

Electronic Supplementary Information for : Optical single molecule characterisation of natural and synthetic polymers through nanopores[†]

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S1: Legends for Movie S1

Legend : Direct optical visualisation of translocation events of λ DNA through a membrane of nanopores $R_p = 45$ nm. The experimental conditions are the same as the one presented in the main text, in the section **Influence of the DNA molecular weight**. For the image acquisition, the binning of the image has been fixed to 1, and the frequency of acquisition is 33 Hz.

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S2: Model for the real length of a molecule at the exit of a nanopore

The direct visualisation of the translocation of one polymer through a nanopore give us access to the measurement of the optical length of the molecule, which is the time of transport of the molecule through a plane times the known local velocity. As we know the local hydrodynamic shear stresses, we can also compute the theoretical size of a blob at the plane, and the theoretical instantaneous length is the number of blobs times the size of one blob. The real length of a molecule is not measured directly, and is more complex to be computed theoretically as the molecule feels decreasing hydrodynamic shear on its sections that are further away from the nanopore. The full configuration of the molecule at the exit of the nanopore is a succession of blobs of increasing size. Here we propose a derivation with a few simplification for this length, to be able to compare it with with other relevant length in the system such as the width of the illumination region (see main text, tab. 1). All variable are defined in the main text.

We consider that the polymer is a succession of N_b blobs at positions r_k , and of size $\xi_k(r_k)$. The first blob is at the nanopore, $r_1 = R_p$. A blob k is supposed to be at a distance $2\xi(r_{k-1})$ from the previous blob $k-1$. Remembering that $\xi(r) = r\left(\frac{Q_c}{Q}\right)^{\frac{1}{3}}$, and introducing the notation $\alpha = \left(\frac{Q_c}{Q}\right)^{\frac{1}{3}}$ to alleviate the equations, the position of any blob can be expressed simply by iteration:

$$r_k = r_{k-1} + 2\xi(r_{k-1}), \quad (\text{S1})$$

and their size:

$$\xi(r_k) = \xi(r_{k-1})(1 + 2\alpha) \quad (\text{S2})$$

$$= \xi(R_p)(1 + 2\alpha)^{k-1}. \quad (\text{S3})$$

The real length is then

$$L_r = \sum_{k=1}^{N_b} 2\xi(r_k) \quad (\text{S4})$$

$$= R_p \left((1 + 2\alpha)^{N_b} - 1 \right). \quad (\text{S5})$$

The number of blobs can be computed from the conservation of the number of monomers, from the number of monomers per blobs $g_k = \left(\frac{\xi}{a}\right)^{\frac{5}{3}}$:

$$N = \sum_{k=1}^{N_b} g_k \quad (\text{S6})$$

$$= \left(\frac{\xi(R_p)}{a}\right)^{\frac{5}{3}} \sum_{k=1}^{N_b} \left[(1 + 2\alpha)^{\frac{5}{3}} \right]^{k-1} \quad (\text{S7})$$

$$= \left(\frac{\xi(R_p)}{a}\right)^{\frac{5}{3}} \frac{1 - (1 + 2\alpha)^{\frac{5}{3}N_b}}{1 - (1 + 2\alpha)^{\frac{5}{3}}}. \quad (\text{S8})$$

Then

$$N_b = \frac{3}{5} \frac{\log \left(1 - \left(\frac{R_g}{\alpha R_p} \right)^{\frac{5}{3}} \left(1 - (1 + 2\alpha)^{\frac{5}{3}} \right) \right)}{\log(1 + 2\alpha)}. \quad (\text{S9})$$

The numerical application from Tab. 1 in the main document was obtained by combining equations S5 and S9.

S3: Complementary information for the structure and characterisation of the synthetic polymers

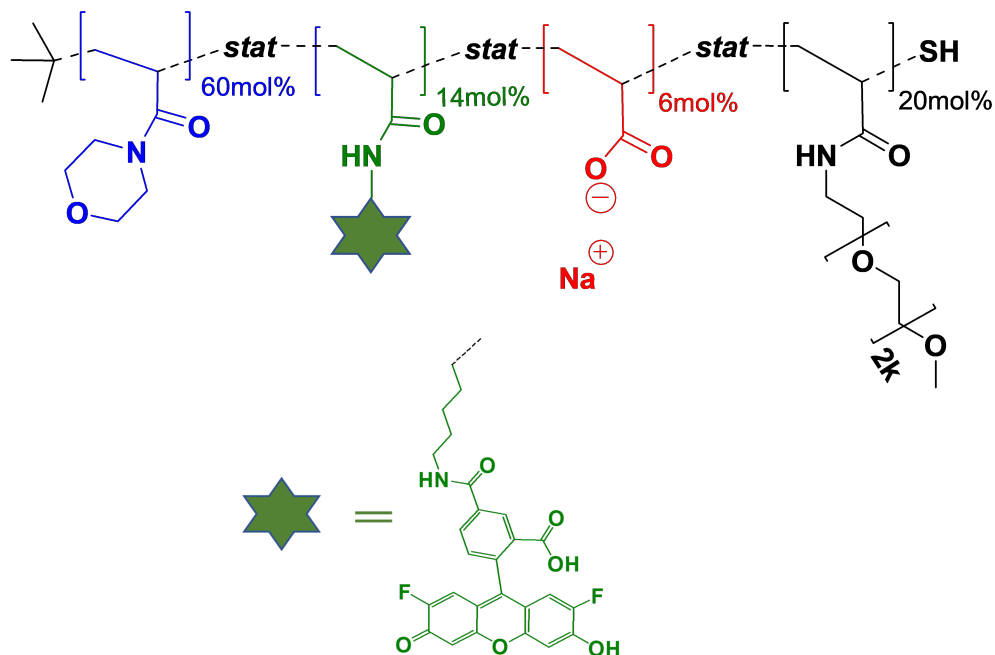


Fig. S1 Structure and composition of the synthetic polymers PolyHD and PolyLD, as well as the structure of the Oregon green fluorophore.

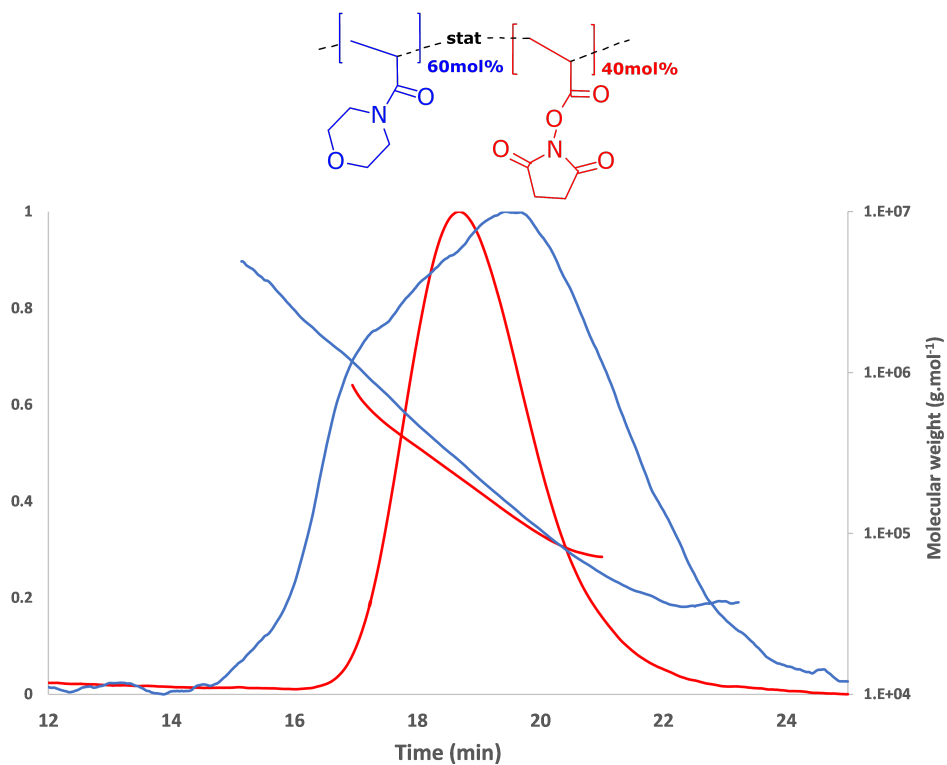


Fig. S2 SEC-MALLS chromatograms of the poly(NAM-stat-NAS) copolymers (structure in insert) synthesized by conventional radical polymerization (blue, high dispersity) and by RAFT controlled radical polymerization (red, low dispersity).

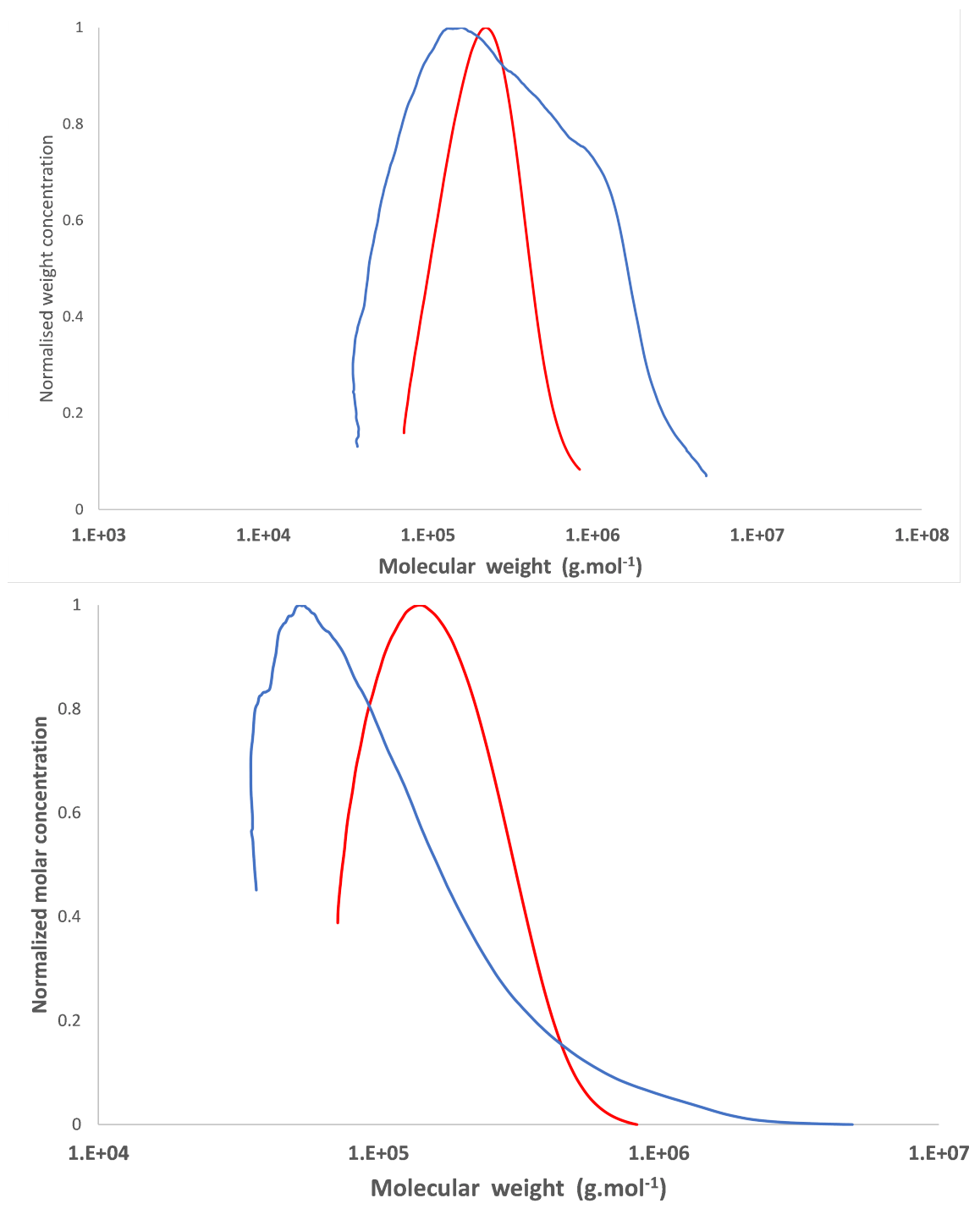


Fig. S3 Normalized weight (Top) and molar (Bottom) vs. molecular weight distribution determined by SEC-MALLS for the poly(NAM-stat-NAS) copolymers synthesized by conventional radical polymerization (blue, high dispersity) and by RAFT controlled radical polymerization (red, low dispersity).

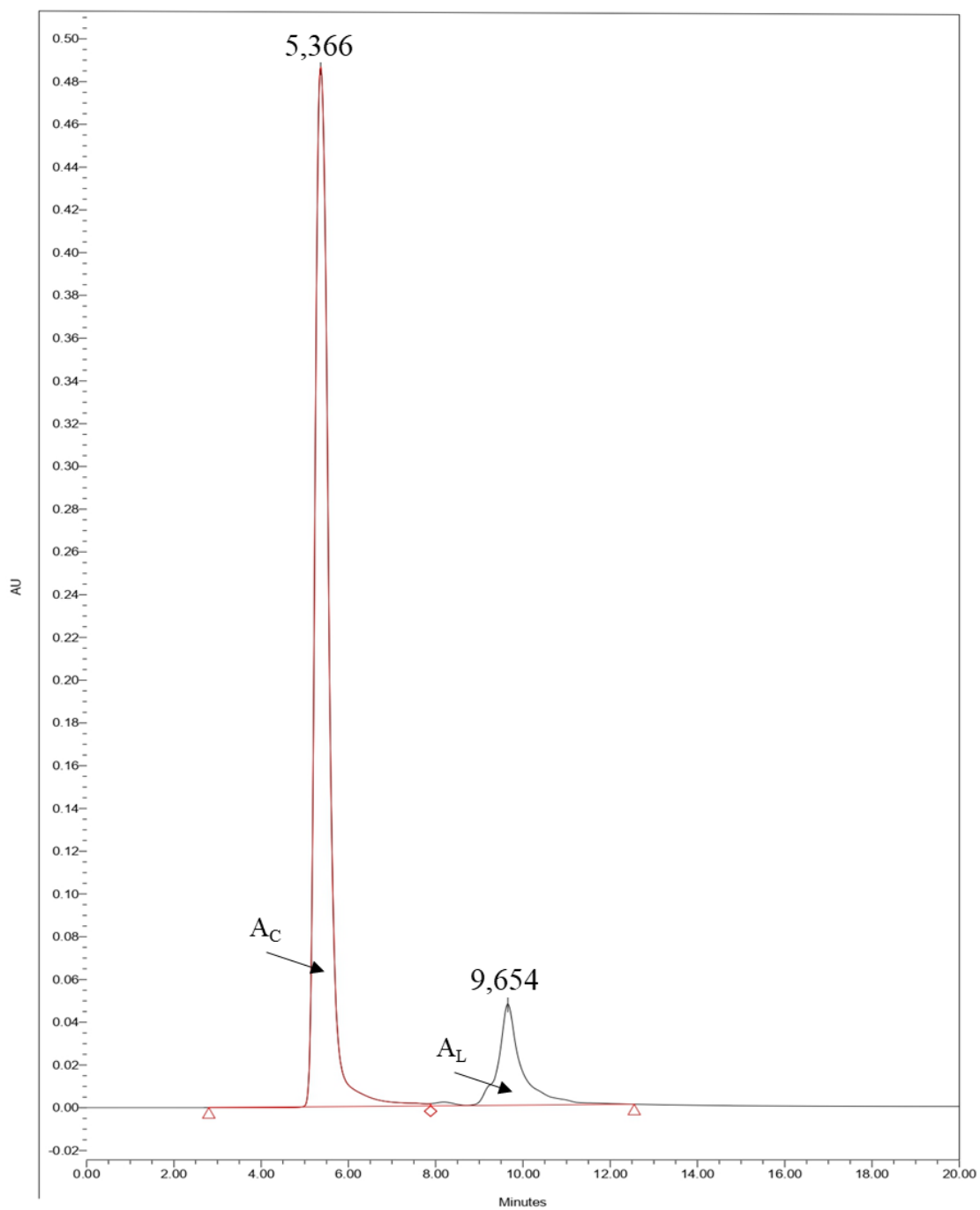


Fig. S4 SEC-UV analysis at 488 nm of the Oregon green fluorophore coupling onto the poly(NAM-stat-NAS) copolymer. AC and AL are the peak areas corresponding respectively to the fluorophore coupled onto the polymer (left peak) and to the free unbound fluorophore (right peak). Coupling yield = $AC / (AC + AL)$.

S4: Complementary discussion on the behaviour of small polymers and the influence of diffusion

As described in the main text, the translocation time of the smaller DNA molecules ΦX and pNEB was slower than predicted by the theoretical model. Experimental observations reported in Fig. 6 of the main text suggested that the model accurately captured the scaling law dependency of the translocation time with the flow rate Q , but it appeared to overlook an effect associated with the size of the polymers.

This effect is likely related to the fact that the theoretical model neglects the effect of diffusion which plays a more substantial role in the transport of smaller molecules ($Pe < 1$). However, understanding this effect is intricate, as one might expect diffusion to favour faster transport for smaller molecules, contrary to observations in the experiments. We suspect that diffusion may increase the number of accessible trajectories at the exit of a nanopore, including trajectories close to the membrane, potentially enabling interactions between the molecule and the surface. This scenario is discussed here as a way to provide some insight into possible future experimental and theoretical work.

A plausible scenario for the effect of diffusion would be the following:

1. At the exit of a nanopore, the flow streamlines extend in various directions, ranging from perpendicular to parallel to the membrane.
2. In the absence of diffusion, a molecule at the nanopore exit is directed by the flow and follows the shortest trajectory, which is perpendicular to the membrane. This trajectory has been considered in the transport model described earlier for the computation of t_{ex} and t_{ej} .
3. As diffusion becomes significant compared to transport by advection, the molecule's position becomes randomized inside and at the nanopore exit. This randomness leads to the molecule following alternative streamlines, resulting in a longer trajectory for exiting the nanopore or reaching the ejection plane.
4. Their path may also keep them closer to the surface of the membrane, favouring interactions that might further slow down the polymer.

In that scenario, the diffusion causes the molecules to take longer times to be transported through the illumination regions.

For future work, based on these experimental observations, we suggest that constructing a theoretical framework that includes the effect of diffusion may require focusing on combining two physical phenomena: (i) the distribution of accessible trajectories for a small polymer at the exit of a nanopore of finite size, based on the known hydrodynamic flow field¹; and (ii) the computation of a friction coefficient between a polymer and a surface. The latter has been previously investigated^{2,3}, particularly in the case of voltage-driven DNAs in very small nanopores when the hydrodynamic flow field can be neglected. An in-depth understanding of this phenomenon will require further investigations that go beyond the scope of the present study.

S5: Complementary data for the measure of the dispersity of synthetic polymer samples

Note that the average intensities of the distribution are strongly dependent on the experiment (laser intensity, background intensity, noise), but that the measures of the distribution are not.

Table 1 Measurement of the intensity dispersity for independent realisation (different days, different membranes) of the translocation of PolyLD grafted with PEG, or not grafted, through a membrane of nanopores $R_p = 45$ nm, as described in the main text.

Exp. Number	1	2	3	4	5	6
PEG grafting	no	no	no	yes	yes	yes
I_n ($\times 10^3$)	3.93	2.25	1.95	4.10	2.07	1.73
I_w ($\times 10^3$)	4.69	2.60	2.24	4.75	2.60	1.99
\mathfrak{D}_I	1.19	1.15	1.15	1.16	1.26	1.15

By analogy with the quantities commonly used from the molar mass distribution, we define the mean intensity in number $I_n = \frac{1}{N} \sum_N I$, the mean intensity in weight $I_w = \frac{1}{N} \sum_N I^2 / \sum_N I$ and the intensity dispersity $\mathfrak{D}_I = \frac{I_w}{I_n}$.

Table 2 Measurement of the intensity dispersity for independent realisation (different days, different membranes) of the translocation of PolyHD grafted with PEG, or not grafted, through a membrane of nanopores $R_p = 45$ nm, as described in the main text.

Exp. Number	1	2	3	4	5	6
PEG grafting	no	no	yes	yes	yes	yes
I_n ($\times 10^3$)	1.37	3.52	2.24	2.60	3.90	1.95
I_w ($\times 10^3$)	1.54	5.51	3.77	4.28	4.96	2.67
\mathfrak{D}_I	1.13	1.57	1.68	1.65	1.27	1.37

By analogy with the quantities commonly used from the molar mass distribution, we define the mean intensity in number $I_n = \frac{1}{N} \sum_N I$, the mean intensity in weight $I_w = \frac{1}{N} \sum_N I^2 / \sum_N I$ and the intensity dispersity $\mathfrak{D}_I = \frac{I_w}{I_n}$.

Notes and references

- 1 Z. Dagan, S. Weinbaum and R. Pfeffer, *Journal of Fluid Mechanics*, 1982, **115**, 505.
- 2 M. Wanunu, J. Sutin, B. McNally, A. Chow and A. Meller, *Biophysical Journal*, 2008, **95**, 4716–4725.
- 3 J. Sarabadani, T. Ikonen, H. Mökkönen, T. Ala-Nissila, S. Carson and M. Wanunu, *Scientific Reports*, 2017, **7**, 7423.