

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

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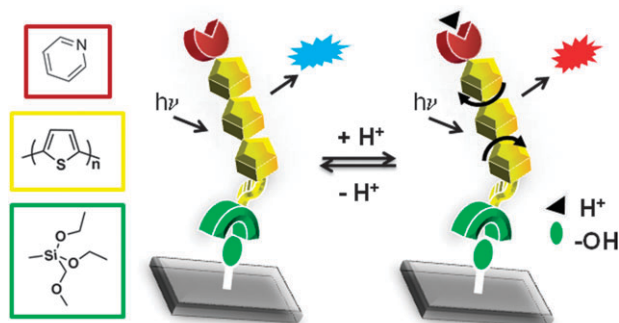
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We present a new strategy to realize self-assembled monolayers (SAMs) on quartz and silicon with a multicolour fluorescence pattern starting from a single, proton sensitive oligothiophene dye exposed at a defined pH. Fine tuning of the SAMs emission color over the entire visible range, including white, is demonstrated. Finally, integration of SAMs in patterned thin layer cells (TLCs) is exploited to demonstrate cation sensing potential in real devices.

Surface functionalization is a key element for the tuning of interfacial chemical and physical properties.<sup>1</sup> It is recognized that the fabrication of self-assembled monolayers (SAMs) on surfaces is one of the most reliable approaches to realize ideal platforms for both (opto)electronic, and clinical applications.<sup>1,2</sup> Furthermore, integration of SAMs with functional molecules allows for the bottom-up fabrication of devices in which the responsiveness of individual molecular components is extended to the entire substrate.<sup>3</sup> In particular, due to multiple recognition sites, high signal-to-noise ratio and fast response time, SAMs integrated with fluorescent moieties could find prominent applications as chemo- and biosensors.<sup>4,5</sup> Given this great potential, simple routes to access responsive SAMs with bright and multicolour emission are highly desirable. In this realm, formation of fluorescent SAMs on silicon substrates<sup>2</sup> by grafting a range of dyes such as rhodamine, cumarine, Ru(II) complexes, and porphyrins<sup>4,5</sup> is receiving increasing attention.

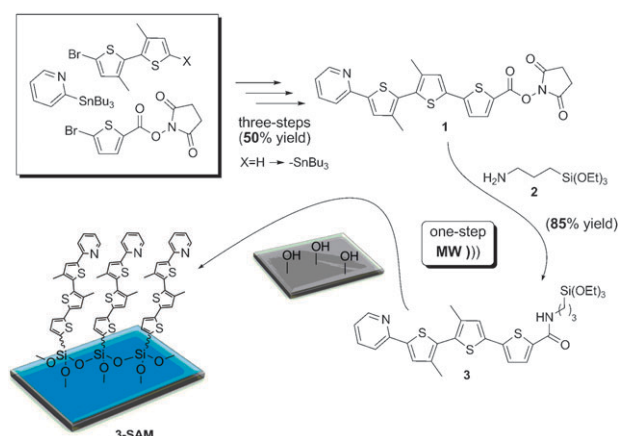
As a part of our ongoing interest in the design and development of new thiophene-based materials for organic electronics and sensing,<sup>6</sup> we reasoned that the combination of unique photophysical characteristics and synthetic flexibility could candidate such functional materials as dyes to realize fluorescent SAMs. In particular, we considered that the optical properties of a conformationally-flexible thiophene-based  $\pi$ -conjugated building block could be altered by protonation of a conjugated sensitive site capable to reversibly capture protons as well as by changes in solvent dielectric.<sup>7</sup> Finally, a



**Fig. 1** Molecular design strategy for the pH sensitive SAM. Red: proton antenna, yellow: fluorescent block, green: surface anchoring groups.

proper end-functionalization would allow for the efficient covalent grafting to silicon/glass substrates (Fig. 1).

To realize such a molecular architecture a pyridine ring (pH sensitive unit)<sup>8</sup> was condensed to the  $\pi$ -fluorescent oligothiophenyl block bearing  $\beta$  methyl groups on the inner thienyl rings in order to increase the solubility and to prevent strong  $\pi$ -aggregations. Optimized stannylation/bromination and Stille coupling reactions between 2-tributylstannylpyridine, 2-bromo-thiophene 5-succinimidyl ester thienyl and 2-bromo-(3,3'-dimethyl-bithiophene) furnished the succinimidyl ester **1** in satisfying yield (see ESI†). The reaction performances were improved starting from the stannyl-pyridine derivative rather than the bromopyridine analogue. Then, the desired compound **3** was prepared by condensing **1** with aminopropyltriethoxysilane **2** (85% yield) by using the already described procedure (Scheme 1).<sup>9</sup>



**Scheme 1** Synthetic route to triethoxysilane-ended dye **3** and to correspondent SAMs (details in ESI†).

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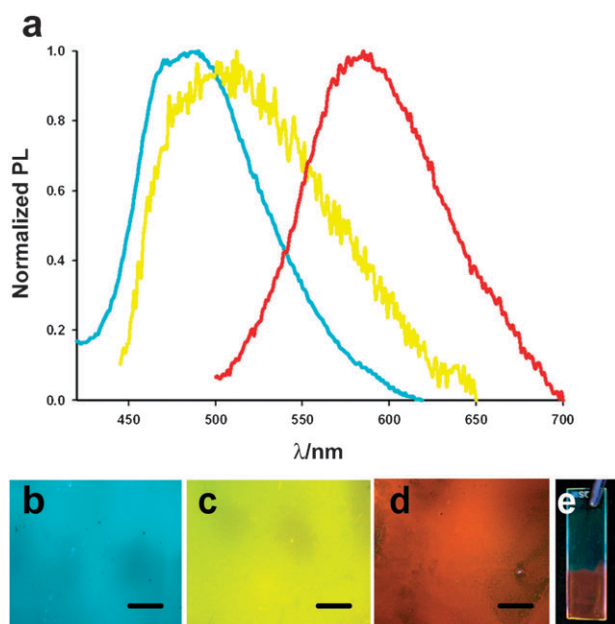
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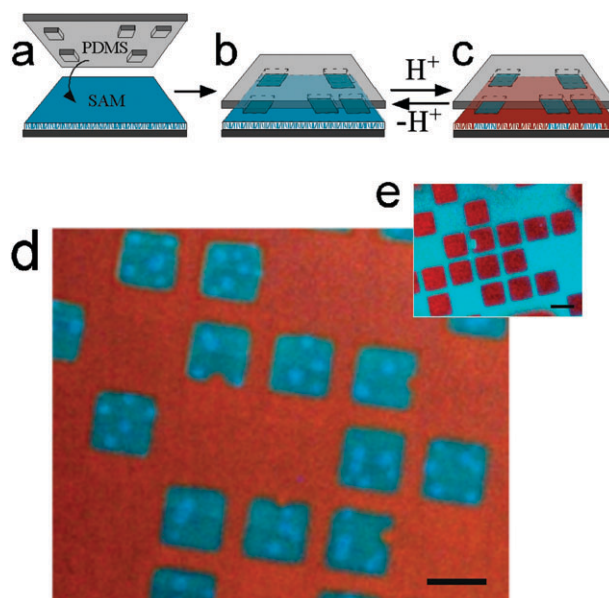
† Electronic supplementary information (ESI) available: Synthesis and characterization of molecular compounds, AFM, contact angle, UV-vis and PL spectra. See DOI: 10.1039/c0cc04478d

SAM's of **3** were finally prepared by microwave (MW)<sup>10</sup> irradiation of the substrates immersed in a dry toluene solution of **3** (50 min, 80 °C, 300 W, see ESI† for details). The substrates were then washed several times with fresh toluene and ethanol until no emission was observed from the residual washing solvents. The water contact angle changes from  $\theta = 16.3^\circ$  to  $\theta = 69.0^\circ$  upon functionalization as a consequence of the strong hydrophobic character of the grafted conjugated molecule. AFM investigation of functionalized substrate shows a uniform smooth surface with a roughness comparable to that of the unfunctionalized substrate (rms roughness  $\sim 0.5$  nm). The thickness, estimated by scratching the SAM with a sharp metallic tip, is  $<1$  nm fully compatible to a monolayer formation. Accordingly, a surface density of  $\rho = 1.1$  molecules  $\text{nm}^{-2}$  was estimated by the absorption spectra of the functionalized quartz.<sup>11</sup>

The fluorescence spectrum of **3**-SAMs on quartz (Fig. 2a) shows a maximum emission band at 480 nm ( $\lambda_{\text{exc}} = 360$  nm), slightly red shifted with respect to that of **3** in EtOH ( $\lambda_{\text{max}} = 472$  nm) and strongly blue-shifted with respect to that of a thick cast film ( $\lambda_{\text{max}} = 500$  nm, broad band).<sup>12</sup> When exposed to an acid solution, even vapours, the fluorescence exhibits a strong reversible red shift (Fig. 2,  $\lambda_{\text{max}} = 508$  nm and 582 nm at pH = 5 and pH = 1, yellow, red curves, respectively). The fluorescence spectrum of the SAM at pH 5 is not a linear combination of the spectra of the protonated and unprotonated oligothiophene dye **3** as it would be expected in the case of the absence of electronic interaction between the different molecular components. Hence, a relevant perturbation of the excited states of the acid and basic forms due to the mutual interaction of the two species simultaneously

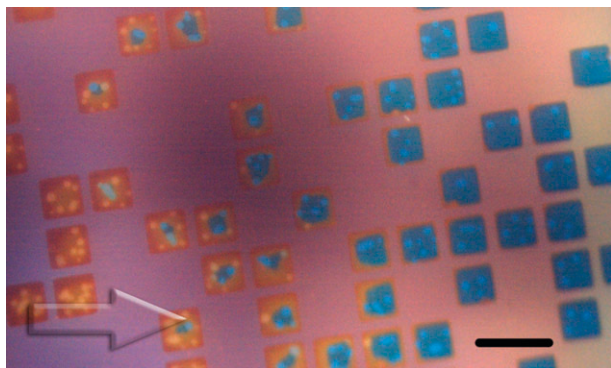


**Fig. 2** (a) PL spectra of **3**-SAMs ( $\lambda_{\text{exc}} = 360$  nm) as obtained (cyan curve) and upon treatment with HCl, pH  $\sim 5$  (yellow curve) and pH  $\sim 1$  (red curve). (b, c, d) Fluorescence microscopy images (Hg lamp,  $\lambda_{\text{exc}} = 330$ – $380$  nm) of functionalized 300 nm thick  $\text{SiO}_x$  at different pH. Bar size 250  $\mu\text{m}$ . (e) Functionalized quartz (4 cm  $\times$  1.2 cm) partially dipped in HCl/EtOH (pH = 1).



**Fig. 3** (a–c) Scheme of the device used for optical sensing of acidity. The PDMS mold is placed onto the emission-tailored **3**-SAM functionalized  $\text{SiO}_x$ . (d) Fluorescence image during infilling by HCl (pH 1, bar 20  $\mu\text{m}$ ) and (e) of the device treated with HCl (pH = 1) during infilling by TEA (pH 8, bar 20  $\mu\text{m}$ ).

present occurs in the bi-dimensional network in such pH conditions. Treatment with triethylamine (TEA) shifted the fluorescence back to the pristine one. Therefore, the sensing mechanism relies not on a simple fluorescence on–off, as generally observed for SAM based chemosensors,<sup>4b,5,13</sup> but on a reversible multicolour emission switch. Fig. 2b–e show the homogeneous and intense fluorescence of the functionalized 300 nm thick  $\text{SiO}_2$  and quartz at different pH (fluorescence microscope at low magnification, 10 $\times$ , no colour corrections). Noteworthy, the SAMs were stable even after several acid–base cycles. The potential of **3**-SAMs as optical device for pH sensing was tested in the thin layer cell (TLC) depicted in Fig. 3a–c by using ethanol solution at known pH. Here, a PDMS mold  $\sim 500$   $\mu\text{m}$  thick (Scriba Nanotechnologie), whose motif consists of square pillars (20  $\mu\text{m}$  size and 4  $\mu\text{m}$  spacing), is placed in contact with the surface to form the target TLC. The use of PDMS allowed us to fabricate a device transparent to visible light, thus easily detectable by optical/fluorescence microscopy at low magnification. When deposited on the functionalized substrate, the square pillars of the mold come in contact with the substrate affording protected areas under them and determining the cell thickness ( $\sim 1$   $\mu\text{m}$ ). When a solution is poured at the open end of the TLC, the liquid spontaneously fills the channels under the effect of capillary pressure.<sup>14</sup> During the infilling no reaction takes place under the pillars, thus such areas remain unperturbed. Consequently, when an acid solution is used the cyan fluorescence of the background turns to red, while it does not change under the pillars which act as reference (Fig. 3d). Therefore, the patterned TLC intrinsically allows us to by-pass the problem of colour calibration, which is an important issue for sensors based on colour reading (usually based on Charge Coupled Device, CCD) often affected by false colour and saturation



**Fig. 4** Fluorescence image of the device exposed to a pH gradient obtained by fluxing HCl/EtOH. Bar size 50  $\mu\text{m}$ .

effects. Since the colour of the reference can be tuned by treatment of 3-SAM with calibrating acid/basic solution. As example, Fig. 3e shows fluorescence images of the pattern obtained using HCl/EtOH treated SAM (red squares) and infilling the TLC by TEA/EtOH (pH = 8, cyan background).

In this configuration the reference areas are stable for several minutes (2–10 min, depending on the nature of the solution) after that the solution infills also the protected areas (see also Fig. 4). It must be noted as if an extra pressure is applied on the mold a complete quenching of the fluorescence is observed under the pillars (see Fig. S7, ESI $\dagger$ ). This can be likely ascribed to aggregation effects between adjacent molecules in the SAM network, induced by the increased pressure. Further investigation is currently under way to elucidate this phenomenon.

Besides the use as sensor, such TLC configurations offer us a great opportunity to create a continuous-flow microreactor allowing to investigate the evolution of the (de)protonation reaction *via* local colour detection during the flux of analytes (HCl or TEA). Indeed, since the analytes diffusion inside the cell is slow, direct visualization of the chemical process can be obtained taking advantage of the reference colour under the pillars. As an example, Fig. 4 shows a large area fluorescence image during infilling of HCl/EtOH solution. The colour changes from blue to red on going from basic to acidic pH (8.5  $\rightarrow$  1).

In conclusions, we have presented a new approach based on smart molecular engineering and microwave assisted surface functionalization to realize a new type of SAMs on glass and silicon substrates, based on oligothiophene materials, with tuneable, bright and homogeneous fluorescence over centimetre squares scale. To the best of our knowledge, no example of single dye SAMs with *multicolour fluorescence* by pH changes have been reported so far. Studies on the applicability of our method to the sensing of biologically active metal cations by exploiting the pyridine coordination capability<sup>15</sup> are currently under way. Moreover, the possibility of real-time monitoring of the distribution of such analytes on the SAMs surface opens

perspectives for studying the kinetics of confined (bio)chemical reactions in continuous flow glass microreactors.

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