Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Mechanical behavior of contractile gels based on light-driven molecular motors

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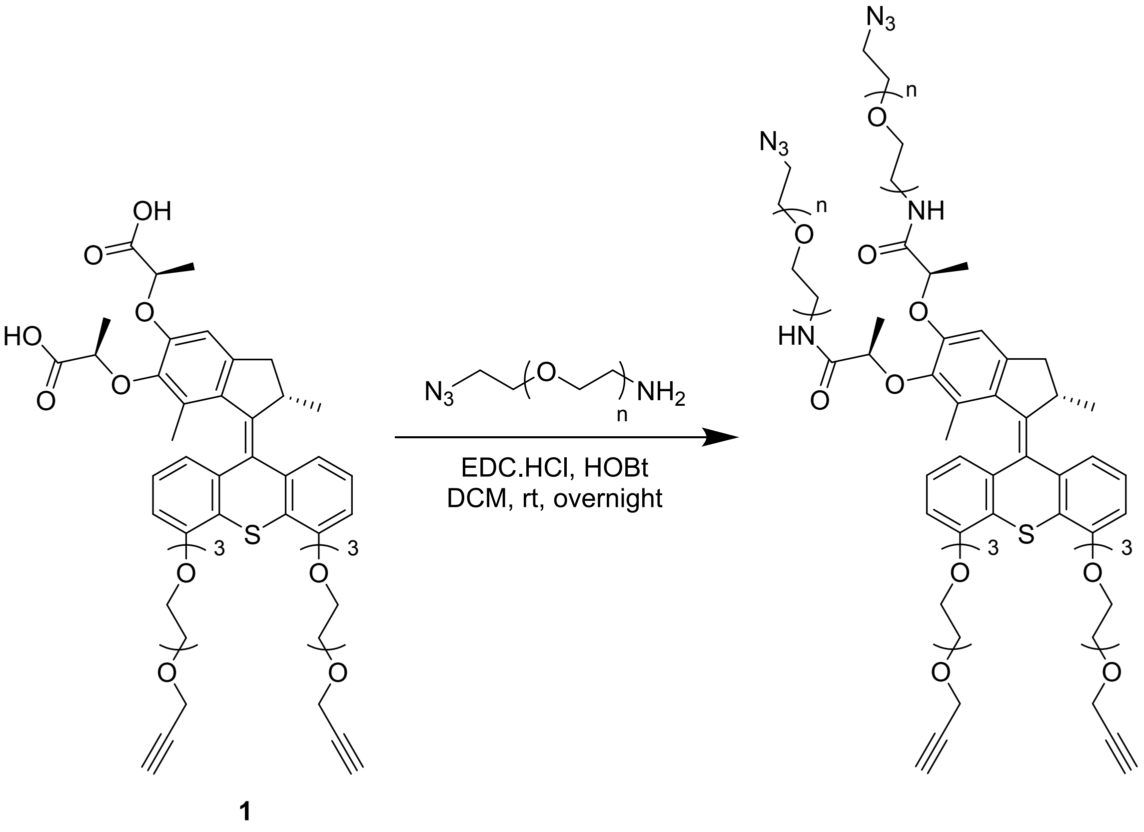
1. *General Methods*

All reagents and solvents were purchased at the highest commercial quality and used as received (Sigma Aldrich, Acros and TCI). N_3-PEG$_{3000}$-NH_2 (M_w= 3073 Da), N_3-PEG$_{5000}$-NH_2 (Mw= 4790 Da), N_3-PEG$_{10000}$-NH_2 (M_w=10526 Da) and N_3-PEG$_{20000}$-NH_2 (M_w=17479 Da) were purchased from Iris Biotech. All reactions were carried out under an Argon atmosphere with dry solvents unless otherwise noted. Dry solvents were obtained using a double column SolvTech purification system. Water was deionized by using a milli-gradient system (Millipore, Molsheim, France). Yields refer to purified spectroscopically (^{1}H NMR) homogeneous materials. Ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) was recorded on a Waters Acquity UPLC-SQD machine equipped with a PDA detector (190-500 nm, 80 Hz), using a reverse phase column (Waters, BEH C18 1.7 µm, 2.1 × 50 mm), MassLynx 4.1 – XP software for analysis, and a water/acetonitrile/0.1% formic acid mixture gradient as eluent. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer with working frequencies of 400 MHz for ^{1}H nuclei. ^{1}H NMR spectra were internally referenced to the residual proton solvent signal (CD_3OD: 3.31 ppm) and all chemical shifts are given in ppm. Coupling constants J are listed in Hz. The following notation is used for the ^{1}H NMR spectral splitting patterns: singlet (s), doublet (d), triplet (t), quadruplet (q), multiplet (m), large (l). Irradiation experiments were performed using a Vilber model VL-6.LC lamp at λ = 366 nm. The gel's contractions were followed by video and were recorded using VideoVelocity Free software and USB connected camera. Screenshots from the video of the gel's contraction were taken at different times during the irradiation and the surface area of the gels was measured using ImageJ software. The relative percent volume was then estimated by comparison of the initial surface area (S*i*) and of the surface area at time = t (S*t*) and the exponent scaling factor: $(S_t/S_i)^{3/2}$ assuming an isotropic contraction event. This isotropic contraction was verified on several samples before assuming it is true for all of them. We measured the change in thickness of the film using a micrometer screw gauge mounted on the piezoelectric rheometer. The thickness was determined for the minimum distance leading to a measurable rheological effect.

2. *Synthesis of motor-polymer conjugates*

Motor **1** was prepared using literature protocols.[30]



Supplementary Scheme 1 | Synthesis of bis-azide bis-alkyne motors.

Synthesis of Motor-PEG Conjugate:

PEG_{3000} conjugate (n=67): To a solution of bis-acid motor (10 mg, 0.011 mmol) in CH_2Cl_2 (0.4 mL) was added HOBt (5 mg, 0.028 mmol) and EDC (18 mg, 0.090 mmol) at room temperature. After 15 min, a solution of N_3-PEG_{3000}-NH_2 (M_w= 3073 g.mol^{-1}, 68 mg, 0.022 mmol) in CH_2Cl_2 (0.6 mL) was added. The mixture was stirred at room temperature for 21 h. After removal of the solvent, the residue was then purified by reversed phase column chromatography (C18, Merck, MeOH:water=3:7→8:2) affording the product as a slightly white solid (30 mg, 38 % yield).

^{1}H NMR (CD_3OD, 400 MHz, 298 K) δ 7.41 (d, *J*=7.6 Hz, 1H), 7.32 (t, *J*=8.0 Hz, 1H), 7.05 (t, *J*=8.0 Hz, 1H), 6.94 (d, *J*=8.0 Hz, 1H), 6.89 (d, *J*=8.4 Hz, 1H), 6.75 (s, 1H), 6.65 (d, *J*=7.2 Hz, 1H), 4.73 (q, *J*=6.8 Hz, 1H), 4.43 (q, *J*=6.8 Hz, 1H), 4.34-4.16 (m, 7H), 3.98-3.94 (m, 2H), 3.84-3.79 (m, 6H), 3.74-3.36 (m, 559H), 2.86 (t, *J*=2.4 Hz, 1H), 2.85 (t, *J*=2.4 Hz, 1H), 2.42 (d, *J*=15.2 Hz, 1H), 1.54 (d, *J*=6.8 Hz, 3H), 1.30 (d, *J*=6.8 Hz, 3H), 1.18 (s, 3H), 0.63 (d, *J*=6.8 Hz, 3H).

Motor-PEG$_{5000}$ conjugate (n=107): To a solution of bis-acid motor (15.1 mg, 0.0170 mmol) in CH_2Cl_2 (0.4 mL) was added HOBt (7.2 mg, 0.052 mmol) and EDC (24.5 mg, 0.128 mmol) at room temperature. After 15 min, a solution of N_3-PEG$_{5000}$-NH_2 (Mw= 4790 g.mol^{-1}, 170.8 mg, 0.0357 mmol) in CH_2Cl_2 (0.6 mL) was added. The mixture was stirred at room temperature over the weekend. After removal of the solvent, the residue was then purified by reversed phase column chromatography (C18, Merck, MeOH:water=3:7→8:2) affording compound as a slightly white solid (89 mg, 50 % yield).

1H NMR (CD_3OD, 400 MHz, 298 K) δ 7.41 (d, *J*=7.6 Hz, 1H), 7.32 (t, *J*=8.0 Hz, 1H), 7.05 (t, *J*=8.0 Hz, 1H), 6.94 (d, *J*=8.0 Hz, 1H), 6.89 (d, *J*=8.0 Hz, 1H), 6.76 (s, 1H), 6.65 (d, J=7.6 Hz, 1H), 4.73 (q, *J*=6.8 Hz, 1H), 4.43 (q, *J*=6.8 Hz, 1H), 4.34~4.16 (m, 9 H), 3.98-3.94 (m, 4H), 3.84-3.79 (m, 12H), 3.74-3.36 (m, 869H), 2.85 (t, *J*=2.4 Hz, 1H), 2.84 (t, *J*=2.4 Hz, 1H), 2.42 (d, *J*=15.2 Hz, 1H), 1.54 (d, *J*=6.8 Hz, 3H), 1.30 (d, *J*=6.8 Hz, 3H), 1.18 (s, 3H), 0.63 (d, *J*=6.4 Hz, 3H).

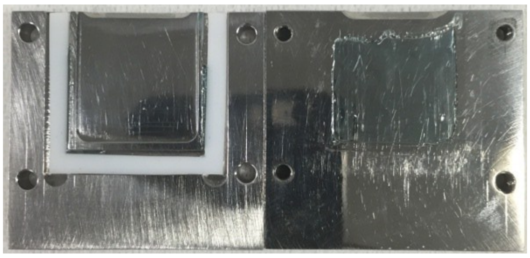
Motor-PEG$_{10000}$ conjugate (n=237): To a solution of bis-acid motor (10 mg, 0.0112 mmol) in CH_2Cl_2 (0.4 mL) was added HOBt (5 mg, 0.028 mmol) and EDC (18 mg, 0.09 mmol) at room temperature. After 15 min, a solution of N_3-PEG$_{10000}$-NH_2 (M_w=10526 g.mol^{-1}, 236 mg, 0.022 mmol) in CH_2Cl_2 (0.6 mL) was added. The mixture was stirred at room temperature overnight. After removal of the solvent, the residue was then purified by reversed phase column chromatography (C18, Merck, MeOH:water=3:7→8 :2) affording compound as a slightly white solid (90 mg, 36 % yield).

1H NMR (CD_3OD, 400 MHz, 298 K) δ 7.41 (d, *J*=7.6 Hz, 1H), 7.32 (t, *J*=8.0 Hz, 1H), 7.05 (t, *J*=8.0 Hz, 1H), 6.94 (d, *J*=8.0 Hz, 1H), 6.89 (d, *J*=8.0 Hz, 1H), 6.76 (s, 1H), 6.65 (d, *J*=7.6 Hz, 1H), 4.74 (q, *J*=6.8 Hz, 1H), 4.43 (q, *J*=6.8 Hz, 1H), 4.34-4.16 (m, 12 H), 3.96-3.36 (m, 1922H), 2.87 (t, *J*=2.4 Hz, 1H), 2.86 (t, *J*=2.4 Hz, 1H), 2.42 (d, *J*=15.2 Hz, 1H), 1.54 (d, *J*=6.8 Hz, 3H), 1.30 (d, *J*=6.8 Hz, 3H), 1.18 (s, 3H), 0.63 (d, *J*=6.4 Hz, 3H).

Motor-PEG$_{20000}$ conjugate (n=396): To a solution of bis-acid motor (5 mg, 0.0056 mmol) in CH_2Cl_2 (0.4 mL) was added HOBt (2.5 mg, 0.014 mmol) and EDC (9 mg, 0.045 mmol) at room temperature. After 10 min, a solution of N_3-PEG$_{20000}$-NH_2 (M_w=17479 g.mol^{-1}, 196 mg, 0.011 mmol) in CH_2Cl_2 (1 mL) was added. The mixture was stirred at room temperature for 1 h. Then another portion of HOBt (2.5 mg, 0.014 mmol), EDC (9 mg, 0.045 mmol) and N_3-PEG$_{20000}$-NH_2 (10 mg) was added. After another 3.5 h, the solvent was removed in vacuum and the residue was then purified by reversed phase column chromatography (C18, Merck, MeOH:water=3:7→8:2) affording the product as a slightly white solid (106 mg, 53 % yield).

^{1}H NMR (CD_3OD, 400 MHz, 298 K) δ 7.41 (d, *J*=7.6 Hz, 1H), 7.31 (t, *J*=8.0 Hz, 1H), 7.05 (t, *J*=8.0 Hz, 1H), 6.94 (d, *J*=8.4 Hz, 1H), 6.89 (d, *J*=8.0 Hz, 1H), 6.76 (s, 1H), 6.65 (dd, *J*=7.2, 0.8 Hz, 1H), 3.80-3.40 (m, 3208H), 2.83 (t, *J*=2.4 Hz, 1H), 2.82 (t, *J*=2.4 Hz, 1H), 2.42 (d, *J*=15.2 Hz, 1H), 1.54 (d, *J*=6.8 Hz, 3H), 1.30 (d, *J*=6.8 Hz, 3H), 1.18 (s, 3H), 0.63 (d, *J*=6.8 Hz, 3H).

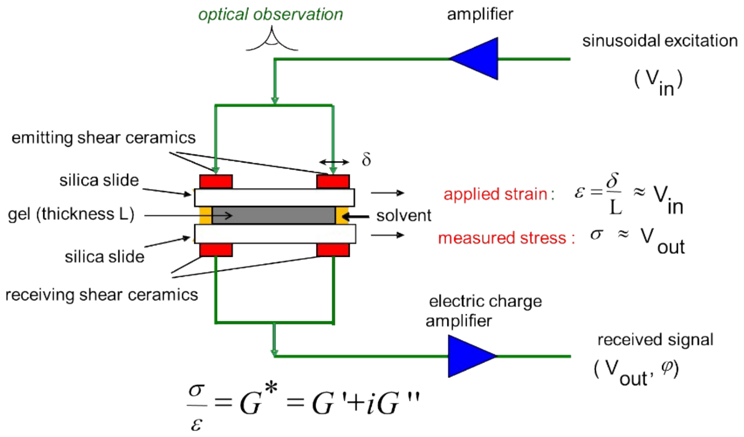
Gels preparation: Every gel was prepared in a home-made metal mold (2 cm×2 cm×458 µm, See Supplementary Figure S1). Typical procedure: in a vial, the motor-polymer conjugated was dissolved in DMF (~0.2 mL, close to the volume of the mold. Also, it needs to adjust this volume according to the volume of PMDETA. In any case, $V_{DMF}+V_{PMDETA}=0.2$ mL). Then CuBr (2 eq.), PMDETA (2 eq.) was added into this solution. This mixture was then transferred into the mold using a pipette. The mold was then heated up to 80 °C in a sand path for 30 min and cooled down to room temperature for another 30 min. After unmolding, a picture of the surface of the gel was taken in order to measure its surface. The formed gel was washed with EDTA, EtOH and water extensively until there is no large aggregates by checking the optical microscopy. Using acetonitrile, the gel was brought into toluene gradually. Another picture of the gel was taken after the final swelling.



Supplementary Figure 1. Unmolding of a gel (on the right) after formation.

3. *Rheological measurements*

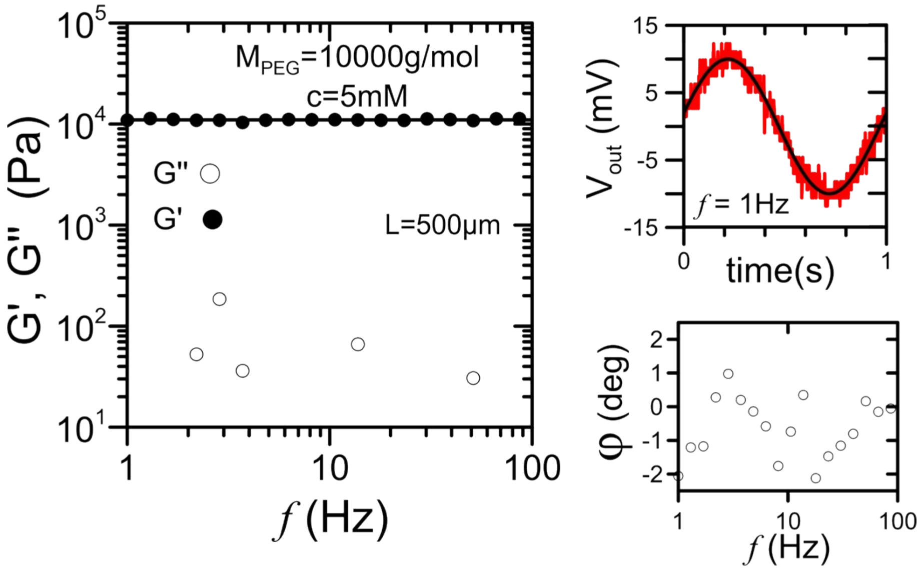
Typical procedure for rheological measurements: From the pictures of the gel's surfaces taken after formation and after swelling was calculated the thickness of the sample. The gel was then placed in a piezo-rheometer, the space between the slides was adjusted from the calculated thickness. In order to avoid the drying of the sample during the shear measurements, a small volume of toluene was introduced between the slides all around the sample. The measurements were performed at frequencies ranging from 1 Hz to 100 Hz at room temperature.



Supplementary Figure 2. Block diagram of the piezo-rheometer.

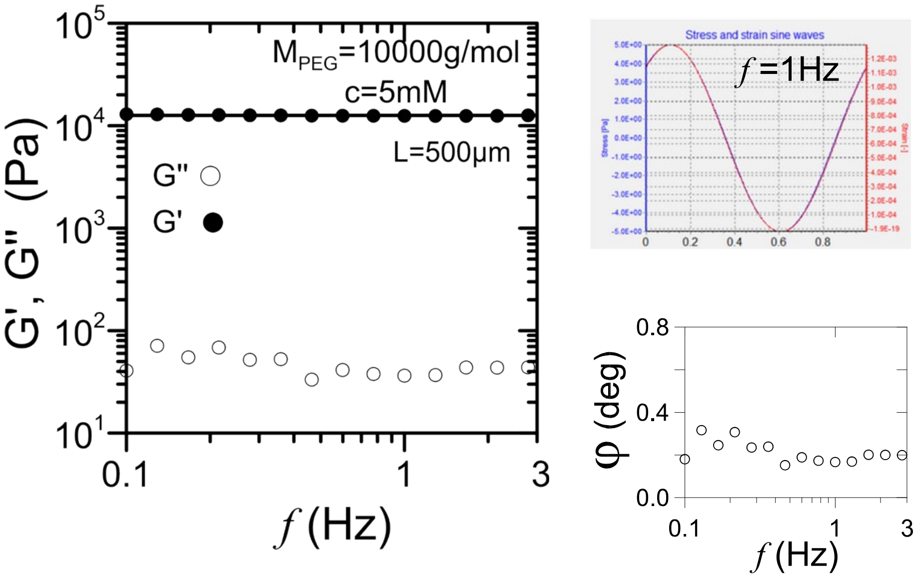
<u>Details on the instrument:</u> The mechanical response of the gels was determined using a shear piezo-rheometer. This rheometer, which induces the sample deformation by a translational movement of one of the bearing plate, allows shear measurements to be carried out on a sample whose area does not necessarily cover the full area of the bearing plates, unlike the usual rheometers. By choosing this device, data can thus be acquired on the same sample, before and after contraction of the gel. However, this rheometer working with piezoelectric elements do not allow applying high deformations. For thick samples (~ 500 µm thick), the

strain applied ε cannot excess $2x10^{-4}$ and therefore this rheometer cannot detect with accuracy a phase lag φ for soft solids. Under these conditions, the weak signal delivered by the receiving piezoelectric elements (Supplementary Figure 3) lead to φ-values close to zero, and randomly distributed in between -2° and + 2° with the frequency. The low negative φ-values does not mean that the G”-values are negative but indicates that these ones lie beyond the device's accuracy and are too weak to be measured. From this piezo-rheometry experiment, an estimation of the G”-value can however be done. The G”-values lies necessarily below ~ 2% of the G’-values, *i.e.* $G" \leq 2x10^2$ Pa.



Supplementary Figure 3. Variation of G', G” and signal phase shift of a non-irradiated sample as a function of frequency measured on a piezo-rheometer.

To go further, shear measurements were performed on the same sample with two different devices: the piezo-rheometer and a conventional rheometer (Haake, Mars III). As expected, the results obtained with the conventional device (Supplementary Figure 4) lead to the same G’-value. For G”, the higher deformation ($\varepsilon \sim 10^{-3}$) applied with this one enables to determine with accuracy the φ-values. This φ-value is lower than 0.5° and lead to G”-values of a tens of Pascal. This result is in good agreement with our previous estimation based on piezo-rheometry experiment ($G" < 2\ 10^2$ Pa). The rheological response of these molecular motor gels is therefore consistent, in the range of our accuracy, with the response observed in the low frequency regime for chemically crosslinked gels.[S1]



Supplementary Figure 4. Variation of G', G” and signal phase shift of a non-irradiated sample as a function of frequency measured on a classical rheometer.

4. *Irradiation of gels*

Typical procedure for gel irradiation: Gel irradiation studies were conducted in dry, degassed toluene. The gel was swollen in a glass vial filled with toluene. All gel manipulations were performed in a room with fluorescent light bulbs emitting over 400 nm so as not to activate the rotation of the motor. The sample was placed 1 cm above a 6W UV lamp (to avoid any heating) equipped with a tube centered around 365 nm (maximum of absorption of the motor), with a camera on top of the vial. The gel contraction was followed by video and stopped when a maximum of contraction was reached (see supplementary video for a typical contraction of a 1 cm^2 piece of gel observed over a 2 hours UV irradiation).

Supplementary Figure 5. Setup for recording gel contraction. See a typical recording of the contraction process in the supplementary video furnished.

5. *Small Angle Neutron Scattering (SANS)*

SANS experiments were performed on the D11 beamline at Institut Laue-Langevin at Grenoble (ILL, France). An incident wavelength, λ, of 6 Å was used for 3 sample-to-detector distances (1.4, 8 and 40 m), allowing to access a total range for the magnitude of the scattering vector q varying between 0.003 $Å^{-1}$ and 0.3 $Å^{-1}$. Data were corrected for empty cell scattering, electronic background and detector uniformity and then converted into absolute scale (cm^{-1}) using normalization by the attenuated direct beam classical method.

The scattering vector q is defined as:

$$q = \frac{4\pi}{\lambda} sin\frac{\theta}{2} \tag{5}$$

Where, θ is the scattering angle. The absolute neutron scattering intensity, I in cm^{-1}, combines the form factor of the scattered objects, $P(q)$, and the interparticles scattering factor, $S_2(q)$:

$$I(q) = \frac{1}{V}\frac{d\sigma}{d\Omega} = (\Delta\rho)^2(V_{scattered\ objects}\Phi_{Vol}P(q) + S_2(q)) \tag{6}$$

Where $\Delta\rho$ is the contrast difference per unit volume between the scattered objects and the solvent, $V_{scattered\ objects} = N\,v\,m \times 1.66 \times 10^{-24}$ is the volume of the N monomers or unimers with mass m in an object (e.g. N monomers in a polymer chain), v their specific volume, and Φ_{Vol} their volume fraction. The respective scattering length densities are given in Table S1.

When the scattering length scale is larger than the overall size of the scattered objects, the experimental curve has been analyzed using the Guinier approximation. The intensity can then be described by:

$$I(q) = I_0 e^{-\frac{q^2 R_g^2}{3}} \tag{7}$$

where R_g is the gyration radius of the objects.

The total scattering curve of the gels made by rotary motors (see Figure 4) has been fitted according to the following equation:

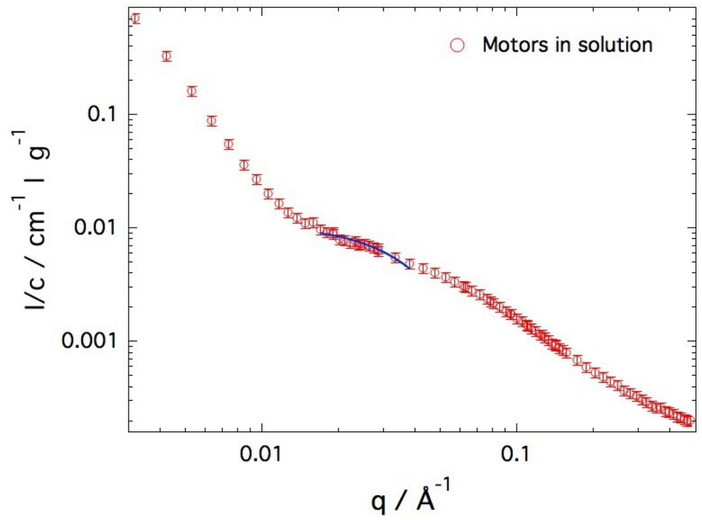
$$I(q) = I(0)_L \frac{1}{(1 + (D + 1/3)q^2 a_1^2)} + I(0)_G e^{-\frac{q^2 a_2^2}{2}} + B \quad (8)$$

Two contributions ($I(0)_L$ and $I(0)_G$) have been considered to take in account two characteristic length scales: a shorter correlation length a_1 representing the mesh size of the polymer network, and a longer one a_2 representing larger structures such as heterogeneities. The latter is described by a simple Guinier function: $a_2^2 = \frac{R_g^2}{3}$, while the first is described by a Lorentzian function. B is the background and D is the fractal exponent.

	Density / g cm^{-3}	SLD / 10^{-6} Å^{-2}	$\Delta\rho^2$ / 10^{-11} Å^{-4}	
Total	1.1	0.711	2.45	$C_{540}H_{1040}O_{256}N_8S$
PEG chain	1.126	-0.492	3.80	C_2H_6O
Central part of rotary motor	1.1	1.93	1.39	$C_{30}H_{26}O_4N_2S$
d-Toluene	0.943	5.664	-	C_7D_8

Supplementary Table 1. Densities, scattering length densities, and contrast per unit volume of the studied system, the PEG chain, the central unit of the motor, and solvent.

Supplementary Figure 6 displays the scattering profile of PEG$_{5000}$ dilute motors before the click reaction. The intermediate q range can be described by a Guinier variation associated with the finite mass and size of the non-connected and isolated motors. The best fit of the data using equation S3 gives a radius of gyration equal to $R_g = 4.3$ nm. The low q upturn is due to a population of aggregated motors. At high q the signal arises from the polymer chain, see main text.



Supplementary Figure 6: Scattering profile of a solution of isolated motors before the click reaction. The continuous line represents the best fit of the data using a Guinier model. Concentration is 43.6 g/L.

6. Additional references

[S1] S. K. Patel, S. Malone, C. Cohen, J. R. Gillmor, R. H. Colby, *Macromolecules* **1992**, *25*, 5241.