# Electronic Supporting Information

## Additive, modular functionalization of reactive self-assembled monolayers: toward the fabrication of multilevel optical storage media

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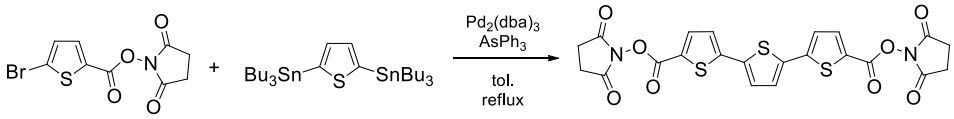
### Synthesis

- Synthesis of *bis(2,5-dioxopyrrolidin-1-yl) [2,2':5',2''-terthiophene]-5,5''-dicarboxylate*, **1**.

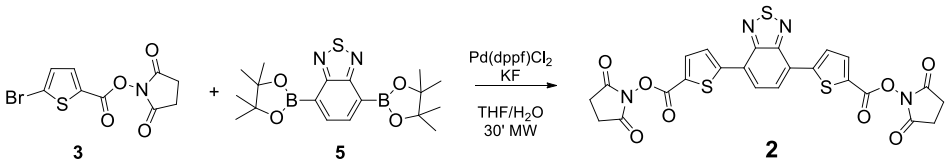



**3** **4** **1**

**Scheme S1**

Tris(dibenzylideneacetone)dipalladium-chloroform adduct (21 mg, 0.02 mmol) and triphenylarsine (51 mg, 0.17 mmol) were dissolved in 4 ml of dry toluene under $N_2$ atmosphere and the solution was refluxed until the color turned to dark brown. 2,5-dioxopyrrolidin-1-yl 5-bromothiophene-2-carboxylate **3** (213 mg, 0.7 mmol) in 1.8 ml of dry toluene was added and then 2,5-bis(tributylstannyl)thiophene **4** (232 mg, 0.35 mmol) in 1.4 ml of dry toluene was added dropwise. The solution was refluxed for 24 h. The solvent was removed and the precipitate washed several times with pentane. The crude was purified by flash chromatography on silica gel (eluent petroleum ether : AcOEt) yielding compound 1 as a yellow powder (122 mg, Y=66%). EI-MS m/z 530 ($M^+$); UV-Vis ($CH_2Cl_2$) $\lambda_{max}$ = 403 nm, PL ($CH_2Cl_2$, $\lambda_{exc}$ = 403 nm) $\lambda_{em}$ = 481 nm; $^1$H-NMR ($CDCl_3$, TMS/ppm) δ 7.94 (d, $^3J$ = 4.0, 2H), 7.31 (s, 2H), 7.26 (d, $^3J$ = 4.0, 2H), 2.91 (s, 8H). $^{13}$C-NMR ($CDCl_3$, TMS/ppm) δ 169.0, 157.0, 146.3, 137.5, 136.8, 127.1, 125.1, 124.9, 25.6. Anal. Calcd for $C_{22}H_{14}N_2O_8S_3$ (529,99): C, 49.80; H, 2.66. Found: C, 49.76; H, 2.59.

- Synthesis of *bis(2,5-dioxopyrrolidin-1-yl) 5,5'-(benzo[c][1,2,5]thiadiazole-4,7-diyl)bis(thiophene-2-carboxylate)*, **2.**

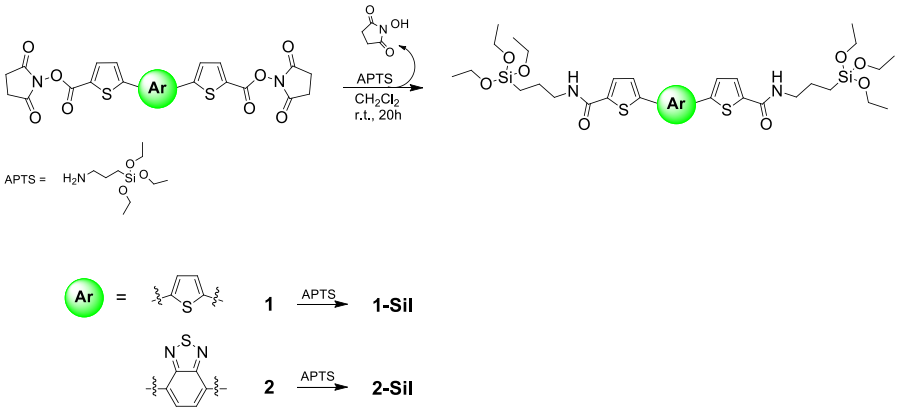



**Scheme S2**

The MW vessel was charged with 2,5-dioxopyrrolidin-1-yl 5-bromothiophene-2-carboxylate (**3**, 100 mg, 0.32 mmol), 4,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[c][1,2,5]thiadiazole (**5**, 127 mg, 0.32 mmol), Pd(dppf)$Cl_2$ (5 mol %, 15 mg, 0.016 mmol), KF (76 mg, 1.31 mmol) and a THF/$H_2O$ mixture (2:1, 3 ml tot.). The vessel was capped and the mixture irradiated with MW for 40 min at 80 °C.

The reaction was quenched with $H_2O$ and the organic layer extracted 3 times with $CH_2Cl_2$. The solvent was evaporated under vacuum and the crude was then purified by flash chromatography on silica gel (eluent $CH_2Cl_2$ : AcOEt). After crystallization from toluene, compound **2** wasobtained as red powder (70 mg, Y= 75%). EI-MS m/z 582 ($M^+$); UV-Vis ($CH_2Cl_2$) $\lambda_{max}$ = 442 nm, PL ($CH_2Cl_2$, $\lambda_{exc}$ = 442 nm) $\lambda_{em}$ = 525 nm; $^1$H-NMR ($CDCl_3$, TMS/ppm) δ 8.16 (d, $^3J$ = 4.0, 2H), 8.11 (d, $^3J$ = 4.0, 2H), 8.07 (s, 2H), 2.93 (s, 8H). Anal. Calcd for $C_{24}H_{14}N_4O_8S_3$ (582,00): C, 49.48; H, 2.42. Found: C, 49.55; H, 2.48.

- General procedure for compounds N5,N5''-bis(3-(triethoxysilyl)propyl)-[2,2':5',2''-terthiophene]-5,5''-dicarboxamide (**1-Sil**) and 5,5'-(benzo[c][1,2,5]thiadiazole-4,7-diyl)bis(N-(3-(triethoxysilyl)propyl)thiophene-2-carboxamide) (**2-Sil**) preparation.




**Scheme S3**

Succinimidyl ester precursor (**1** or **2**, 0.1 mmol) was dissolved in 3.5 ml of dry $CH_2Cl_2$ under $N_2$ atmosphere and aminopropyltriethoxysilane (APTS, 0.22 mmol) was added dropwise. The mixture was stirred for 20 h at room temperature and then the solvent was evaporated

under vacuum. The precipitate was stirred for 10 min in dry cyclohexane, filtrated and washed several times with pentane. The precipitate was dissolved in dry $CH_2Cl_2$ and the mixture stirred for other 10 min. The insoluble fraction was removed by filtration and the solvent was evaporated, giving the triethoxysilane derivative as a glassy powder.

**N5,N5''-bis(3-(triethoxysilyl)propyl)-[2,2':5',2''-terthiophene]-5,5''-dicarboxamide (1-Sil).** Yellow powder (Y>99%). UV-Vis ($CH_2Cl_2$) $\lambda_{max}$ = 378 nm, PL ($CH_2Cl_2$, $\lambda_{exc}$ = 378 nm) $\lambda_{em}$ = 468 nm; $^1$H-NMR ($CDCl_3$, TMS/ppm) δ 7.39 (d, $^3J$ = 4.0, 2H), 7.16 (s, 2H), 7.12 (d, $^3J$ = 4.0, 2H), 6.40 (t, 2H, NH), 3.84 (m, 12H), 3.45 (q, 4H), 1.75 (q, 4H), 1.23 (m, 18H), 0.71 (t, 4H). $^{13}$C-NMR ($CDCl_3$, TMS/ppm) δ 161.5, 140.8, 137.8, 136.5, 128.5, 125.6, 124.0, 58.6, 42.1, 22.9, 18.3, 7.8. Anal. Calcd for $C_{32}H_{50}N_2O_8S_3Si_2$ (742,23): C, 51.72; H, 6.78; Si, 7.56. Found: C, 51.80; H, 6.84; Si, 7.61.

**5,5'-(benzo[c][1,2,5]thiadiazole-4,7-diyl)bis(N-(3-(triethoxysilyl)propyl)thiophene-2-carboxamide) (2-Sil) .** Orange powder (Y=74%). UV-Vis ($CH_2Cl_2$) $\lambda_{max}$ = 449 nm, PL ($CH_2Cl_2$, $\lambda_{exc}$ = 449 nm) $\lambda_{em}$ = 563 nm; $^1$H-NMR ($CDCl_3$, TMS/ppm) δ 8.08 (d, $^3J$ = 3.6, 2H), 7.95 (s, 2H), 7.58 (d, $^3J$ = 3.6, 2H), 6.46 (t, 2H, NH), 3.85 (m, 12H), 3.48 (m, 4H), 1.79 (m, 4H), 1.24 (m, 18H), 0.74 (t, 4H). Anal. Calcd for $C_{34}H_{50}N_4O_8S_3Si_2$ (794,23): C, 51.36; H, 6.34; Si, 7.06. Found: C, 51.29; H, 6.37; Si, 7.10.

**EXPERIMENTAL SECTION**

**Samples preparation**

*Microcontact printing:* The PDMS stamps are inked with 10 µL of ink solution (1 mM solution of the molecules **1** or **2** in toluene), dried with nitrogen, brought and left in contact with the silicon oxide surface for 30 min. Afterwards, the stamp is removed and, before characterization, the sample is left to react in air overnight, then rinsed in toluene and dried in a stream of nitrogen. Using the same procedure, a second printing process can be performed on the sample.

Reference samples were prepared by µCP of compound **1- Sil** and **2- Sil** on bare Si/SiO2.

*Materials:* Spectroscopic grade quality solvents and (3-aminopropyl)triethoxysilane) (**APTS**) were purchased from Sigma Aldrich. Sylgard 184 (polydimethylsiloxane) silicone elastomer base and curing agent were purchased from Dow Corning.

*Substrate cleaning and functionalization:* silicon wafers with a thermally grown silicon dioxide layer (200 nm thick) are used as substrates. All substrates were cleaned by sonication in acetone for 15 min, then in 2-propanol for 15 min, dried in a stream of nitrogen and treated in UV-ozone plasma cleaner (5 min, 60 W), prior to use. For APTS-functionalized substrates, silicon wafers were immersed overnight in an APTS solution (1 mM) in toluene, then rinsed in toluene and dried in a stream of nitrogen.

Polydimethylsiloxane (PDMS) substrates were prepared by spin coating 250 µl of PDMS spinning at 500 r.p.m. for 3 minutes on glass substrate.

*Soft stamp preparation:* the PDMS stamps were prepared by replica molding of a pre-paterned Si/$SiO_2$ master fabricated by photolithography. After curing for 6 h at 60°C, PDMS stamps were peeled off, cleaned by sonication in ethanol for 10 min, and dried in $N_2$.

**Laser scanning confocal fluorescence microscopy and fluorescence lifetime imaging (FLIM)**

Laser scanning confocal fluorescence microscopy was performed on an inverted Nikon Ti-E microscope (Nikon Co., Shinjuku, Japan). The confocal fluorescence microscope system Nikon A1 is equipped with a 405 nm pulsed/CW diode laser (PicoQuant GmbH, Berlin, Germany). Fluorescence images of 521x512 or 1024x1024 pixels were collected using a Nikon Plan Apo VC 60X oil immersion objective with NA 1.40 or a Nikon Plan Apo VC 20X with NA 0.75. Zooming factor of 4 has been applied in some cases. With this imaging configuration, pixel side dimension ranged from 160

to 310 nm. In the case of fluorescence intensity imaging filters were set to detect contemporarily the fluorescence in the 425-475 nm range, 500-550 nm range and 570-620 nm range.

Spectral imaging has been performed using the Nikon A1 spectral detector module consisting of a multi-anode photomultiplier with an array of 32 anodes. A wavelength band width of 6 or 10 nm per anode has been applied.

Fluorescence lifetime imaging was performed on the Nikon A1 microscope system integrated with PicoHarp 300 electronics (PicoQuant GmbH, Berlin, Germany) for time-correlated single photon counting (TCSPC) measurements using the 405 nm diode laser in pulse mode. The repetition rate of the pulsed excitation at 405 nm was 40 MHz. TCSPC was set to 16 ps per channel and photons collected in 1564 channels. 512x512 pixel images were collected using the 20X objective with NA 0.75 applying a zooming factor of 4. Two single-photon avalanche diode (SPAD) detectors, each with a bandpass filter centered at 480 nm (40nm) and 585 nm (40nm) respectively, were used for this scope. The instrument response function of the system is approximately 200 ps. A tail fit was performed on the fluorescence decay histogram calculated for a region of interest (ROI) of the sample image. The fluorescence decay profiles of the selected ROI were analyzed with a least-squares method, using multi-exponential decay functions provided by Picoquant SymPhoTime software.

The fitting function used is

$I(t) = b + \Sigma_j a_j e^{(-t/\tau_j)}$ with j ranging from 1 to 3

The fractional intensity and the average fluorescence lifetime are calculated according to the following equations:

$f_i = a_i\tau_i / \Sigma_j a_j\tau_j$ $\qquad\qquad$ $\tau_{av} = \Sigma_j f_j\tau_j$

Finally the FLIM image was obtained fitting the decay of each pixel of the image with the same function used for ROI fitting keeping the lifetimes fixed to the values calculated for the selected ROI. Only the preexponential factor of each lifetime was calculated to create the image.

**Fluorescence lifetime measurements of compounds 1,2 1/2-Sil in solution**

Fluorescence decays were measured in air-equilibrated solutions for excitation at 373 (**1** and **1**-**Sil**) nm or 465 nm (**2** and **2**-**Sil**) (1 MHz repetition rate) using a time-correlated single photon counting system (TCSPC) (IBH Consultants Ltd., Glasgow, UK) with a resolution of 55 ps per channel. Photons were detected in right angle configuration at 480 nm and at 560 nm with a 435 nm cut-off filter. Fluorescence decay profiles were analyzed with a least-squares method, using multiexponential decay functions and deconvolution of the instrumental response function. The software package was provided by IBH Consultants Ltd.

In the tables below $\tau$ (Table S2, S3)is the lifetime (ns), b is the pre-exponential factor and a is the fractional intensity.

**Fluorescence microscopy**

Fluorescence images were recorded with a Nikon i-80 microscope equipped with epi-fluorescence (FM) using FM filters: Ex 330-380 (BP), Dm 400, Em 420 nm (LP); and Ex 400-440 (BP), Dm 455, Em 475 (LP) nm. The FM images were recorded using a commercial CCD camera (Nikon Nikon CCD DS-2 Mv). The illumination was performed by a 100 W halogen lamp at fixed power (i.e. tension 12 V) and with fixed time of acquisition of the CCD.

**Surface Morphometry**

AFM imaging was performed on a Multimode 8 microscope equipped with a Nanoscope V controller and type J piezoelectric scanner (Bruker, USA). Samples were scanned at 0.5 Hz/line in PeakForce mode using Scanasyst-Air probes (Bruker, USA) in air, imposing an applied force of 2.5 nN. Background interpolation and quantitative surface characterization were performed with Gwyddion 2.37 (http://gwyddion.net/). SAM thicknesses and root mean squared area roughness ($S_q$) values were determined by averaging at least three different 9 $\mu m^2$ areas, using the standard deviation of these measures as the uncertainty.

**X-ray photoelectron spectroscopy**

The XPS investigations were carried out by means an ESCALAB 250Xi spectrometer (Thermo Scientific – UK). XPS was used to demonstrate the amidic bond occurred in the printed structure of compound **1** and **2** on APTS. The N 1s signal acquired on **1**-APTS-SAM, shown in

the figure, was characterized by the presence of a peak positioned at BE = 402.0 eV, while the N 1s signal acquired on **2**-APTS-SAM sample, was characterized by the presence of two contributions, positioned at BE = 399.8 eV and 401.6 eV, respectively. To optimize the assignments, the signals were compared with the N 1s signals of compounds powders and APTS on Si substrate. In this contest, the discussion will be limited only to the comparison with reference samples of compound **2**. As it concerns the APTS sample, the peak-fitting procedure was performed by using of two components, which intensity ratio was 1.3: component a', positioned at BE = 399.9 eV, was assigned to amine group, and b', positioned at BE = 401.6 eV, was assigned to protonated amine group, respectively [Robert G. Acres, Amanda V. Ellis, Jason Alvino, Claire E. Lenahan, Dmitriy A. Khodakov, Gregory F. Metha, Gunther G. Andersson, J. Phys. Chem. C 2012, 116, 6289-6297]. As it concerns the compound 2 powder, there were found two components, which intensity ratio was 1.5: component a, positioned at BE = 399.2 eV, was assigned to imidic and thiadiazol group , and b, positioned at BE = 401.6 eV , was assigned to protonated imidic group. As it concerns the **2**-APTS-SAM sample, the N 1s signal was characterized by the presence of two components, which intensity ratio was 0.2: component a, positioned at BE = 399.8 eV, was assigned to amine group, while component b, positioned at BE = 401.6 eV, was assigned to protonated amine and amidic group. It is worth to note that the contribution assigned to imidic group at BE = 399.2 eV disappeared, indicating that the reaction occurred by the formation of the amidic bond.

**FIGURE S 1. FLUORESCENCE IMAGE OF A FULL TAG.**

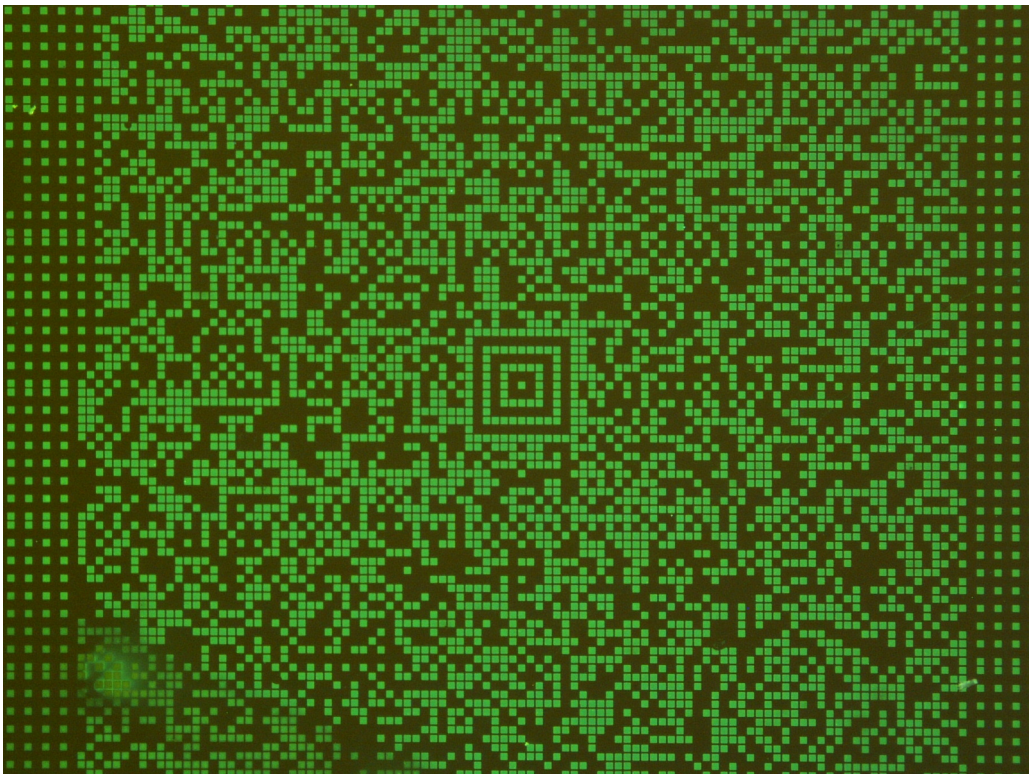
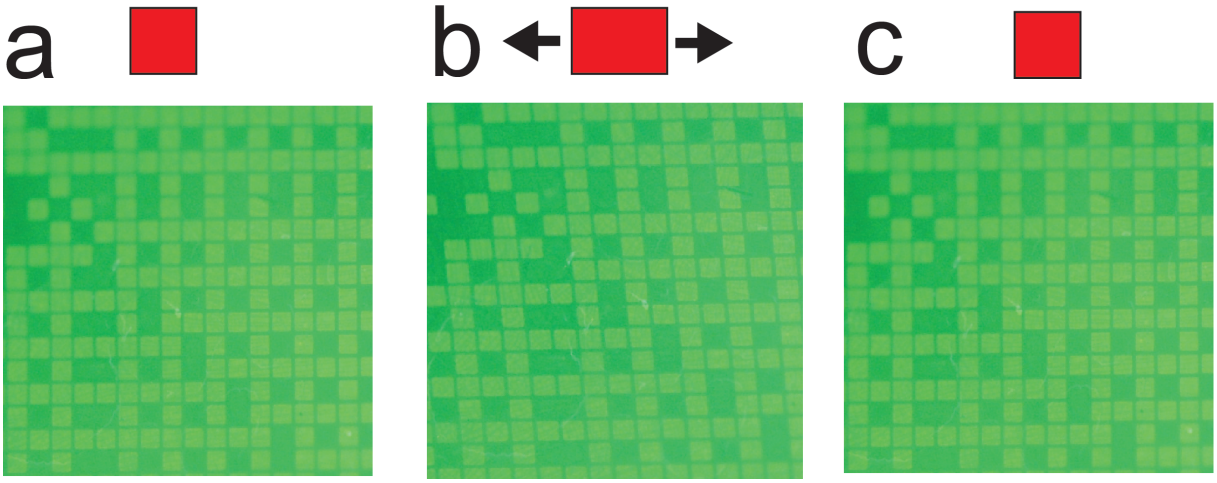
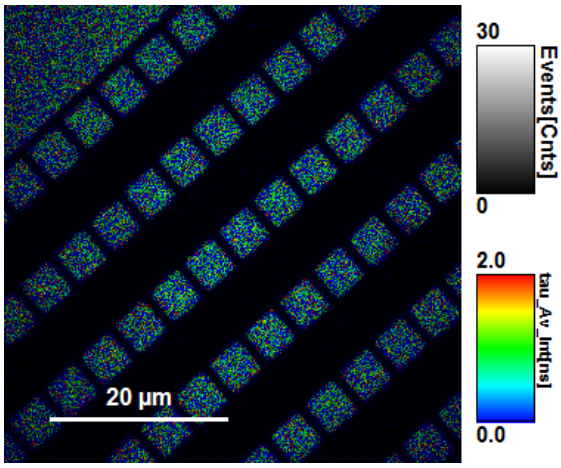


**FIGURE S2. EFFECT OF STRETCHING IN FLUORESCENCE IMAGE OF TAG PRINTED ON PDMS**

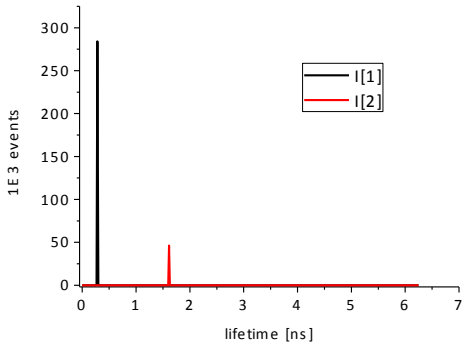



a) Fluorescence microscopy (FM) image of TAG as printed on PDMS. B) FM image of TAG recorded during the stretching (deformation obtained by elongation of 30 % of the original length of the sample along one direction). C) FM mage of TAG printed on PDMS after stretching.

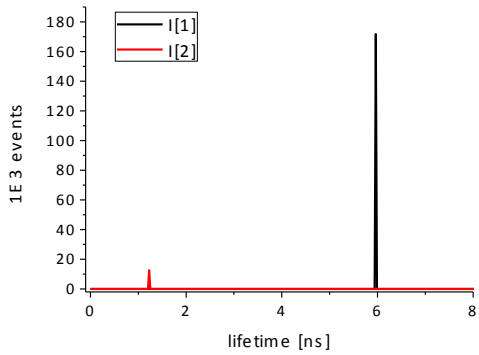
**FIGURE S3: 1/2-APTS-SAM**




FLIM image showing the average lifetime of the **1**-APTS-SAM excited at 405 nm; emission collected in the 460-500 nm range.

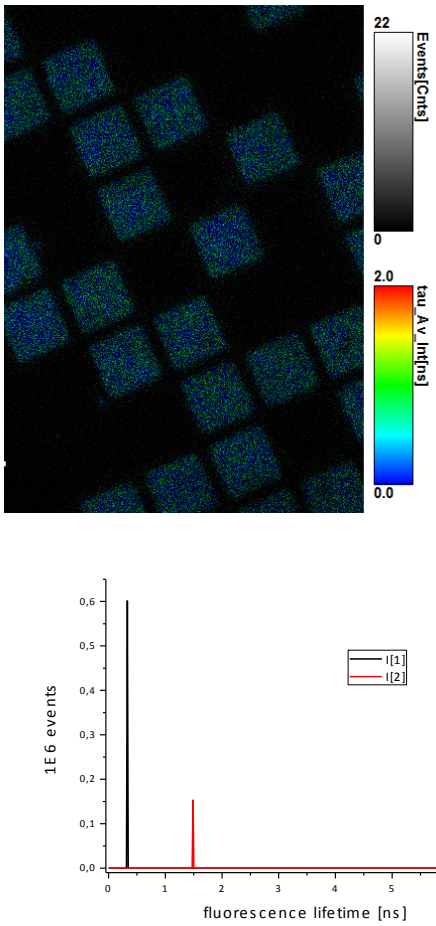



Histogram of the frequency of the lifetimes of **1**-APTS-SAM excited at 405 nm; emission collected in the 460-500 nm range

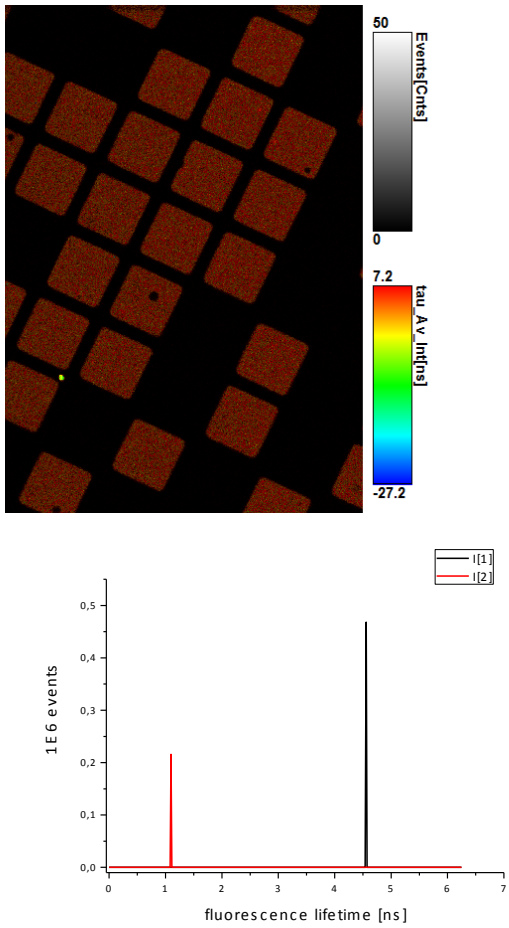



Histogram of the frequency of the lifetimes of **2**-APTS-SAM excited at 405 nm; emission collected in the 565-605 nm range

**FIGURE S4: 1/2-Sil-SAM ( printed on $SiO_2$ substrate)**




Average fluorescence lifetime image of **1-Sil** on $SiO_2$ substrate and histogram of the frequency of the lifetimes of **1**-Sil-SAM TAG excited at 405 nm and collecting in the 460-500 nm range.




Average fluorescence lifetime image of **2-Sil** on $SiO_2$ substrate and histogram of the frequency of the lifetimes of **2**-Sil-SAM TAG excited at 405 nm and collecting in the 565-605 nm range.

The data of **1**-APTS-SAM are very similar to those obtained for **1**-Sil-SAM. In the case of **2**-APTS-SAM data are somewhat different compared to data obtained for **2**-Sil-SAM. Compound **2**-Sil does not solubilize well and likely tends to aggregate. This influences the characteristics of the TAG of **2**-Sil. Indeed both the confocal spectra with an intense shoulder at 620 nm and the long lifetime shortened to 4.6 ns with reduced relative frequency are indicative of aggregated fluorophores in **2**-Sil-SAM.

**FIGURE S4.** X-RAY PHOTOELECTRON SPECTROSCOPY

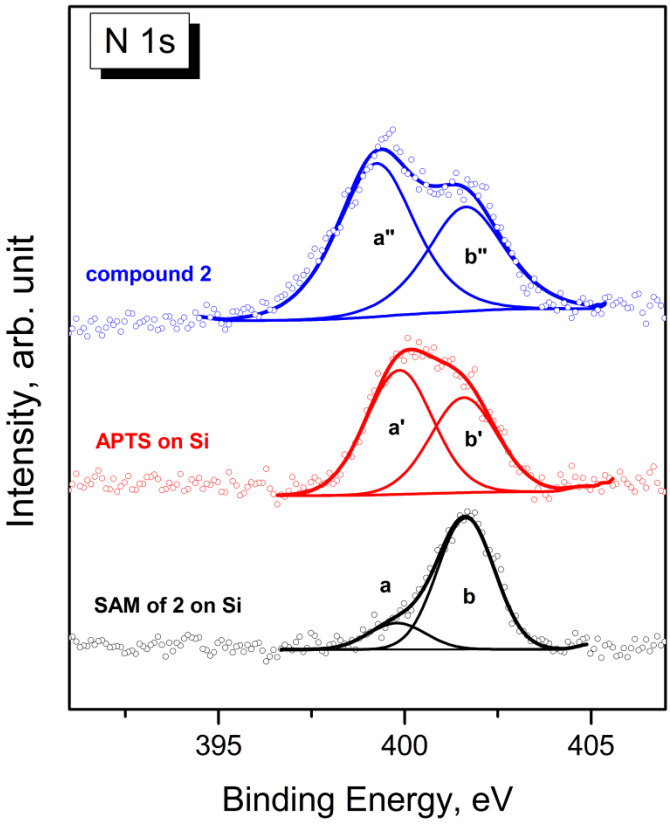
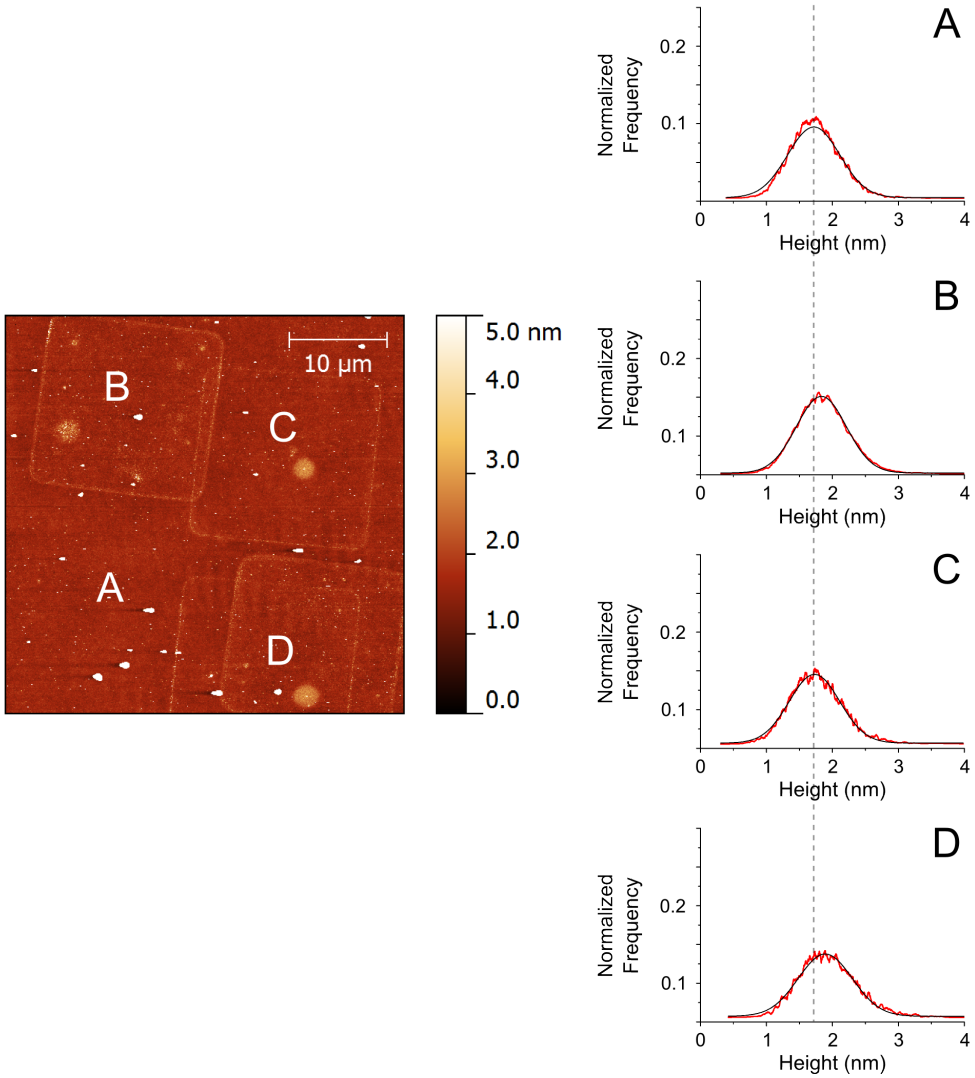
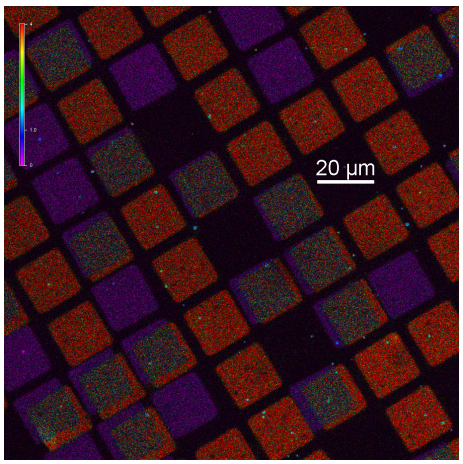


**FIGURE S 5. AFM MORPHOLOGY**

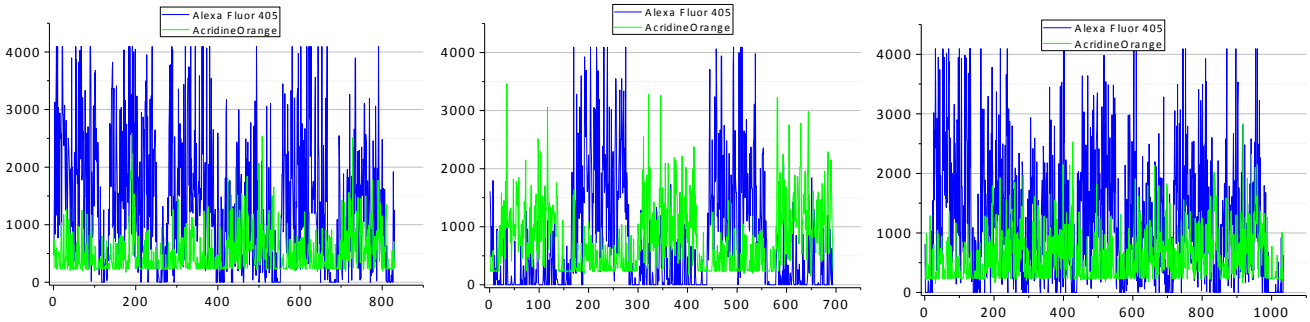


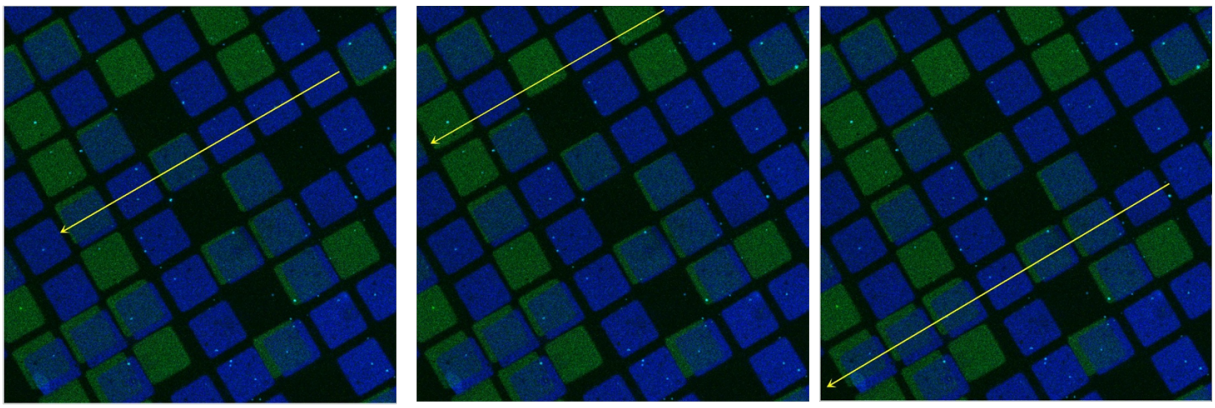

Height distributions of different zones in a doubly-printed TAG: (A) unprinted APTS areas, (B) first TAG, (C) second TAG, (D) mixed areas. The slight height increments observed in type B and D areas with respect to type A areas are not statistically significant and are, if present, in any case smaller than 0.1 nm.

**FIGURE S6: FLUORESCENCE INTENSITY IMAGING OF 1+2-APTS-SAM**




The picture shows a ratio view for the **1+2-APTS-SAM** (**multi-TAG**): the color scale corresponds to the 480 nm/520 nm intensity ratio ranging from 0 (pink) to 4 (red). We have clear discrimination of the different areas in the multi-TAG with single and double fluorophores.



The graphs above show the intensity profiles for the 480 nm and 520 nm channels along the arrows indicated in the figures below. (the x value 0 corresponds to the arrow start); These graphs show that the pixel intensity along the arrows is on average lower in the areas with two fluorophores close to each other.

**FIGURE S7: FLUORESCENCE LIFETIME IMAGING OF 1+2-APTS-SAM (multi-TAG)**

The picture below represents an image in function of the average lifetime; the scale ranges from 0 (blue) to 8 ns (red). To obtain the fluorescence lifetime image we considered photons collected on both SPADs 1 (585 nm) and 2 (480 nm) as well as the complete area scanned. A tri-exponential function yielded a satisfactory fit. The image was then calculated keeping the three lifetime values fixed, 0.3, 1.6 and 5.9 ns and fitting the pre-exponentials factors.

Fluorescence lifetime image, color scale corresponds to average lifetime

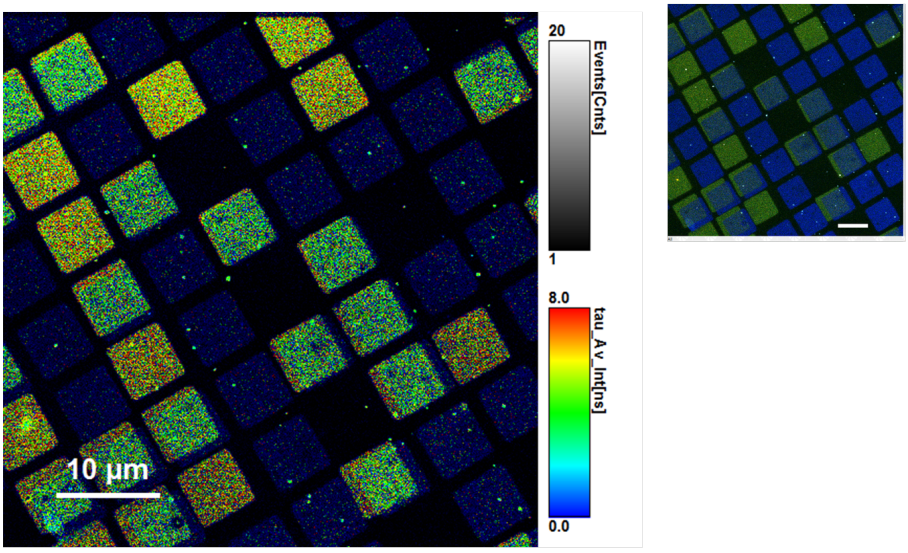


**FIGURE S 8. FLUORESCENCE IMAGE OF MULTI-TAG RECORDED USING DIFFERENT FILTERS AND EXPOSITION TIME**

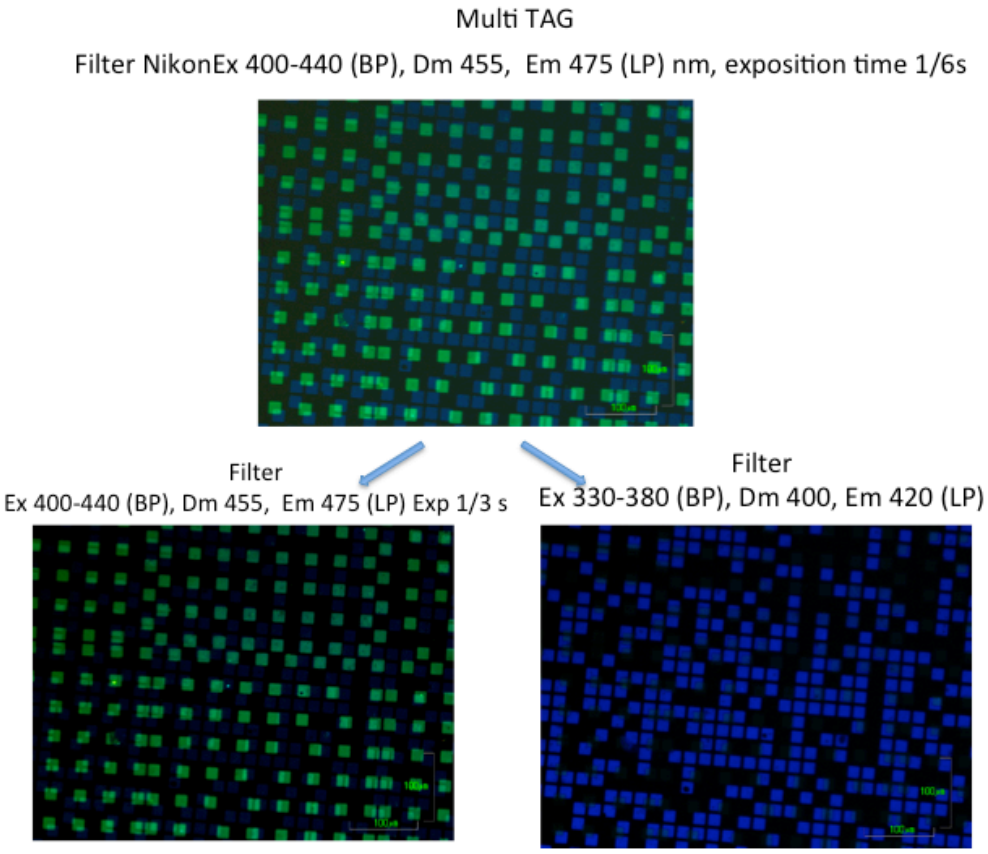
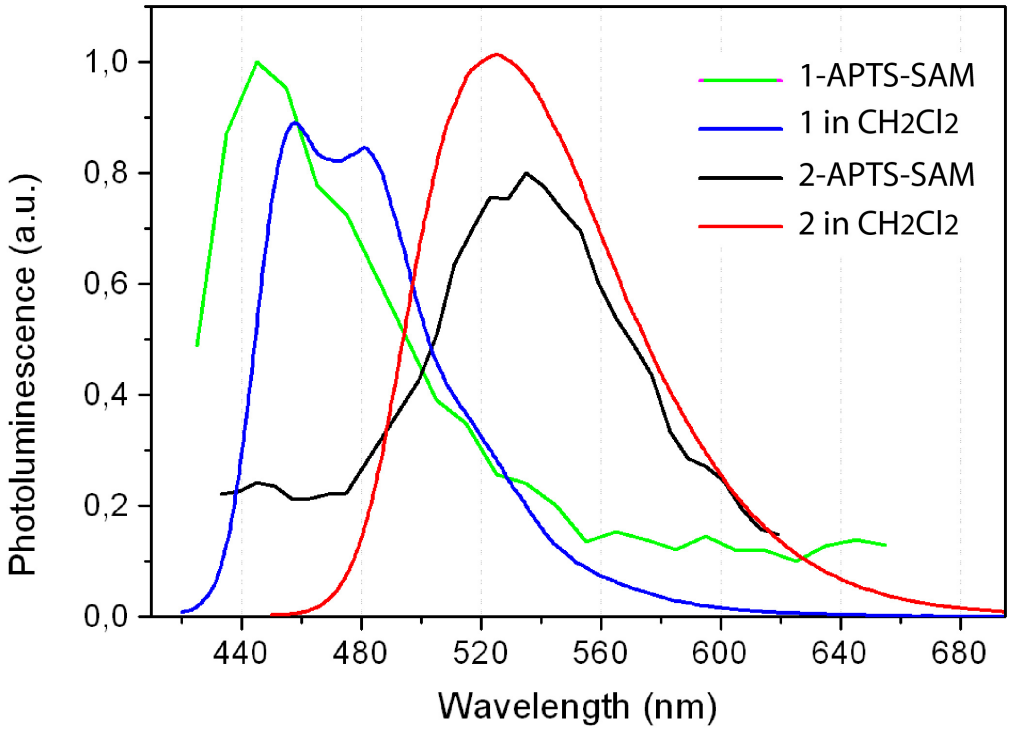


**FIGURE S9 FLUORESCENCE SPECTRA IN SOLUTION COMPARED TO PRINTED COMPOUNDS**




Fluorescence spectra of printed 1(2) APTS-SAM in solution compared to printed compounds

**Table S1 XPS parameters**

| | Name | Peak BE | Area CPS.eV | FWHM fit param (eV) | L/G Mix (%) Product |
|---|---|---|---|---|---|
| 2-APTS-SAM | a | 399.8 | 613.43 | 2.0 | 30 |
| | b | 401.6 | 3049.44 | 2.0 | 30 |
| APTS-SAM on $SiO_2$ | a' | 399.9 | 3429.52 | 2.19 | 28.45 |
| | b' | 401.6 | 2624.45 | 2.19 | 28.45 |
| compound 2 | a'' | 399.2 | 5148.01 | 2.55 | 62.42 |
| | b'' | 401.6 | 3500.89 | 2.55 | 62.42 |

**Table S2: Multi-TAG FLIM data**

| analyzed area | emission | $\tau_1$ (ns) | frequency$_1$ (kCts) | $\tau_2$ (ns) | frequency$_2$ (kCts) | $\tau_3$ (ns) | frequency$_3$ (kCts) |
|---|---|---|---|---|---|---|---|
| Complete image | 480 nm<br>585 nm | 0.3 | 80 | 1.6 | 120 | 5.90 | 600 |
| 1+2-APTS-SAM | 480 nm | 0.29 | 56 | 2.44 | 8 | | |
| 1+2-APTS-SAM | 585 nm | 0.80 | 57 | 4.44 | 105 | | |
| 1-APTS-SAM | 480 nm | 0.32 | 56 | 2.06 | 1 | | |
| 2-APTS-SAM | 585 nm | 5.96 | 170 | 1.2 | 10 | | |

**Table S3**

| sample | $\tau_1$ | $b_1$ | $a_1$ | $\tau_2$ | $b_2$ | $a_2$ | $\tau^2$ |
|---|---|---|---|---|---|---|---|
| **1** | 0.25±0.01 | 0.228±0.001 | 0.91 | 0.73±0.01 | 0.008±0.001 | 0.9 | 0.89 |
| **1-Sil** | 0.26±0.01 | 0.23±0.01 | 0.90 | 2.21±0.01 | 0.003±0.001 | 0.10 | 1.12 |
| **2** | 0.98±0.01 | 0.022±0.001 | 0.08 | 4.79±0.01 | 0.050±0.001 | 0.92 | 0.93 |
| **2-Sil** | 1.75±0.17 | 0.014±0.001 | 0.06 | 8.08±0.01 | 0.046±0.001 | 0.94 | 0.98 |