ACS Macro Letters

Letter

pubs.acs.org/macroletters

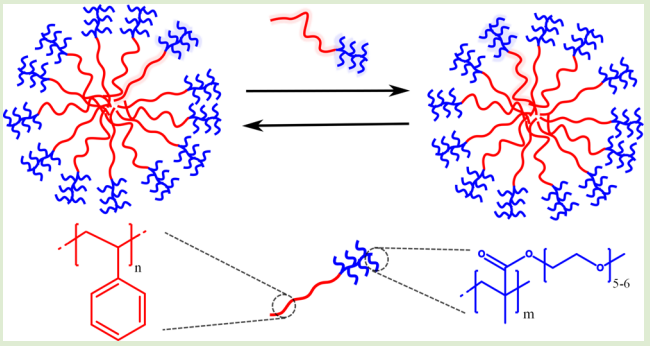
Molecular Exchange Kinetics of Diblock Copolymer Micelles Monitored by Fluorescence Correlation Spectroscopy

David Schaeffel, Andreas Kreyes, Yi Zhao, Katharina Landfester, Hans-Jürgen Butt, Daniel Crespy, and Kaloian Koynov*

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

Supporting Information

ABSTRACT: We investigated the equilibrium chain-exchange kinetics of amphiphilic diblock copolymer micelles, using a new method based on fluorescence correlation spectroscopy. The micelles were formed from polystyrene-*block*-poly[oligo(ethylene glycol) methyl ether methacrylate] (PS−POEGMA) in different solvents and studied at various temperatures. This linear-brush copolymer was chosen as a model system, forming micelles with short and bulky corona. Depending on the applied solvent, fast exchange could be observed even at temperatures well below the nominal glass transition of the core-forming PS block. The effect is caused by swelling of the core and allows extensive tuning of the chain-exchange rate by adding to the system minor amounts of good or bad solvent for the core block.



Amphiphilic block copolymers can self-assemble in aqueous solutions and form supramolecular structures like micelles or vesicles. In addition to numerous further applications, such self-assemblies are considered among the most promising candidates for drug carrier systems. In particular, block copolymer micelles with functional, nonimmunogenic hydrophilic corona and a hydrophobic core that can accommodate hydrophobic drugs have attracted growing interest.[1,2] Recently, the first polymeric micelle based drug carrier systems have entered clinical trials.[3] To further develop, tune, and optimize such carriers with respect to drug loading capacity, stability, long circulation times, and controlled release, it is of paramount importance to have a good knowledge of the physical processes governing the formation, the structure, and the kinetic stability of block copolymer micelles. However, while the static properties of block copolymer micelles have been extensively studied and are relatively well understood,[4−6] much less is known about their dynamic behavior, in particular for the process of chain exchange between micelles in equilibrium. Yet, such an exchange may have important effects on the micelle drug carrier properties, e.g., on their loading capacity, stability, and controlled release.

The chain exchange between block copolymer micelles at equilibrium was studied theoretically by Halperin and Alexander.[7] They derived an analytical model predicting that the exchange of individual chains through expulsion−insertion is the dominating mechanism, and eventual fission and fusion of polymer aggregates (micelles or "submicelles") plays only a secondary role. This result was further confirmed by dissipative particle dynamics simulations performed by Li and Dormidontova.[8] On the other hand, experimental studies are relatively rare, and the number of investigated block copolymer micelle systems remains extremely limited. The main reason is the lack of appropriate and easily accessible experimental techniques. Indeed, while methods based on fluorescence quenching,[9] sedimentation,[10] or cryo-TEM[11] have been used to study the exchange kinetics, to date the most important, quantitative results that could be compared with the theoretical predictions were obtained by time-resolved small-angle neutron scattering (TR-SANS) experiments performed by Richter and co-workers[12−16] and Bates, Lodge, and co-workers.[17−20] Clearly, the availability of new methods based on broadly accessible tabletop equipment shall boost the related studies and help to improve our understanding in this important field.

Fluorescence correlation spectroscopy (FCS) is a sensitive and selective method for investigating the mobility of fluorescent species, such as small molecules, macromolecules, or nanoparticles in various environments.[21] In a typical FCS experiment, the temporal fluorescence intensity fluctuations caused by, e.g., the diffusion of the studied fluorescent species through a small observation volume, are monitored and used to evaluate their diffusion coefficient, size, and concentration.[21] While initially developed and still predominantly used in molecular and cell biology,[22,23] FCS was also established as a powerful tool in polymer, colloid, and interface science.[24,25] Furthermore, it was already successfully used to study

Received: March 21, 2014
Accepted: April 16, 2014
Published: April 17, 2014

   dx.doi.org/10.1021/mz500169n | *ACS Macro Lett.* 2014, 3, 428−432

amphiphilic block copolymer based supramolecular structures. For example, Papadakis and co-workers applied FCS to study the formation of block copolymer micelles and investigate the effect of polymer architecture on the size and critical micelle concentration (CMC) of the micelles.[26,27] The formation of block copolymer vesicles, their loading with drugs, and the process of nanoparticles uptake by such vesicles were also studied by FCS.[28−30]

In this letter, we present a new method for studying the dynamic equilibrium chain-exchange between polymer micelles. The method is based on a variation of the classical FCS technique, called dual-color fluorescence cross-correlation spectroscopy (DC FCCS).[31] Compared to TR-SANS, DC FCCS uses tabletop equipment and fluorescent labeling that make it more easily accessible and applicable to a broad range of supramolecular structures. We apply this method to monitor the chain-exchange between polystyrene-*block*-poly[oligo-(ethylene glycol) methyl ether methacrylate] (PS−POEGMA) micelles and to investigate the effect of temperature and solvent quality on its rate.

The linear-brush block copolymer PS−POEGMA was chosen for two reasons. First, it represents a model system for a micelle-forming copolymer with a short and bulky corona block. The chain exchange between such types of micelles was never studied before. Second, PS−POEGMA is also a model functional polymer because of the very interesting properties displayed by the POEGMA block. Indeed, polymers of OEGMA are thermoresponsive in water and were found to display an antifouling behavior below their lower critical solution temperature and to have no specific interactions with biomolecules, which make them ideal for biomedical applications, e.g., drug delivery.[32] The PS−POEGMA copolymers were synthesized by atom transfer radical polymerization (Supporting Information (SI)). The degree of polymerizations of the hydrophobic PS and the hydrophilic POEGMA blocks were $N_{PS} \approx 47$ and $N_{POEGMA} \approx 10$, respectively. The polydispersity index of the PS block was PDI = M_w/M_n = 1.18 as measured by gel permeation chromatography (GPC). Thus, we expect that the micelles formed by the PS−POEGMA copolymer in polar media should have a rather dense and relatively thin corona. To enable DC FCCS studies, part of the copolymers were labeled either "blue" or "red" by covalent attachment of "blue" or "red" fluorescent BODIPY dyes to the PS block with a Diels−Alder reaction (see SI). The labeled copolymers were mixed with unlabeled ones in a weight ratio of 5:95. The mixture was dissolved in THF that is a good solvent for both blocks. Dispersions of "blue" or "red" micelles were obtained by stirring the copolymer THF solutions while dropping a selective solvent (water or methanol) for 40 min. This process was followed by an immediate quenching with an excess of the selective solvent. The micelle solutions were dialyzed for 3 days to remove the THF.

The formation of micellar structures was confirmed by measuring the hydrodynamic radius of the diffusing fluorescent species in the selective solvent solutions at different copolymer concentrations ranging from 0.01 to 4 μM using classical FCS as described in the SI.[26,27] In methanol, at very low concentrations only single chains with $R_H \approx 2$ nm were observed. However, at higher concentrations significantly larger species representing the formed micelles were recognized (Figure S2, SI). This allowed determination of the CMC of 0.04 μM in methanol. In water the CMC was below 0.01 μM. Thus, all further experiments were conducted at 4 μM polymer concentration, i.e., well above the CMC. The hydrodynamic radii of the micelles were determined to be $R_H = (13 \pm 2)$ nm in methanol and $R_H = (21 \pm 2)$ nm in water. Neither R_H nor the CMC of the micelles were affected by the type of the label, i.e., "blue" or "red", confirming that the labeling has a minor or no effect on the properties of the formed micelles. This is not surprising in view of the small size of the fluorescent labels and the fact that only 5% of the block copolymers were labeled.

To investigate the chain exchange kinetics, independently prepared dispersions of "blue" and "red" labeled PS−POEGMA micelles were mixed at a 1:1 ratio. The relative concentration of the double colored micelles that appeared as a result of chain exchange was measured as a function of time using DC FCCS. Detailed descriptions of the DC FCCS method and our experimental setup, which is based on a commercial FCS apparatus (Olympus and Pico Quant), are given elsewhere.[33] Briefly, two collinear laser beams with different wavelengths ("blue" and "red" for simplicity) are coupled to a confocal microscope and used to create subfemtoliter probing volumes V_b and V_r into the studied micellar solution. Ideally, these volumes are perfectly overlapping to create an efficient observation volume V_{br}.[33] The temporal fluctuations of the "red" and "blue" fluorescence signals $\delta F_b(t)$ and $\delta F_r(t)$ caused by the diffusion of fluorescent species through V_{br} were independently measured and analyzed by a cross-correlation function[31]

$$G_{br}(\tau) = \frac{\langle \delta F_b(t) \delta F_r(t+\tau) \rangle}{\langle F_b(t) \rangle \langle F_r(t) \rangle} \quad (1)$$

The amplitude of this function, $G_{br}(0)$, is directly proportional to the concentration of dual-colored species in the studied solution. Thus, if the fraction of dual-colored micelles increases with time $G_{br}(0)$ should also rise. This is illustrated in Figure 1 which shows experimental cross-correlation curves measured for a mixture of "red" and "blue" labeled PS−PEOGMA in methanol at T = 23 °C at different times after mixing. Furthermore, in addition to the cross-correlation function $G_{br}(\tau)$, two autocorrelation functions $G_{bb}(\tau)$ and $G_{rr}(\tau)$ can be defined using equations analogous to eq 1. By

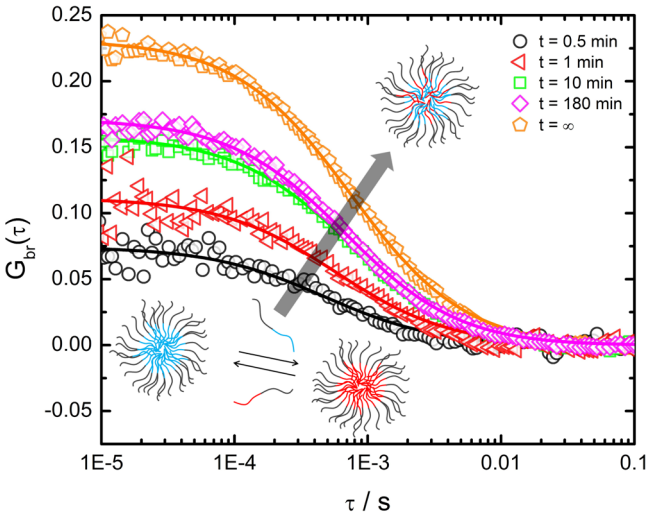


Figure 1. Cross-correlation curves measured at 23 °C in methanol at different times. The figure delineates that with progressing time the cross-correlation amplitude rises revealing an increase in the fraction of dual-colored micelles.

dx.doi.org/10.1021/mz500169n | ACS Macro Lett. 2014, 3, 428−432

fitting experimental auto- and cross-correlation functions with an analytical model for freely diffusing species,[21] the hydrodynamic radii and the concentrations of single and dual-colored micelles can be evaluated.[21,33] In particular the concentration of dual-colored micelles is given by $C_{br} = (G_{br}(0) - 1)V_{br}/(G_{bb}(0) - 1)V_b(G_{rr}(0) - 1)V_r$, and their relative fraction is $f_{br} = (C_{br}/C_b + C_r - C_{br})$. Finally, with the purpose of describing the exchange of polymers between the micelles and thus the transition of single-colored micelles to dual-colored ones in terms of a relaxation process similar to that used in TR-SANS experiments,[14,17] we define the experimental relaxation function as

$$R_{exp}(t) = \frac{f_{br}(\infty) - f_{br}(t)}{f_{br}(\infty) - f_{br}(0)} \quad (2)$$

Figure 2 (upper inset) shows typical relaxation functions $R_{exp}(t)$ measured for PS−POEGMA micelles in methanol at

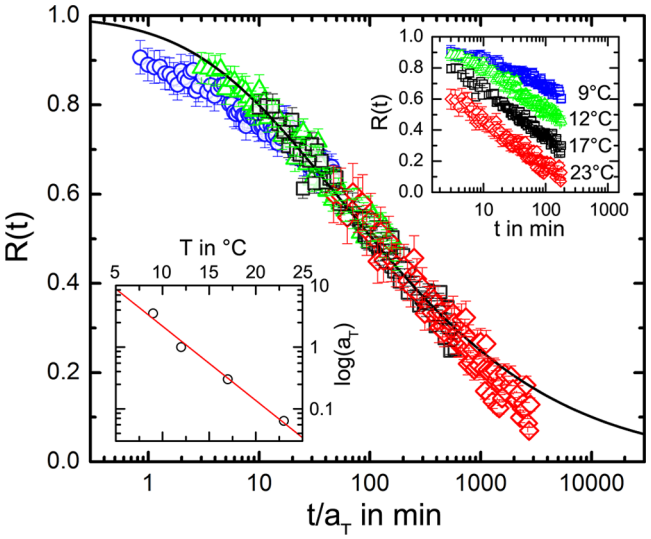


Figure 2. Relaxation functions of the chain exchange kinetics of PS−POEGMA micelles in methanol as measured with DC FCCS. A master curve is constructed by horizontal shifting of the individual relaxation functions measured at different temperatures (upper inset) to the reference temperature T_{ref} = 12 °C. The lower inset demonstrates that the temperature dependence of the shift factors follows the WLF equation. The solid line in the main figure represents a fit with eqs 3−5 (see text for details).

temperatures of 9, 12, 17, and 23 °C. An almost logarithmic time dependence of $R_{exp}(t)$ was observed, a result that agrees with earlier TR-SANS findings for star-shaped micelles.[14,17] This similarity is significant given the fact that the block copolymers studied here have short and bulky corona blocks and thus are expected to form thin corona micelles. The relaxation curves (Figure 2) display a trend to faster decay, i.e., faster exchange kinetics, at higher temperatures. As shown by Choi et al.[17] this effect is related to the temperature dependence of the chain relaxations of the PS blocks forming the micelle cores. The time−temperature superposition principle often used, e.g., for rheological data can be applied to create a "master curve". Such a master curve was constructed by horizontally shifting the relaxation curves measured at 9, 17, and 23 °C with respect to the curve measured at 12 °C (Figure 2). The results could be nicely superimposed, and the temperature dependence of the corresponding shift factors (lower inset in Figure 2) followed the classical Williams−Landel−Ferry (WLF) equation.[34]

Next, we compared our results with existing theoretical models to confirm their validity with respect to FCS-based experiments and obtain quantitative information on the relevant parameters for the studied PS−POEGMA micelles. As discussed above, there is an agreement[7,12−20,35] that the exchange of individual copolymer chains between micelles is the major relaxation mechanism. Fusion or fission processes have only little influence. Under this assumption, the chain exchange kinetics is almost solely governed by the expulsion of the block forming the core (PS in our case) from the micellar core through the corona into the solvent.[7,35] A time correlation function for the copolymer exchange can be defined as[14,17]

$$K(t, N_{Core}) = \exp\left[-\frac{t}{\tau}\exp\left(\frac{-A\gamma V_m^{2/3} N_{Core}^{\beta}}{kT}\right)\right] \quad (3)$$

Here k is the Boltzmann constant; T is the temperature; τ is the characteristic relaxation time; γ is the interfacial tension between the core forming polymer and solvent; and N_{Core} and V_m are degree of polymerization and monomeric volume of the core-forming polymer, respectively. A and β are parameters, which describe the conformation of the core polymer during the expulsion process as discussed below. The polydispersity of the core-forming block plays an important role[17] and was taken into account by convolving eq 3 with a Schulz−Zimm distribution

$$P(N_{Core}) = \frac{\xi^{\xi+1}}{\Gamma(\xi+1)}\frac{N_{Core}^{\xi-1}}{\langle N_{Core}\rangle^{\xi}}\exp\left(\frac{\xi N_{Core}}{\langle N_{Core}\rangle}\right) \quad (4)$$

where $\xi = 1/(\mathrm{PDI} - 1)$, to finally obtain a relaxation function that has the form

$$R(t, N_{Core}) = \int_1^{\infty} K(t, N_{Core})P(N_{Core})\mathrm{d}N_{Core} \quad (5)$$

We used eqs 3−5 to fit the experimental results (Figure 2). Not all parameters need to be varied, and many of them may be estimated from independent measurements or by using simple considerations. For example, the polydispersity of the core block and hence the parameter ξ were determined with GPC (SI). The second exponent in eq 4, $A\gamma V_m^{2/3} N_{Core}^{\beta}$, represents the activation energy in terms of creation of new interfacial area between the core polymer and solvent after expulsion. We estimated the interfacial tension between PS and methanol from the extended Fowkes equation[36,37] to be $\gamma \approx 7.5$ mN/m. The parameters A and β describe the conformation of the core's blocks. For a totally collapsed, solvent-free, globular conformation, A should be $(36\pi)^{1/3}$ and $\beta = 2/3$. For completely stretched chains, $A = (8\pi)^{1/3}$ with $\beta = 1$.[14,15] Since the number of repeat units of the core's block of our micelles is relatively low ($N_{PS} \approx 47$) and a dense corona is formed by the short bulky POEGMA blocks, a stretched polymer conformation of the PS during the expulsion process and thus $\beta = 1$ can be expected.[17−19] Furthermore, leaving β free to vary between 2/3 and 1, when fitting our experimental data, always resulted in $\beta \approx 1$. Therefore, to reduce the number of fit parameters we fixed $\beta = 1$ and used only A as a fit parameter describing the conformation of the core-forming chains. In addition, any further change of the activation energy, e.g., due to the penetration of solvent into the micelle core, can also be accounted for via the parameter A. The last unknown

dx.doi.org/10.1021/mz500169n | ACS Macro Lett. 2014, 3, 428−432

parameter describing the relaxation function (eq 3) is the characteristic time τ. Since the PS blocks of our micelles are short, the micelle core can be considered as an unentangled and partially swollen polymer melt. We followed Choi et al.[17] and chose the Rouse time τ_R as the characteristic time of the process. Thus, $\tau = \tau_R = 6\pi^2 kT/(N_{Core}^2 b^2 \zeta)$ with b and ζ being the monomer segment length and monomeric friction, respectively.

The value of b = 0.67 nm is known from literature reports[17,38] and was used as a fixed parameter. However, there are no available data for the monomeric friction coefficient ζ, e.g., from rheological measurements, because the PS was probably swollen to an unknown extent by methanol. Therefore, we used the monomeric friction coefficient ζ as a second fit parameter. Moreover, to account for the temperature dependence of ζ, we used eqs 3−5 to fit directly the "master curve" of experimental data (Figure 2). Therefore, our fit results correspond to the reference temperature T_{ref} = 12 °C. This approach fitted the experimental data reasonably well (Figure 2, solid line) and yielded $A = 1.25 \pm 0.01$ and $\zeta = (105 \pm 5)\cdot 10^{-5}$ Ns/m. This value of ζ is similar to that obtained by rheology for bulk nonentangled PS slightly above its glass transition.[38] As the master curve is constructed for a reference temperature of 12 °C, we estimated that the glass transition of the micelle's core should be around 5−8 °C. Although the low molecular weight of the PS chains implies a T_g of about 75 °C for this polymer,[38] only an additional swelling of the PS core with the surrounding methanol can explain the strong reduction of T_g. Here the possibility that a small amount of remaining THF is causing the core swelling can be ruled out since no chain exchange was observed for micelles formed in water as discussed below (inset Figure 3). The fact that the fit produced a value of A = 1.25, which is lower than $A = (8\pi)^{1/3} \approx 2.93$ expected for stretched PS chains, further indicates that the methanol has penetrated into the micelle's core and lowered the energy required for the polymer expulsion. As the exact extent of the core's swelling cannot be determined accurately, we roughly estimated it by applying the Fox equation.[34] Using the value of ≈−98 °C for the glass transition of methanol and ≈75 °C for PS we calculated that the cores of our PS−POEGMA micelles are swollen with roughly 25 wt % methanol.

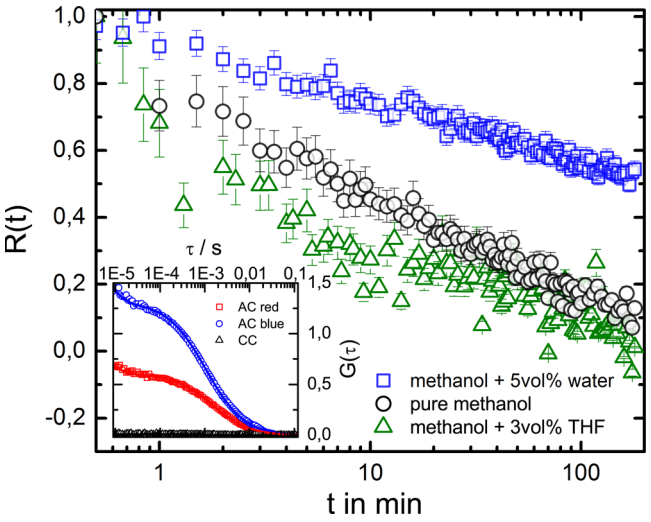


Figure 3. Relaxation curves of the exchange kinetics in methanol, methanol−water, and methanol−THF mixtures at 23 °C. The inset shows the auto- and cross-correlation curves of the micelles in water after several weeks. The amplitude of the cross correlation is practically zero, showing that there is no exchange between micelles in pure water at this temperature.

To examine further the effect of solvent on the chain-exchange kinetics, we studied micelles formed in methanol mixed with either 5 vol % of water that is a bad solvent for the PS core or 3 vol % of THF as a good solvent for the PS core (Figure 3). For the methanol−THF mixture the relaxation accelerates, indicating that the chain dynamics inside the PS micelle's core becomes faster and the energy required for chain expulsion decreases. The opposite is observed for the methanol−water mixture. The relaxation process slows down, suggesting an increased expulsion energy and slower dynamics inside the PS micelle's core. These findings demonstrate the major role that the solvent quality and the eventual core swelling have on the exchange dynamics. The latter process is especially important as it allows chain exchange at temperatures below the nominal glass transition of the core block. In the absence of core swelling the chain exchange dynamics of the studied PS−PEOGMA copolymer micelles is basically frozen at such temperatures. This is illustrated in the inset in Figure 3 that shows the auto- and cross-correlation curves measured a month after mixing "blue" and "red" labeled micelles formed in pure water and kept at 23 °C. The amplitude of the cross-correlation is practically zero, showing that there was no chain exchange between the micelles even after this extended period of time.

In summary, we have presented a new method for studying the chain exchange kinetics in diblock copolymer micelles by using dual-color fluorescence cross-correlation spectroscopy (DC FCCS). This technique employs tabletop equipment and fluorescent labeling that makes it accessible to a large research community and applicable to a broad range of copolymer systems. We applied the new method to measure the exchange kinetics of micelles formed by a linear-brush copolymer PS−POEGMA, as a model system with short and bulky corona blocks. By varying the temperature and comparing the results with a scaling theory reported earlier,[7,17,35] we were able to quantify the extent of swelling of the PS micelle's core and explain the fast exchange that takes place at temperatures well below the nominal glass transition of PS. Furthermore, we showed that the addition of small amounts of either good or bad solvent for the PS core had a tremendous effect on the exchange kinetics.

■ ASSOCIATED CONTENT

Supporting Information

Description of the FCS setup and principle as well as determination of the CMC. Synthesis and characterization of the fluorescent dyes and polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: koynov@mpip-mainz.mpg.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The financial support from DFG (SFB 1066, Q2) is gratefully acknowledged.

REFERENCES

(1) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug Delivery Rev.* **2001**, *47*, 113−131.
(2) Batrakova, E. V.; Kabanov, A. V. *J. Controlled Release* **2008**, *130*, 98−106.
(3) Matsumura, Y.; Kataoka, K. *Cancer Sci.* **2009**, *100*, 572−579.
(4) Lodge, T. P.; Pudil, B.; Hanley, K. J. *Macromolecules* **2002**, *35*, 4707−4717.
(5) Jain, S.; Bates, F. S. *Science* **2003**, *300*, 460−464.
(6) Halperin, A.; Tirrell, M.; Lodge, T. P. *Adv. Polym. Sci.* **1992**, *100*, 31−71.
(7) Halperin, A.; Alexander, S. *Macromolecules* **1989**, *22*, 2403−2412.
(8) Li, Z. L.; Dormidontova, E. E. *Soft Matter* **2011**, *7*, 4179−4188.
(9) Prochazka, K.; Bednar, B.; Mukhtar, E.; Svoboda, P.; Trnena, J.; Almgren, M. *J. Phys. Chem.* **1991**, *95*, 4563−4568.
(10) Tian, M.; Qin, A.; Ramireddy, C.; Webber, S. E.; Munk, P.; Tuzar, Z.; Prochazka, K. *Langmuir* **1993**, *9*, 1741−1748.
(11) Jain, S.; Bates, F. S. *Macromolecules* **2004**, *37*, 1511−1523.
(12) Willner, L.; Poppe, A.; Allgaier, J.; Monkenbusch, M.; Richter, D. *Europhys. Lett.* **2001**, *55*, 667−673.
(13) Lund, R.; Willner, L.; Stellbrink, J.; Lindner, P.; Richter, D. *Phys. Rev. Lett.* **2006**, *96*, 068302.
(14) Lund, R.; Willner, L.; Stellbrink, J.; Lindner, P.; Richter, D. *Phys. Rev. Lett.* **2010**, *104*, 049902.
(15) Lund, R.; Willner, L.; Pipich, V.; Grillo, I.; Lindner, P.; Colmenero, J.; Richter, D. *Macromolecules* **2011**, *44*, 6145−6154.
(16) Zinn, T.; Willner, L.; Lund, R.; Pipich, V.; Richter, D. *Soft Matter* **2012**, *8*, 623−626.
(17) Choi, S. H.; Lodge, T. P.; Bates, F. S. *Phys. Rev. Lett.* **2010**, *104*, 047802.
(18) Choi, S. H.; Bates, F. S.; Lodge, T. P. *Macromolecules* **2011**, *44*, 3594−3604.
(19) Lu, J.; Choi, S.; Bates, F. S.; Lodge, T. P. *ACS Macro Lett.* **2012**, *1*, 982−985.
(20) Lu, J.; Bates, F. S.; Lodge, T. P. *ACS Macro Lett.* **2013**, *2*, 451−455.
(21) Rigler, R.; Elson, E. *Fluorescence correlation spectroscopy: theory and applications*; Springer series in chemical physics, 65; Springer: Berlin; New York, 2001.
(22) Kim, S. A.; Schwille, P. *Curr. Opin. Neurobiol.* **2003**, *13*, 583−590.
(23) Hess, S. T.; Huang, S.; Heikal, A. A.; Webb, W. W. *Biochemistry* **2002**, *41*, 697−705.
(24) Woll, D. *RSC Adv.* **2014**, *4*, 2447−2465.
(25) Koynov, K.; Butt, H.-J. *Curr. Opin. Colloid Interface* **2012**, *17*, 377−387.
(26) Bonné, T. B.; Ludtke, K.; Jordan, R.; Stepanek, P.; Papadakis, C. M. *Colloid Polym. Sci.* **2004**, *282*, 1425−1425.
(27) Bonné, T. B.; Ludtke, K.; Jordan, R.; Papadakis, C. M. *Macromol. Chem. Phys.* **2007**, *208*, 1402−1408.
(28) Mueller, W.; Koynov, K.; Fischer, K.; Hartmann, S.; Pierrat, S.; Basche, T.; Maskos, M. *Macromolecules* **2009**, *42*, 357−361.
(29) Jaskiewicz, K.; Larsen, A.; Lieberwirth, I.; Koynov, K.; Meier, W.; Fytas, G.; Kroeger, A.; Landfester, K. *Angew. Chem., Int. Ed.* **2012**, *51*, 4613−4617.
(30) Jaskiewicz, K.; Larsen, A.; Schaeffel, D.; Koynov, K.; Lieberwirth, I.; Fytas, G.; Landfester, K.; Kroeger, A. *ACS Nano* **2012**, *6*, 7254−7262.
(31) Schwille, P.; MeyerAlmes, F. J.; Rigler, R. *Biophys. J.* **1997**, *72*, 1878−1886.
(32) Lutz, J.-F. *Adv. Mater.* **2011**, *23*, 2237−2243.
(33) Schaeffel, D.; Staff, R. H.; Butt, H. J.; Landfester, K.; Crespy, D.; Koynov, K. *Nano Lett.* **2012**, *12*, 6012−6017.
(34) Rubinstein, M.; Colby, R. H. *Polymer Physics*; Oxford University Press: USA, 2003.
(35) Dormidontova, E. E. *Macromolecules* **1999**, *32*, 7630−7644.
(36) Fowkes, F. M. *J. Phys. Chem.* **1962**, *66*, 382.
(37) Li, I. T. S.; Walker, G. C. *J. Am. Chem. Soc.* **2010**, *132*, 6530−6540.
(38) Chapman, B. R.; Hamersky, M. W.; Milhaupt, J. M.; Kostelecky, C.; Lodge, T. P.; von Meerwall, E. D.; Smith, S. D. *Macromolecules* **1998**, *31*, 4562−4573.