

Supporting Information for:

Multicolor, large-area fluorescence sensing through oligothiophene-self-assembled monolayers

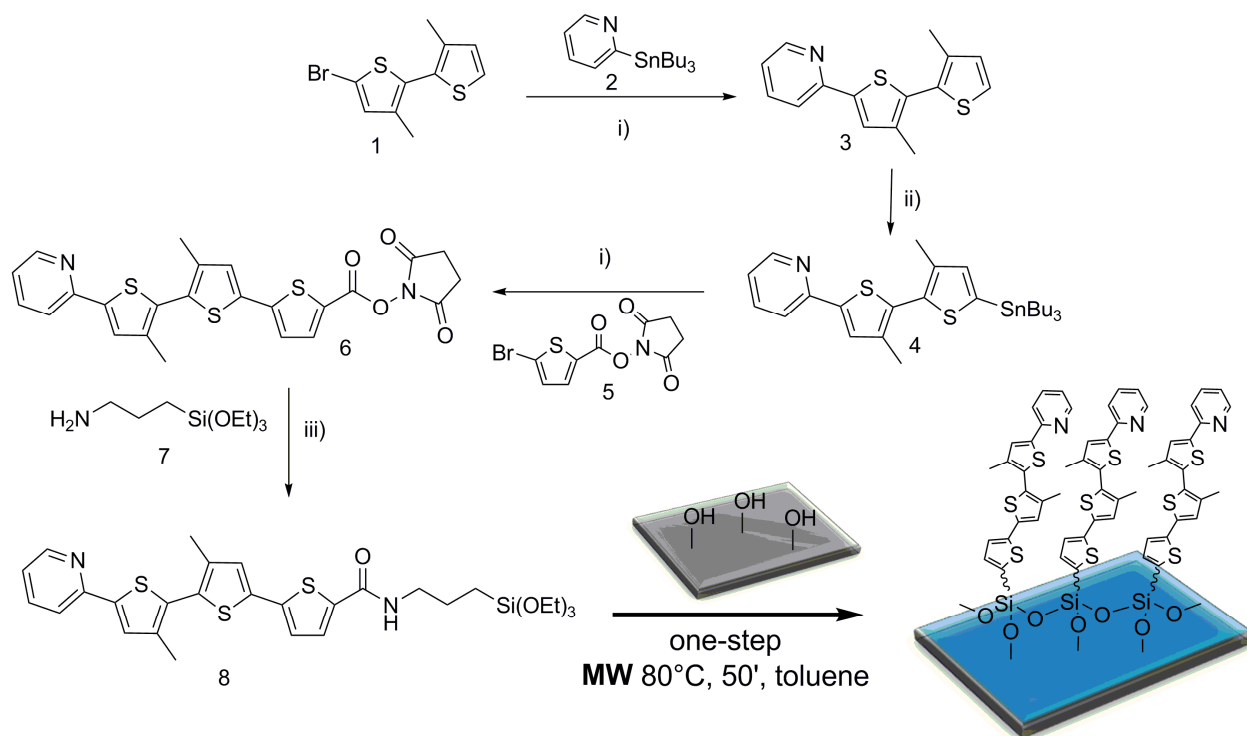
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1. Synthesis

General: All commercially available reagents and solvents were used as purchased without further purification. Microwave experiments were carried out in a Milestone Microsynth Labstation operating at 2450 MHz. The oven was equipped with magnetic stirring, pressure and temperature sensors. Reactions were performed in a glass vessel (capacity 10 mL) sealed with a septum. The microwave method was power controlled (300 W maximum power input) and the samples were irradiated with the required power output (setting at the maximum power) to achieve the desired temperature (80 °C) for 50 min. All ^1H NMR, ^{13}C NMR spectra were recorded at room temperature on a Varian Mercury-400 spectrometer equipped with a 5-mm probe. Melting points were determined on a Kofler bank apparatus and are uncorrected.



Scheme SI_1.

The synthesis of compounds **1** and **5** was performed according to previously reported methods. ^[1]

2-(3,3'-dimethyl-2,2'-bithiophen-5-yl)pyridine (3): Tris(dibenzylideneacetone)dipalladium-chloroform adduct (46 mg, 0.045 mmol) and triphenyl arsine (110 mg, 0.36 mmol) were dissolved in 69 ml of dry toluene under N₂ atmosphere and the solution was refluxed until the colour turned to dark brown. *2-bromo (3,3'-dimethyl-2,2'-bithiophen-5-yl 1* (401 mg, 1.47 mmol) in 3 ml of toluene was added then *2-tributylstannyl pyridine 2* (650 mg, 1.76 mmol) diluted in 4 ml of dry toluene was added drop wise and the mixture stirred overnight. The crude was purified by chromatography (silica gel neutralized by petroleum ether with 4% of triethylamine, pet.eth. +5% AcOEt + 5% CH₂Cl₂) to give 272 mg (68% Y) of compound **3** as a viscous yellow oil.

$m/z = 271$ (M^{+}), UV-Vis $\lambda_{\max} = 327$ nm (CH₂Cl₂), PL ($\lambda_{\text{exc}} = 327$ nm, CH₂Cl₂), $\lambda_{\max} = 421$ nm. ¹H NMR (400 MHz, CDCl₃, TMS/ppm) δ 8.55 (1H, m), 7.65 (m, 2H), 7.28 (d, 1H, $J = 5.2$ Hz), 7.13 (m, 1H), 6.93 (d, 1H, $J = 5.2$ Hz), 2.25 (s, 3H), 2.22 (s, 3H). ¹³C NMR (400 MHz, CDCl₃, TMS/ppm) δ 152.4, 149.6, 143.3, 137.4, 136.6, 131.9, 130.2, 129.4, 128.9, 127.6, 125.1, 121.8, 118.5, Anal. calcd for C₁₅H₁₃NS₂: C, 66.38; H, 4.83 Found C, 66.39; H, 4.87.

2-(3,3'-dimethyl-5'-(tributylstannyl)-2,2'-bithiophen-5-yl)pyridine (4): To a solution of **3** (270 mg, 1 mmol) in anhydrous THF (4.5 mL) cooled to -70 °C was treated dropwise with LDA 2M (0.6 mL, 1.2 mmol). After stirring for 1 h at -70 °C and 1h at room temperature, tributyltin chloride (0.27 ml, 1 mmol) was added dropwise. The mixture was allow to react overnight then the THF was removed. The crude was dissolved in CH₂Cl₂ and washed several time with H₂O. Chromatography on fluorisil, pet.eth.+2% AcOEt) afforded 225 mg (40% Y) of compound **4** as dark yellow oil. $m/z = 561$ (M^{+}), ¹H NMR (400 MHz, CDCl₃, TMS/ppm) δ 8.55 (1H, m), 7.64 (m, 2H), 7.43 (s, 1H, $J = 5.2$ Hz), 7.12 (m, 1H), 6.97 (s, 1H, $J = 5.2$ Hz), 2.28 (s, 3H), 2.24 (s, 3H), 1.58 (m, 6H), 1.35 (m, 6H), 1.11 (m, 6H), 0.91 (t, 9H).

2-(3,3'-dimethyl-2,2':5',2'':terthiophene-5''- carboxylic acid 2,5-dioxopyrrolidin-1-yl ester-5-yl)pyridine (6): To a refluxing solution of tetrakis(triphenylphosphine)palladium [0] (20 mg, 0.017 mmol) and compound **5** (98 mg, 0.32 mmol) in dry toluene (15 ml), stannyl derivative **4** (220mg,

0.38 mmol, 2 ml of toluene) was added dropwise. The mixture was stirred for 6 h at reflux. After chromatography (silica gel neutralized by petroleum ether with 4% of triethylamine, pet.eth. + 20% AcOEt → 50% AcOEt) compound **6** was crystallized by toluene to give a red-orange powder 68 mg (43% Y). m.p. 170°C, m/z = 494 (M^+), UV-Vis $\lambda_{\max}$ = 384 nm (CH_2Cl_2), PL (λ_{exc} = 384 nm, CH_2Cl_2), $\lambda_{\max}$ = 523 nm. ^1H NMR (400 MHz, CDCl_3 , TMS/ppm) δ 8.57 (1H, m), 7.92 (d, 1H, J = 4 Hz), 7.67 (m, 2H), 7.44 (s, 1H), 7.20 (m, 2H), 7.16 (m, 1H), 2.90 (s, 4H), 2.28 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (400 MHz, CDCl_3 , TMS/ppm) δ 169.1, 157.1, 152.2, 149.7, 147.6, 144.1, 138, 137.9, 137.6, 136.7, 134.5, 131.7, 130.6, 129.3, 127.7, 124.1, 124.0, 122.1, 118.6, 25.6, 15.3, 15.1. Anal. calcd for $\text{C}_{24}\text{H}_{18}\text{N}_2\text{O}_4\text{S}_3$: C, 58.28; H, 3.67. Found C, 58.31; H, 3.62.

2- [N-(3-(triethoxysilyl)propyl)- (3,3'-dimethyl-2,2':5',2'':terthiophene-5''-carboxamide-5-yl)]pyridine (8): APTS (20 μl , 0.086 mmol) was added dropwise to a CH_2Cl_2 solution (3.5 ml, dry) of succinimidyl ester precursor **6** (34 mg, 0.069 mmol) under nitrogen atmosphere. After 12 h of vigorous stirring the solvent was removed under vacuum and cyclohexane was added. The solid precipitate so obtained was dissolved in CH_2Cl_2 , the insoluble fraction was removed. Compound **8** was isolated as yellow powder, (35 mg, 85% yield).

m.p. 89-95°C, EI-MS m/z 600 (M^+). ^1H NMR (400 MHz, CDCl_3 , TMS/ppm) δ 8.56 (m, 1H), 7.66 (m, 2H), 7.43 (s, 1H), 7.38 (d, J = 4.4 Hz), 7.14 (m, 1H), 7.09 (m, 2H), 6.35 (m, 1H), 3.84 (q, 6H), 3.45 (q, 2H), 2.26 (s, 3H), 2.25 (s, 3H), 1.75 (m, 2H), 1.23 (t, 9H), 0.71 (t, 2H). ^{13}C NMR (400 MHz, CDCl_3 , TMS/ppm). δ 161.6, 152.2, 149.6, 143.7, 141.3, 137.7, 137.6, 137.3, 136.6, 135.6, 131.1, 129.9, 128.4, 127.9, 127.7, 123.5, 122.0, 118.5, 58.5, 26.9, 22.9, 18.3, 15.2, 15.1, 7.8. Anal. calcd for $\text{C}_{29}\text{H}_{36}\text{N}_2\text{O}_4\text{S}_3\text{Si}$: C, 57.97; H, 6.04. Found C, 57.93; H, 6.09.

SAMs preparation. Quartz substrates were pre-treated with pyranha solution at 70°C for 20 min. Si(100) substrates were cleaned by standard RCA procedure.² SAM's of **8** were prepared by irradiating two pre-cleaned and oxidized substrates immersed in a dry toluene solution of **8** ($C \sim 10^{-6}$ M, filtered on 0.2 μm PTFE filter) in a closed microwave oven reactor (fixed temperature, Max Power = 300W for, 50 min). Then they were extracted and washed with toluene and ethanol until no

emission (by PL spectroscopy analysis) was observed from the washing solvent.

2. Optical measurements

2.1 Absorption and emission of compound 8 in EtOH

UV-Vis spectra were recorded using a Perkin Elmer Lambda 20 spectrophotometer.

Photoluminescence spectra were collected on a Perkin Elmer LS50 spectrofluorometer.

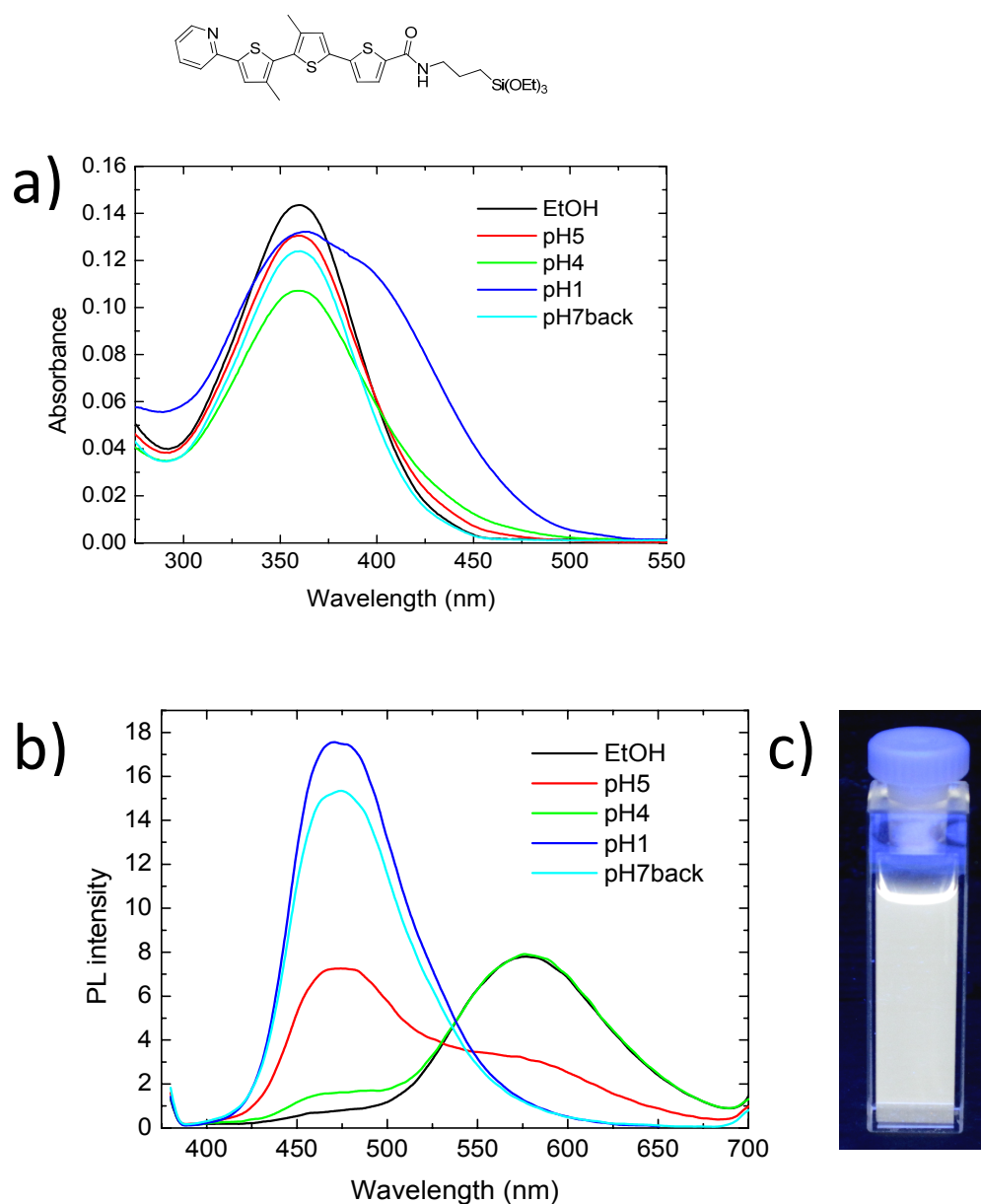


Figure SI_1. a) Absorption spectra of compound 8 in EtOH at different pH and b) corresponding emission upon excitation at $\lambda_{exc} = 365$ nm. c) white emission observed at pH 5 ($\lambda_{exc} = 364$ nm).

2.2 Emission of solution cast film of **8**

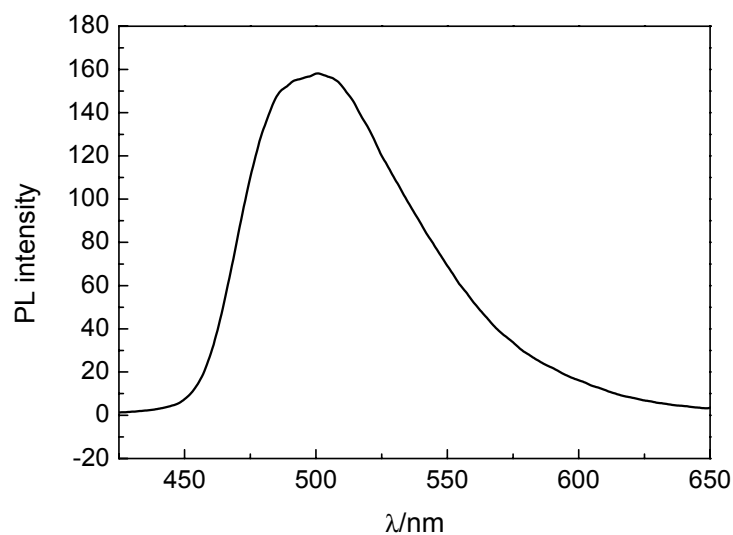


Figure SI_2. Emission spectra of a thin film obtained by solution cast of a solution of compound **8** (EtOH, 1mg/ml).

2.3 Absorption of SAM of compound **8**

Absorption spectra of SAM (figure SI_3) were recorded on a spectrophotometer Perkin-Elmer Lambda 650. For the fluorescence spectra a spectrofluorimeter Fluorolog 3 by Jobin-Yvon in a front-face configuration was used. The surface density ρ (molecules nm^{-2}) of **8** was calculated using the Lambert-Beer law, $\rho = 0.5 N A 10^{-17} \epsilon^{-1}$, where N is the Avogadro constant, A the absorbance at 360 nm and ϵ the molar absorption coefficient ($30600 \text{ M}^{-1} \text{cm}^{-1}$) at that wavelength.

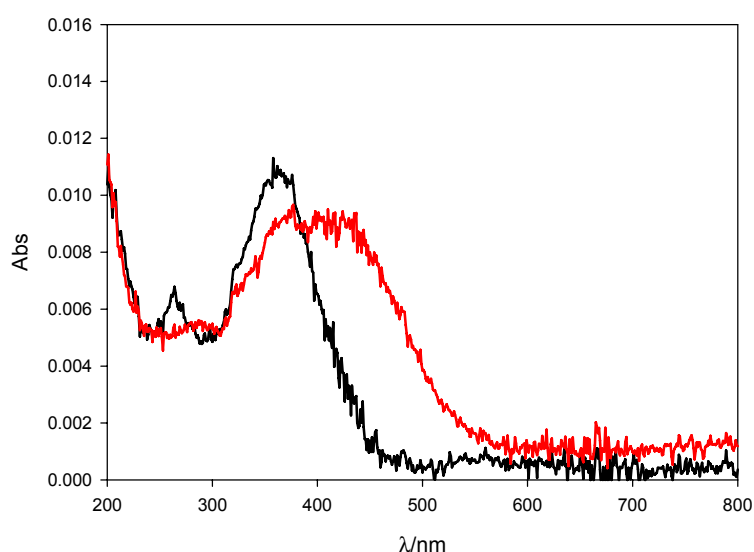


Figure SI_3. Absorption spectra of SAM of **8** as obtained (black curve) and upon acidification with HCl/EtOH , pH 1 (red curve).

3. SAM characterization

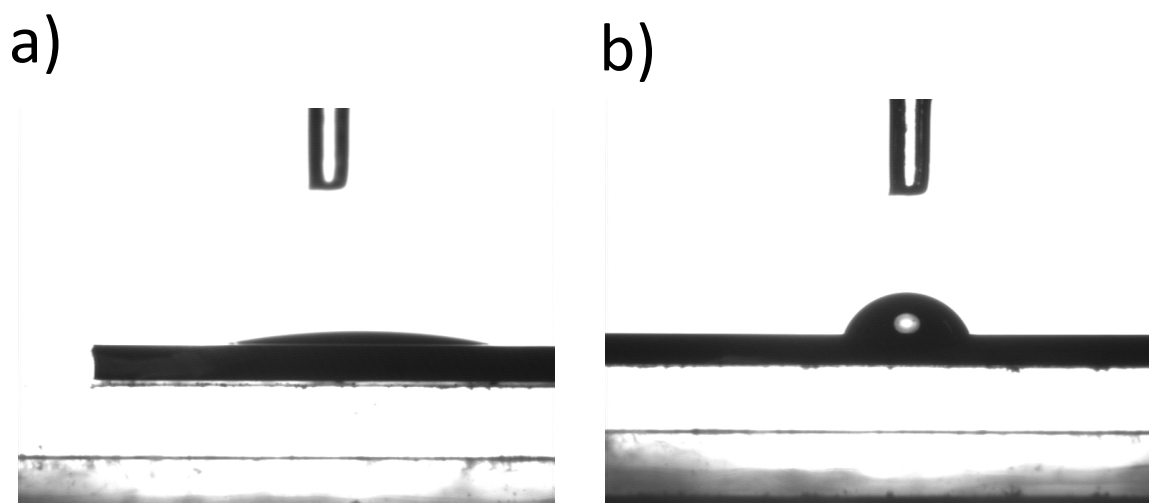


Figure SI_4. Water contact angle images of a) unfunctionalized SiO₂ substrate ($\theta = 16.3^\circ$) and b) of a SAM functionalized one ($\theta = 69.0^\circ$).

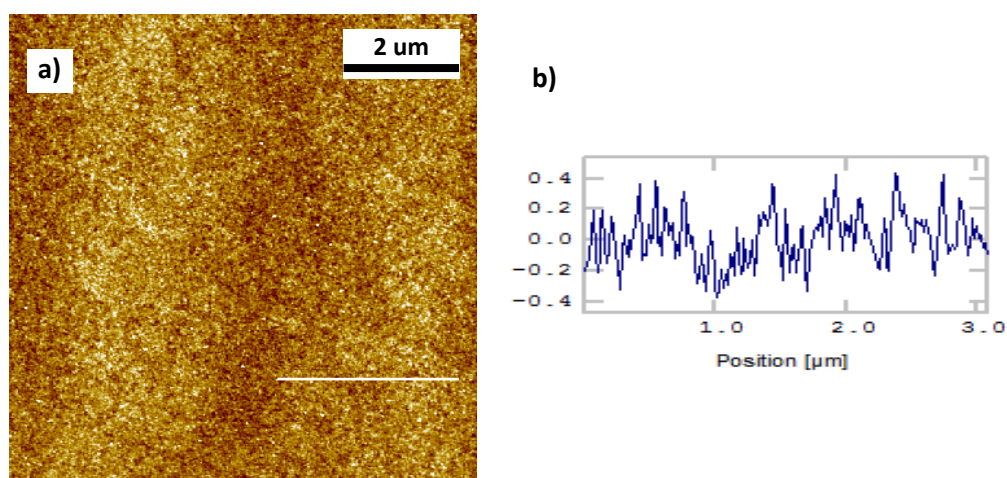


Figure SI_5. a) AFM image of a functionalized substrate, z-range 1.5 nm. b) height profile taken along the white line in a).

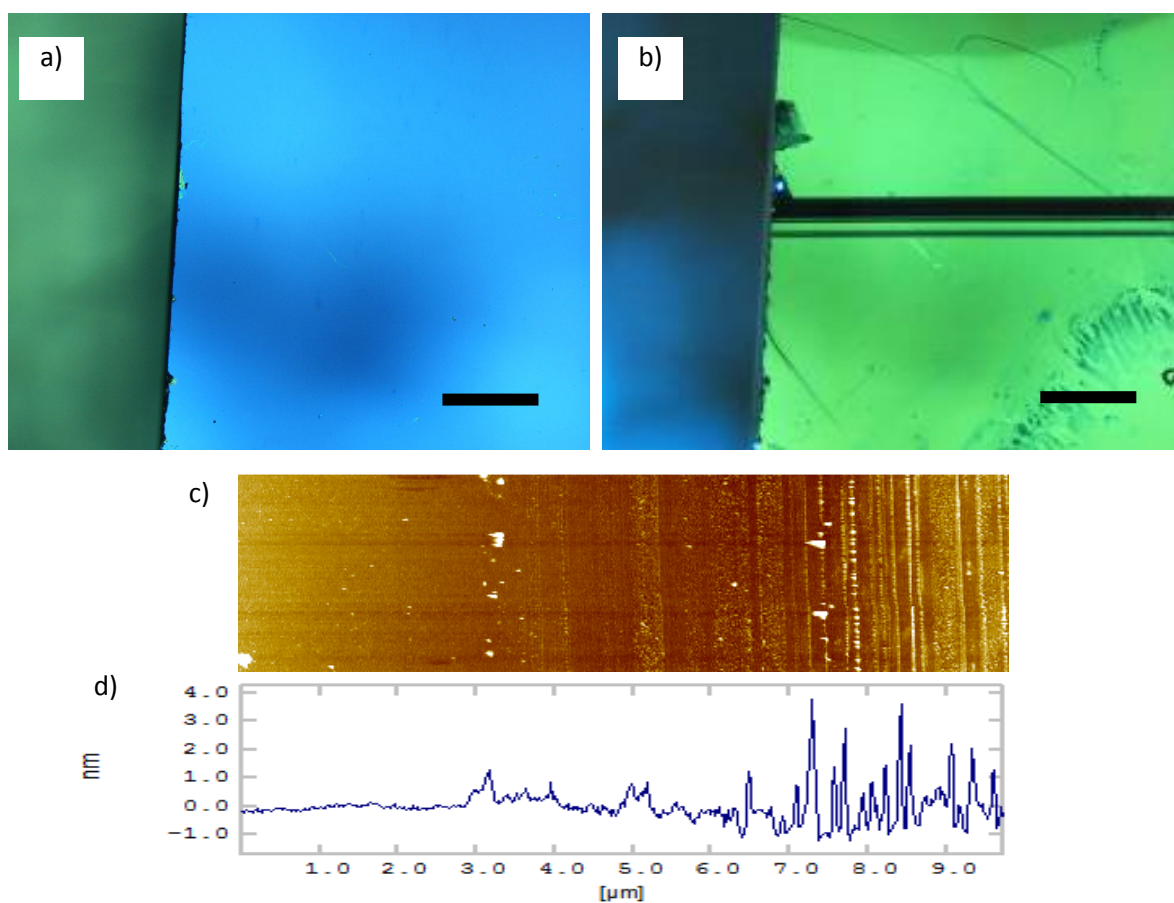


Figure SI_6. a) Reflection and b) fluorescence image of functionalised 300 nm thick SiO_x . Where a scratch has been drawn in the fluorescent SAM using a sharp tip. c) AFM image of the scratch, $10 \times 2.6 \mu\text{m}$, z-range 36 nm. d) averaged profile of the height across the scratch. Bar size is $200 \mu\text{m}$.

By scratching the SAM with a sharp metallic tip (Figure SI_6 a), a complete removal of the fluorescence was attained by removing less than 1 nm of material (Figure SI_6 b), thus suggesting that the SAM thickness is close to one monolayer thick.

4. Patterning

When an extra pressure is applied on the mold, no infilling under the pillars was observed. Furthermore quenching of the fluorescence was observed. Figure SI 7 shows a fluorescence image obtained apply a pressure of $\sim 100 \text{ g/cm}^2$ on the mold and infilling the thin layer cell by a HCl solution at pH 5.

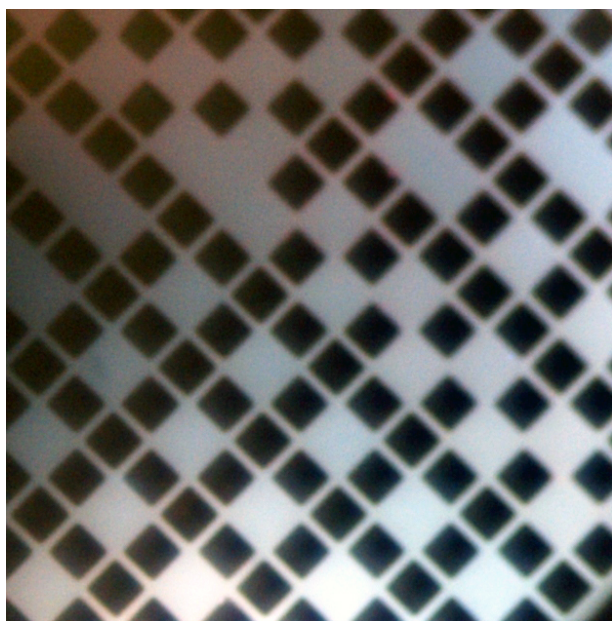


Figure SI_7. Fluorescence image of the SAM calibrated at pH5 showing white emitting background and fluorescence quenching from the areas under the pillars. Square size $20 \mu\text{m}$.

6. Fluorescence switch reversibility

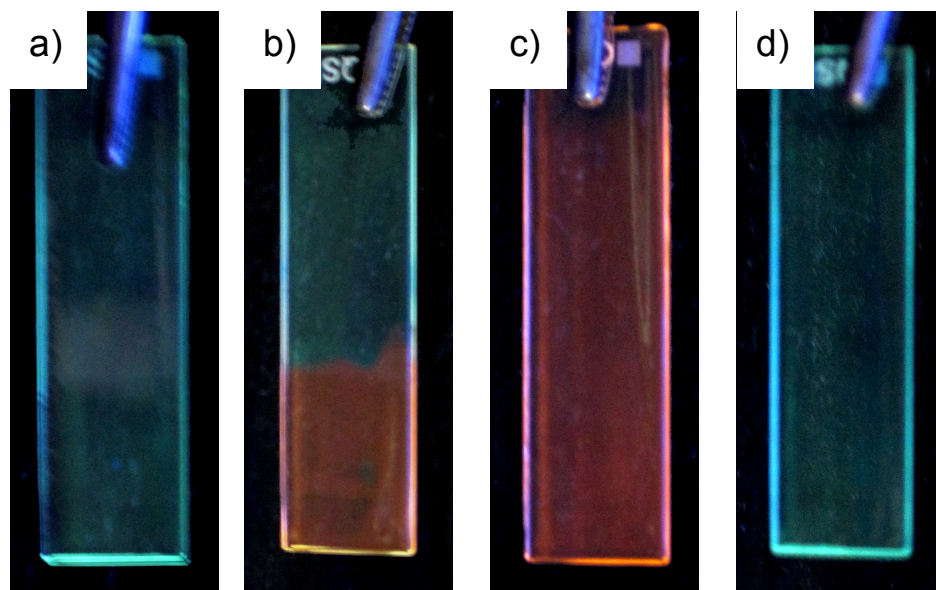


Figure SI_8. Fluorescence microscopy image (Hg lamp, $\lambda_{\text{exc}} = 330\text{-}380\text{ nm}$) of a functionalized quartz (4 cm x 1.2 cm). a) as-obtained quartz, b) the same quartz upon partial dipping in EtOH pH=1, c) upon complete immersion in EtOH pH= 1, d) upon neutralization by EtOH/TEA.

¹ a) M. Melucci, G. Barbarella, G. Sotgiu *J. Org. Chem.* **2002**, 67, 8877-8884, b) A. Cazzato, M. L. Capobianco, M. Zambianchi, L. Favaretto, C. Bettini, G. Barbarella, *Bioconjugate Chem.* **2007**, 18, 318-322.

² W. Kern, D. A. Puotinen, *RCA Review: a Technical Journal* **1970**, 31, 187-206.