

Supporting Information

EPR Study of Spin Labeled Brush Polymers in Organic Solvents

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Experimental Section

Materials. All reagents were obtained from commercial vendors and used as received unless otherwise noted. Styrene was distilled over CaH_2 before use; *p*-chlorostyrene was passed through a plug of neutral alumina. NB-PLA MMs were prepared according procedures we previously published.¹

Characterizations. ^1H and ^{13}C NMR spectra were recorded in CDCl_3 using a Varian Mercury 300 or Varian Inova 500 spectrometer. Chemical shifts are reported in ppm relative to CDCl_3 ($\delta = 7.27$).

High-resolution mass spectra were obtained using an Agilent 6200 series accurate-mass time-of-flight (TOF) LC/MS.

Gel permeation chromatography (GPC) was carried out in THF on two PLgel 10 μm mixed-B LS columns (Polymer Laboratories) connected in series with a DAWN EOS multiangle laser light scattering (MALLS) detector and an Optilab DSP differential refractometer (both from Wyatt Technology). No calibration standards were used, and a dn/dc value of 0.05 mL/g was calculated for PLA brush polymers assuming 100% mass recovery.

Electron paramagnetic resonance (EPR) spectroscopy was performed on a Bruker EMX X-band spectrometer. EPR tubes with O. D. 1 mm were used. The solutions were deoxygenated by bubbling argon then sealed prior to the EPR measurements. Typical parameters used for the EPR

measurements are modulation frequency: 100 KHz; modulation amplitude: 1 G; time constant: 11 ms; conversion time: 86 ms; scan time: 86 s; number of scans: 5. For the quenching experiments, the solutions were prepared in air. The EPR spectra were recorded following addition of the quencher. The peak intensity of the low field peak was used for calculation of the percentage of quenching.

Synthesis of (Norbornene-*exo*-dicarboximido)-*N*-(TEMPO)butamide **2.** To a round-bottom flask was added (5-norbornene-*exo*-2,3-dicarboximido)butanoic acid (500 mg, 2.01 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) (385 mg, 2.01 mmol) and 4-dimethylaminopyridine (DMAP) (25 mg, 0.20 mmol), followed by 8 mL THF. 2,2,6,6-tetramethyl-4-amino-piperidine-1-oxyl (0.25 g, 2.5 mmol) was added as a solution in 2 mL THF. The reaction mixture was allowed to stir under nitrogen at room temperature overnight. The reaction mixture was washed with water (2x 20 mL) and brine (20 mL) and dried over MgSO₄. The solvent was evaporated and the remaining residual was purified by silica gel chromatography (ethyl acetate/hexanes, 3:1 v/v) to give pure **2** as a dark red viscous oil (78% yield). ¹H NMR (500 MHz, CDCl₃): broad peaks at δ 1.1-1.3, 1.4-1.7, 1.8-2.3, 2.5-2.7, 3.1-3.3, 3.3-3.7, 5.2-5.4, 6.2-6.4. ¹³C NMR (125 MHz, CDCl₃): δ 24.9, 36.8, 38.0, 43.1, 45.5, 48.3, 138.2, 171.4, 178.8. HRMS (TOF+) *m/z* calcd for C₂₂H₃₃O₄N₃ [M+H]⁺: 403.24712, found 403.24759. Elemental analysis, theoretical C 65.7%, H 8.0%, N, 10.4%, found C 64.5%, H 7.8%, N 10.2%.

Synthesis of End- and Middle-Labeled Brush Polymers. In a glove box, an oven-dried small vial was charged with the desired amount of Grubbs catalyst **1** in ~100 µl anhydrous DCM and a stir bar. The desired amounts of monomer **2** and PLA macromonomer were added in sequence via syringe or pipette. The sequence of addition controls the sequence of the blocks in the final brush polymers. After each addition, the reaction vial was stirred at room temperature for 10 min to allow complete conversion of the monomer or the macromonomer. After the polymerization was complete, the reaction mixture was quenched with one drop of ethyl vinyl ether. A small sample was withdrawn for GPC measurement. The rest of the reaction mixture was then diluted and precipitated into stirring MeOH at least three times. The resulting brush polymer was dried *in vacuo*.

Synthesis of Peripherally Labeled Brush Polymers. In an oven-dried vial, PLA brush homopolymer (backbone DP=100) was dissolved in anhydrous DCM. *N*-TEMPO-succinamic

acid (0.05 eq), 0.1 eq EDC, and 0.01 eq DMAP were added to the reaction vial. The reaction was stirred under nitrogen at room temperature overnight. The polymer was precipitated into stirring MeOH at least three times to remove all the reagents and any residual TEMPO-succinamic acid. The resulting polymer was dried *in vacuo*.

Synthesis of Polystyrene-*co*-poly(4-chlorostyrene) (PS-PSCI) Random Copolymer by Atom Transfer Radical Polymerization (ATRP). CuBr (62 mg, 0.44 mmol) was added to a two-neck round bottom flask fitted with a stir bar. The flask was sealed with a rubber septum, evacuated, and backfilled with argon. Styrene (5.0 mL, 44 mmol), 4-chlorostyrene (261 μ L, 2.18 mmol), and *N,N,N,N*-pentamethyldiethylenetriamine (PMDETA, 91 μ L, 0.44 mmol) were added via syringe. The mixture was stirred for 5 min to allow formation of the PMDETA-CuBr complex. 1-Bromoethylbenzene (298 μ L, 2.18 mmol) was then added and the flask was placed in a preheated 100 °C oil bath. The reaction was stirred under argon for 6 h. After this time the flask was opened to air and diluted with THF. The solution was passed through a column of neutral alumina, concentrated by rotary evaporation, and precipitated into 10:1 v:v methanol. The polymer precipitate was filtered, re-dissolved in a minimal amount of THF, and precipitated into methanol. This precipitation procedure was repeated 2 more times; after the final precipitation and filtration the fluffy white polymer (3.8 g, 79% yield) was transferred to a round-bottom flask and dried under vacuum for 48 h. M_n (GPC) = 2,480 Da, PDI = 1.09.

Synthesis of Polystyrene-*co*-poly(*p*-hydrazinostyrene) (PS-PSNHNH₂). [Pd(cinnamyl)Cl]₂ (21 mg, 40 μ mol), Mor-DalPhos (28 mg, 60 μ mol), and toluene (2 mL) were combined in a vial in a glovebox. The solution was stirred for 5 min at room temperature. NaOtBu (154 mg, 1.60 mmol), **PS-PSCI** (2 g, 0.8 mmol), and toluene (3 mL) were then added. The vial was capped with a septum and removed from the glovebox. Hydrazine hydrate (79 μ L, 0.77 mmol) was added via syringe while flushing with argon. The reaction mixture was stirred at 110 °C for 3 h under argon atmosphere. After cooling, the reaction mixture was passed through an alumina column with THF eluent, concentrated to < 1 mL, and precipitated into MeOH (30 mL). The precipitate was filtered, re-dissolved in THF (~ 1 mL), and added dropwise to MeOH (30 mL). This procedure was performed once more (for a total of three precipitations), and the filtered polymer was dried under vacuum for 48 h. M_n (GPC) = 2,350 Da, PDI = 1.10. MALDI-TOF clearly indicates the polymer structure containing hydrazine moiety (Figure S5). Determination

of hydrazine content from NMR has been challenging. Elemental analysis found C 90.7%, H 7.7%, N 0.2%, which corresponds to one hydrazine unit per 120 repeat units on average.

Quenching of EPR Signals. Stock solutions of E-TEMPO-Brush, M-TEMPO-Brush, and P-TEMPO-Brush (0.02 mM, [nitroxide] = 0.1 mM), phenylhydrazine (1.5 mM) and PS-hydrazine (1.5 mM) in DMF were prepared at room temperature. The EPR spectrum of each brush polymer was recorded at a concentration of 0.01 mM by diluting the stock solution. For a typical quenching experiment, the stock solution of a brush polymer (40 μ L) was mixed with phenylhydrazine (or PS-hydrazine) (40 μ L) and the resulting solution was quickly transferred to an EPR tube (O.D. 1 mm). The EPR spectrum was recorded over time and the intensity of the low-field peak was used to calculate the percentage of quenching.

Computation of EPR spectra. The computation of the EPR spectra was performed according to a well-known procedure reported by Budil *et al.*² using program NLSL. This program provides an automatic fitting between the computed and the experimental spectra by changing each magnetic or mobility parameter. For simulated EPR spectra, only the hyperfine coupling between the electron spin and the nitrogen nuclear spin was used. The computation allowed us to extract the following parameters: (a) the g_{ii} components of the g tensor for the coupling between the electron spin and the magnetic field (if not specified, these parameters were assumed to be 2.009, 2.006, 2.003, as used in previous studies for nitroxide radicals); (b) the A_{ii} components of the tensor for the hyperfine coupling between the unpaired electron spin and the nitrogen nuclear spin ($I_N=1$) (an increase in these components corresponds to an increase in the environmental polarity of the probes). Generally, in a series of spectra from similar samples, only A_{zz} is modified in the calculation, and it is preferable to compare with the isotropic values $\langle A_N \rangle = (A_{xx}+A_{yy}+A_{zz})/3$. The accuracy of the spectral computation for the A_{ii} and $\langle A_N \rangle$ parameters was ± 0.01 G; (c) the correlation time for the rotational motion, τ . An increase in this parameter indicates an increased strength of interaction of the probe with its environment. We assumed a Brownian rotational diffusional motion with $\tau = 1/(6D)$, where D is the diffusion coefficient. According to the diffusional model and probe geometry, the main mobility parameter is the perpendicular component of the correlation time, τ_{perp} . The accuracy of the spectral computation for this parameter was ± 0.01 ns; (d) the intrinsic line width (ΔH) and the Heisenberg spin-spin exchange frequency (W_{ex}) relate to local concentration of the radical. With increasing

concentration, the line width increases and at a point collision among the radicals leads to collapse of the three hyperfine lines into a single line, whose line width decreases with the increase in the exchange frequency.

In several cases the spectra were constituted by two superimposed signals arising from non-exchangeable probes (on the EPR time scale) in two different environments; subtraction of experimental spectra containing the signals at different relative intensities allowed us to extract the signals, calculate the relative intensities by double integration, and compute each signal. From the intensities of the components compared to the intensities of the overall EPR spectrum, we calculated the percentages (accuracy ± 0.01 %) of the signals which corresponded to the relative percentages of the probes in different environments.

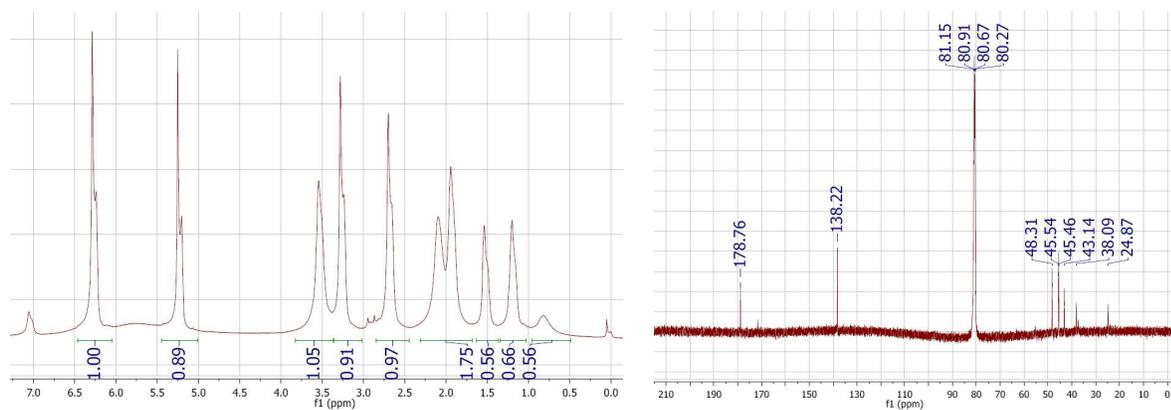


Figure S1. ^1H and ^{13}C NMR spectra of monomer **2** in CDCl_3 .

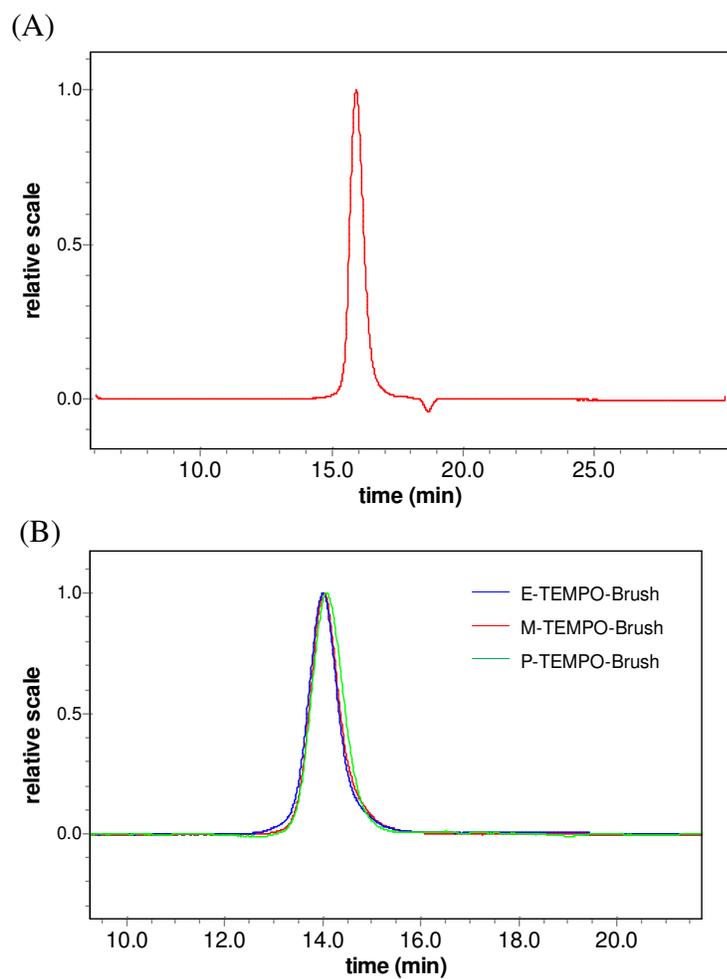


Figure S2. GPC traces of (A) polynorbornene-nitroxide homopolymer and (B) spin-labeled PLA brush polymers.

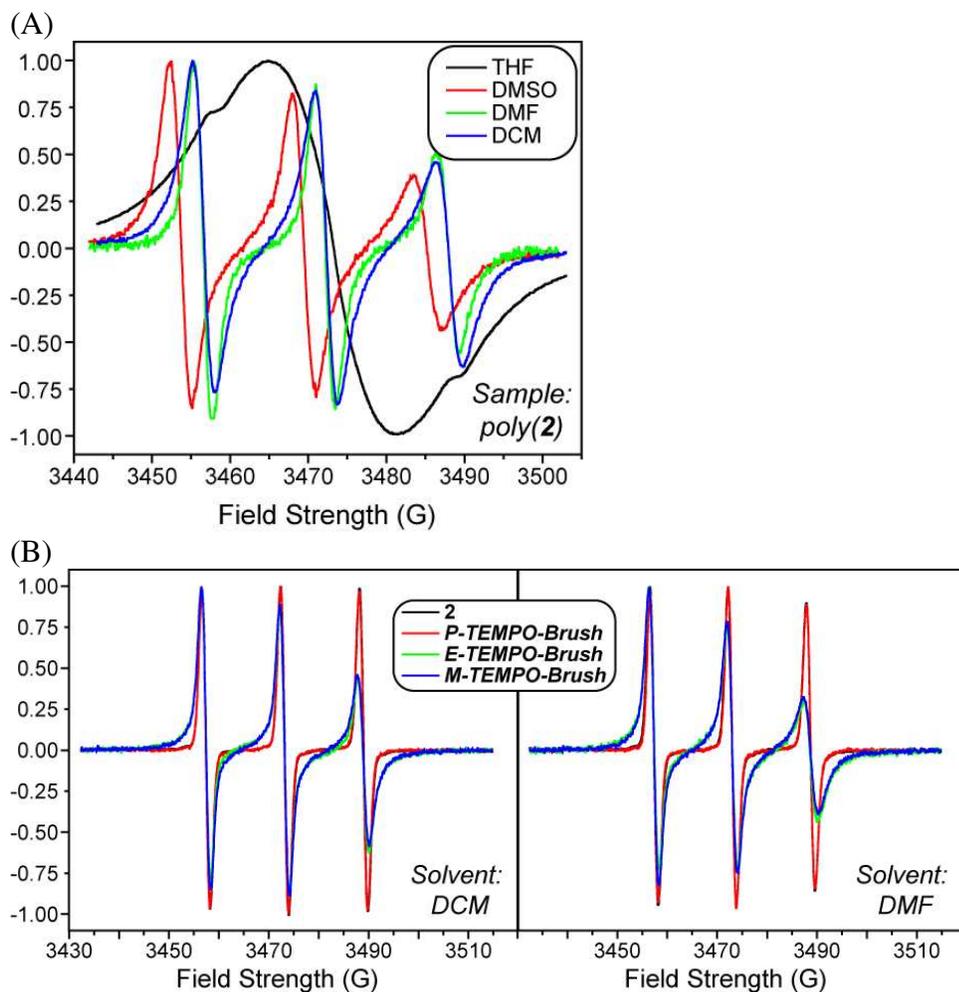


Figure S3. EPR spectra of (A) polynitroxide homopolymer (*poly2*) in DCM, DMF, DMSO, and THF; (B) monomer **2**, P-TEMPO-Brush, E-TEMPO-Brush, M-TEMPO-Brush in DCM and DMF. (In Figure S3B, signals from **2** (black) and P-TEMPO-Brush (red) almost completely overlapped.)

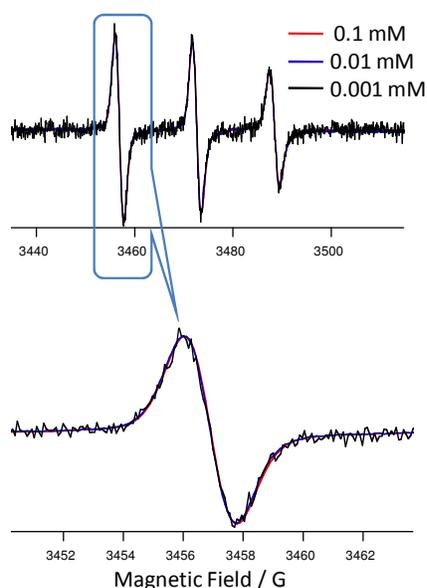


Figure S4. EPR spectra of P-TEMPO-Brush in DMSO at [nitroxide] = 0.001, 0.01, and 0.1 mM. Overlapping signals were obtained, indicating the absence of concentration-dependent EPR line-broadening.

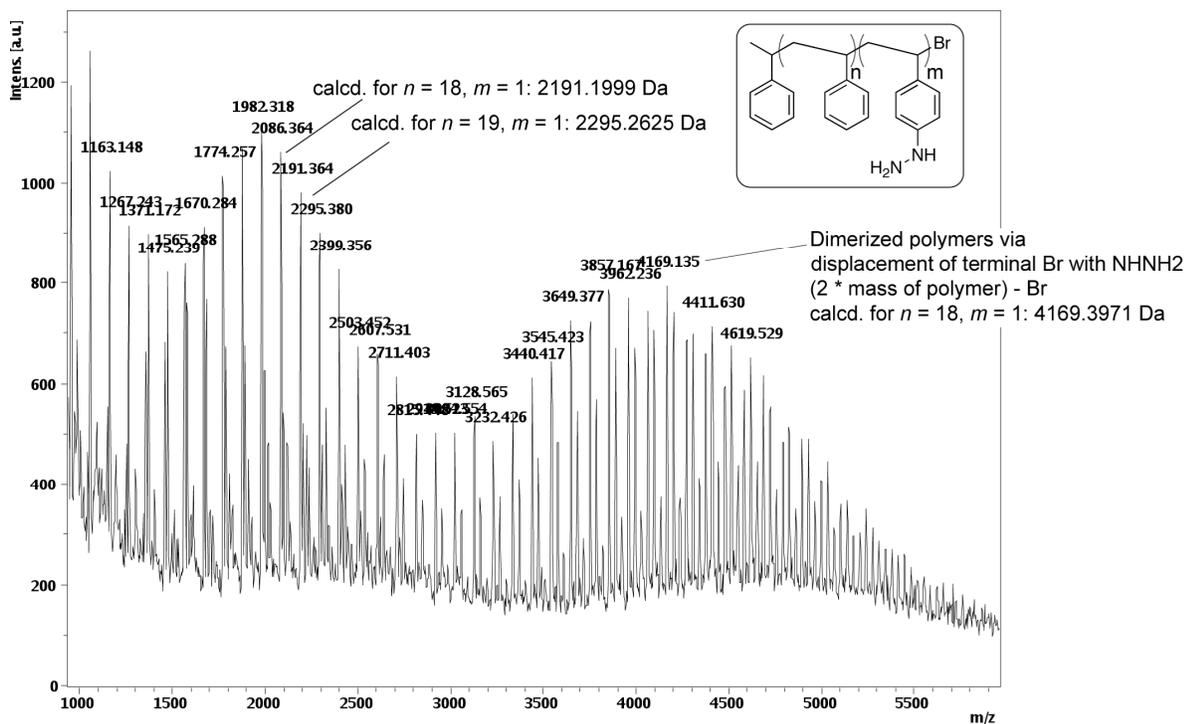


Figure S5. MALDI-TOF spectrum of PS-hydrazine.

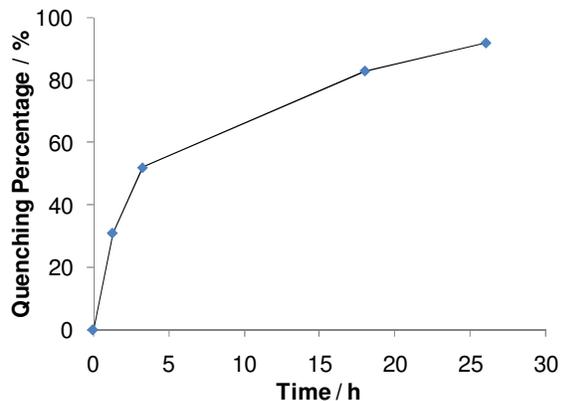


Figure S6. Quenching of the EPR signal for M-TEMPO-Brush by PS-NHNH₂ in degassed DMF. [nitroxide]₀ = 5 × 10⁻⁵ M, room temperature.

	solvent	<A>/G
<i>Monomer 2</i>	DCM	15.80
	DMF	15.69
	DMSO	15.76
<i>E-TEMPO-Brush</i>	DCM	15.75
	DMF	15.66
	DMSO	15.93
<i>M-TEMPO-Brush</i>	DCM	15.75
	DMF	15.66
	DMSO	15.83
<i>P-TEMPO-Brush</i>	DCM	15.78
	DMF	15.69
	DMSO	15.77

Table S1. Hyperfine constant <A> for the coupling between the electron spin and the nitrogen nuclear spin obtained from simulated spectra.

References

- Xia, Y.; Olsen, B. D.; Kornfield, J. A.; Grubbs, R. H. *J. Am. Chem. Soc.* **2009**, *131*, 18525-18532.
- Budil, D. E.; Lee, S.; Saxena, S.; Freed, J. H. *J. Magn. Reson. A*, **1996**, *120*, 155-189.