

**Heterogeneous Diffusion in Thin Polymer-Films as observed by
High-Temperature Single Molecule Fluorescence Microscopy**

Bente M. I. Flier¹, Moritz Baier¹, Johannes Huber¹, Klaus Müllen³, Stefan Mecking¹,
Andreas Zumbusch¹ and Dominik Wöll^{1,2*}

¹ Fachbereich Chemie, Universität Konstanz, Universitätsstr. 10, 78464 Konstanz, Germany.

² Zukunftskolleg, Universität Konstanz, Universitätsstr. 10, 78464 Konstanz, Germany.

³ Max-Planck-Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany.

Tel: +49 7531 88 2024, Email: dominik.woell@uni-konstanz.de

SUPPORTING INFORMATION

1. Single Molecule Microscopy Setup
2. Heating device
3. Sample preparation
4. Structure of PDI derivative used
5. Temperature-dependence of S/N-ratio of PDI
6. Distinction between mobile and immobile molecules based on R_g
7. Determination of the localization accuracy by a comparison between experiment and simulation
8. Random Walk Simulations at constant dye concentration
9. Random Walk Simulations with dye concentration profile
10. Simulations with reduced mobility at the polymer-glass interface
11. Movie to demonstrate R_g analysis
12. Sample movies

1. Single Molecule Miocroscopy Setup

A 561 nm solid state laser (Cobolt Jive, 75 mW) was used to excite the dye molecules. The laser power was adjusted by neutral density filters. To obtain homogeneous illumination, the laser was coupled into a multi-mode fibre (NA 0.22 ± 0.02 , Optronis) which was shaken to destroy coherence and suppress interference effects detrimental to homogeneous sample illumination. The end of the fibre was imaged onto the sample to a spot of 50 μm diameter yielding an intensity of about 1-4 kW/m². The samples were placed on a home-built heating stage which fits into a commercial microscope (Leica DMI 6000B). The fluorescence was collected using a 63x/0.75 NA air objective (Leica HI Plan), separated from the laser light by a dichroic mirror (AHF, z561 660rpc) and a longpass filter (AHF, RazorEdge LP 568RU), further magnified by two photo objectives (Nikkor 28 mm and 86 mm) and imaged onto an EMCCD camera (Andor iXON). The total magnification resulted in an image size of 86 nm per pixel of the CCD camera.

2. Heating Device

A heating stage was developed which allows for single molecule measurements up to 200 °C. The device consists of an ITO-coated cover slip, an isolated ground plate which fits into the microscope stage, a computer controlled power supply, and an infrared thermometer with RS232 interface. The samples were spin cast on the glass side of the ITO coated cover slip. To contact the ITO layer on the bottom surface, conductive copper tape was attached to the slip. A voltage was applied to the ITO layer by two copper contact clips which were connected to the computer controlled power supply. The IR thermometer was placed above the substrate such that the temperature measurement spot of about 0.9 mm was localized on the sample directly over the microscope objective. Both thermometer and power supply were connected by RS232 interfaces to a computer which runs a temperature control software. The

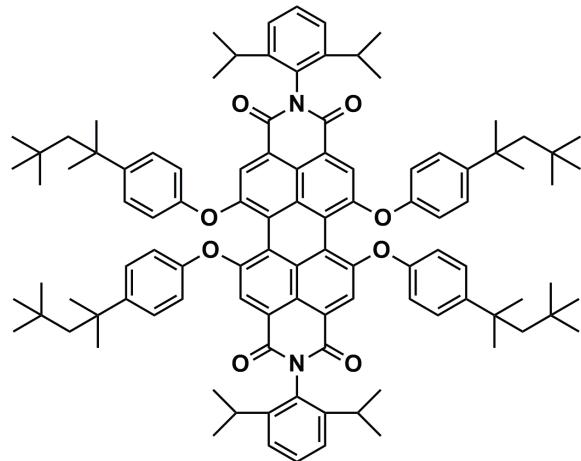
heating power was calculated with a modified proportional–integral–derivative algorithm. The developed heating stage possesses a high temperature stability (± 0.5 °C) with low fluctuations in the heating voltage (± 0.2 V) and an excellent heating speed. A sample can be heated from room temperature to 150 °C in less than 10 s. Since the glass cover slides are very thin (170 μ m), also fast cooling of the sample can be achieved.

3. Sample Preparation

Samples were prepared on ITO-coated cover slides (SPI supplies, 8-12 ohm/sq) allowing the heating of the sample. The substrates were cleaned by ultrasonication in isopropanol (spectroscopic grade, Acros organics) and in an oxygen plasma (Plasmacleaner Femto, Diener Electronics). Polystyrene of molecular weight $M_w = 3000$ g/mol (Polymer Source Inc., P8921-S, $M_n = 2800$ g/mol, polydispersity 1.07) and a perylene diimide derivative (PDI, IUPAC name: N,N'-bis(2,6-diisopropylphenyl)-1,6,7,12-tetra[4-(1,1,3,3-tetramethylbutyl)phenoxy]-perylene-3,4:9,10-tetracarboxylic diimide, see 4.) were dissolved in toluene (99+%, spectroscopic grade, Acros organics) to obtain solutions between 0.5 wt% and 10 wt%. These polymer concentrations were chosen in order to produce films of thicknesses between 30 and 250 nm. The solutions were spin cast at 2000 rpm. Subsequently the samples were dried under vacuum for 24 h at 80 °C to remove any remaining toluene from the films and relax stress introduced by spin-coating.^[S1] The film thickness of the samples was measured after single molecule measurements by scratching a cross into the polymer film and measuring the depth of the scratch by AFM. Wide-field movies were measured not too far from this cross to ensure that the thickness was the one measured by AFM.

4. Structure of PDI derivative used

N,N'-bis(2,6-diisopropylphenyl)-1,6,7,12-tetra[4-(1,1,3,3-tetramethylbutyl)phenoxy]-perylene-3,4:9,10-tetracarboxylic diimide^[S2]



5. Temperature-dependence of S/N-ratio of PDI

In order to determine the suitability of PDI molecules for high temperature single molecule measurements, we embedded them in high molecular weight PS films ($M_n = 250$ kg/mol) and investigated the S/N-ratio at different temperatures. Figure S1 shows mean values and standard deviations of the S/N-ratio for up to 250 molecules per temperature, an integration time of 6 s per image and an excitation power of ca. 1–4 kW / m², the same conditions as used for the experiments described in the main part of the paper. The fluorescence intensity, and thus the fluorescence quantum yield decreases only slightly with temperature. Even at 400 K, single molecules can be easily observed and localized. Apart from the emission intensity, the photostability does not significantly decrease with increasing temperature. We could not observe earlier photobleaching at 380 K compared to 298 K. The high S/N-ratio and photostability at high temperatures makes PDI very suitable for single molecule measurements throughout the entire observable temperature range.

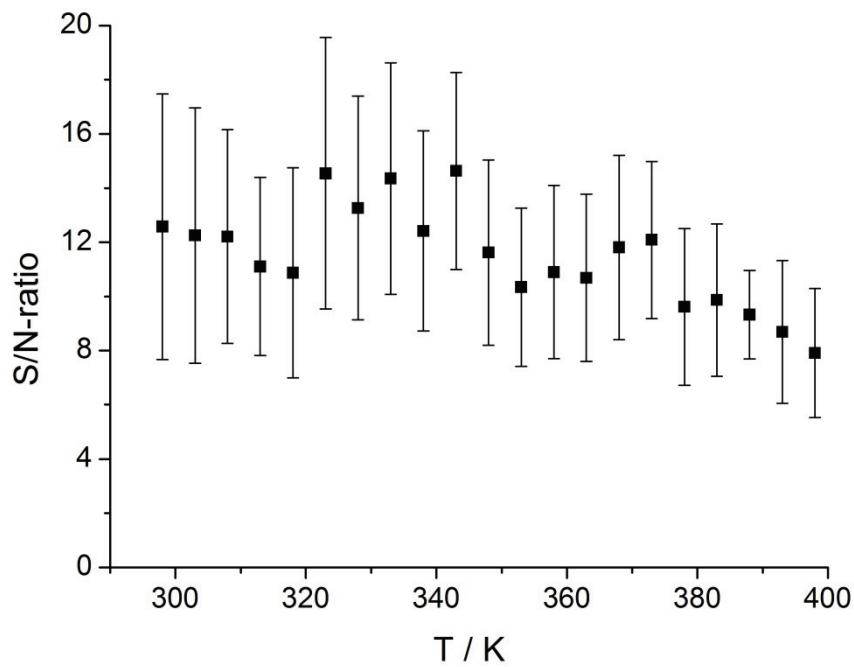


Figure S1. Temperature dependence of the S/N-ratio of the fluorescence of single PDI molecules including standard deviations.

6. Distinction between mobile and immobile molecules based on R_g

The localization-limit for our investigations was determined by measurements of single molecule fluorescence in thick (> 500 nm) PS films ($M_n = 250$ kg/mol, bulk- $T_g = 373$ K) at room temperature, ca. 80 K below their bulk glass transition temperature, i.e. a temperature range where translational motion of the dye molecules can be excluded. Thus, only localization inaccuracies cause fluctuations in the single molecule positions determined in different frames of the movies. An analysis of their R_g gave values which were, with few exceptions, smaller than 0.6 pixels with a distribution as presented in Figure S2. Thus molecules with $R_g < 0.6$ pixels (52 nm) are defined as immobile and molecules with $R_g > 0.6$ pixels as mobile.

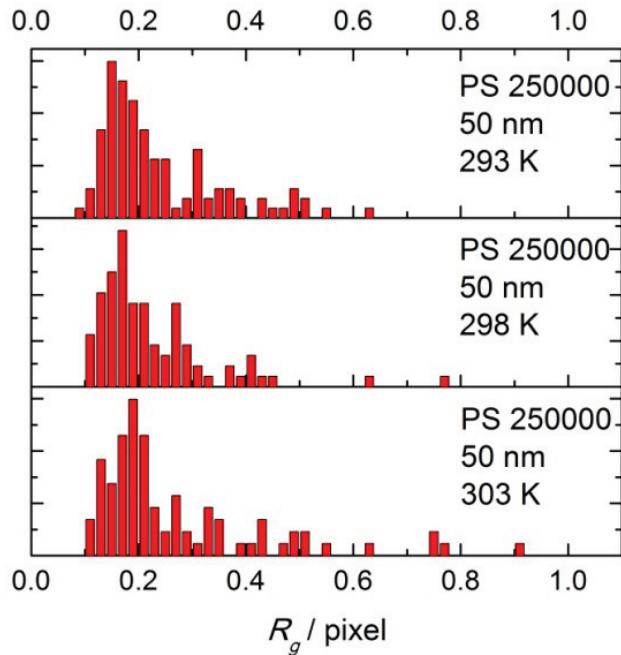


Figure S2. R_g -distribution of immobile PDI molecules in 50 nm thick films of PS

($M_n = 250$ kg/mol, bulk- $T_g = 373$ K) far below T_g .

7. Determination of the localization accuracy by a comparison between experiment and simulation

A comparison between the R_g values of measured and simulated data allows for an estimation of the localization accuracy of our experiments. Single molecule microscopy movies of dye molecules in high molecular mass PS ($M_n = 250$ kg/mol) were recorded at room temperature and the R_g determined according the procedure described above (see Figure S2). The distributions were compared to simulated data of immobile molecules with a diffusion coefficients of 0 (no motion) and different localization accuracies (see Figure S3). 500 molecules with 25 frames/molecule were simulated choosing each x - and y -position from a Gaussian normal distribution of different widths (standard deviations from 5 to 30 nm). The

distributions of simulated data are considerably more narrow than experimental distributions since they are not broadened by inhomogeneities or inaccuracies in the localization procedure. The distribution of measured R_g 's (see Figure S3) resembles basically the simulated distributions with a localization accuracy between 10 and 15 nm. Thus, the localization accuracy of our experiment is approx. 10 to 15 nm.

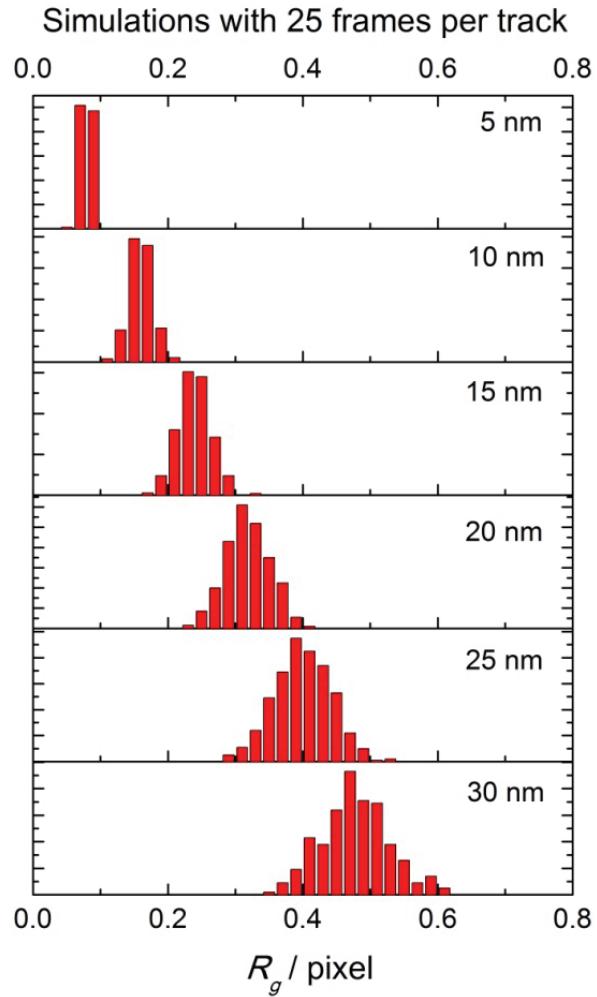


Figure S3. Determination of R_g -distributions for simulations of 500 immobile molecules ($D = 0$) for different localization accuracies as indicated on the right side of each histogram.

8. Random Walk Simulations at constant dye concentration

Before each simulated displacement step, the corresponding diffusion coefficient was determined using the Vogel-Fulcher-Tamman relationship.

$$D(z, T) = D_0 \exp\left(-\frac{B}{R \cdot (T - T_0(z))}\right)$$

with the high temperature diffusion coefficient limit $D_0 = 10^{-12} \text{ m}^2 \text{ s}^{-1}$, $B = 6100 \text{ J}$ as determined by SM diffusion measurements in thick PS3000 films and the gas constant R . The dependence of the Vogel temperature T_0 on the distance from the film surface (z -position) was taken from literature^[S3] as

$$T_0(z) = \frac{T_{0,\infty} \left(1 + \frac{h_0}{z}\right)}{\left(1 + \frac{h_0}{2z}\right)^2}$$

where h_0 is a distance which sets the length scale for surface dependent T_g and was set to 0.82 according to Herminghaus et al.^[S4] $T_{0,\infty}$ is the critical temperature in bulk which was set to $T_g - 49 \text{ K} = 293 \text{ K}$ as also used by Tsui and coworker.^[S5] Random numbers were chosen from a Gaussian normal distribution which was cut at $3 \times$ the standard deviation σ to avoid unrealistically large steps and multiplied by the factor $\sqrt{2 \cdot D(z, T) \cdot t_{step}}$ to take into account the T_g -profile.

The time t_{step} for each step of the simulations was chosen so that the maximum displacement per step was smaller than 1.5 nm, i.e. the time per step t_{step} was 10^{-3} s for $D(z, T) < 10^{-16} \text{ m}^2 \text{ s}^{-1}$, 10^{-5} s for $10^{-16} \text{ m}^2 \text{ s}^{-1} \leq D(z, T) < 10^{-14} \text{ m}^2 \text{ s}^{-1}$, and 10^{-7} s for $10^{-14} \text{ m}^2 \text{ s}^{-1} \leq D(z, T)$. Overall a time window of 150 s was simulated, from which positions were determined each 0.3 s, equivalent to the measurements.

9. Random Walk Simulations with dye concentration profile

The Random Walks described above were also extended to a situation where dye molecules are preferentially located at the polymer-air surface due to a decreased polymer density in this zone. For this, we multiplied a Gaussian distribution function, which was used to determine subsequent diffusion step lengths and shifted according to the current position of the molecule, with the probability functions shown in Figure S4. The probability functions were estimated according to monomer density profiles.^[S6] Two different profiles (see below) were chosen to investigate their influence on single molecule mobility.

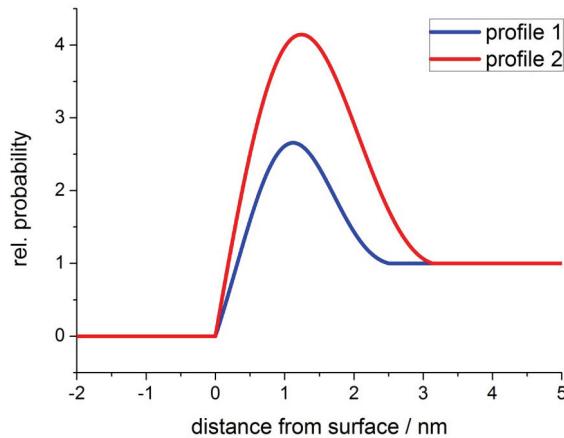


Figure S4. Functions used to simulate an increased probability for molecules to stay at the surface. (blue, lower curve: profile 1; red, upper curve: profile 2).

Functions chosen for the profiles:

$$\text{profile 1: } p(x) = \begin{cases} 3 \cdot \exp\left(-\frac{(x-1)^2}{s^2} - 1.1 + \frac{x}{1.4}\right) & \text{for } x < 2.50 \\ 1 & \text{for } x > 2.50 \end{cases}$$

$$\text{profile 2: } p(x) = \begin{cases} 7 \cdot \exp\left(-\frac{(x-1)^2}{s^2} - 4.48 + \frac{x}{0.688}\right) & \text{for } x < 3.13 \\ 1 & \text{for } x > 3.13 \end{cases}$$

10. Simulations with reduced mobility at the polymer-glass interface

In order to verify that the effect of a lower T_g at the polymer surface is clearly dominating the influence of the polymer-glass interface with a T_g assumed to be higher than in bulk, we performed Random Walk Monte Carlo simulations with the same Matlab routine which has already been described in the main text and paragraph 8 of the Supporting Information, but using diffusion coefficient profiles as shown in Figure S5 (solid lines).

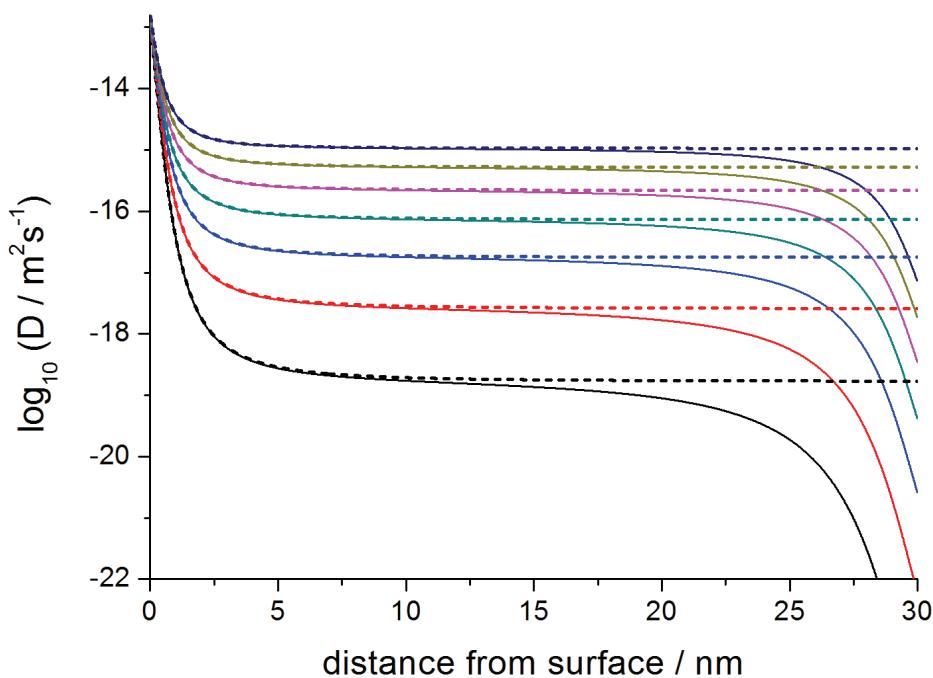


Figure S5. Modification of the diffusion coefficient profile at the polymer-glass interface (solid lines) compared to the profile used for the simulations in the paper (dashed lines) for temperatures between 340 K (lowest curve) and 400 K (highest curve) in steps of 10 K. (See also Figure 5 in main document). The saturation value of the diffusion coefficient of the dashed line at far distance from the surface is the $D_{\text{bulk,sim}}$ used for Figure S6.

Such a profile results in the accumulation of dye molecules in vicinity of the polymer-glass interface where the diffusion coefficients are significantly reduced. For thick films with initially homogeneously distributed probes this effect is negligible at low average D since most molecules do not reach the interface within our simulation time. However, for higher

temperatures i.e. higher average diffusion coefficients, many molecules reach the surface and remain a considerable amount of time there, thus reducing the fraction of molecules defined as mobile. The magnitude of this effect depends strongly on film thickness as shown in Figure S6. The effect of the polymer-glass interface, i.e. a shift to a lower fraction of mobile molecules for thinner films, is contrary to our experimental observations. Thus, we can deduce that the polymer-glass interface has a minor effect on the fraction of mobile molecules, and the reduced T_g at the polymer surface dominates single molecule motion in thin polymer films.

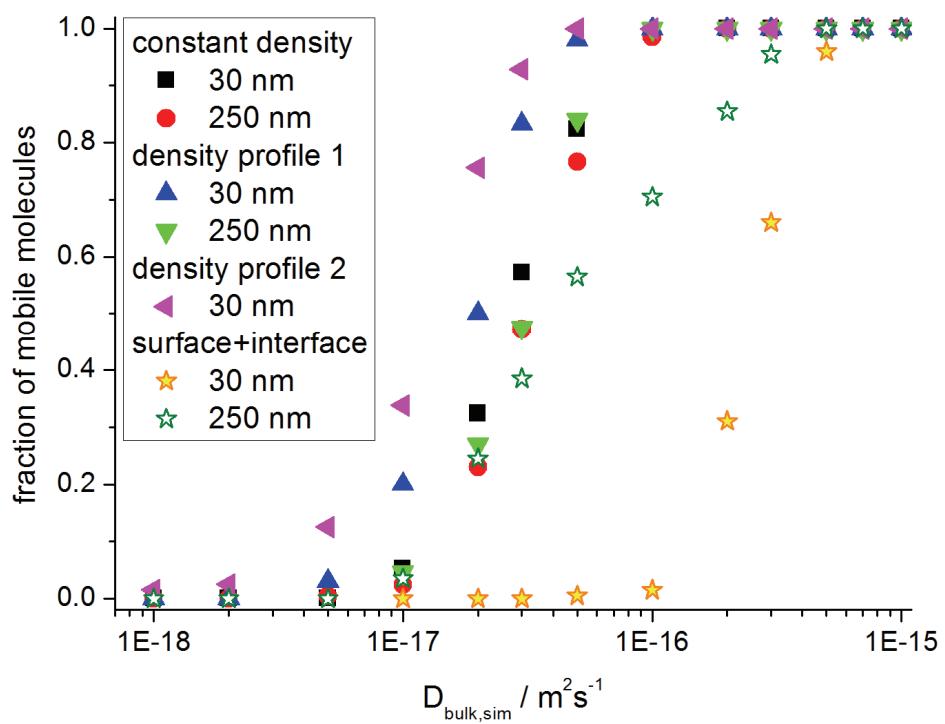


Figure S6. Comparison of the fraction of mobile molecules for the simulations described in the main text (solid symbols, see also Figure 5) and simulations with reduced mobility at the polymer-glass interface (open stars) for 30 nm and 250 nm thick films.

11. Movie to demonstrate R_g analysis

Movie “**ja208581r_si_006.avi**” can be found in the supporting material.

12. Sample movies

The following movies are available in the supporting material:

- ja208581r_si_004.mpg:** movies of 400 nm thin polystyrene film at 75 °C, 95 °C, 115 °C and 135 °C, integration time per frame 0.2 s, unprocessed movie.
- ja208581r_si_002.mpg:** movies of 30 nm thin polystyrene film at 70 °C, 80 °C, 90 °C and 100 °C, integration time per frame 6 s, movie processed with 2 pixel median filter, intensity scale optimized.
- ja208581r_si_003.mpg:** movies of 250 nm thin polystyrene film at 70 °C, 80 °C, 90 °C and 100 °C, integration time per frame 6 s, movie processed with 2 pixel median filter, intensity scale optimized.

- [S1] Zhang, X. H.; Yager, K. G.; Kang, S. H.; Fredin, N. J.; Akgun, B.; Satija, S.; Douglas, J. F.; Karim, A.; Jones, R. L. *Macromolecules* **2010**, *43*, 1117.
- [S2] Qu, J. Q.; Zhang, J. Y.; Grimsdale, A. C.; Müllen, K.; Jaiser, F.; Yang, X. H.; Neher, D. *Macromolecules* **2004**, *37*, 8297.
- [S3] Peter, S.; Meyer, H.; Baschnagel, J.; Seemann, R. *J. Phys.: Condens. Matter* **2007**, *19*, 205119.
- [S4] Herminghaus, S.; Jacobs, K.; Seemann, R. *Eur. Phys. J. E* **2001**, *5*, 531.
- [S5] Yang, Z.; Fujii, Y.; Lee, F. K.; Lam, C.-H.; Tsui, O. K. C. *Science* **2010**, *328*, 1676.
- [S6] Peter, S.; Meyer, H.; Baschnagel, J. *J. Chem. Phys.* **2009**, *131*, 014902.