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Supporting Information

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Radical Polymerization tracked by Single Molecule Spectroscopy

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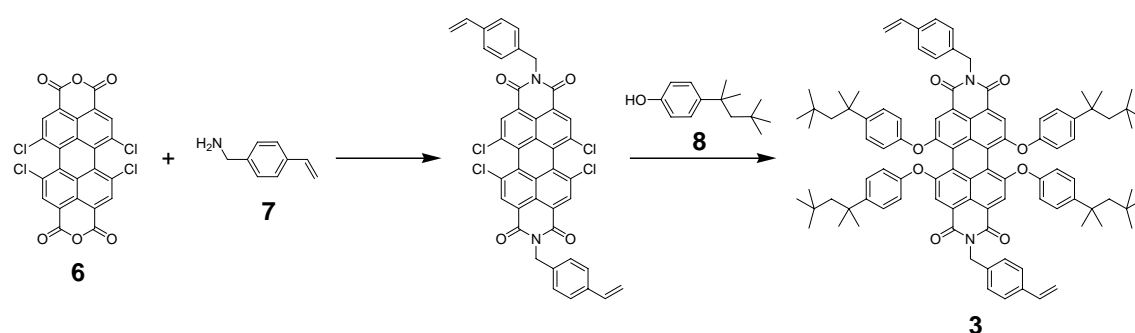
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1. Synthesis

Synthesis of N,N'-(4-vinylbenzyl)-1,6,7,12-tetrakis[4'-(1'',1'',3'',3''-tetramethylbutyl)phenoxy]perylene-3,4:9,10-tetracarboxydiimide (3)

General: Unless stated otherwise, all reagents were obtained from commercial sources and were used without further purification. The solvents were used in HPLC grade purity as purchased. ¹H- and ¹³C-NMR spectra were recorded on a Bruker AC300 NMR spectrometer. Field desorption (FD) mass spectra were recorded on a VG-Instruments ZAB 2-SE-FDP using 8 kV accelerating voltage. UV/Vis absorption spectra were taken on a Perkin-Elmer Lambda 9.



Scheme S1. Synthesis of **3**.

1,6,7,12-Tetrachloro-3,4:9,10-perylene-tetracarboxydiimide (**6**, 2.65 g, 5.00 mmol) and (4-vinylphenyl)methanamine^[S1] (**7**, 4.00 g, 30.00 mmol) were dissolved in propanoic acid (60 mL). After stirring for 16 h at 150 °C, the reaction mixture was allowed to cool to room temperature and water was added. The precipitated product was filtered under suction, washed thoroughly with water and dried under vacuum. Without further purification, a part of the crude product (3.04 g), *tert*-octylphenol (**8**, 8.24 g, 40.0 mmol) and K₂CO₃ (2.80 g, 20.0 mmol) were dissolved in *N*-methylpyrrolidone (100 mL). After stirring for 16 h at 90 °C under argon atmosphere the reaction mixture was allowed to cool to room temperature and poured into aqueous hydrochloric acid (1 L, 2 M). The precipitated product was filtered under suction, washed thoroughly with water and dried under vacuum. The product was purified by column chromatography on silica gel with petrol ether/CH₂Cl₂ (1/1) as eluent to give **3** (1.93 g, 26%) as a red solid.

UV/VIS (CHCl₃): λ_{max} (ε) 588 nm (39945 M⁻¹ cm⁻¹), 547 nm (23828 M⁻¹ cm⁻¹), 451 nm (15003 M⁻¹ cm⁻¹)

¹H-NMR (300 MHz, CD₂Cl₂, 298 K): δ 8.09 (s, 4 H), 7.37 – 7.28 (m, 16 H), 6.87 (d, ³J = 8.7 Hz, 8 H), 6.68 (dd, ³J = 10.9 Hz, ³J = 17.6 Hz, 2H), 5.60 (dd, ³J = 17.6 Hz, ²J = 0.9 Hz, 2 H), 5.16 (s, 4 H), 5.10 (dd, ³J = 10.9 Hz, ²J = 0.9 Hz, 2 H), 1.66 (s, 8 H), 1.28 (s, 24 H), 0.69 (s, 36 H).

¹³C-NMR (75 MHz, CD₂Cl₂, 298 K): δ 163.6, 156.6, 153.2, 147.2, 137.4, 137.2, 136.8, 133.1, 129.0, 128.1, 126.5, 122.9, 120.6, 120.0, 119.9, 119.7, 114.0, 57.3, 43.7, 38.7, 32.7, 32.0, 31.7

IR (KBr pellet, cm⁻¹): ν = 3038, 2952, 2902, 1701, 1661, 1587, 1502, 1433, 1414, 1352, 1286, 1214, 1171, 1019, 926, 835, 572

MS (FD, 8kV) m/z (%): 1439.8 (100%) [M⁺] (calcd for C₉₈H₁₀₆N₂O₈ 1439.9)

2. Materials

Polymerization experiments

Styrene (>99%) was purchased from Aldrich and purified by distillation under reduced pressure. 1,4-Divinylbenzene (95%, **5**) was purchased from Sigma and used without further purification. The radical initiator **4** (V70) was obtained from Wako Chemicals and used without further purification. Toluene (99+%) was purchased from Acros.

In a typical experiment, 1% (w/w) of **4** and **5** (0%, 1% or 3% w/w) were dissolved in styrene (ca. 2 g), and one drop of dye solution in toluene added so that the final dye concentration was between 10^{-8} M and 10^{-9} M. The solution was subjected to four freeze-pump-thaw cycles to remove oxygen and was kept under nitrogen. For measurements, samples at different stages of polymerization were spotted on a glass slide. At the point when the solution became very viscous, one drop of the sample was closely sandwiched between two glass slides with a Teflon spacer (100 μ m thick) and the polymerization reaction was allowed to proceed in this device.

The initiation rate constant in all experiments was $k_{\text{ini}} = 1.5 \times 10^{-5} \text{ s}^{-1}$ and equals a decay time of 19 h. Therefore, it took several hours for the equilibrium radical concentration to be reached. The propagation and termination rate constant of styrene at room temperature are $k_p = 69 \text{ M}^{-1} \text{ s}^{-1}$ and $k_t = 8.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, respectively^[2]. After the initial stage, instead of slowing down, U increased linearly. The gel effect, even though not very pronounced in the polymerization of styrene, was responsible for this phenomenon. This is in accordance with the literature for the polymerization of styrene at 70 °C^[20]. Finally, at $U > 0.90$, the polymerization rate slows down because the monomers become less accessible.

For the experiment with no crosslinker the synthesized polymer was analysed by gel permeation chromatography. The number and average weighted molecular mass of the synthesized polymer was 71571 g mol^{-1} and $412165 \text{ g mol}^{-1}$, respectively, which resulted in a polydispersity of 5.76.

IR analysis for determination of the conversion U

For a comparison between the performed experiments, it was essential to determine the conversion U , defined as the number of polymerized styrene molecules divided by the total number of (unreacted and polymerized) styrene molecules. FT-IR spectroscopy was used for this purpose, even though other methods like NIR spectroscopy and gravimetry are also described in the literature^[20]. Samples taken from the polymerizing solution were sandwiched between two NaCl-crystals and their IR spectra recorded. The evolution of the IR absorbances of the C-H stretch vibrations for the different sets of experiments described in the main text is shown in Figure S1. The polymerization of styrene results in IR bands appearing at 2849 cm^{-1} and 2924 cm^{-1} .

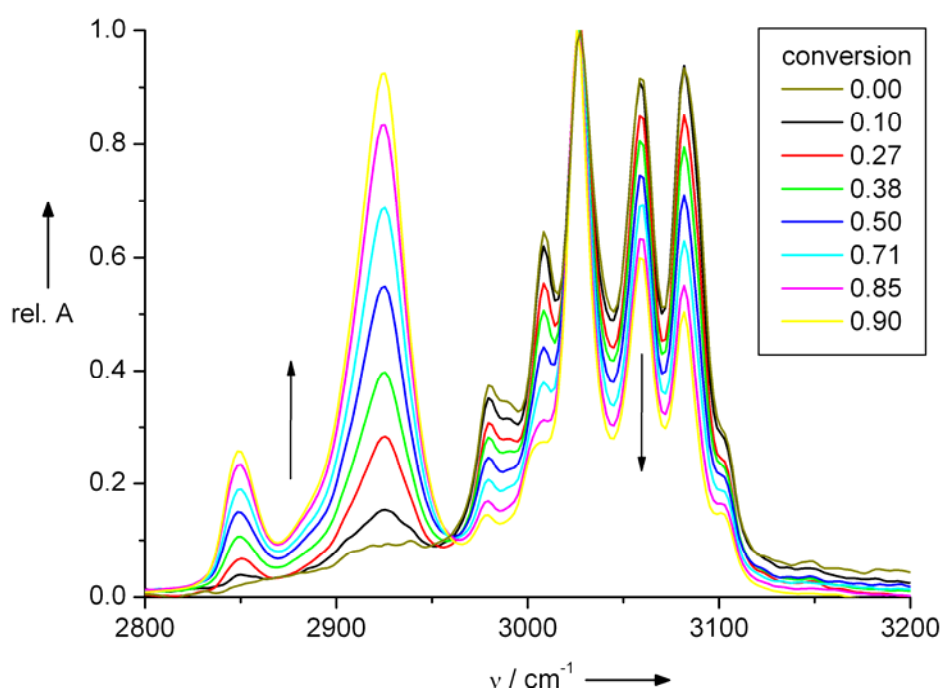


Figure S1. Evolution of the relative IR absorbances (normalized to 1 at 3026 cm^{-1}) during polymerization. Representative IR changes during the polymerization of styrene probed with **1** and without crosslinker are shown.

The conversion U was determined by the ratio of the baseline-corrected heights I_a and I_b of the peak at 2924 cm^{-1} and the peak at 3026 cm^{-1} according to equation S1 (as reference the corresponding terms for polystyrene denoted as $I_{a,PS}$ and $I_{b,PS}$ were used). The latter peak appeared during the polymerization of styrene whereas the former was abundant in both monomer and polymer.

$$U = \frac{I_a}{I_a + I_b} \cdot \frac{I_{b,PS}}{I_{a,PS}} \quad (\text{S1})$$

The time-dependence of the conversion for all the experiments is shown in Figure S2. It was found to be similar before the mixtures became solid and U could not be determined any more.

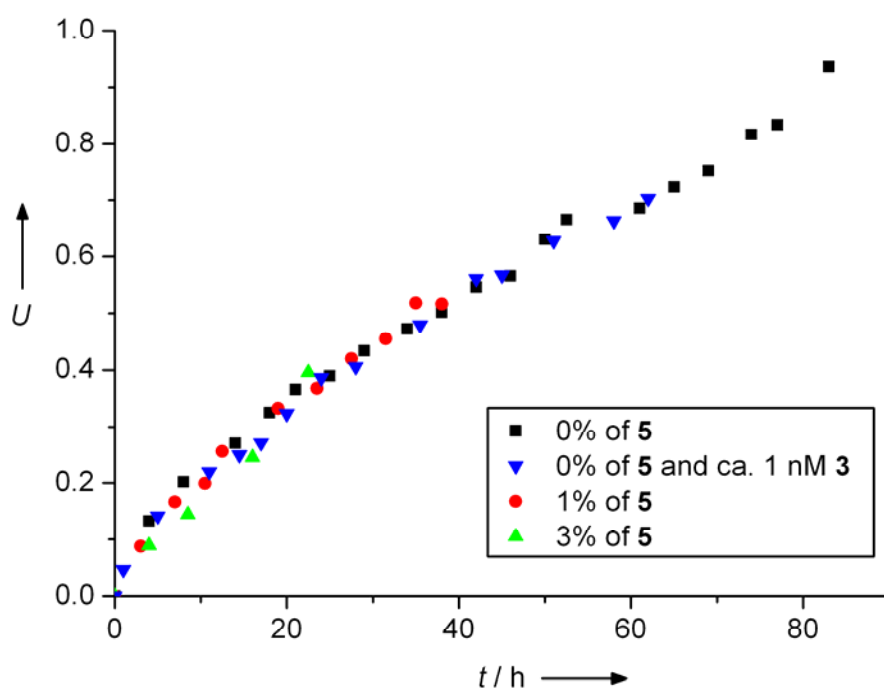


Figure S2. Dependence of conversion U on polymerization time.

3. Methods

Single Molecule Spectroscopy

Imaging of the single molecules was performed using a wide-field fluorescence microscope consisting of an inverted optical microscope (IX71, Olympus) equipped with a 1.3 numerical aperture, 100x oil immersion objective (Plan Fluorite, Olympus) and a highly sensitive cooled CCD camera with 512 x 512 pixels (cascade 512B, Princeton Instruments Inc.) with a pixel size of $16 \times 16 \mu\text{m}^2$. For excitation, the 532 nm light from a diode-pumped solid state laser (CDPS532M-50, JDS Uniphase Co.) was used. The wide-field illumination for excitation was achieved by focusing the expanded and collimated laser beam onto the back-focal plane of the objective (Köhler illumination mode). The polarization of the excitation light in the sample plane was carefully tuned to be circular using zero-order $\lambda/4$ and $\lambda/2$ waveplates in order to compensate for the polarization shift of the dichroic mirror. The power density of the plane was adjusted to 1-10 kW/cm². The emission of molecules which were situated ca. 10 μm above the glass surface was collected by the same objective and imaged by the CCD after passing through a dichroic mirror (z532rdc, Chroma Technology Co.) and an additional spectral filter (HQ542LP, Chroma Technology Co.) removing the excitation light. The image was further magnified 3.3 times with a camera lens in front of the CCD camera, resulting in a maximum field of view of $24.6 \times 24.6 \mu\text{m}^2$ ($48 \times 48 \text{ nm}^2$ per pixel). For the Supporting Information of this paper, the wide-field movies were processed using the video codec Cinepak (SuperMatch).

For the FCS measurements the 543 nm-light of a He-Ne-laser (Melles Griot) was used. After passing through a bandpass filter (BP543, Chroma Technology Co.), the beam was expanded and coupled into an inverted optical microscope (IX71, Olympus) equipped with dichroic mirror (z543RDC, Chroma Technology Co.), a 1.3 numerical aperture and a 100x oil immersion objective (Plan Fluorite, Olympus). The light intensity at the objective was 110 μW . The focus of the illuminated area was ca. 5 μm above the glass surface. The fluorescence light was sent through a pinhole (diameter 100 μm) and two filters (HQ635/90m and S600/40m) into the single photon avalanged diode (Picoquant) whose surface area (50 μm) acted as an additional pinhole. The single photons were counted with a SPC630 card and processes by software developed in our group.

FCS autocorrelation curves and analysis

The FCS experiments were analysed by calculating the intensity autocorrelation curves $I(\tau)^{[S2]}$ which were fitted with one diffusion time according to equation S2^[S3, S4]. Here, N is the average number of fluorescing molecules in the focal volume, τ_D the diffusion time of dye molecules and $\omega = w_z/w_{xy}$ the ratio of the dimensions w_z and w_{xy} of the sampling volume. In the cases for which a fit with one diffusion time was unacceptable on basis of the residuals, equation S3^[S5] was used to fit the data. In this case, f is the fraction of molecules with diffusion constant $\tau_{D,1}$ (the other molecules are assumed to move with diffusion constant $\tau_{D,2}$). The diffusion constant D (or D_n) was calculated from the corresponding diffusion time τ_D (or $\tau_{D,n}$) by using sulforhodamine 101 ($D = 2.0 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$) as a reference^[S6] (equation S4). In order to present the contribution f of

the two different diffusion times, an average diffusion constant as defined in equation S5 is shown in the diagrams.

$$I(\tau) = \frac{1}{N} \left(1 + \frac{\tau}{\tau_D} \right)^{-1} \left(1 + \frac{\tau}{\omega^2 \tau_D} \right)^{-\frac{1}{2}} + 1 \quad (\text{S2})$$

$$I(\tau) = \frac{1}{N} \left(f \cdot \left(1 + \frac{\tau}{\tau_{D,1}} \right)^{-1} \left(1 + \frac{\tau}{\omega^2 \tau_{D,1}} \right)^{-\frac{1}{2}} + (1-f) \cdot \left(1 + \frac{\tau}{\tau_{D,2}} \right)^{-1} \left(1 + \frac{\tau}{\omega^2 \tau_{D,2}} \right)^{-\frac{1}{2}} \right) + 1 \quad (\text{S3})$$

$$D = \frac{\langle w_{xy}^2 \rangle}{4} \cdot \frac{1}{\tau_D} \quad \text{or} \quad D_n = \frac{\langle w_{xy}^2 \rangle}{4} \cdot \frac{1}{\tau_{D,n}} \quad (\text{S4})$$

$$D = f \cdot D_1 + (1-f) \cdot D_2 \quad (\text{S5})$$

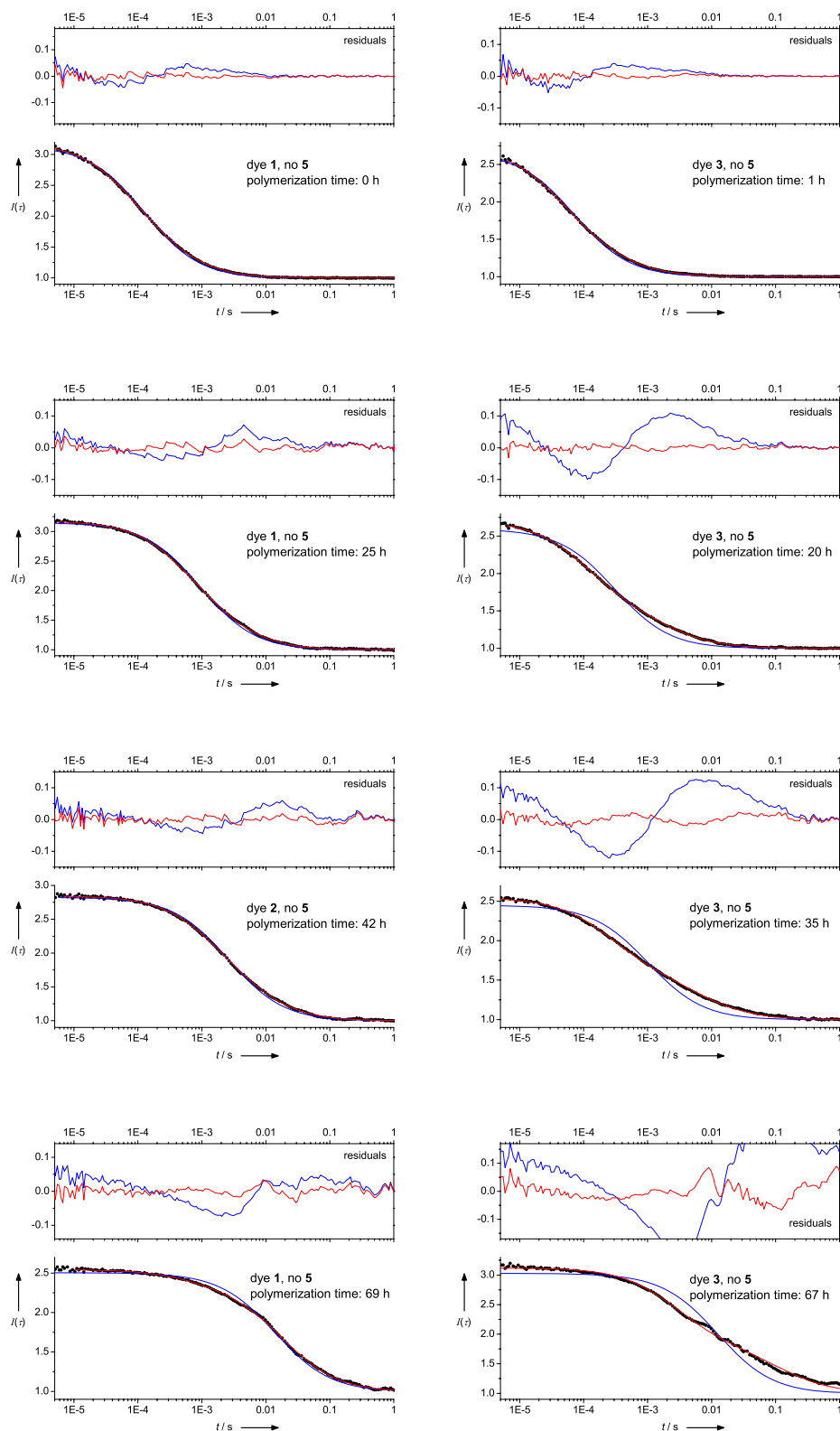


Figure S3. Representative FCS autocorrelation curves for (left) the polymerization of styrene probed with dye **1** and (right) the polymerization of styrene and dye **3**. Additionally, the fits and their residuals according to equation S1 (blue) and equation S2 (red) are shown. In both cases no crosslinker **5** was present.

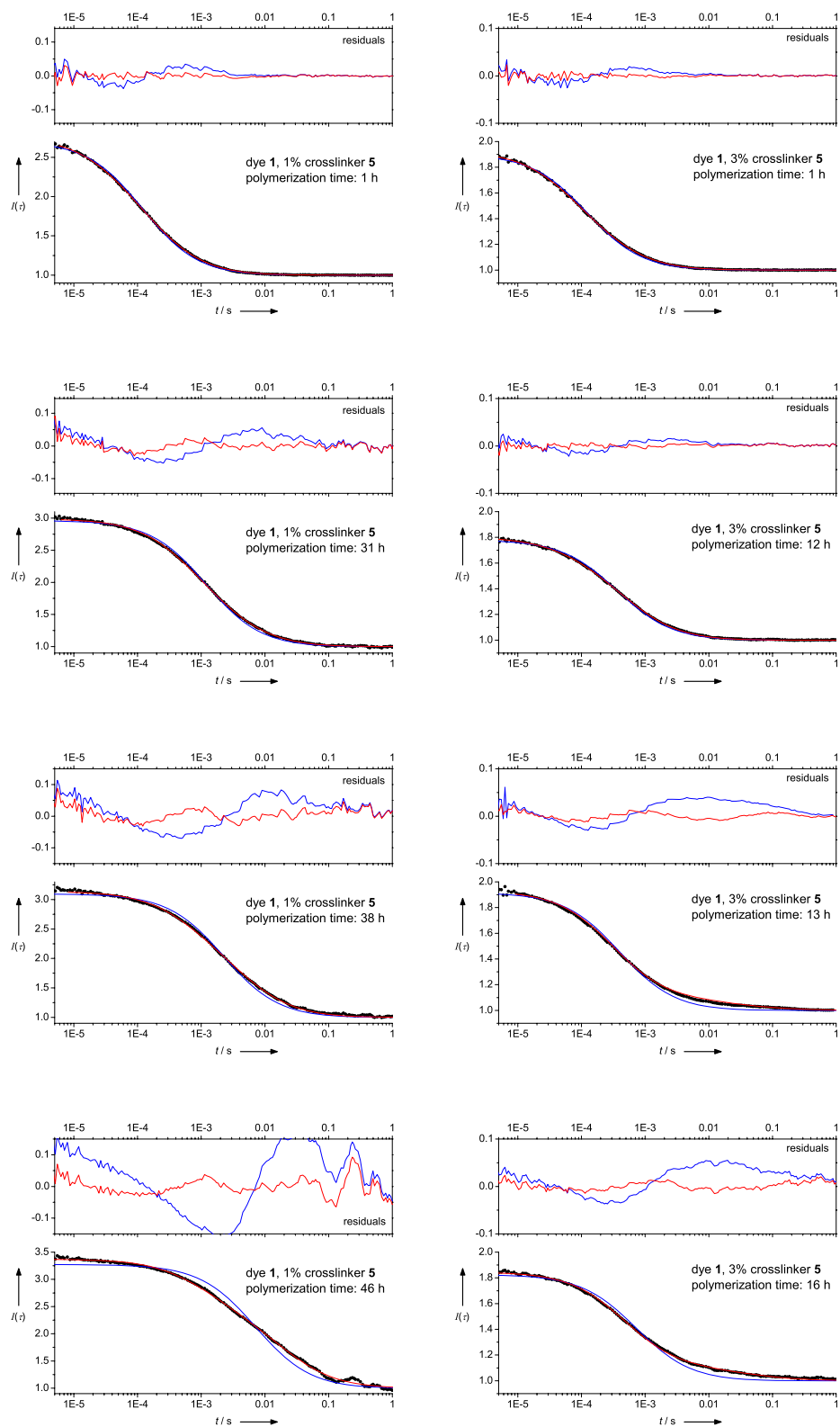


Figure S4. Representative FCS autocorrelation curves for the polymerization of styrene and crosslinker **5** in a concentration of 1% (left) and 3% (right), respectively, probed with dye **1**. Additionally, the fits and their residuals according to equation S1 (blue) and equation S2 (red) are shown.

Wide-field tracking

Single particle tracking was performed on wide-field images using a home-written routine in MatLab[®]. The position of the molecules was determined with 2D Gaussian fitting of the fluorescence spots.^[S7, S8, S9] Typical trajectories of single molecules are shown in Figure 2a, 2b and S5. As an example, in Figure S5 the decrease in step length of the probing dye molecules when going to high conversions is visualized.

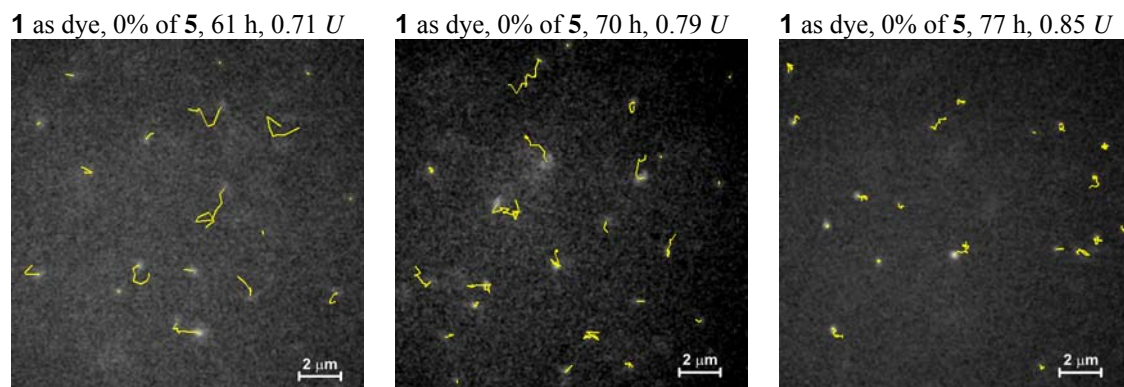


Figure S5. WFM pictures for **1** during the polymerization of styrene without crosslinker at high conversions U including tracking for up to 20 steps (see also Figure 2b).

Step length distribution analysis at ca. 0.64 conversion

The step length distributions for different time lags t_{lag} are shown in Figure S6. They were obtained by calculating the step length distribution for each single-molecule, summation of all these normalized single-molecule distributions and division by the number of observed molecules. This procedure evenly weighted small and long tracks. The step length distributions were fitted by the following equation:

$$P(r, t_{\text{lag}}) = \sum_i \frac{2}{4D_i t_{\text{lag}}} \cdot r \cdot \exp\left(-\frac{r^2}{4D_i t_{\text{lag}}}\right) \quad (\text{S6})$$

Analysis of these distributions according to literature^[23, S7, S8] results in the diagram shown in Figure S7. Here, the mean square displacement $\langle r^2 \rangle$ is plotted versus different time lags t_{lag} . For the experiments with free **1** during linear polymerization and incorporated dye **3**, the distributions could be fitted with one diffusion constant (one component in equation S6). The values are $1.14 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ and $0.261 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$. In case of tracking **1** in the experiment with 1% of crosslinker **5**, a fit with two diffusion constants was used (two components in equation S6). One constant is close to 0 and does not show any increase when going to higher time lags. It originates from the immobile fraction of molecules. The other component shows a linear increase with t_{lag} and can be attributed to the fraction of free molecules. Its diffusion constant was determined to be $0.469 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$.

For the three types of experiments, a comparison between the diffusion constants D obtained from WFM and FCS shows that D obtained from WFM corresponds reasonably with the slow fraction of the diffusion constants obtained by FCS ($3.89 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$, $0.435 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ and $0.528 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$, respectively). However, especially for the experiment with **1** and no crosslinker, D obtained from WFM is lower due to the

fact that detection of the fast motion of dye molecules is limited by the time resolution of our CCD camera, i.e. fast molecules are not as easily tracked as slow ones.

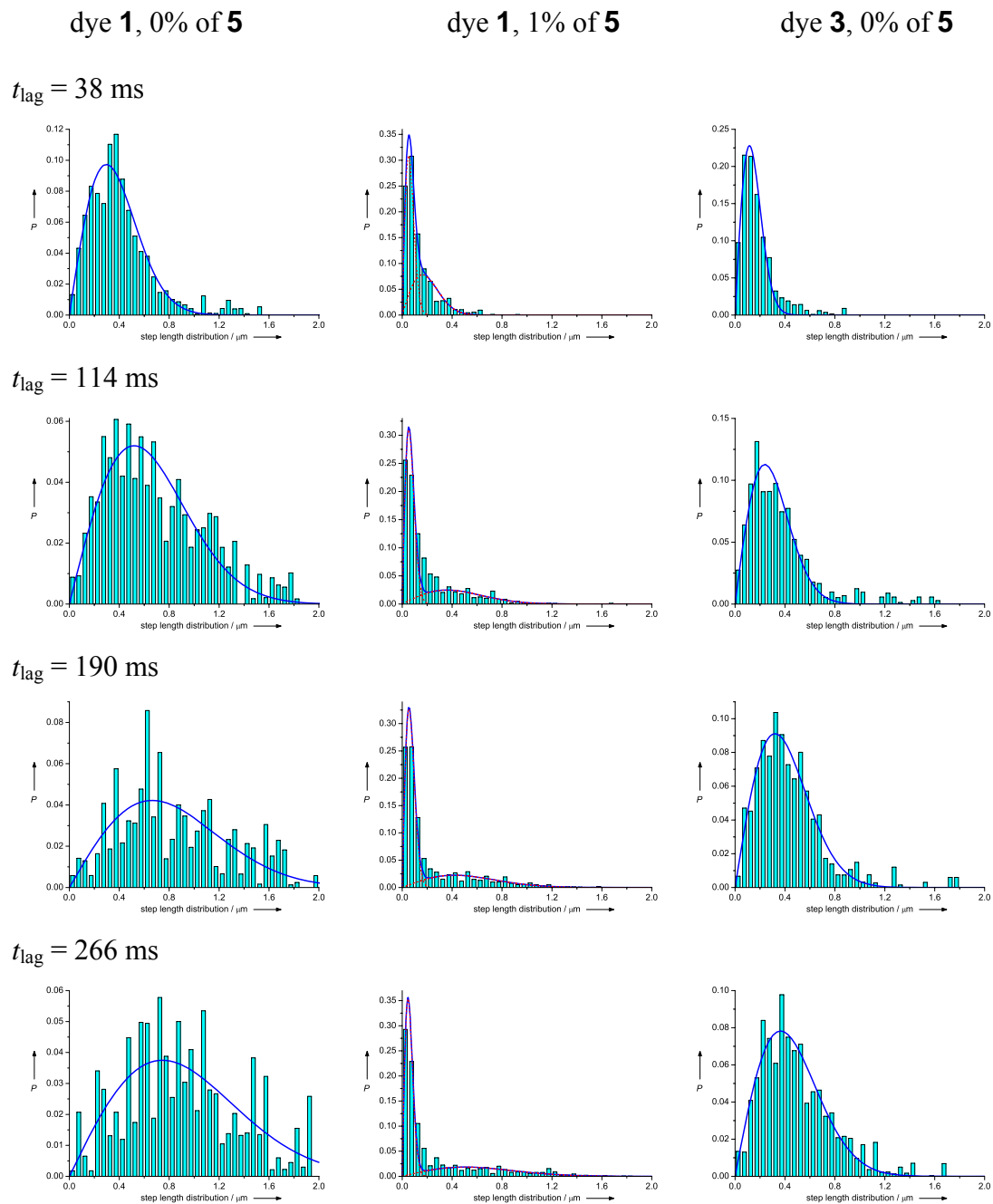


Figure S6. Step length distributions for different time lags for the three different types of experiments discussed in the main text.

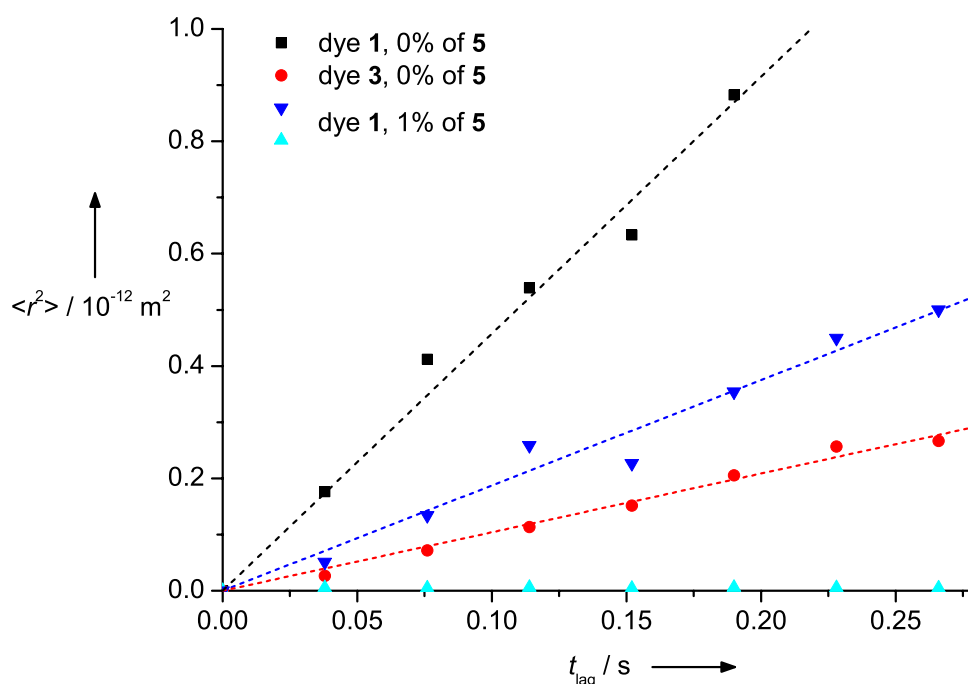


Figure S7. Plot of the mean square displacement $\langle r^2 \rangle$ versus different time lags t_{lag} .

4. List of movies

The videos are in real-time and encoded using Cinepak (SuperMatch).

1 as dye, 0% of crosslinker **5**: [Movie1.mpg](#)

(after 25 h, 0.42 U ; after 53 h, 0.64 U ; after 70 h, 0.79 U ; after 83 h, 0.90 U)

1 as dye, 1% of crosslinker **5**: [Movie2.mpg](#)

(after 35 h, 0.48 U ; after 38 h, 0.50 U ; after 63 h* ; after 69 h*)

1 as dye, 3% of crosslinker **5**: [Movie3.mpg](#)

(after 13 h, 0.25 U ; after 16 h, 0.30 U ; after 42 h* ; after 47 h*)

3 as dye, 0% of crosslinker **5**: [Movie4.mpg](#)

(after 28 h, 0.42 U ; after 51 h, 0.62 U ; after 72 h, 0.80 U ; after 87 h, 0.94 U)

Comparison between the experiments **1** as dye / 0% of crosslinker **5**, **1** as dye / 1% of crosslinker **5** and **3** as dye / 0% of crosslinker **5** at ca. 0.64 conversion U : [Movie5.mpg](#)

* The conversion U could not be determined in these solutions of crosslinked polymer.

Literature of Supporting Information

- [S1] **7** was synthesized according to the literature procedure described in: V. Bertini, S. Alfrey, M. Pocci, F. Lucchesini, N. Picci, F. Iemma, *Tetrahedron* **2004**, 60(50), 11407-11414.
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