VIP Polymerization

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Radical Polymerization Tracked by Single Molecule Spectroscopy**

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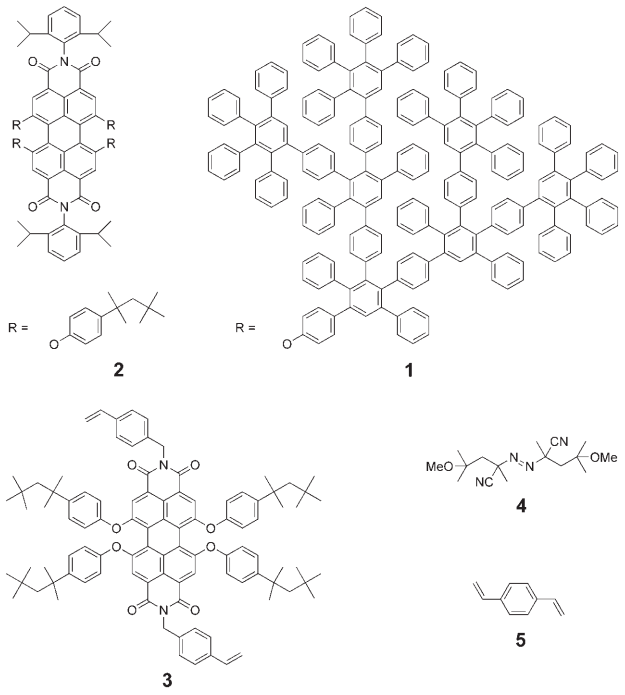
Products from polymerization have influenced our daily lives tremendously over the past hundred years. It is therefore not surprising that enormous efforts have been and are being made to fully understand each detail of the polymerization process. Prominent examples of analytical techniques used are ESR spectroscopy,[1] pulsed-laser-initiated polymerization in conjunction with size-exclusion chromatography[2] and mass spectrometry,[3] as well as NMR[4] and fluorescence spectroscopy.[5–11] With these techniques, very detailed knowledge about polymerization kinetics could be gained. However, most of these techniques cannot probe over a large extent of conversion and all average over an ensemble of molecules. As a consequence, none of these techniques can detect heterogeneities occurring at molecular level during polymerization, a phenomenon which influences the final polymer properties. In contrast to ensemble techniques, single molecule spectroscopy (SMS) can elucidate such heterogeneities.

SMS has already been used to study the dynamics of single molecules[12–14] or single polymer chains[15,16] in a polymer matrix. In particular, fluorescence correlation spectroscopy (FCS) allowed the study of diffusion in polymer solutions, gels with different cross-linker concentrations,[17] poly(acrylic acid) grafted on poly(ethylene terephthalate) films,[15] and thrombin-induced fibrin aggregation.[18]

Herein we present SMS measurements performed for the first time during bulk radical polymerization in the absence and presence of a cross-linker. In particular, we follow polymerization by detecting changes of the diffusion constant D of dye molecules acting as probes. Changes in D can be related to the freedom of molecules to move within the monomer solution or matrix formed by the polymer. FCS measures the time diffusing molecules remain within a defined volume and allowed determination of $D > 10^{-13}\ m^2 s^{-1}$ up to high conversions U before motion became too slow and thus limited the applicability of this method. Wide-field microscopy (WFM) directly visualizes the position of fluorescent molecules and is a suitable method to track slow moving molecules ($D < 10^{-12}\ m^2 s^{-1}$) and even to detect molecules which are immobilized. Both methods, therefore, complement each other, and in combination, they permit following translational motion of dyes for the entire polymerization process. The detection of heterogeneities is an important advantage over determination of an average value of D from viscosity measurements using the Stokes–Einstein relation.

Perylenediimide derivatives were used as probing dyes. Using dye molecules **1** or **2**, the polymerization of styrene in absence and presence of a cross-linker was studied. The dye **1** was of particular interest as it moves more slowly because of its large size and, therefore, allows WFM detection already at lower U. Additionally, perylenediimide derivative **3** bearing two styrenyl groups (for the synthesis, see the Supporting Information) allowed for the formation of polystyrene with the dye acting as a potential cross-linker which is covalently



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incorporated into the polymer.[19] Dye **2** is of similar size as **3** and was used as reference.

All polymerizations were initiated with the thermal radical initiator **4** and were performed at room temperature. Three sets of experimental conditions were selected: the polymerization of styrene probed with **1** or **2**, the polymerization of styrene with cross-linker **5** probed with **1**, and the polymerization of styrene with **3**. The kinetics of the polymerizations are presented in the Supporting Information.

For the polymerization probed with **1** and no cross-linker, FCS autocorrelation curves were fitted with one diffusion time τ (Equation S2, Supporting Information) up to high conversion (0.83). The resulting diffusion constants D are shown as gray circles in Figure 1a and Figure 1b. The reasonable fits with one D value indicate that translational diffusion of the dyes is rather homogeneous. Even at higher conversion U, a lot of freedom remains for the dye molecules to move because the surrounding polymer chains are loosely entangled and are able to move along each other. The motion of dye molecules with D below about $3\times10^{-12}\,m^2s^{-1}$ can be directly observed in WFM (for movies see the Supporting Information). However, at early stages of polymerization, the dyes move too fast for the time resolution of the CCD camera (an integration time of 38 ms per frame (26 Hz) was used for all reported WFM experiments). Even though no dye molecules could be localized at this stage, their traces can be recognized in the WFM movies at $0.42\,U$. The progress of polymerization causes the translation of the dye molecules to gradually slow down. The movie taken at $0.64\,U$ shows that molecules become occasionally detectable for a few hundreds of milliseconds before they move out of the focus (see Figure 2b, left). It takes until about $0.70\,U$ before the motion of most molecules slows down sufficiently for tracking (see Figure S5 and movies in Supporting Information). At the final stage of polymerization, the slow translational diffusion, still detectable at $0.85\,U$, finally stops at about $0.90\,U$. At that stage, the strongly hindered motion of molecules results in very low polymerization rates.[20]

A second set of experiments focused on the diffusion in polymer networks. For that purpose, the above mentioned experimental conditions were repeated, but with addition of 1,4-divinylbenzene (**5**) as cross-linker in a concentration of 1% and 3%, respectively (Figure 1a and b). At low U, autocorrelation curves, the quality of their fits, and diffusion constants were similar to the experiment without **5**. However, when gelation started, the FCS curves could only be fitted acceptably with two diffusion constants (Equation S3, Supporting Information) as indicated by triangles (fast: green, slow: red; black circles: weighted average given by Equation S5, Supporting Information). The fast component of D shows a decrease relative to that of D for **1** in the experiment without cross-linker (gray circles). In contrast, at approximately the reaction time when gelation was observed, the slow component of D dropped by about one order of magnitude for a cross-linker concentration of 1% and circa two orders of magnitude for 3%. At 3%, gelation also started earlier than at 1% (observed ratio of gelation times, 0.47/0.25 $\approx$1.9, is close to the theoretical value of $\sqrt{3}$[21]). The WFM movies (see Supporting Information) show that before

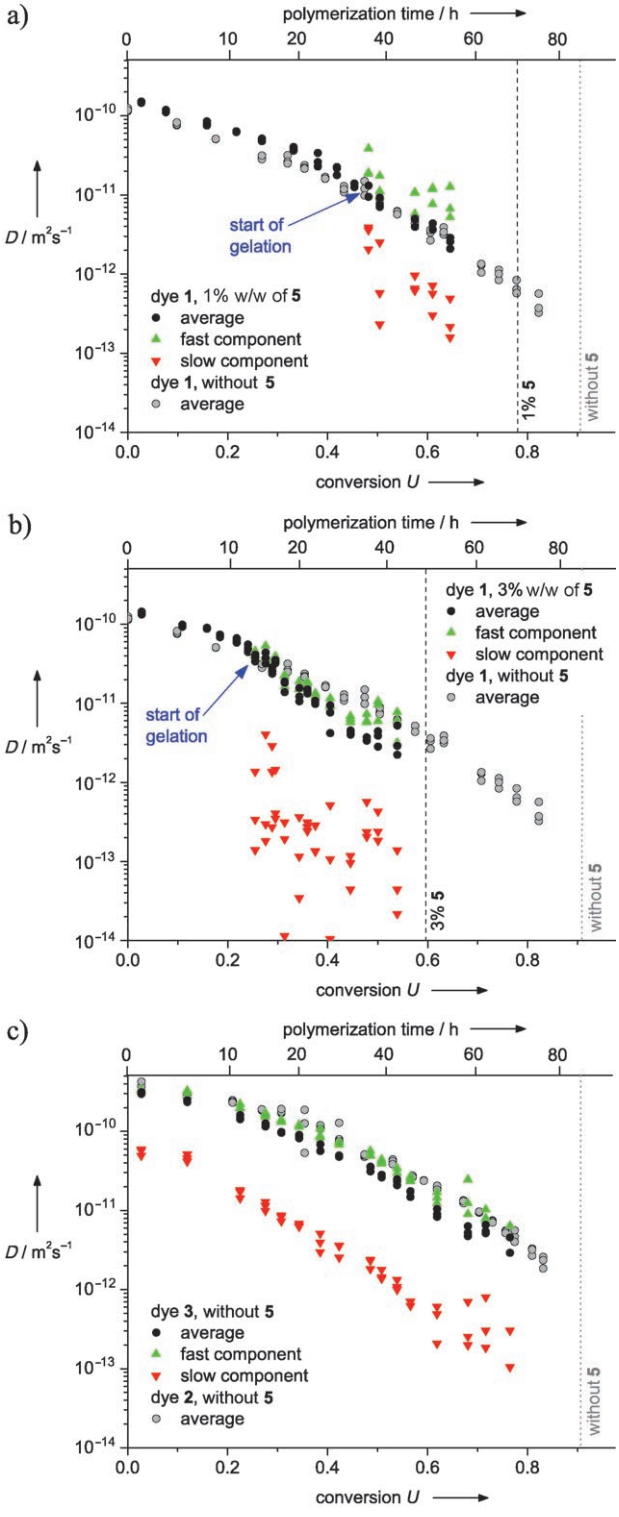

Figure 1. Dependence of the diffusion constant D obtained by FCS on U and on the polymerization time: a,b) for polymerization of styrene probed with **1** and 0%, 1%, and 3% w/w of cross-linker **5**. The correlation between polymerization time and conversion is valid until $0.9\,U$, $0.6\,U$, and $0.4\,U$, respectively (Figure S2, Supporting Information) c) for the polymerization of styrene with **3** and as comparison of styrene probed with **2**. Each measurement was repeated three times at different positions. The dashed line indicates the point at which all molecules were immobilized as determined by WFM.

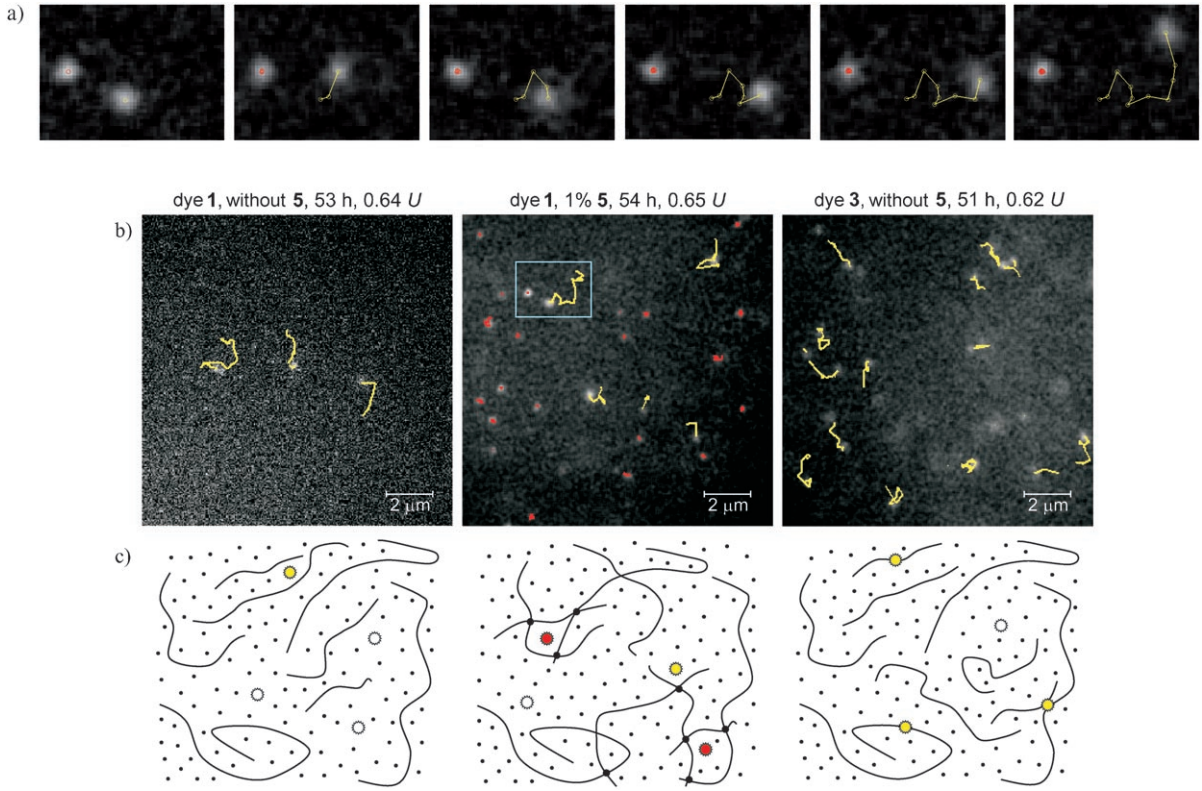

Figure 2. a) Movie extract from the indicated region in Figure 2b (middle, gray rectangle) with one immobilized and one moving dye (only every second frame is shown). b) WFM pictures at circa 0.64 *U* including tracks for up to 20 steps for the three types of experiments discussed in the text. c) Schematic representation of dyes in their surrounding. Dyes are shown as circles for which the color indicates their current velocity (white: too fast for detection by WFM, yellow: slow enough for WFM, red: very slow/immobilized).

gelation, more than 90% of the molecules move too fast to be localized owing to our WFM time resolution. However, some dye molecules move very slowly or are even immobilized. We assume that these molecules are situated in regions in which a polymer network has already formed and therefore are hindered in their movement. With gelation the number of slow and immobile molecules increases significantly. The heterogeneity arising from the concomitant presence of fast, slow and immobilized dyes is not only obvious in WFM but also causes the FCS autocorrelation fits with one diffusion constant to become unacceptable. In the course of the further polymerization, the diffusion of all molecules becomes slower and the concentration of immobilized molecules gradually increases, as seen in the WFM movies. Finally, translational motion stops entirely. This cessation happens earlier in time for higher cross-linker concentrations.

Dye **3** with two styrenyl groups can be incorporated with one or two ends into the growing polymer chain. Therefore, the translational motion of free and incorporated dye can be studied simultaneously. Dye **3** was added in nanomolar concentrations to a solution of styrene and initiator. At this low concentration an influence on viscosity or other properties of the reaction mixture can be neglected. The FCS autocorrelation curves at all times had to be fitted with two diffusion constants. At any time and with comparable conditions, the fast component corresponds well with the diffusion constant of free **2**, a dye similar in size to **3** (Figure 1c), suggesting that this fraction represents the dye which has not been incorporated into the polymer chains. The slow component has a diffusion constant about one order of magnitude smaller and can be associated with **3** incorporated into polystyrene. The dye can be incorporated into chains of different length, each of them having a characteristic *D*, but only an average value could be determined by FCS. In comparison to the experiment with the even larger dye **1**, for a similar dye concentration, the fraction of dyes detectable in WFM is higher at any time. This can be explained by the incorporation of **3** into the growing polymer chains which slows down their motion already at an earlier stage. As in the experiment with **1** or **2**, motion (of both incorporated and free dye **3**) can no longer be detected at ca. 0.90 *U*.

The WFM measurements were compared at ca. 0.64 *U*. At this conversion clear differences in lateral diffusion can be observed (see Figure 2 and Movie 5 in the Supporting Information). To quantify the WFM observations, the step-length distributions[22,23] of the lateral motion of single dye molecules between frames was measured and presented in a histogram[24] (see Figure 3a, and Figure S6 in the Supporting Information). In the polymerization of styrene without **5** and probed with **1**, the dye molecules move fast and therefore tracking can be done only for a few molecules that occasionally slow down. On average, however, even these "slow"

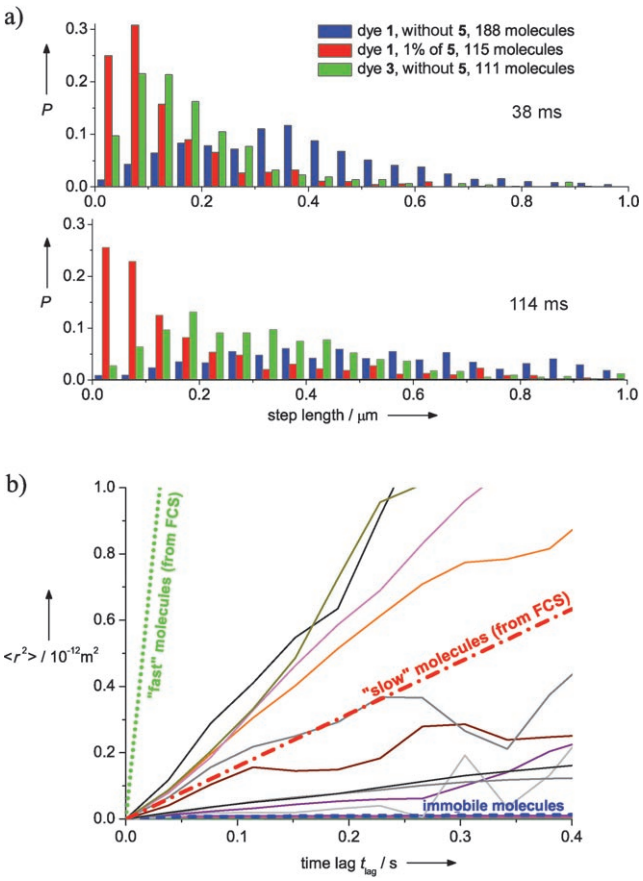

Figure 3. a) Step-length distributions for time lags of 38 and 114 ms for the three types of experiments mentioned in the text; b) $\langle r^2 \rangle$ for several single molecules of **1** at 0.65 *U* in the experiment with 1% of **5**.

moving molecules diffuse more freely with larger steps compared to the other two experiments ($D = 1.14 \times 10^{-12}\ m^2 s^{-1}$).

The situation is different in case of the growing network polymer. Significant heterogeneity of the motion of dyes was observed. The step-length distributions reveal two fractions (see Supporting Information, Figure S7). One fraction shows free diffusion with $D = 0.469 \times 10^{-12}\ m^2 s^{-1}$ and can be assigned to molecules moving in areas where the polymer network is less dense. For the other fraction the step length does not significantly increase at longer time lags, and thus it can be attributed to molecules immobilized in the network. Further evidence that the observed heterogeneity is due to different fractions of molecules with a different motion is presented in Figure 3b. The mean square displacement $\langle r^2 \rangle$ is plotted against time lag for several single molecules. The immobile fraction of dyes shows values of $\langle r^2 \rangle$ which remain close to zero for all time intervals. Another fraction diffuses normally and can be related to the slow fraction detected in FCS. However, in contrast to FCS, WFM reveals a distribution of $\langle r^2 \rangle$ for different molecules as can be seen from the spread in Figure 3b. The fast fraction detected in FCS is too quick for the WFM time resolution used.

In the experiment using **3**, free dyes and incorporated dyes are present simultaneously. The former are in most cases too fast for a localization by WFM. However, the motion of dyes incorporated into polymer chains is much slower and can be tracked. *D* was found to be $0.261 \times 10^{-12}\ m^2 s^{-1}$. Differences between dyes incorporated into chains of different length or even cross-linked dyes could not be evaluated quantitatively.

In conclusion, we present a novel way to follow radical polymerization over an extensive conversion range. Our method is based on the detection of single dye molecules and the fact that their mobility changes during polymerization. In absence of a cross-linker, **1** and **2** diffuse freely in the surrounding medium, but in presence of **5** the influence of heterogeneity that arises during the formation of a network on the motion of reporter molecules could be verified. Furthermore, using **3** in low concentration for example (ca. 10^{-9} M), the incorporation of the monomer units into the growing polystyrene chains could be visualized.

Our investigations can be extended to other polymerization systems such as interpenetrating networks and nanocomposites during their formation process and in particular will provide a deeper understanding of heterogeneities in molecular motion. With this knowledge a better control over polymerization and the properties of the resulting polymers and polymer networks might be gained.

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