# **Supporting Information**

# Diffusion of single molecular and macromolecular probes during the free radical bulk polymerization of MMA – towards a better understanding of the Trommsdorff effect on a molecular level

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# 1 Distributions of diffusion coefficients during the autoacceleration period



**Fig. S1**: Distributions of diffusion coefficients for the four different probes used in this paper for times within the autoacceleration period. The reference time  $t_R$  is the time where an average diffusion coefficient of log  $(D / m^2 s^{-1}) = -13.5$  was found (see paper). It should be noted that, due to better visibility the scaling of **PDI0**, **PDI-PMMA13k** and **PDI-PMMA150k** changes at  $t_R$ -1 min ,  $t_R$ -2 min and  $t_R$ -1 min, respectively.

# 2 Evaluation of the distributions of diffusion coefficients

A statistical analysis of the distributions shown in Figure S1 is presented in Table S1. For evaluation of the standard deviations, trajectories with the same lengths exhibiting normal diffusion (Monto Carlo random walks) with the average diffusion coefficient of the measured data were simulated and the distribution of their diffusion coefficients analysed. It is obvious that the measured distributions are significantly broader reflecting the heterogeneity appearing during the polymerizations.

**Tab. S1**: Statistical analysis of the distributions presented in Fig. S1. \*The movies of these polymerization times contain several probes too fast to be tracked. \*\*Insufficient statistics to determine a reliable standard deviation.

probe	time with respect to t <sub>R</sub> in Min	mean of log10(D)	standard deviation of log10( <i>D</i> )	standard deviation of simulated data	number of trajectories
	-9	-15.5	**	**	1 *
	-6	-14.8	**	**	2 *
	-3	-13.4	0.34	0.30	6 *
	-2	-13.4	0.63	0.32	55 *
<u> </u>	-1	-13.4	0.49	0.42	689
	0	-13.5	0.41	0.34	1293
<u> </u>	1	-14.0	0.36	0.23	754
	2	-14.8	0.36	0.17	536
	3	-15.5	0.51	0.15	387
	5	-15.7	0.62	0.14	304
	6	-15.7	0.71	0.13	336
	-6	-13.6	0.73	0.38	169 *
	-5	-13.4	0.56	0.38	268 *
	-4	-13.5	0.54	0.44	343 *
	-2	-13.5	0.56	0.35	358 *
<u>.</u>	-1	-13.5	0.54	0.38	587
	0	-13.7	0.50	0.32	747
	1	-13.9	0.41	0.27	665
	2	-14.1	0.45	0.26	607
	3	-14.4	0.47	0.21	542
	4	-14.8	0.40	0.20	440
	6	-15.6	0.52	0.17	369
	-9	-13.7	0.87	0.48	38 *
ž	-7	-13.5	0.69	0.35	89 *
Ξ	-6	-13.7	0.77	0.38	108 *
A A	-5	-13.6	0.47	0.31	79 *
	-2	-13.5	0.71	0.37	389 *
6	0	-13.6	0.53	0.27	672
그	1	-14.2	0.54	0.25	515
	2	-15.3	0.59	0.16	274
	4	-15.4	0.69	0.17	280
	5	-15.3	0.74	0.19	298
×	-11	-15.1	0.97	0.17	149 ^
0	-6	-15.0	1.05	0.15	1/2 ^
15	-5	-15.0	1.14	0.20	199 *
I ₹	-3	-14.3	1.21	0.28	279 "
Σ	-1	-13.7	0.80	0.32	1204
N	0	-13.8	0.73	0.23	1099
느	4	-15.0	0.60	0.15	331
0	5	-15.7	0.68	0.15	340
<u>д</u>	/	-15.5	0.72	0.18	417
	Ö	-15.5	0.72	0.10	428

## **3** Time-conversion curves as determined by Raman spectroscopy

The time-conversion curves were determined by Raman spectroscopy using the ratio between two characteristic Raman bands as reported previously.<sup>[1]</sup> Time-conversion curves for four different polymerization experiments with different fluorescence probes are presented in Figure S2. Since the probes are present in nanomolar amounts their presence is not expected to influence the polymerization kinetics. The violet curve of this figure was taken as the basis to convert time data to conversions. The reference points with an average diffusion coefficient of log (D / m<sup>2</sup> s<sup>-1</sup>) = -13.5 are marked with dashed lines in the corresponding colors.



Fig. S2: Time-conversion curves for four different polymerization experiments with different probes. The Trommsdorff effect region in these experiments varied slightly by some minutes presumably due to slight variations in temperature. In order to obtain a reliable master curve (see violet curve), the data were slightly adjusted by addition/substraction of some minutes so that their steepest point converge.

# 4 Synthesis of perylendiimide labeled PMMA chains

### Materials

Methyl methacrylate (MMA, Sigma Aldrich) was purified via filtration through inhibitor removers (Sigma-Aldrich). N-Ethyldiisopropylamine (DIPEA), maleic anhydride, and toluene was purchased from Fluka. 1-Hydroxybenzotriazole hydrat (HOBT), 3-chloro-1-propanol, *n*hexane, ethyl acetate, sodium azide, 4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid, 2-Cyano-2-propyl benzodithioate (Sigma-Aldrich), DEAD in toluene, and PPh<sub>3</sub> were purchased from Sigma Aldrich. N,N,N',N'-Tetramethyl-O-(1*H*-benzotriazol-1-yl)uroniumhexafluoro-phosphat (HBTU) was purchased from ABCR, and dimethyl formamide from Güssing.

### Synthesis of RAFT agent 3



Synthesis was performed via an adapted literature approach.<sup>[2]</sup> To a solution of 4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid 1 (504 mg, 1.25 mmol) in anhydrous THF (50 mL) were added PPh<sub>3</sub> (491 mg, 1.87 mmol) and 3-azido-1-propanol 2 (190 mg, 1.87 mmol) under argon. The mixture was cooled to 0 °C and diethyl azodicarboxylate (DEAD, 0.84 mL, 40 wt%, 1.87 mmol) in toluene was added, and the solution stirred overnight. Afterwards, DCM (25 mL) was added, and the solution washed with a saturated solution of NaHCO<sub>3</sub> (25 mL). The organic layer was separated and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed and the product **3** purified via column chromatography (*n*hexane/EtOAc, 20:1 v:v). A yellow oil (247 mg, 41 % yield) was obtained.

<sup>1</sup>H-NMR (toluene-*d*8, 300 MHz):  $\delta = 4.20$  (*t*, *J* = 6 Hz, 2H, CH<sub>2</sub>, e), 3.41 (t, J = 6.6 Hz, 2H, CH<sub>2</sub>, g), 3.33 (t, J = 7.5 Hz, 2H, CH<sub>2</sub>, a), 2.64 (m, 2H, CH<sub>2</sub>-S, d), 2.52 (m, 1H, CH<sub>2</sub>, c'), 2.43–2.32 (m, 1H, CH<sub>2</sub>, c''), 1.93 (m, 2H, CH<sub>2</sub>, f), 1.88 (s, 3H, CH<sub>3</sub>, b), 1.66 (m, 3H, CH<sub>3</sub>, d), 1.40–1.26 (m, 20 H, CH<sub>2</sub>, a), 0.88 (m, 3H, CH<sub>3</sub>, a).

<sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>):  $\delta = 216.87$  (CS<sub>3</sub>), 171.34 (CO<sub>2</sub>), 125.51 (CN), 61.97 (e-CH<sub>2</sub>-), 48.12 (g-CH<sub>2</sub>-), 46.33 (b-C), 37.08 (a-CH<sub>2</sub>-), 34.23 (c-CH<sub>2</sub>-), 33.83 (c'-CH<sub>2</sub>-), 31.91 (a-CH<sub>2</sub>-), 30.32 (a-CH<sub>2</sub>-), 29.73 (d-CH<sub>2</sub>-), 29.61 (a-CH<sub>2</sub>-), 29.54 (a-CH<sub>2</sub>-), 29.41 (a-CH<sub>2</sub>-), 29.33 (a-CH<sub>2</sub>-), 29.06 (a-CH<sub>2</sub>-), 28.92 (c-CH<sub>2</sub>-), 28.05 (f-CH<sub>3</sub>), 27.67 (a-CH<sub>2</sub>-), 25.61 (b-CH<sub>3</sub>), 24.92 (b'-CH<sub>3</sub>), 22.69 (a-CH<sub>2</sub>-), 21.18 (a-CH<sub>2</sub>-), 14.12 (a-CH<sub>3</sub>).

ESI-MS (ESI, m/z): 487.222 (Calc.: 487.223 (M+H)), 509.204 (Calc.: 509.205 (M+Na)).



Synthesis of PDI-Amin 5

Synthesis via activation with HOBT and HBTU:<sup>[3]</sup> Under argon, HOBT (47.8 mg, 0.15 mmol) in DMF (0.5 mL), HBTU (109.9 mg, 0.14 mmol) in DMF (0.5 mL) and DIPEA (167 mg, 0.14 mmol) in DMF (0.3 mL) were added to a solution of carboxylic acid-functionalized PDI **4** (202.4 mg, 0.14 mmol) in DMF (10 mL). The reaction mixture was allowed to stir for 5 minutes before an excess of ethylene diamine (0.6 mL) was added. The reaction was stirred overnight at ambient temperature. The dye was precipitated in 1 M HCl (50 mL) and filtered off. The solid was washed with deionized water (50 mL) and after drying dissolved in acetone (10 mL). After removal of the solvent the resulting dye mixture was purified via column chromatography (*n*-hexane/EtOAc, 10:1, v:v) yielding 90.8 mg of **5** as a red solid. Also, 67.4 mg of the starting material could be reisolated.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta = 8.16$  (*s*, 2H, CH, PDI-core), 8.06 (*s*, 2H, CH, PDI-core), 7.40 (*s*, 1H, CH, arom.), 7.26–7.23 (m, 10H, CH, arom.), 6.82 (*m*, 8H, CH, arom.), 4.34 (m, 10H, CH, arom.), 3.49 (*m*, 2H, CH<sub>2</sub>, (CO)<sub>2</sub>NCH<sub>2</sub>), 3.12 (*m*, 2H, CH<sub>2</sub>, CONHCH<sub>2</sub>), 2.77–2.56 (*m*, 4H, 2×CH<sub>2</sub>), 1.69 (*m*, 8H, 4×CH<sub>2</sub>), 1.32 (*m*, 24H, 4×2×CH<sub>3</sub>), 1.10 (*m*, 12H, 2×2×isopropyl-CH<sub>3</sub>), 0.73 (*m*, 36H, 4×3×CH<sub>3</sub>).

ESI-MS (ESI, m/z): 1482.8 (Calc.: 1482.9)

# Synthesis of PDI-Maleimid 6

PDI-Amine **5** (90.8 mg, 61  $\mu$ mol) was dissolved in DCM (12 mL). NaOAc (8.4 mg, 100  $\mu$ mol) and maleic anhydride (15.5 mg, 158  $\mu$ mol) were added and the solution stirred for 3 h. The solvent was removed and acetic anhydride (6.5 mL) and NaOAc (6.0 mg, 73 $\mu$ mol) added. The mixture was stirred in the dark for 16 hours at 90 °C. The solvent was removed and the crude product purified via filtration trough Allox (DCM/MeOH, 9:1, v:v) yielding 88.2 mg of **6** as a red solid (overall yield over two steps: 40%).

<sup>1</sup>H-NMR(CDCl<sub>3</sub>, 300 MHz) δ = 8.20 (*s*, 2H, CH, PDI-core), 8.13 (*s*, 2H, CH, PDI-core), 7.42 (*s*, 1H, CH, arom.), 7.33–7.23 (m, 10H, CH, arom.), 6.87 (*m*, 8H, CH, arom.), 6.68 (*s*, 2H, 2×maleimide-H), 4.24 (m, 10H, CH, arom.), 3.85 (*m*, 2H, (CO)<sub>2</sub>NCH<sub>2</sub>), 3.72 (*m*, 2H, CONHCH<sub>2</sub>), 2.74–2.64 (*m*, 4H, 2×CH<sub>2</sub>), 1.72 (*m*, 8H, 4×CH<sub>2</sub>), 1.35 (*m*, 24H, 4×2×CH<sub>3</sub>), 1.12 (*m*, 12H, 2×2×isopropyl-CH<sub>3</sub>), 0.75 (*m*, 36H, 4×3×CH<sub>3</sub>).

ESI-MS (ESI, m/z): 1583.9 (Calc.: 1583.9 (M+Na))

#### Synthesis of PMMA13k (7) by RAFT polymerization

To ABIN (62.0 mg, 0.38 mmol), 2-Cyano-2-propyl benzodithioate (211.4 mg, 0.96 mmol), MMA (18.9 g) and toluene (11.2 g) were added and the solution was degassed via three freeze-pump-thaw circles. In a glovebox, the samples were transformed into small sample flask and polymerized at 60 °C in a heating block. After certain time intervals a flask was removed and the reaction quenched by cooling down at 0 °C. The conversion was determined gravimetrically and the polymer analyzed via SEC. A polymer sample with  $M_n = 13395 \text{ g mol}^{-1}$ ,  $M_w = 15149 \text{ g mol}^{-1}$  and D = 1.13 was obtained and used for further modification.

#### Synthesis of PMMA150k (8) by RAFT polymerization

To ABIN (10.1 mg, 61 µmol), RAFT agent **3** (19.7 mg, 39 µmol), MMA (30.1 g) and toluene (10.0 g) were added and the solution was degassed via three freeze-pump-thaw circles. In a glovebox the samples were transformed into small sample flask and polymerized at 60 °C in a heating block. After certain time intervals a flask was removed and the reaction quenched by cooling down at 0 °C. The conversion was determined gravimetrically and the polymer analyzed via SEC. From this synthesis a polymer sample with  $M_n = 155530 \text{ g mol}^{-1}$  and  $M_w = 209090 \text{ g mol}^{-1}$ , D = 1.34 was obtained and used for further modification.

#### Polymer labeling of the RAFT polymers (7 and 8) with perylene diimide derivative 6



The polymer was reacted with the perylene diimide derivative 10 via a thiole-ene reaction.<sup>[4,5]</sup> The polymer was dissolved in 2 mL of DMF and flushed with argon for 10 min. *n*-Butylamine (0.1 mL) was added under argon and the reaction was quenched after 15 min by addition of acetic acid (0.15 mL). Subsequently, a solution of the dye (1 mg) in DMF (1 mL) was added under argon. After 10 min, the reaction tube was released from the argon stream and shaken overnight. The polymer was precipitated in *n*-hexane (70 mL), filtered off and washed once with *n*-hexane (50 mL). Due to the small amounts, it was chosen to dissolve the polymer from the filter with acetone. For the **PDI-PMMA13k** polymer, after evaporation of the solvent, the polymer was dissolved in DMF (2 mL) and the precipitation procedure repeated several times until the amount of free dye was below 1%, as determined via GPC with fluorescence detection (see Figure S3). For the **PDI-PMMA150k** polymer, ethyl acetate (10 mL) was added and the polymer precipitated in *n*-hexane as described for the **PDI-PMMA13k** polymer until the amount of free dye was below 1%, as determined via GPC with fluorescence detection (see Figure S3).



**Fig. S3**: Molecular mass distribution determined via GPC: (left) for the 13k polymer and (right) for the 150k polymer. The refractive index measured after GPC is presented in black whereas the fluorescence at an excitation wavelength of 580 nm measured at a wavelength of 620 nm is drawn in red. The high value of the refractive index at low molecular weight in the 13k sample is caused by a baseline shift.



## **5** Fluorescence spectra of the probes

**Fig. S4**: Fluorescence spectra of the probes measured in toluene solution with an excitation wavelength of 543 nm. The spectra were normalized to 1 at their emission maximum.



# 6 Quality of the scaling law fits of equation (1) to FCS data

Fig. S5: Evaluation of the fits of Fig. 1 according to the scaling law presented in equation (1). On the left side the relative mean square deviations between data points and fitted values are presented for different values of  $\alpha$  and  $\nu$ . Blue regions present good fits, dark red regions worse fits. On the right side representative fits are presented for different "good" points. The darker the color of the fit, the lower is its mean square deviation.

The values fitted according to equation (1) are not totally independent from each other. In the left graphs of Fig. S5, we show how simultaneous variation of both fitting values,  $\alpha$  and  $\nu$ , can result in similar fitting quality. We set the scale so that mean square deviations with up to 10% higher values than the optimum are plotted in dark red. The regions with the lowest mean square deviations are shown in dark blue. The  $\alpha$ - and  $\nu$ -values of the point with the lowest mean square deviation was taken as optimum. The standard deviation was determined from the region with mean square deviations within 10% of the optimum.

# 7 Supporting widefield fluorescence microscopy movies

The supporting widefield movies are presented in a video file in the Supporting Material. Kinetic series of fluorescence microscopy movies were recorded using the following setup and acquisition parameters: A Cobolt Jive laser (75 mW) was operated at a wavelength of 561 nm to excite the fluorophores. It was coupled into a DMI6000 B microscope (Leica Microsystems GmbH) via a multimode glass fiber (NA= $0.22 \pm 0.02$ ). The fiber was shaken (Vortex, 2000 UPM) in order to suppress speckles by averaging over time. The fiber end was projected into the sample using an HCX PL APO 100 1.40 - 0.70 oil immersion objective (Leica Microsystems GmbH). Fluorescence light was collected with the same objective and separated from the originating laser wavelength by a dichroic filter z561/660rpc (Chroma Technology Corp.). After passing a long pass filter (RazorEdge LP568 RU), the signal was imaged by two photo objectives (Nikkor 28 mm f2.8,  $f = \infty$ ; Nikkor 85mm f2.0,  $f = \infty$ ) onto the chip of an Andor Ixon DV887DCS-BV EMCCD camera. The chip was cooled to -80 °C. As soon as the reaction mixture had reached an appropriate viscosity, recording of the movies was started. The focal plane conjugated to the fiber end was projected into the sample at a distance of 8 µm from the cover glass. Each measurement was taken at a different spot to minimize bleaching effects. Parameters of the EMCCD camera were as follows: acquisition mode: kinetics; number in kinetics series: 300; EM-DAC level: 255; exposure time: 100 ms; pre-amplifier gain: 5; readout mode: full resolution image 512 by 512 pixels. The conjugated length scale in the sample was calculated to 52.3 nm per pixel.

### 8 Supporting Literature

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