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# Electronic Supplementary Information

## Probing the mobility of catenane rings in single molecules

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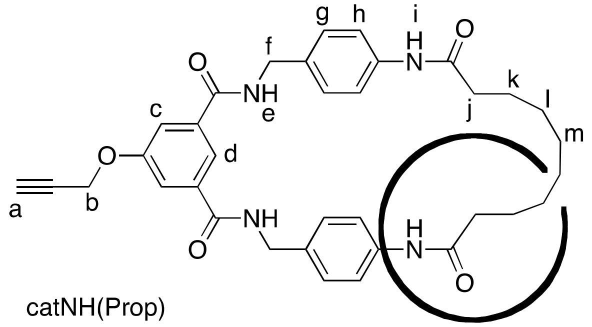
### 1. Synthesis

***Materials and methods***

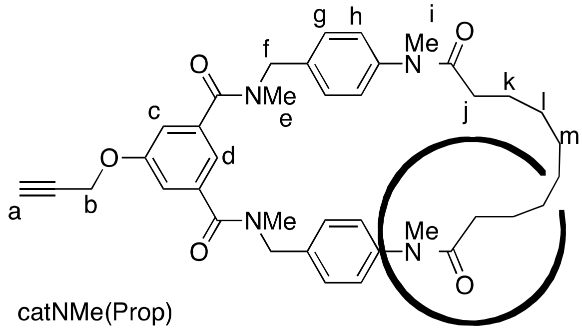
Nuclear magnetic resonance spectra (NMR) were recorded on 300 MHz or 500 MHz Bruker Avance II spectrometers. The $^1$H spectra were recorded in deuterated chlorofom ($CDCl_3$) or deuterated dimethyl sulfoxide ($D_6$-DMSO). The deuterated solvent was used as an internal reference for proton and carbon NMR. The chemical shifts (δ) are expressed in parts per million (ppm).

The solvents used were of analytical grade and distilled before use. DMF and THF were distilled on $CaH_2$ under argon. Triethylamine was dried over KOH pellets. Poly(ethylene oxide) mono methyl ether ($PEO_{45}$-OH, Mn= 2,000 g/mol, PDI = 1.04) was purchased from Polymer Source Inc. and was dried by azeotropic cycles with toluene prior to use. Potassium carbonate ($K_2CO_3$, 99%) was dried 24 h at 100°C before use. The two catenanes were synthesized as previously described.[S1]

***General procedure for the benzylic amide [2]catenane functionalization.*** In a dried 25 mL round bottom flask was added the benzylic amide catenane (1 eq.), $K_2CO_3$ (6 eq.) and freshly dried DMF. The solution was stirred for 1 hour at 70°C under Ar. A solution of propargyl bromide (4 eq.) in dry DMF was then added dropwise. The solution was stirred overnight at 70°C under Ar. The solvent was removed under vacuum and water was added to the remaining slurry, the mixture was stirred for 10 minutes. The yellow precipitate was filtered and dried under vacuum to yield the desired product.



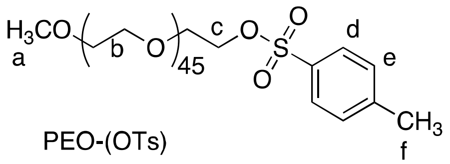
**catNH(Prop):** yield: 89%, $^1$H-NMR ($D_6$-DMSO): δ = 0.78 (s, 18H, $H_m$ and $H_l$), 1.28 (s, 8H, $H_k$), 2.13 (s, 8H, $H_j$), 3.16 (s, 2H, $H_a$), 4 (s, 8H, $H_f$), 4.84 (s, 4H, $H_b$), 6.90 (d, 8H, $H_g$), 7.31 (d, 8H, $H_h$), 7.52 (s, 4H, $H_c$), 7.76 (s, 4H, $H_d$), 8.46 (s, 4H, $H_e$), 9.75 (s, 4H, $H_i$).



catNMe(Prop)

**catNMe(Prop)**: yield 83%, $^1$H-NMR ($D_6$-DMSO): δ = 0-0.712 (multipeaks, 16H, $H_l$ and $H_m$), 0.71 (s, 8H, $H_k$), 1.09 (s, 8H, $H_m$), 2.79 (s, 12H, $H_i$), 3.07 (s, 12H, $H_e$), 3.63 (s, 2H, $H_a$), 4.50 (s, 8H, $H_f$), 4.92 (s, 4H, $H_b$), 7.13-7.47 (multipeaks, 22H, $H_{c,d,g,h}$).

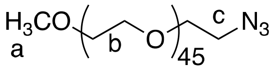
***Tosylate end-functionalized poly(ethylene oxide):*** To a solution of hydroxy end-functionalized poly(ethylene oxide) (1 eq.) and para-toluene sulfonyl chloride (10 eq.) in dry THF was added dry $Et_3N$ (20 eq.). The solution was stirred for 4 hours at 35°C. Another portion of para-toluene sulfonyl chloride and (10 eq.) and $Et_3N$ (20 eq.) were then added again and the mixture was heated overnight. The reaction mixture was then partitioned between water and $CH_2Cl_2$, and the organic layers was then dried over $Na_2SO_4$ and concentrated in vacuo. The resulting solid was precipitated from THF into diethyl ether. This last operation was repeated twice.



PEO-(OTs)

**PEO-(OTs)**: yield 93%, $^1$H-NMR ($CDCl_3$) : δ = 2.43 (s, 3H, $H_f$), 3.36 (s, 3H, $H_a$), 3.68 (s, 180H, $H_b$), 4.14 (t, 2H, $H_c$), 7.28 (d, 2H, $H_e$), 7.77 (d, 2H, $H_d$)

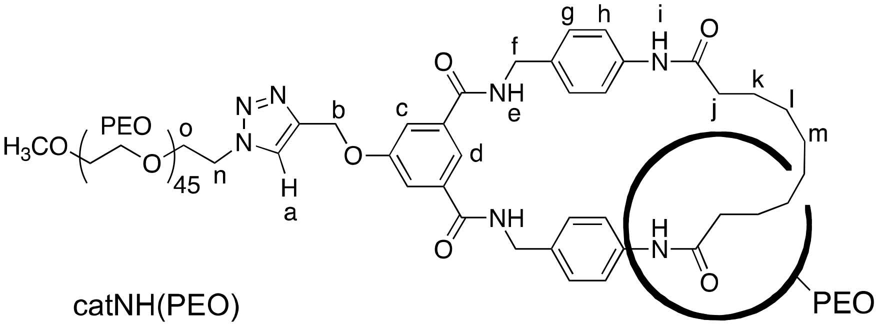
***Azide end-functionalized poly(ethylene oxide):*** To a solution of tosylate end-functionalized poly(ethylene oxide) (1 eq.) in dry DMF was added $NaN_3$ (20 eq.). The mixture was stirred for 20 hours at room temperature, under Ar. The solvent was removed under vacuum and the slurry taken up into $CH_2Cl_2$. The organic layers were washed with water, dried over $Na_2SO_4$ and concentrated in vacuo. The resulting solid was precipitated from THF into diethyl ether to yield the desired product as a white powder.



PEO-($N_3$)

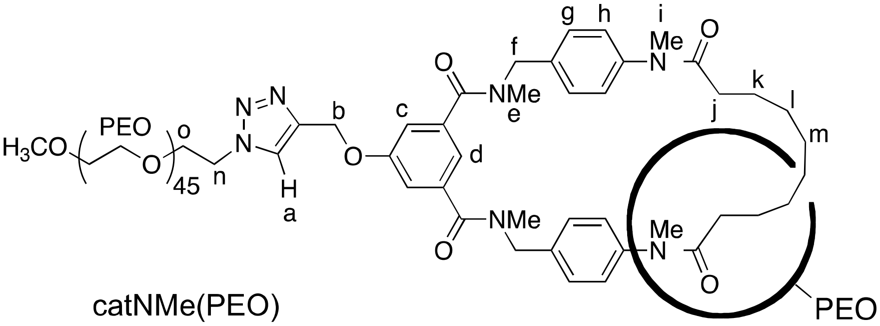
**PEO-($N_3$)**: yield 91%, $^1$H-NMR ($CDCl_3$): δ = 3.37 (s, 3H, $H_a$), 3.4 (s, 2H, $H_c$), 3.63 (s, 180H, $H_b$).

***General procedure for the synthesis of the catenane containing polymers:*** Propargyl functionalized [2]catenanes (catNH(Prop) or catNMe(Prop)) (1 eq.), azide end-functionalized poly(ethylene oxide) (PEO-$N_3$) (2.2 eq.), $Me_6$-Tren (2.4 eq.) and DMF were introduced in a Schlenk tube. In another Schlenk tube, was introduced CuBr (0.6 eq). Two freeze-pump-thaw cycles were applied on the tube containing the DMF solution before transferring this solution to the Schlenk tube containing CuBr. After two additional freeze-pump-thaw cycles, the Schlenk tube was filled with Ar and transferred in a preheated oil bath at 35°C. The reaction was carried out for 36 hours. The solvent was then removed in vacuo and the slurry taken up into $CH_2Cl_2$. The organic layers were washed twice with a solution of aqueous EDTA, then dried over $Na_2SO_4$, filtered and the solvent removed in vacuo. The catenane-containing polymers were then purified on a Bio Beads SX1 column using DMF as solvent to remove the excess PEO.



catNH(PEO)

**catNH(PEO)**: yield: 43%, $^1$H-NMR (D$_6$-DMSO): δ = 0.78 (s, 16H, $H_{l,m}$), 1.28 (s, 8H, $H_k$), 2.13 (s, 8H, $H_j$), 3.59 (m, 360H, $H_{PEO}$), 3.83 (s, 4H, $H_o$), 4.08 (s, 4H, $H_f$), 4.56 (t, 4H, $H_n$), 5.04 (s, 4H, $H_b$), 6.92 (d, 8H, $H_g$), 7.31 (d, 8H, $H_h$), 7.52 (s, 4H, $H_c$), 7.73 (s, 2H, $H_d$), 8.2 (s, 2H, $H_a$), 8.4 (s, 4H, $H_i$), 9.69 (s, 4H, $H_e$).



catNMe(PEO)

**catNMe(PEO)**: yield: 35%, $^1$H-NMR (D$_6$-DMSO): δ = 0.52, 0.71, 1.07, 1.77 (broad multipeak, 32H, $H_{j,\ k\ ,l\ \text{and}\ m}$), 2.79 (s, 12H, $H_i$), 3.07 (s, 12H, $H_e$), 3.5 (m, 360H, $H_{PEO}$), 3.83 (s, 4H, $H_o$), 4.50 (s, 12H, $H_{f,\ n}$), 5.24 (s, 4H, $H_b$), 6.67-7.46 (m, 22H, $H_{c,\ d,\ g,\ h}$), 8.24 (s, 2H, $H_a$).

## 2. AFM-based single molecule force spectroscopy

### *Surface preparation.*

Silicon substrates were cleaned by UV-ozone treatment for 15 minutes (UV-ozone cleaner, Model 42, Jelight Company Inc) before being rinsed with acetone followed by ethanol. Filtered solutions (0.2 μm) of the polymers in methanol at a concentration of 0.1 mmol/L were drop-casted onto these substrates. The excess of solution was removed by gentle blowing with a nitrogen flux on the surface.

### *AFM-SMFS experiments*

Before starting the experiments, all the AFM cantilevers were cleaned by UV-ozone treatment for 10 min. The AFM-SMFS experiments were performed with a PicoPlus 5500 microscope (Agilent Technologies, Inc.) equipped with a closed-loop scanner. Force curves were realized using silicon nitride cantilevers with a nominal spring constant of 0.05 $Nm^{-1}$ (MLCT series, Veeco Instruments, Inc.) and a loading rate of 1000 pN $s^{-1}$. All the curves were realized at the same loading rate for comparison. The tip was gently pressed against the substrate with a maximum force of 300 pN, which is low enough to avoid any damage to the molecules. During the contact, the PEO chain can physisorb onto the tip and bridge the substrate and the tip. When moving the tip away from the substrate, the bridging molecule will be stretched. The stretched molecule exerts a restoring mechanical force on the cantilever producing its deflection. The experimental result is a force-extension curve that reflects the elasticity of a single molecule and reveals structural changes induced in the molecule by the external force. This method relies on the ability of most polymers to adhere nonspecifically to the AFM tip. Multiple bridges can form when the tip comes into contact with the surface, but it is possible to limit the number of molecules adsorbed to the tip by using surfaces with low surface density of the adsorbed molecules.

The extension, $x$, of the molecule being stretched is determined from the separation distance between the AFM tip and the substrate. This separation is calculated as the difference between the travel of the piezo scanner from the reference position, $Z$, and the deflection of the cantilever, $d$ ($x = Z - d$). The force $F$ experienced by the molecule is measured by the deflection $d$ of the cantilever and is calculated by using Hooke’s law as $F = k_C.d$, where $k_C$, is the spring constant of the cantilever (calibrated by the thermal noise method).

***Fitting with the worm-like chain (WLC) model***

Stretching a polymer chain causes two kinds of restoring forces. At small displacements, entropic forces dominate. In solution, the molecule describes a random coil, which upon stretching loses entropy, inducing a restoring force. At larger displacements tension appears in the polymer backbone, the bond angles being deformed and the bonds being stretched in the direction of the pulling force. The force profiles were fitted using the worm-like chain model (WLC),[S2] predicting the relationship between the extension of a linear polymer and the entropic restoring force. The force required to stretch a WLC with persistence length *lp* and contour length *L* to a distance *D* is given by:

$$F(D) = \frac{k_B T}{l_p}\left[\frac{D}{L} + \frac{1}{4(1-D/L)^2} - \frac{1}{4}\right]$$

The adjustable fit parameters are the persistence length and contour length. As the elasticity of a linear polymer scales with its contour length, whereas the intrinsic properties like the persistence length are independent of the chain length, all force traces originating from different polymer chains of identical structure superimpose when scaled to the same contour length (this is routinely used as a criterion for single molecule detection). Thus the only relevant fit parameter is the persistence length, which dictates the shape of the profile at small forces.

We used two methods to fit the experimental curves: an automatic and a manual one. Both methods have their limitations. When the fit is performed automatically (least squares method), it is possible that some random noise, spikes of noise, or particular characteristics of the curve play an important role and compromise the obtained fit. When the fit is performed manually, it is possible to completely disregard the perturbation of the signal coming from the noise, but in this case the evaluation of the best WLC fit could be biased by the user. It is also important to note that a careful positioning of the origin of the force-extension curve is crucial to avoid important mistakes into the fitting results. By using an automatic fit, it is more difficult to avoid this problem.

The agreement of the results coming from the two methods is a good test to be sure that a user bias has not been accidentally introduced, and that some noise has not mislead the analysis.

Moreover, the superimposition after normalisation of the curves with different persistence length values can qualitatively confirm that the results are real and not biased by the user (see Figure 3 in the main text).

## 3. References

[S1] C. A. Fustin, C. Bailly, G. J. Clarkson, P. DeGroote, T. H. Gallow, D. A. Leigh, D. Robertson, A. M. Z. Slawin, K. K. Y. Wong, *J. Am. Chem. Soc.* **2003**, *125*, 2200.

[S2] Flory, P.J. *Statistical Mechanics of Chain Molecules* (Hanser, Muenchen, 1989).