Mechano-responsive fluorescent polydiacetylene-based materials: towards quantification of shearing stress at the nanoscale

Supporting Information

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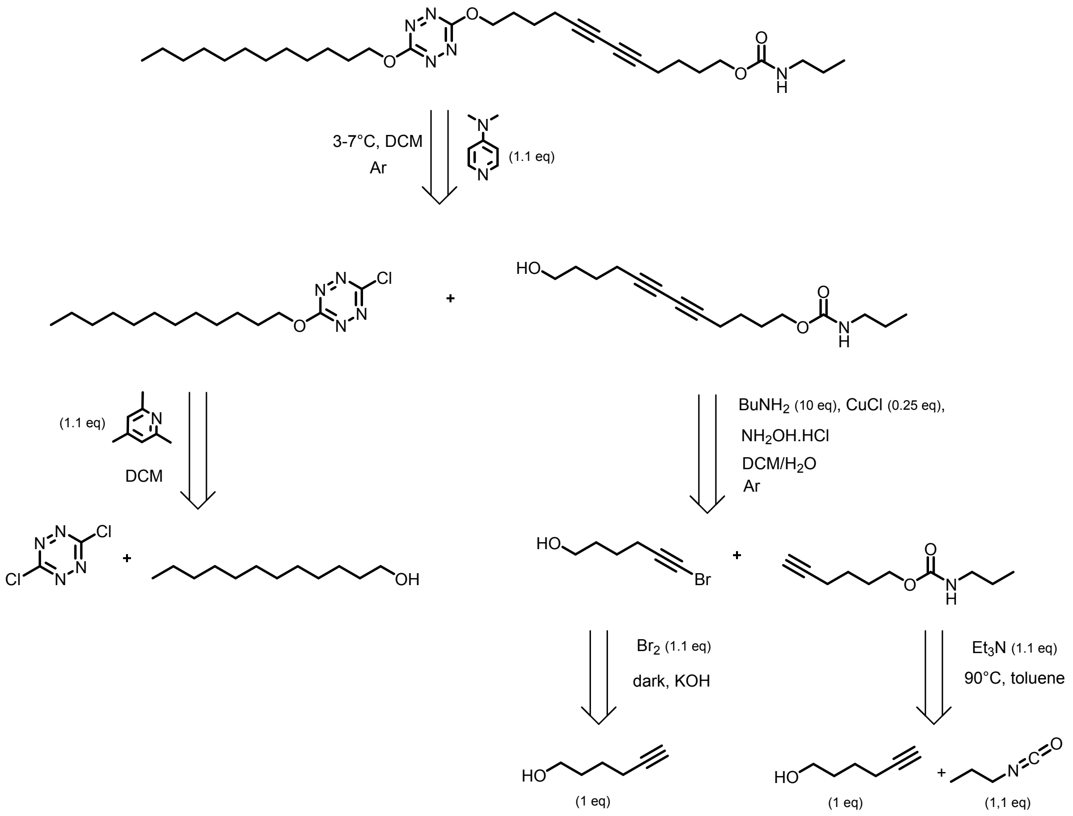
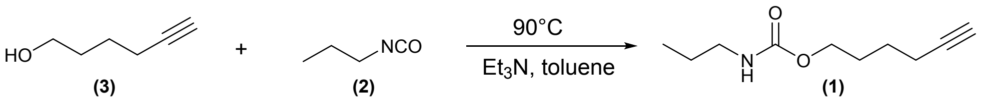
I. Synthetic protocols and characterizations

All reagents were purchased from Sigma Aldrich Chemical Co., and used without further purification. Anhydrous solvents were dried and purified by passage through alumina columns. All reactions were monitored by thin-layer chromatography (TLC) using E Merck TCL silica gel 60 F254, thickness 0.2 µm, and visualized under UV 254 nm or $KMnO_4$ revelation. ^1H and ^{13}C NMR spectra were recorded on a JEOL JMS ECS 400MHz spectrometer (100MHz for 13C). High resolution mass spectroscopy was performed at the CNRS Imagif platform. Melting points were measured with a Stuart apparatus SMP10.

TzDA was synthesized according to the following retrosynthesis scheme:



Hex-5-yn-1-yl propylcarbamate (1)



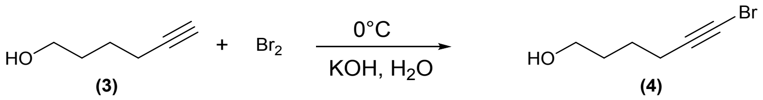
2.21 mL (1.96 g, 20 mmol) of 5-hexyn-1-ol **(3)** are dissolved in 75 mL of toluene and 3.20 mL (2.32 g, 23 mmol, 1.15 eq.) of triethylamine are added to the solution. 2.20 mL (2.00 g, 23.4 mmol, 1.17 eq.) of propylisocyanate **(2)** are added afterwards. The solution is brought to reflux under stirring overnight.

The product solution is washed 3 times with 20 mL of H_2O and the water phase is washed 3 times with 20mL of ethyl acetate. The organic phases are joined, dried on Na_2SO_4 and concentrated. The brown oily solution obtained is purified by SiO_2 column chromatography with 9/1 petroleum ether/ethylacetate, to give 3.01 g of **(1)**, (yield = 82%).

^1H NMR ($CDCl_3$): δ = 0.89 (3H, t, *J* = 7.2 Hz); 1.43 – 1.54 (2H, m); 1.54 – 1.62 (2H, q, *J* = 7.2 Hz); 1.65 – 1.77 (2H, m); 1.94 (1H, t, *J* = 2.3 Hz); 2.20 (2H, t(d), J_1 = 7.2 Hz, J_2 = 2.3 Hz); 3.1 (2H, q, *J* = 6.4 Hz); 4.04 (2H, t, *J* = 6.2 Hz*);* 4.73 (1H, s) ppm.

^{13}C NMR ($CDCl_3$): δ = 11.3; 18.2; 23.5; 25.0; 28.3; 42.8; 64.2; 68.8; 84.1; 156.9 ppm;

6-bromohex-5-yn-1-ol (4)



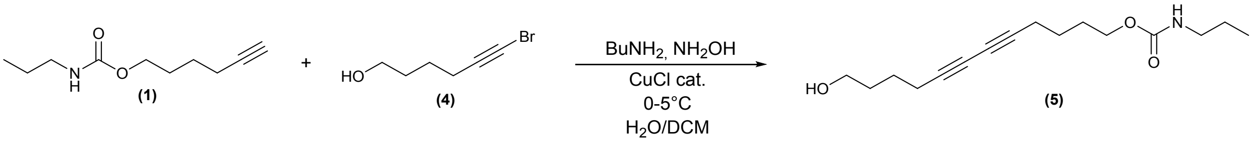
3.12 mL (3.58 g, 22.4 mmol, 1.1 eq.) of Br_2 are added to a thermostated at 0-5°C solution of 4.50 g (80 mmol, 3.9 eq.) of KOH in water. 0.89 mL (2.00 g, 20.4 mmol) of 5-Hexyn-1-ol **(3)** are added dropwise to the solution. The reaction is let run for 2h protected from light. TLC shows the complete consumption of the starting compound **(3)**. The product solution is extracted 3 times with diethylether, the organic phases are joined, washed 3 times with $NaCl_{sat}$, dried on $MgSO_4$ and concentrated to give 3.50 g of **(4)**, (yield = 95%).

^1H NMR ($CDCl_3$): δ = 1.50 – 1.70 (4H, m); 2.22 (2H, t, *J* = 6.9Hz); 2.30 (1H, s); 3.61 (2H, t, *J* = 6.0 Hz) ppm.

^{13}C NMR ($CDCl_3$): δ = 80.1, 62.0, 38.1, 31.6, 24.6, 19.5 ppm.

The material thus obtained satisfactorily matched previously reported results.[1]

12-hydroxydodeca-5,7-diyn-1-yl propylcarbamate (5)



10.8 mL (7.97 g, 109 mmol, 10 eq.) of n-butylamine are dissolved in 55mL of water and N_2 is bubbled in the solution. Temperature is controlled to 0-5°C with an ice bath. Under N_2, a catalytic amount of CuCl (0.270 g, 2.72mmol, 0.25 eq.) and 40 mL of dichloromethane are added to the solution. Hydroxylamine is added until complete disappearance of the blue colour due to the presence of cupric ions. 2.00 g (10.9 mmol) of **(1)** are added to the reaction solution and 1.93 g (10.9 mmol) of **(4)** are first dissolved in 15 mL of dichloromethane and added dropwise to the reaction mixture. The system is let at 0-5°C under stirring for 1h and

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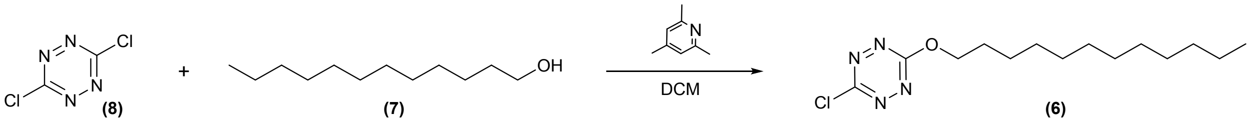
[1] J. M. Montierth, D. R. DeMario, M. J. Kurth and N. E. Schore, *Tetrahedron*, 1998, **54**, 11741-11748.

let react at room temperature overnight. TLC (8/2 petroleum ether/ethyl acetate) shows the presence of a new UV-active product. The reaction solution is diluted with 50 mL of dichloromethane and washed 3 times with 50 mL of NH_4Cl_{sat}. The organic phases are joined, dried on $MgSO_4$ and concentrated. The yellow-brownish oily solution obtained is purified by SiO_2 column chromatography with 8/2 petroleum ether/ethyl acetate, to give 1.94 g of a blue powder (yield = 63.7%) that should be stored at low temperature to avoid thermal polymerization.

^{1}H NMR ($CDCl_3$): δ = 0.92 (3H, t, *J* = 7.3 Hz); 1.45 – 1.75 (10H, m); 2.30 (4H, m); 3.13 (2H, q, *J* = 6.6 Hz); 3.66 (2H, t, *J* = 6.2 Hz); 4.06 (2H, t, *J* = 6.2 Hz); 4.65 (1H, s) ppm.

^{13}C NMR ($CDCl_3$): δ = 11.4; 19.0, 19.1; 23.4; 27.4, 24.9; 28.3; 31.9; 42.7; 62.5; 64.2; 65.7; 77.4; 156.8 ppm

3-chloro-6-(dodecyloxy)-1,2,4,5-tetrazine (6)

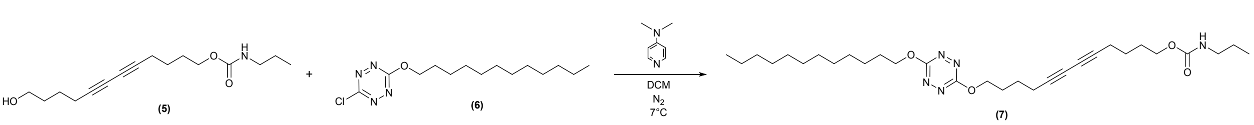


1.863 g (10 mmol) of dodecanol **(7)** and 1.510 g (10 mmol) of dichlorotetrazine **(8)** are dissolved in 70 mL of dichloromethane. 1.59 mL (1.47 g, 11 mmol) of collidine are added under stirring. After 3h, TLC 9/1 petroleum ether/dichloromethane shows the complete disappearance of dichlorotetrazine. The solution is concentrated and purified by column chromatography 9/1 petroleum ether/dichloromethane, to give 2.497 g of **(6)**, (yield = 83%).

^{1}H NMR ($CDCl_3$): δ = 0.88 (3H, t, *J* = 6.7 Hz); 1.26 (16H, m); 1.37 (2H, m); 1.94 (2H, m); 4.65 (2H, t, *J* = 6.6 Hz) ppm.

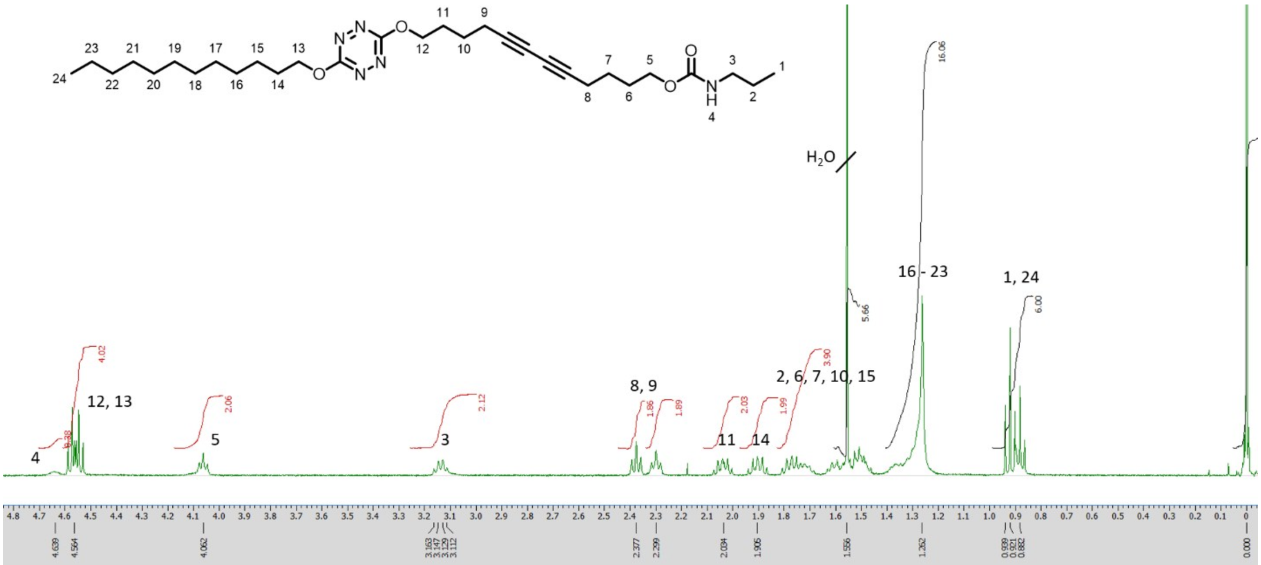
^{13}C NMR ($CDCl_3$): δ = 14.3; 22.8; 25.8; 28.6; 29.3; 29.5; 29.6; 29.7; 29.8 (2C); 32.1; 71.3; 164.3; 166.9 ppm.

12-((6-(dodecyloxy)-1,2,4,5-tetrazin-3-yl)oxy)dodeca-5,7-diyn-1-yl propylcarbamate (TzDA)

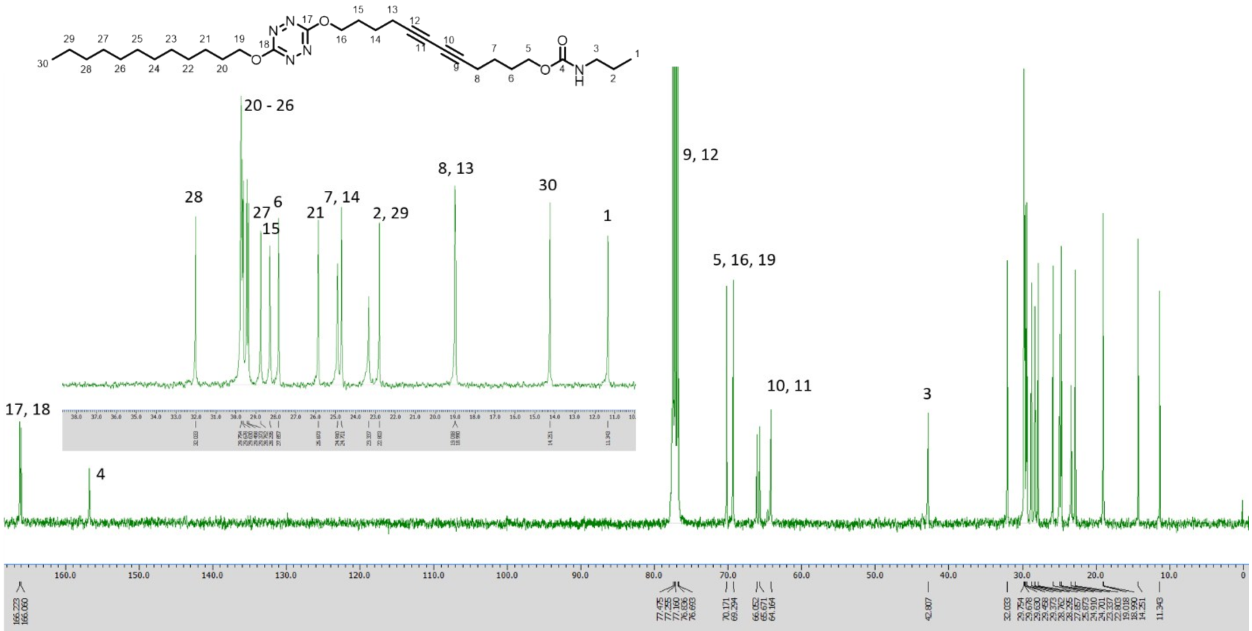


0.150 g (0.537 mmol) of **(5)**, 0.177 g (0.591 mmol, 1.1 eq.) of **(6)** and 0.072 g (0.591 mmol, 1.1 eq.) of DMAP are added in a Schlenk that is previously dried under vacuum to remove any trace of humidity. The system is brought under nitrogen and 8 mL of dichloromethane are introduced in the flask through a syringe. The reaction is let run under inert atmosphere at thermostated 7°C over night. The product solution is concentrated on rotavapor and purified by SiO_2 column chromatography 9/1 petroleum ether/ethyl acetate to give 0.990 g of **(TzDA)**, (yield = 33.9%).

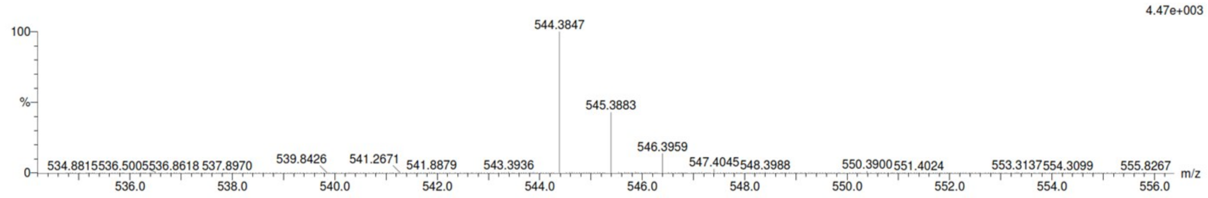
^{1}H NMR ($CDCl_3$): δ = 0.89 – 0.94 (6H, m); 1.26 (16H, m); 1.45 – 1.82 (10H, m); 1.91 (4H, qt, *J*=6.8Hz); 2.00 – 2.08 (2H, m); 2.30 (2H, t, *J*=6.8Hz); 2.38 (2H, t, *J*=6.8Hz); 3.14 (2H, q, *J*=6.6Hz); 4.06 (2H, t, *J*=6.4Hz); 4.52 – 4.60 (4H, m); 4.64 (1H, s) ppm



^{13}C NMR ($CDCl_3$): δ = 11.34; 14.25; 19.00; 19.02; 22.80; 24.70; 24.91; 25.87; 27.86; 28.30; 28.76; 29.37; 29.46; 29.63; 29.68; 29.74; 29.75; 32.03; 42.8; 64.16; 65.67; 66.05; 69.29; 70.17;156.76; 166.06; 166.22 ppm



HRMS (ESI)



$[M + H]^+$ calculated = 544.3857

$[M + H]^+$ found = 544.3847

mp = 84.4°C

II. Photophysical studies and characterizations

II.1. Equipment

The UV/Vis absorption spectra were recorded on a double beam spectrophotometer Cary 5000 equipped with deuterated/halogen lamps for solid samples and on a double beam spectrophotometer Cary 100 for solutions, both from Agilent technologies. The emission spectra were recorded on the Fluorolog FL3-221 with xenon light source for solid samples and on a Fluoromax 3 for solutions, both from Horiba Jobin-Yvon.

Irradiation at 254 nm was performed by a Hg/Xe LC8 light source from Hamamatsu. An interferential filter from Semrock is added to select the 254nm mercury line. The power of the irradiation was calculated with an Ophir powermeter (PD300-UV), using a Schott long pass filter GG385 to measure IR irradiation that is let through the Semrock filter, considering a 90% transmittance: $P_{254} = TotalPower - [{}^{10}/_{9}(PowerLP385)]$.

II.2 Fluorescence quantum yield and molar extinction coefficient of TzDA in acetonitrile

Fluorescence quantum yields in solution were measured using rhodamine 6G in ethanol (literature $\Phi_F{}^R$ = 95%) as the reference. All solutions were diluted to have an absorbance < 0.1. The fluorescence quantum yields of the samples Φ_F (x) were calculated using the following equation:

$$\Phi_F(x) = \Phi_F(0)\,\frac{1-10^{-A_0}}{1-10^{-A_x}}\,\frac{S_x}{S_0}\left(\frac{n_x}{n_0}\right)^2$$

with Φ_F (0) and Φ_F (x) the fluorescence quantum yields, S_0 and S_x the integrated fluorescence, A_0 and A_x the values of the absorbance at the chosen excitation wavelength, n_0 and n_x the refraction indices, for the reference and the sample solutions, respectively. It was obtained ϕ_F = 8.5 % for **TzDA** in acetonitrile.

Molar extinction coefficient (ε) was calculated for **TzDA** monomer at 520 nm in acetonitrile. It was obtained ε_{520} = 500 L mol^{-1} cm^{-1}, which is in good accordance with alkoxy-substituted tetrazines found in literature.[2]

II.3. Coverglasses cleaning and thin film preparation

The coverglasses were preliminary cleaned before film deposition by several steps of sonication in the following solvents: 1% Hellmanex water solution, acetone, ethanol, 1M sodium hydroxide water solution and distilled water, then dried with a heat gun.

The deposition of thin films was carried out using a Leybold Univex 300 vacuum evaporator. 11.2 mg of the pure organic powder obtained from the synthesis was introduced in the crucible, and thin films of 80 nm were obtained. The crucible was heated by means of a tungsten filament source and the temperature was controlled by a thermocouple. A heating rate of 10°C·min^{-1} was used from 25 to 120°C. Then the rate was reduced to 4°C·min^{-1} from 140-160°C. When the temperature reached 160°C, the speed of deposition was manually controlled to be 1Å·s^{-1} maximum by adjusting the furnace temperature. Film thickness and growth rate were monitored in situ by a piezoelectric quartz crystal system. Film thickness was measured by manually performing a scratch and measuring by AFM the step height in 5 different points along the scratch. The obtained mean value is $d = 80 \pm 12$ nm.

II.4. Estimation of the maximum percentage of polymerization

For a thin film of average thickness 80 nm, an OD_{exp} of 6.5×10^{-2} is observed when the polymerization plateau is reached, after substraction of the initial spectrum of the non-irradiated sample in order to remove contributions from scattering. A typical molar absorptivity for PDA derivatives is in the order of 2×10^5 cm^{-1} (please note that this value is only estimated from values reported in the literature for other PDA derivatives).[3]

If the film was entirely polymerized, we would have:

$$OD_{100\%} = (2 \times 10^5/2.3) \times 80 \times 10^{-7} = 0.7$$

The percentage of polymerization can thus be estimated as $OD_{exp}/OD_{100\%} = 10\%$

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[2] Y. H. Gong, F. Miomandre, R. Meallet-Renault, S. Badre, L. Galmiche, J. Tang, P. Audebert and G. Clavier, *Eur. J. Org. Chem.*, 2009, 6121-6128.

[3] S. Spagnoli, J.-L. Fave and M. Schott, *Macromolecules*, 2011, **44**, 2613-2625.

II.5. Low temperature fluorescence spectra

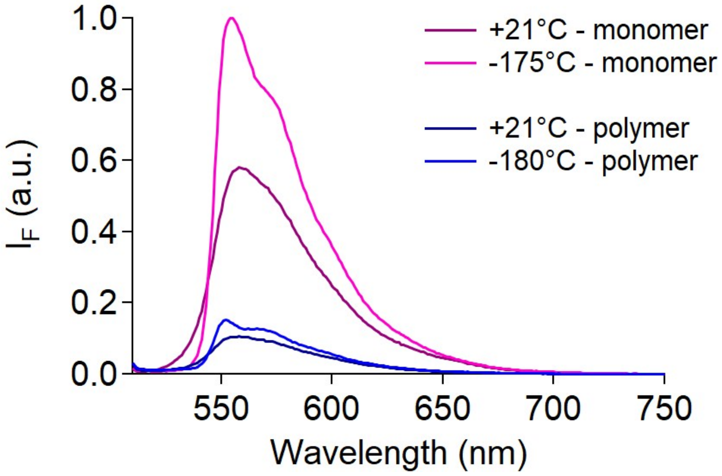

Figure S1. Fluorescence spectra of vacuum evaporated TzDA monomer at ambient temperature (purple line) and at -175°C (pink line); fluorescence spectra of the same TzDA film, polymerised by UV irradiation at 254 nm for 10 min (P254 = 1,37 mW·cm^{-2}), at ambient temperature (dark blue line) and at -180°C (light blue line). λexc = 488 nm

When irradiated at 254 nm in the solid state, **TzDA** efficiently polymerises in the **poly-TzDA** form, which quenches the fluorescence of the attached fluorophore, tetrazine (Tz). Additional information on the quenching mechanism can be obtained by performing fluorescence spectra at ambient and low temperature both in presence and absence of the quencher (blue-PDA). Freezing the system allows to suppress vibrational relaxation and all deactivation processes that depend on spatial rearrangements of the molecule in the excited state like electron transfer. Figure 1 shows that the increase of fluorescence intensity in the **poly-TzDA** (dark blue/light blue lines) is comparable and even lower to the monomer case (purple/pink lines), excluding a quenching by electron transfer thus confirming an energy transfer mechanism. The measurements were performed on vacuum deposited **TzDA** on glass slides inserted in an optical cryostat by Oxford instruments (Optistat DN) on the Fluorolog FL3-221 spectrofluorometer.

II.6. Fluorescence microscopy

The images acquired by fluorescence microscopy were recorded on an inverted microscope (Nikon, Eclipse Ti-U) used in wide field. The samples were excited by a Lumencor Spectra X light engine at 488 nm. The fluorescence signal is collected in epifluorescence by the objective lens Nikon Plan Apo λ 60x/0.95. The fluorescence signal is separated and filtered from the incident excitation beam by Semrock optics (LF488/LP-B-000 dichroic mirror, BLP01-488R emission filter) and directed to an EMCCD (Andor; iXon Ultra 897). All the images were analysed by the Solis software of Andor. Polymerisation by irradiation at 254 nm was performed in situ, by selecting the mercury line of Hg/Xe light source.

II.7. AFM Measurements

AFM images were acquired with a JPK NanoWizard® 3 AFM. The head of the AFM was designed to be coupled with the Nikon microscope, allowing the correlation of object position, size and shape with its photophysical properties. AFM images were recorded in tapping mode under ambient conditions. Silicon cantilevers with diamond-like carbon coating (Budgetsensors, Tap300DLC) were used after calibration of their spring constant with thermal noise method from JPK instruments. The tip's half cone angles are 20°-25° along the cantilever axis, 25°-30° from side and 10° at the apex, with a tip radius < 15 nm. The cantilever nominal force constant is given at 40 N·m^{-1}.

To perform the force measurements, the same silicon cantilevers were used in contact mode to shear the surface of the films with forces ranging from 20 nN to 500 nN and with a scan rate of 0.9 Hz (6.1 µm s^{-1}). The direction of scan was from bottom to top and left to right as shown in Figure 2. It has to be noticed that each line is scanned twice (trace and retrace).

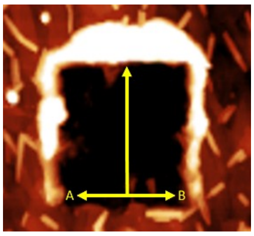

Figure S2. Zoom on a 3x3 µm^2 sheared area. Yellow arrows are traced to show the direction of scan

The tip starts from point A and goes to point B at 6.1 µm s^{-1} (trace). It goes back from point B to point A (retrace) and moves to the next line towards the top of the selected area. This movement is the reason why sheared-off material tends to be found on the sides and of the top of the sheared zone.

The scan rate is an important parameter that affects the shearing mechanical stimulation, which is thus not only defined by the vertical nominal force (in nN). The quality of the fluorescent response (Figure S3) was assessed at different tip velocities at the same nominal force of 150 nN in the following experiment. Using slow rates (0.1 – 0.7 Hz) increases the chance of material attachment to the tip as shown in detail in Figure S4, while fast rates do not ensure a homogenous application of the nominal force along the scan lines, since the feedback loop of the Z piezo needs to be very fast as well (1.1 – 1.5 Hz). The most homogeneous response in fluorescence along with the certainty of applying the chosen vertical nominal force was found for scan rates between 0.7 and 1.2 Hz. The intermediate value 0.9 Hz (corresponding to 6.1 µm·s^{-1} for a 3x3 µm^2 scan area) was then chosen as default speed of scan.

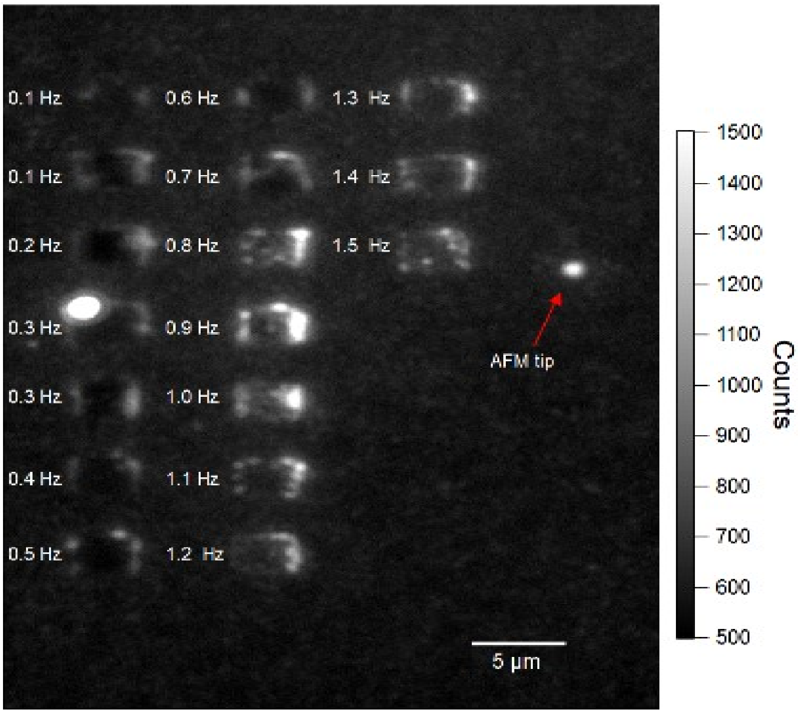

Figure S3. Fluorescent image obtained after mechanical stimulation performed at 150 nN with increasing scan rates, from 0.1 Hz up to 1.5 Hz

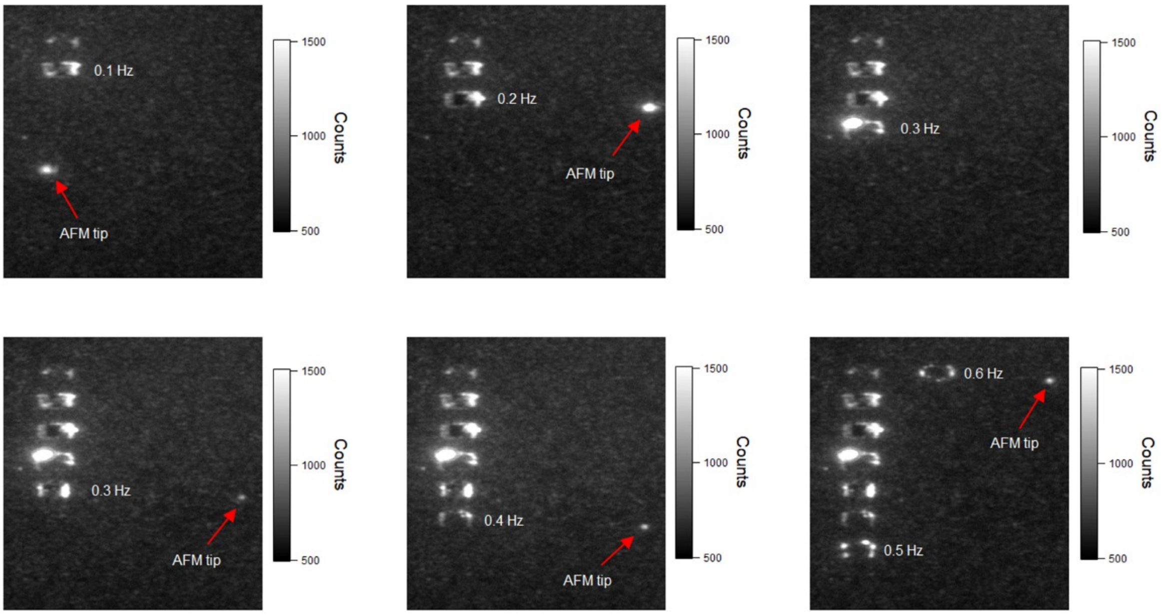

Figure S4. Consecutive images obtained at constant vertical force of 150 nN and increasing tip velocities.

II.8. Fluorescence spectrum of an AFM sheared area

The fluorescence spectrum of an AFM sheared area recorded under the microscope with a CCD-spectrograph (Princeton Instruments Acton SP2356 with Spec-10 400Br/LN-eXcelon CCD detector) is shown in figure S5.

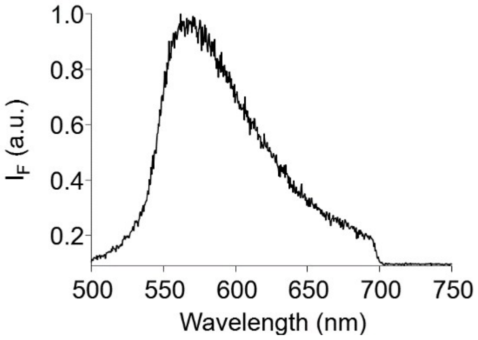

Figure S5. Fluorescence spectrum of poly-TzDA sheared area

The emission maximum at 560 nm corresponds to the tetrazine fluorescence, with a consistent spectral shape. However, quantification of fluorescence restoration is not easily accessible by spectroscopy measurements, due to material displacement over a large area. Therefore, the most reliable method to quantify the fluorescence level before and after mechanical stimulation consists in the analysis of fluorescence images, as described in the main text.

II.9. Morphology study of vacuum evaporated TzDA

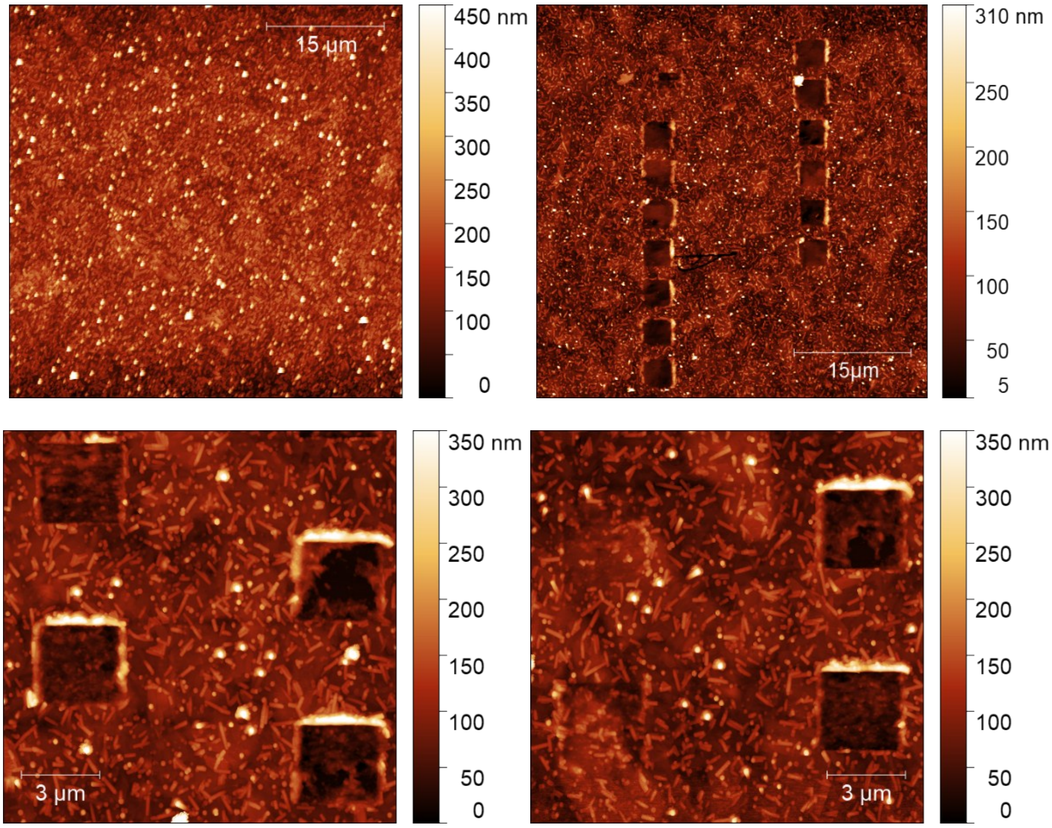

Figure S6. AFM images obtained in tapping mode in different zones of the vacuum evaporated sample of **TzDA** after polymerization and after shearing by contact mode scanning.

AFM images obtained in tapping mode in different zones of the vacuum evaporated sample of **TzDA** show that the same morphology is retained (Figure S6). Moreover, images of sheared areas show that a similar morphology change is induced by contact mode scanning in different areas of the sample.

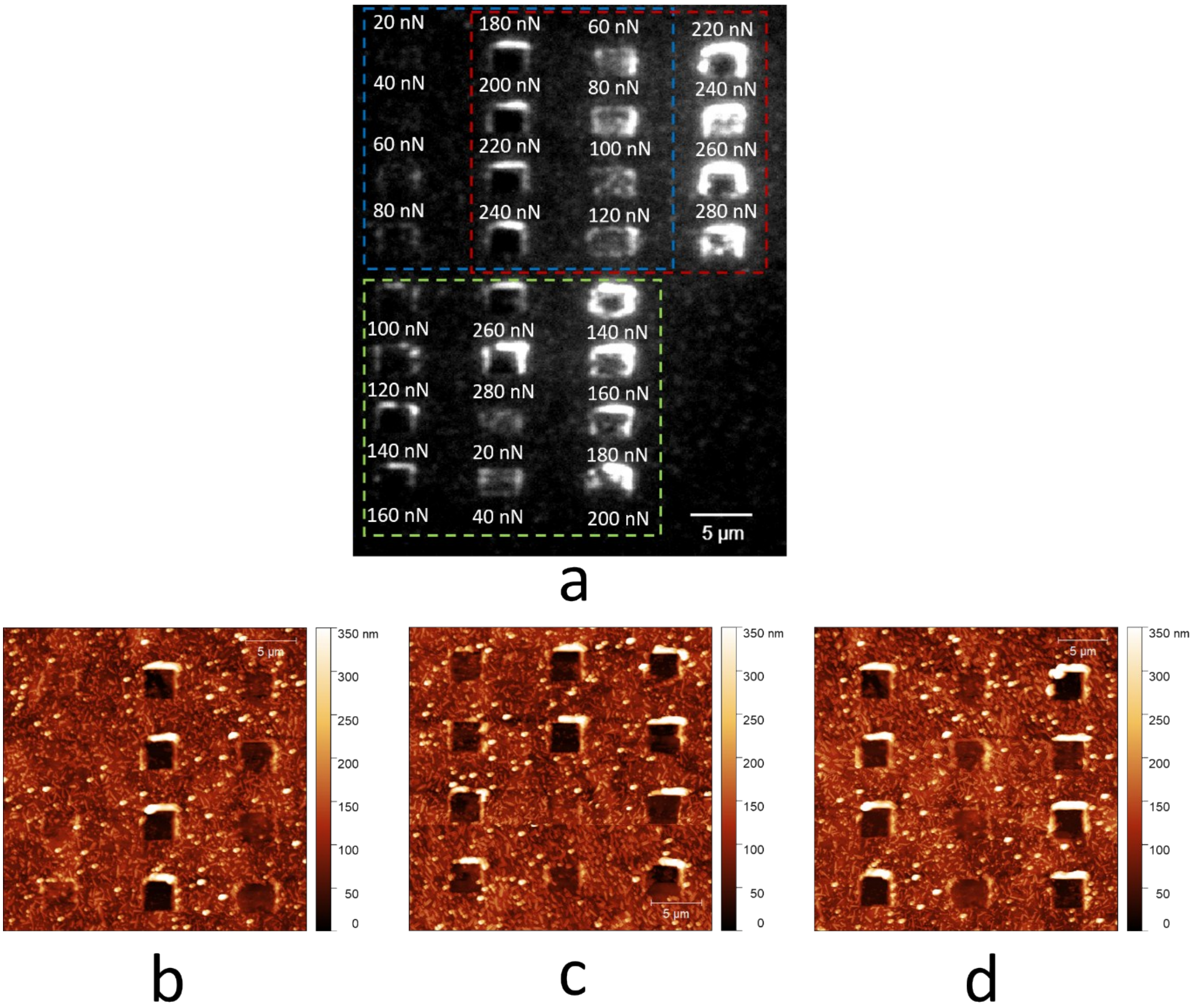

Figure S7: Fluorescence and AFM images of sheared TzDA films. a) Fluorescence image; b) AFM morphology image of blue area in a; c) AFM morphology image of green area in a; d) AFM morphology image of red area in a;

The images presented in figures 4C (AFM) and 4D (fluorescence) are part of a bigger area that has been scanned several times at different forces in that range. The entire area of interest is shown in figure S7.

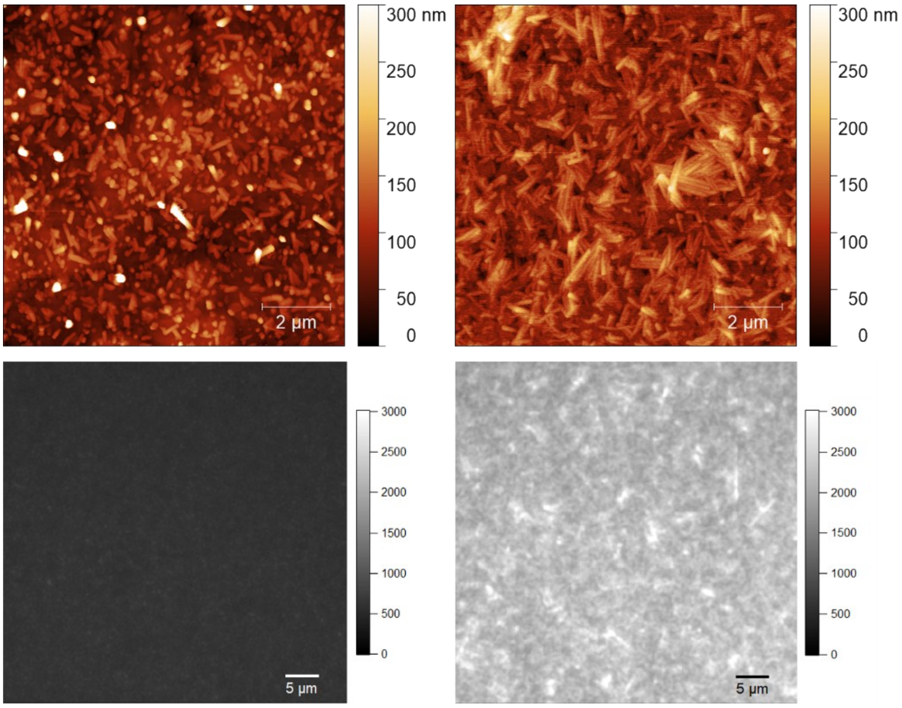

Figure S8. Top. Tapping mode AFM images of **poly-TzDA** before (left) and after (right) heating at 75°C. Bottom. Corresponding fluorescence microscopy images.

Tapping mode images of sample of poly-TzDA before and after heating (at 75°C thus below the melting point of the monomer) show that the morphology is affected by heating. As a consequence, the fluorescence is restored (Figure S8).