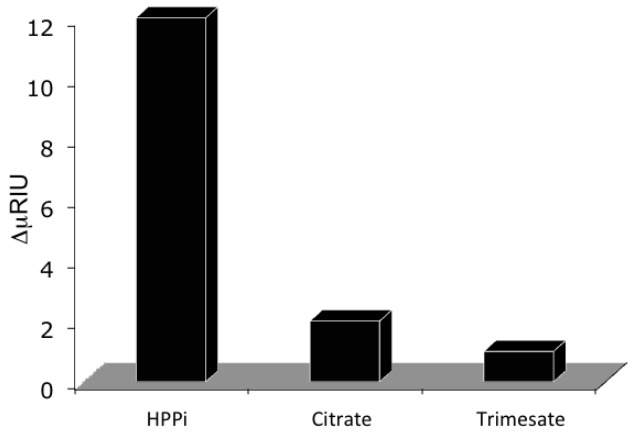

Figure S11. Selectivity of **1·SAM** towards trivalent anions. The concentration used was 10^{-9}M for HPPi and 10^{-7}M for the rest of the anions (100-fold excess)

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·SAM** is severely reduced. Nevertheless, while the signal towards hydrogen pyrophosphate is still good, the response showed for the possible competing anions decreased abruptly.

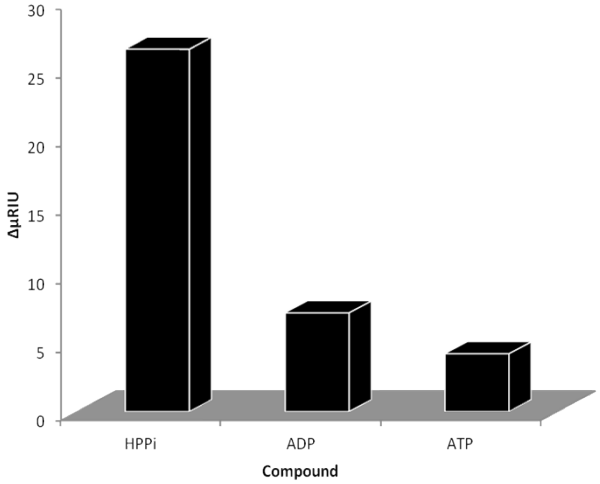

Figure S12. Selectivity of **1·SAM** in 20 mM HEPES towards several phosphate anions. The concentration used for all anions were 10^{-7} M.

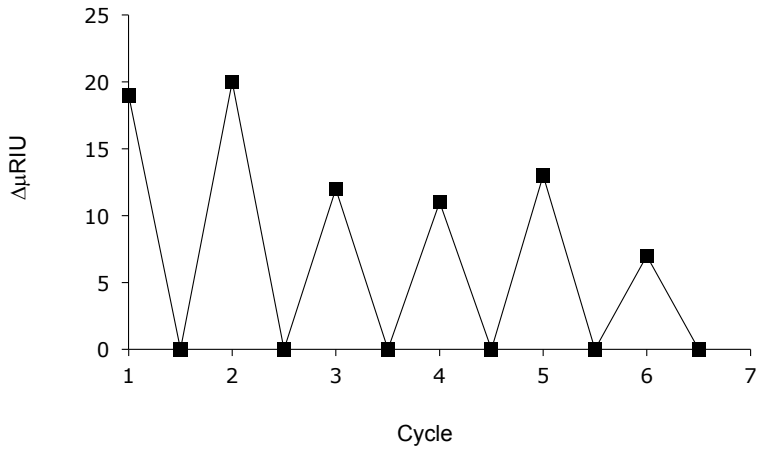

Figure S13. Regeneration tests of **1·SAM** in 20 mM HEPES saline buffer using $[HP_2O_7^{3-}] = 10^{-9}$ M.

Scheme 1. Synthesis of compound **1**.