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Self-protective action in multicomponent fluorescent self-assembled monolayers

Marianna Barbalinardo, Denis Gentili, Marco Brucale, Francesco Valle, Ilse Manet, Giulia Foschi, Massimo Zambianchi, Manuela Melucci and Massimiliano Cavallini**

Electronic Supplementary Information

Experimental Section

Samples preparation: First (3-aminopropyl) triethoxysilane (APTS) functionalized substrates were prepared by immersing silicon wafers for 16 hours into APTS solution (1 mM) in toluene, then rinsed in toluene and dried in a stream of nitrogen.

Conventional fluorescent SAMs were prepared by immersion APTS functionalized silicon wafers for 16 hours into **1** solution (1 mM) in toluene, then rinsed in pure toluene and dried in a stream of nitrogen.

Printed fluorescent SAMs were prepared by micro-contact printing: The PDMS stamps are inked with 10 $\mu L/cm^2$ of ink solution (1 mM solution of the molecules 1 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.

Protected multicomponent SAMs were prepared printing OTS on fluorescent SAMs by using the same procedure, used for fluorescent SAMs using 10 mM solution of the OTS in hexane and maintaining the stamp in contact with the surface for 5 minutes.

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

DPBS, DMEM and Tris(hydroxymethyl)aminomethane hydrochloride buffer (TrisHCl) were purchased from Sigma Aldrich and used as received.

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.

Soft stamp preparation: the PDMS stamps were prepared by replica molding of a pre-patterned or feature-less 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. [15]

Laser scanning confocal fluorescence microscopy: 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 CW argon ion laser as well as a 485 nm pulsed/CW diode laser (PicoQuant GmbH, Berlin, Germany). 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. Images of 1024*1024 or 512*512 pixels were collected using a Nikon Plan Apo VC 20X objective with NA 0.75 applying a zoom factor of 3. Filters were set to register the SP-**1**-SAM fluorescence in the 500-550 nm or 570-620 nm range.

Fluorescence lifetime imaging was performed with integrated PicoHarp 300 electronics (PicoQuant GmbH, Berlin, Germany) for TCSPC measurements. A single-photon avalanche diode detector with a 565-605 nm band-pass filter was used for this scope. The repetition rate of the pulsed excitation at 485 nm was 40 MHz. The instrument response function of the system is approximately 300 ps. A deconvolution fit using AA software calculated IRF was performed on the histogram calculated for a region of interest of the sample. Fluorescence decay profiles were analyzed with a least-squares method, using bi- or triexponential decay functions provided by Picoquant SymPhoTime software.

Fluorescence decay profiles were analyzed with a least-squares method, using multiexponential decay functions provided by Picoquant SymPhoTime software and deconvolution of the instrumental response function calculated by the software. The fitting function used is:

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

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

$f_i = a_i\tau_i / \sum_j a_j\tau_j$

$\tau_{av} = \sum_j f_j\tau_j$

Fluorescence microscopy: Fluorescence images were recorded with a Nikon i-80 microscope equipped with epi-fluorescence using FM filters: Ex 400-440 (BP), Dm 455, Em 475 nm (LP). The FM images were recorded using a commercial CCD camera (Nikon Nikon CCD DS-2 Mv). The illumination was performed by a 103W/2 W lamp at fixed power (i.e. tension 22.5 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. Nanoscratching tests were performed in contact mode with an imposed force of 10.0 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 3 x 3 μm^2 areas, using the standard deviation of these measures as the uncertainty.