

pH-Sensitive Radical-Containing-Nanoparticle (RNP) for the L-Band-EPR Imaging of Low pH Circumstances

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S1. Materials

2,2'-Azobisisobutyronitrile (AIBN) (Kanto Chemical Co., Inc., Tokyo, Japan) was purified by recrystallization from methanol. Chloromethylstyrene (CMS) was kindly provided by Seimi Chemical Co., Ltd. (Kanagawa, Japan) and purified on a silica gel column to remove the inhibitor, followed by vacuum distillation under a nitrogen atmosphere. 2,2,6,6-Tetramethylpiperidinyloxy (TEMPO), 4-amino-TEMPO, 4-hydroxy-TEMPO (TEMPOL) (Aldrich Chemical Co., Inc., U.S.A.), 2-propanol, diethyl ether, dimethyl sulfoxide (DMSO) and N,N-dimethylformamide (DMF) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used without further purification. PEG possessing a methoxy group at the α -chain end and a sulfanyl group at the ω -chain end (MeO-PEG-SH) (NOF CORPORATION Co., Ltd., Japan) was used without further purification.

S2. Synthesis of MeO-PEG-*b*-PCMS

Method: After 0.2 mmol (1 g) of MeO-PEG-SH ($M_n = 5,000$) and 0.1 mmol of AIBN were weighed into a flask, the inside of the reactor was degassed and filled with nitrogen gas. The degassing-N₂-purge cycle was repeated three times. Ten millimoles (1.38 mL) of CMS and 10 mL of benzene were then added to the flask. Polymerization was conducted for 24 h at 60 °C in an oil bath. The reaction mixture was recovered by precipitation into 40 mL of hexane, followed by freeze-drying with benzene. The reacted polymer was washed with diethyl ether three times in order to eliminate the PCMS homopolymer, followed by freeze-drying with benzene. The yield of the obtained MeO-PEG-*b*-PCMS copolymer was 87.5% (1.4 g).

Results: MeO-PEG-*b*-PCMS was prepared by the radical telomerization of CMS using MeO-PEG-SH ($M_n = 5,000$) as a telogen, according to the previous report, as shown in Scheme 1. The molecular weight distribution of the MeO-PEG-*b*-PCMS thus prepared was evaluated by SEC, as shown in Figure S1a. The SEC profile of MeO-PEG-*b*-PCMS after polymerization showed a bimodal distribution similar to that of previously reported acetal-PEG-*b*-PCMS. In the previous study, ¹H NMR analysis performed after the two peaks were separated by SEC fractionation showed that the composition ratios of PEG to PCMS in the two fractions were almost the same. The M_n

value of PCMS was determined by the ^1H NMR data based on the M_n of PEG ($M_n = 5,000$). From the SEC profile, it can be seen that the number-average molecular weight (M_n) of the first fraction was two times higher than that of the second fraction. Based on the M_n value of PEG as determined by SEC, the M_n values of the PCMS segments for the two fractions were determined to be 3,300 and 6,600, respectively. The products were a mixture of di-block and tri-block copolymers possessing the same composition, as reported previously. The ratios of di-block to tri-block copolymer were determined to be 59 % and 41 %, respectively, based on the chromatogram.

S3. Synthesis of the radical-containing blockcopolymer (MeO-PEG-*b*-PCTEMPO)

Method: After 177 μmol (1.25 g) of the obtained MeO-PEG-*b*-PCMS ($M_n = 8,000$) were weighed into a 100 mL flask, a DMSO solution (40 mL) of 4-amino-TEMPO (17.7 mmol, 3 g) was added to the flask and stirred for 12 h at room temperature. The reacted polymer was recovered by precipitation into 80 mL of cold 2-propanol ($-15\text{ }^\circ\text{C}$) and centrifugation for 30 min at 5,000 rpm ($4,500 \times g$). In order to purify the obtained polymer, the precipitation-centrifugation cycle was repeated three times, followed by freeze-drying with benzene. The yield of the obtained MeO-PEG-*b*-PCTEMPO copolymer was 74.4 % (1.26 g).

Results: The chloromethyl groups in the PCMS segment of the block copolymer MeO-PEG-*b*-PCMS were converted to stable radicals via the amination of MeO-PEG-*b*-PCMS with 4-amino-TEMPO in DMSO (Scheme S1). When PEG-*b*-PCMS was reacted with 4-amino-TEMPO, the CH_2Cl signal of the PCMS segment in the ^1H NMR spectrum disappeared. After the TEMPO radicals were reduced by phenylhydrazine, the entire ^1H NMR spectrum was examined, as shown in Figure S2b. After the reducing reaction, it was confirmed that the signal for the methylene protons of the chloromethyl group in the PCMS unit at 4.5 ppm completely disappeared. On the basis of these results, it was confirmed that the TEMPOs were successfully introduced into the polymer.

S4. Preparation of the RNP

Results: The size of the obtained RNP after dialysis was estimated by DLS measurement. As shown in Figure S3, the RNP thus obtained showed a unimodal distribution in the histogram analysis. The average diameter and polydispersity factor (μ_2/Γ^2), as determined by the cumulant method, were about 50 nm and 0.133, respectively.

S5. Calculation method for the rotational correlation time, τ_c

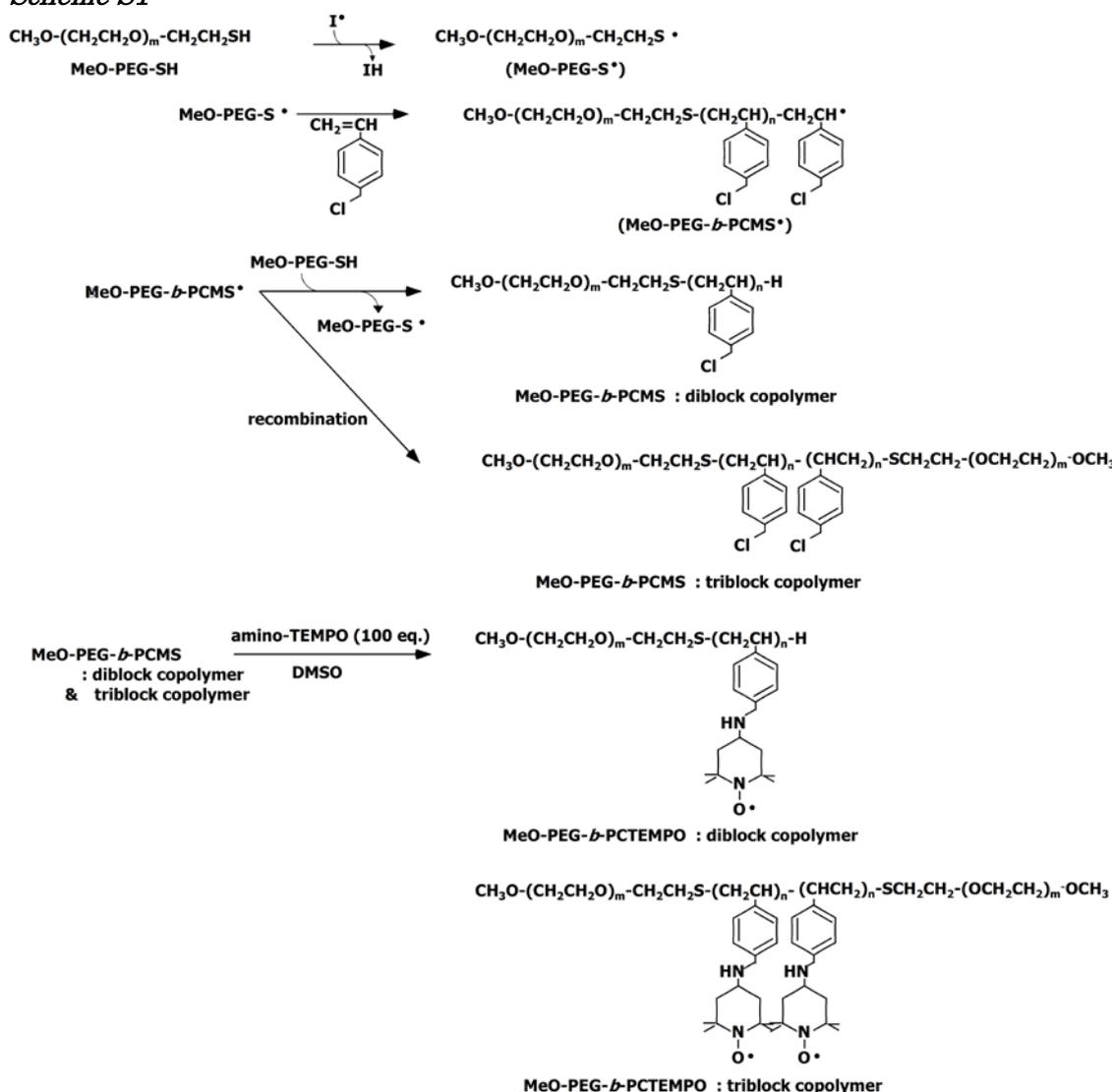
The rotational correlation time, τ_c (s), which is the time taken for the axis of the nitroxyl group to rotate one radian, is highly sensitive to the motion of the TEMPO radicals on the PCTEMPO segment, and is calculated using the following equation¹:

¹ Yoshioka, H., Haga, H., Kubota, M., Sakai, Y., and Yoshioka, H. (2006) Interaction of (+)-Catechin with a Lipid Bilayer Studied by the Spin Probe Method. *Biosci. Biotechnol. Biochem.* 70, 395-400.

$$\tau_c = B \times \Delta H_{(m=+1)} [(I_{m=+1} / I_{m=-1})^{1/2} - 1] \quad (1)$$

Here, B is a constant determined from the principal values of A and the g factor; the value of 6.6×10^{-10} s/Gauss is used. $\Delta H_{(m=+1)}$ is the peak-to-peak width in gauss of the low-field lines, as shown in Figure S4. $I_{(m=+1)}$ and $I_{(m=-1)}$ are the peak-to-peak heights in arbitrary units of the low- and high-field lines, respectively. The value of τ_c reflects the mobility of the probe and the rigidity of the surroundings of the probe. An increase in τ_c means that the motion of the probe is suppressed.

Scheme S1



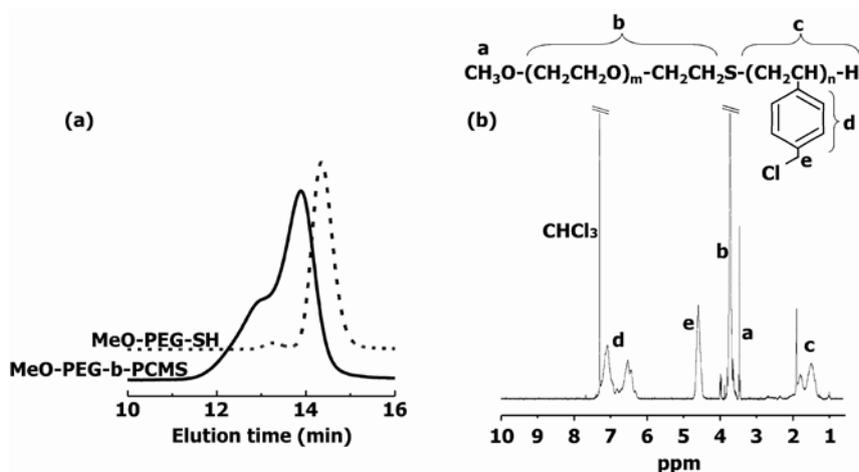


Figure S1. SEC diagram (a) and ^1H NMR spectrum (b) of MeO-PEG-*b*-PCMS. (SEC apparatus, TOSOH HLC-8120GPC; detector, RI; column, TOSOH SUPER HZ4000+3000; solvent, THF (containing 2 w% triethylamine); flow rate, 0.35 mL/min; calibration, standard PEGs.)

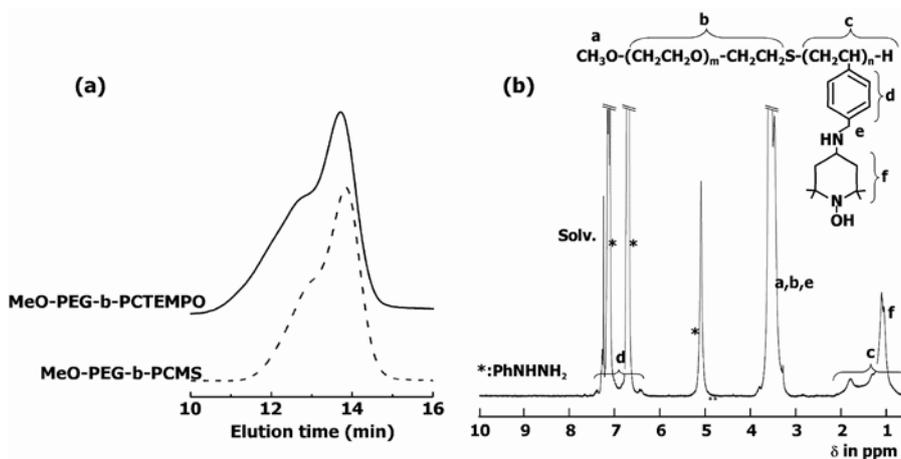


Figure S2. SEC diagram (a) and ^1H NMR spectrum (b) of MeO-PEG-*b*-PCTEMPO. (SEC apparatus, TOSOH HLC-8120GPC; detector, RI; column, TOSOH SUPER HZ4000+3000; solvent, THF (containing 2 w% triethylamine); flow rate, 0.35 mL/min; calibration, standard PEGs.)

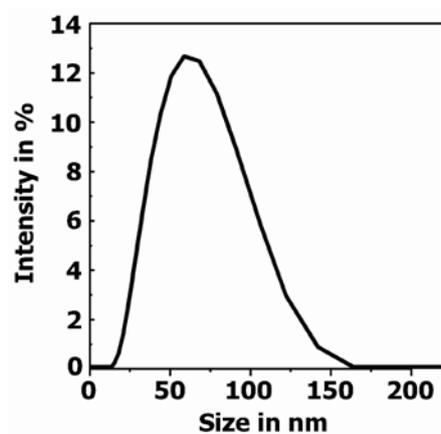


Figure S3. Size distribution of the RNP analyzed by DLS.

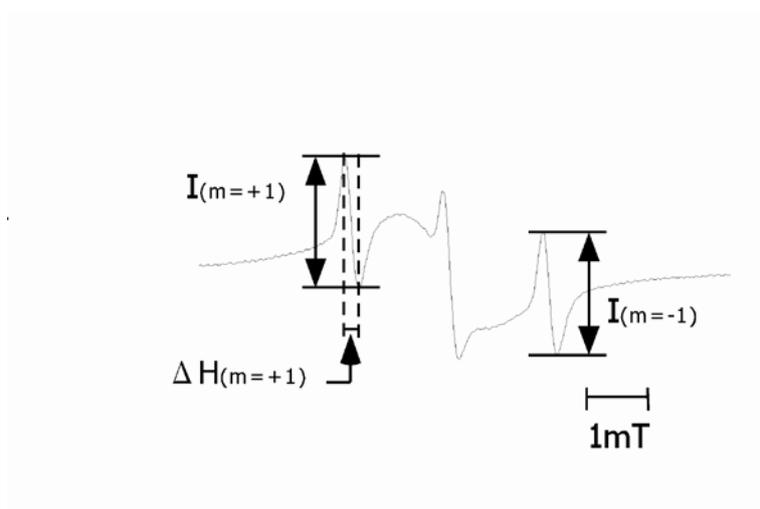


Figure S4. EPR spectrum of the RNP at pH 5.5 and the peak-to-peak height and width of line.