

1 **Supplementary Information**

2 **Molecular mechanisms of cell cryopreservation with polyampholytes**
3 **studied by solid-state NMR**

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5 Kazuaki Matsumura,^{1*} Fumiaki Hayashi,² Toshio Nagashima,² Robin Rajan,¹ and Suong-Hyu Hyon³

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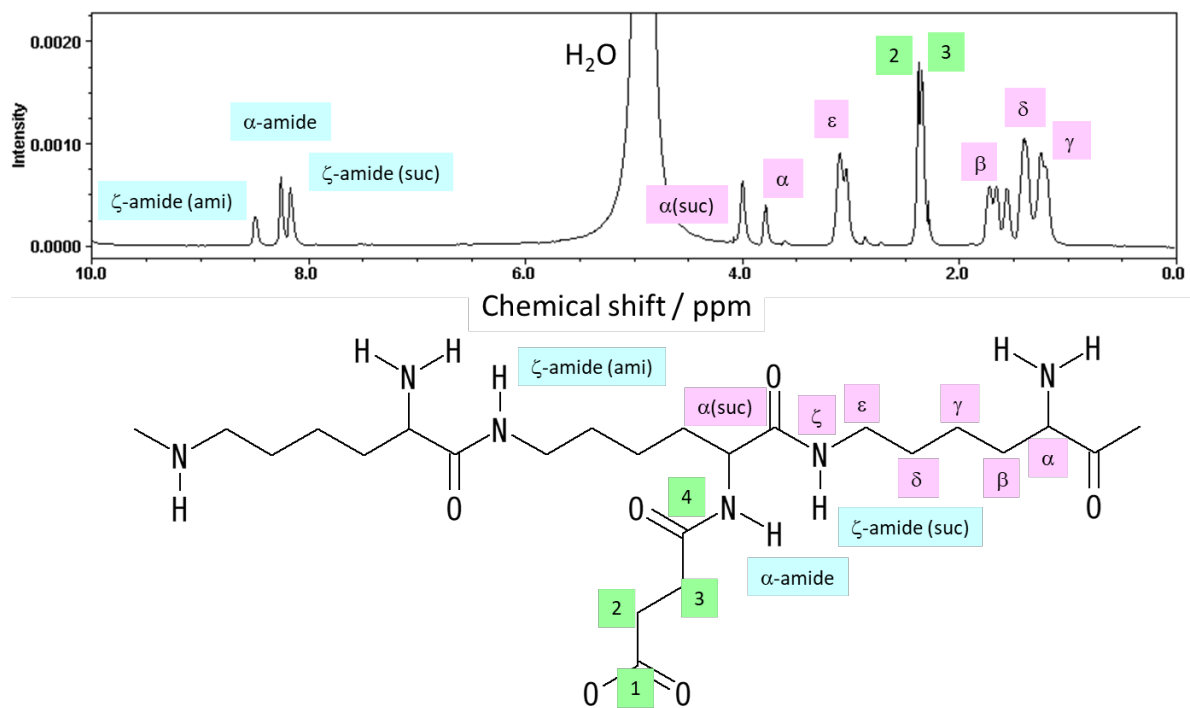
7 ¹School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai,
8 Nomi, Ishikawa 923-1292, Japan.

9 ²Advanced NMR Application and Platform Team, NMR Research and Collaboration Group, NMR
10 Science and Development Division, RIKEN SPring-8 Center, 1-7-22 Suehiro-cho, Tsurumi-ku,
11 Yokohama City, Kanagawa 230-0045, Japan.

12 ³Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24, Korimoto, Kagoshima 890-
13 8580, Japan.

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Supplementary Fig. 1. ^1H NMR signal assignments for COOH-PLL in D_2O .

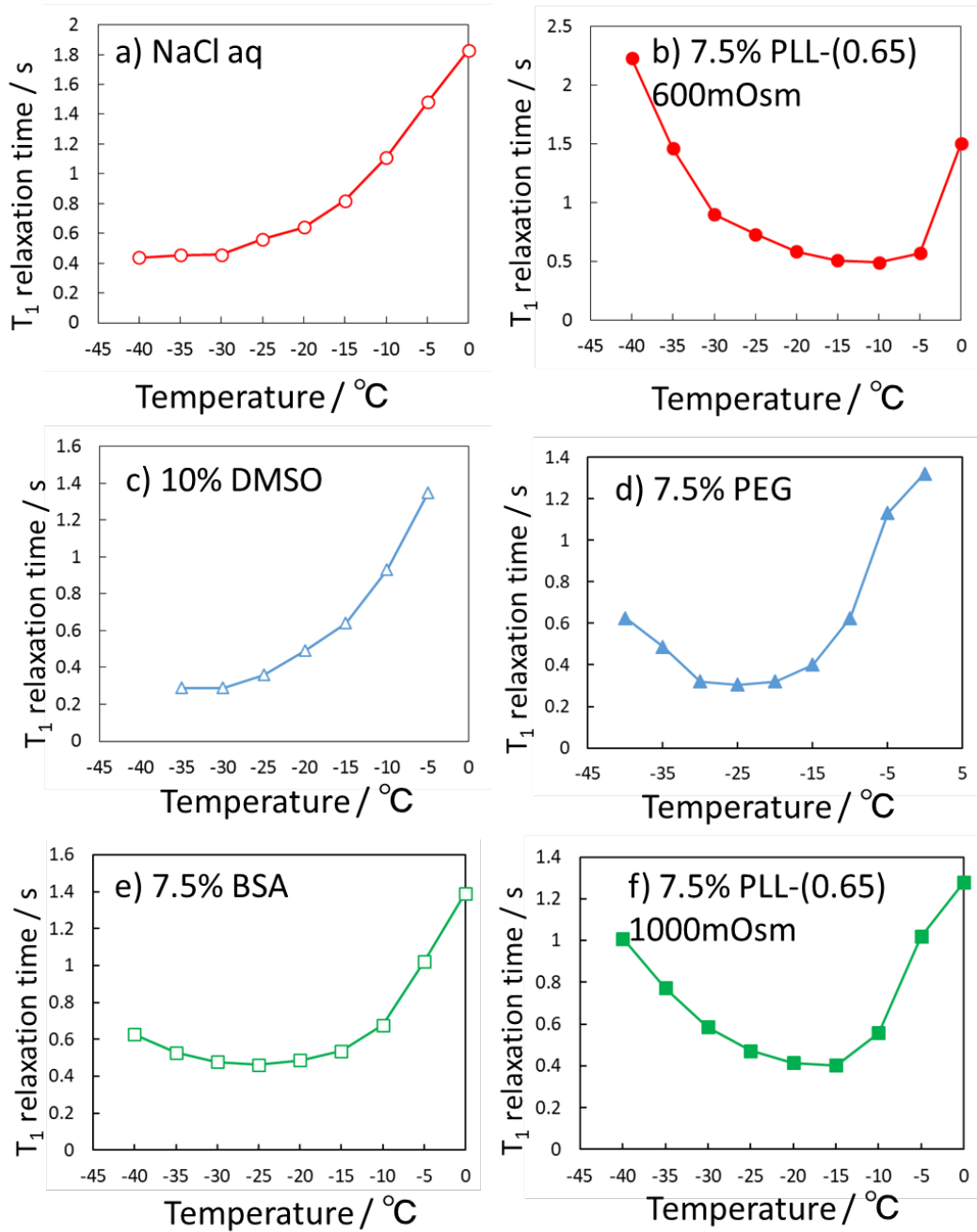
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19 **Supplementary Table 1.** Carboxylation rates of various COOH-PLLs determined by ^1H NMR
20 spectroscopy and the TNBS assay

Carboxylated poly-L-lysine	Carboxylation rate calculated by ^1H NMR spectroscopy	Carboxylation rate calculated using the TNBS assay
PLL(0)	0	0
PLL(0.2)	0.21 ± 0.03	0.19 ± 0.03
PLL(0.4)	0.43 ± 0.01	0.42 ± 0.07
PLL(0.65)	0.63 ± 0.02	0.62 ± 0.05

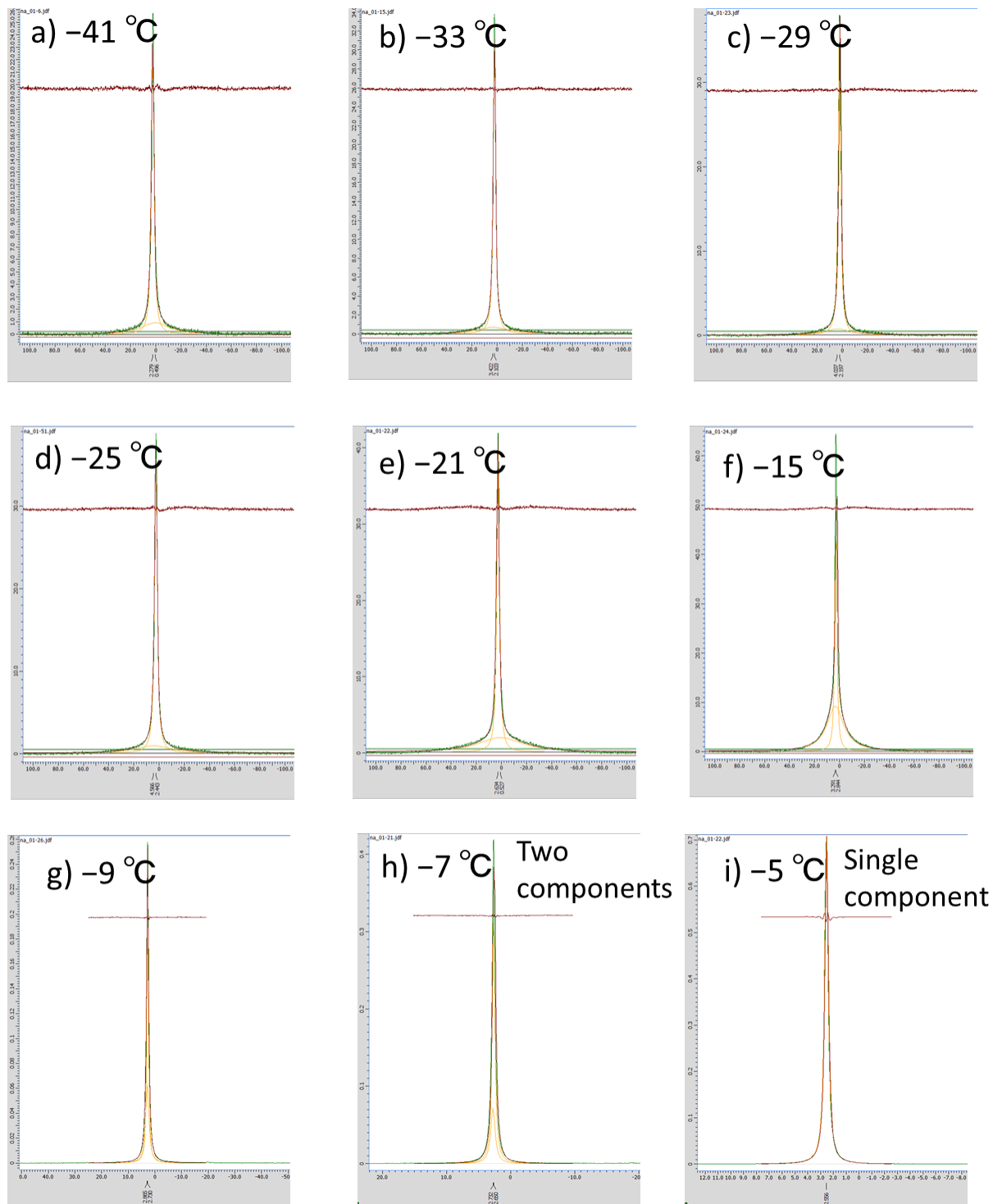
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Proton T_1 relaxation time



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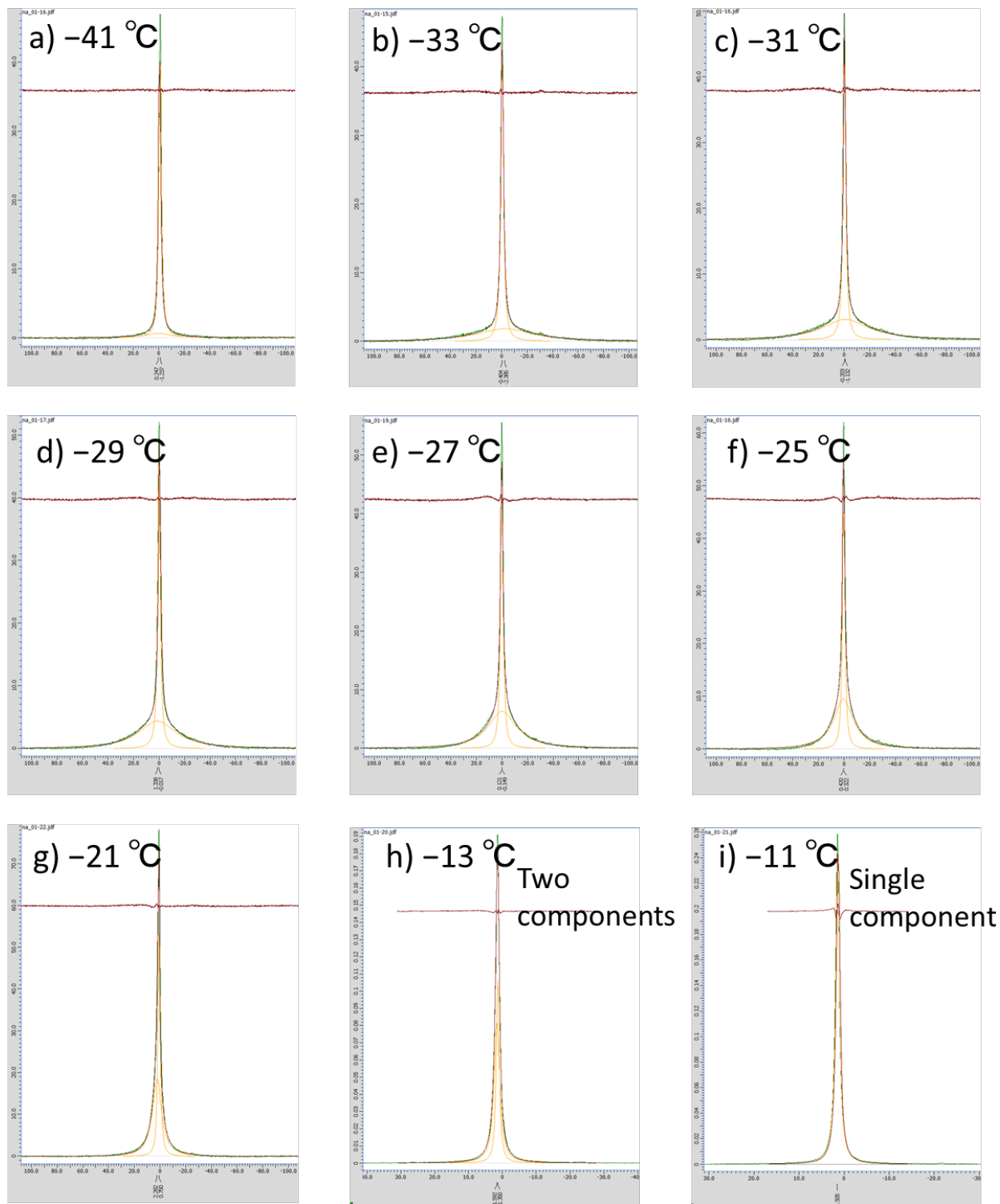
23 **Supplementary Fig. 2. Water proton T_1 relaxation times as functions of temperature.** (a) The saline
 24 solution, (b) 7.5% PLL-(0.65) at 600 mOsm, (c) 10% DMSO, (d) 7.5% PEG, (e) 7.5% BSA, and (f) 7.5%
 25 PLL-(0.65) at 1000 mOsm.



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27 **Supplementary Fig. 3. Peak resolutions of the Na signal of the PLL-(0.65) solution during freezing.**

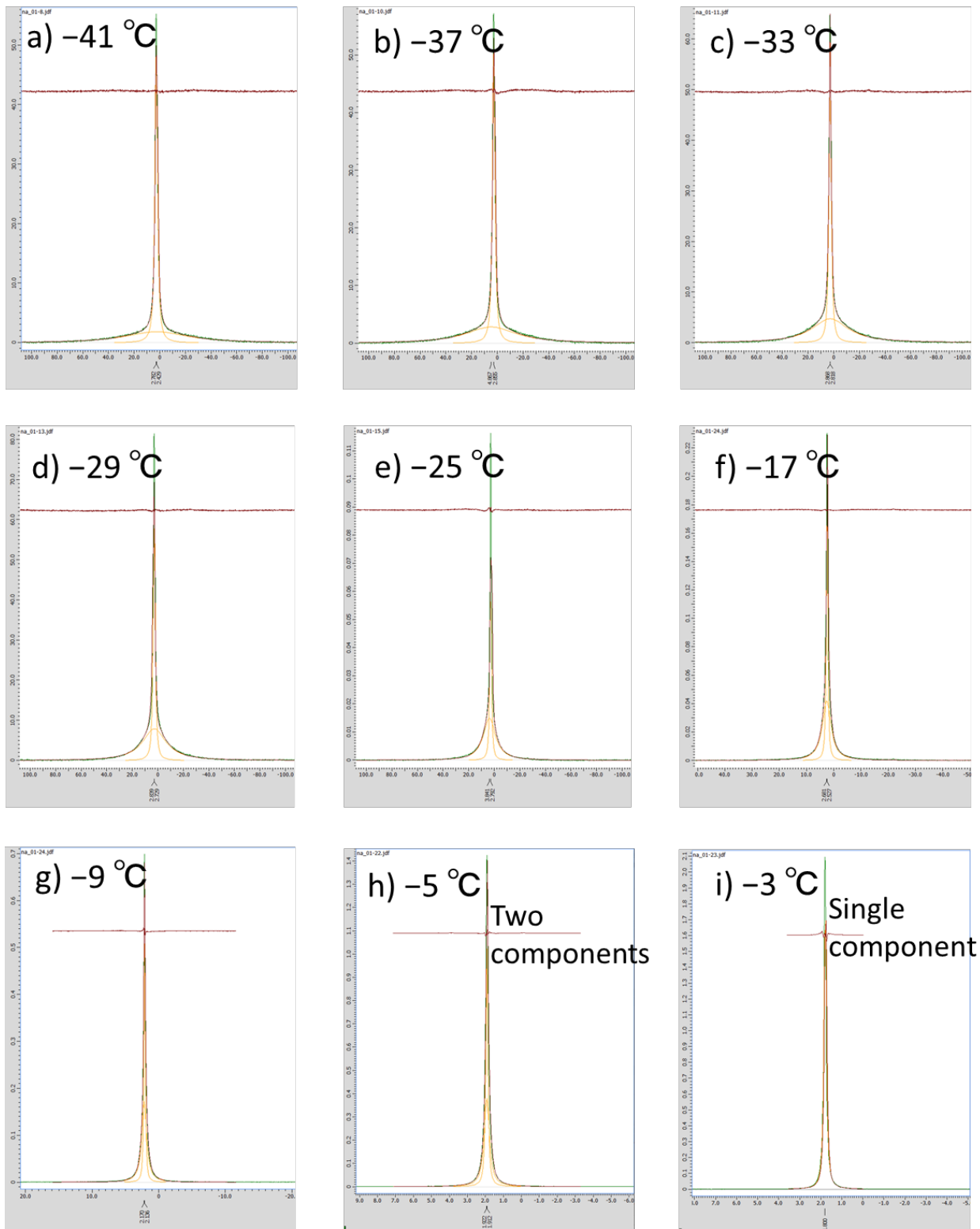
28 a) At $-41\text{ }^{\circ}\text{C}$ through to i) $-5\text{ }^{\circ}\text{C}$.



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30 **Supplementary Fig. 4. Peak resolutions of the Na signal of the PEG solution during freezing. a) At**

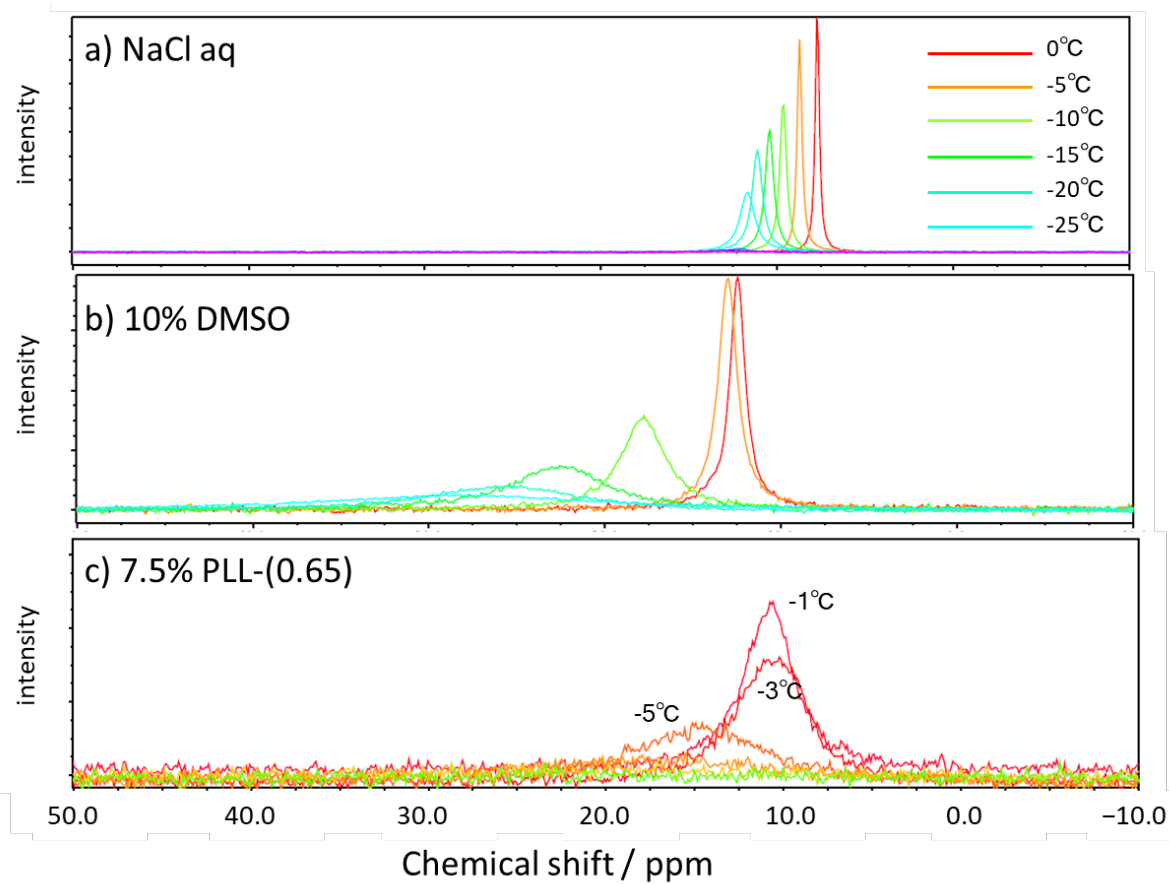
31 $-41\text{ }^{\circ}\text{C}$ through to i) $-11\text{ }^{\circ}\text{C}$.



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33 **Supplementary Fig. 5. Peak resolutions of the Na signal of the BSA solution during freezing. a) At**

34 $-41\text{ }^{\circ}\text{C}$ through to i) $-3\text{ }^{\circ}\text{C}$.



Supplementary Fig. 6 Analyzing Cl-ion behavior during freezing. Solid state ^{35}Cl NMR spectra in (a) saline solution, (b) 10% DMSO, and (c) 7.5% PLL-(0.65).

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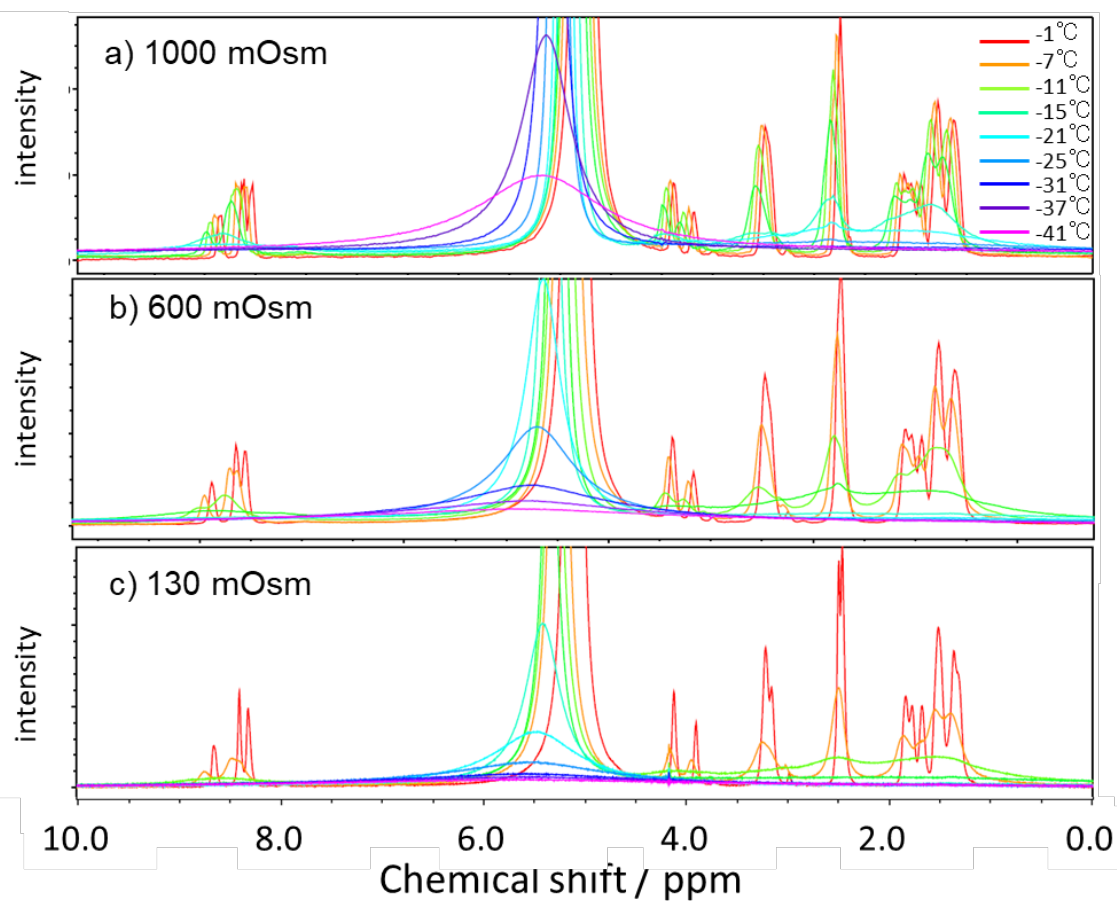
44 **Supplementary Table 2** H₂O/Na ion ratio at the temperature at which local maximum of the sharp
 45 component in ²³Na NMR spectrum in various polymer solutions.

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	Temperature at which a local maximum of the sharp component is observed in the ²³ Na NMR spectrum / °C (from Figs. 3f, 4c)	Concentration of Na ions / M	Unfrozen water / M	H ₂ O/Na ion Ratio (Na conc./ unfrozen water)
PLL-(0.65) 600 mOsm	−25	0.358	4.02	11.2
PEG	−35	0.3	0.26	14.2
BSA	<−41	0.3	3.25	10.8
PLL-(0.65) 1000 mOsm	−31	0.558	5.31	9.5

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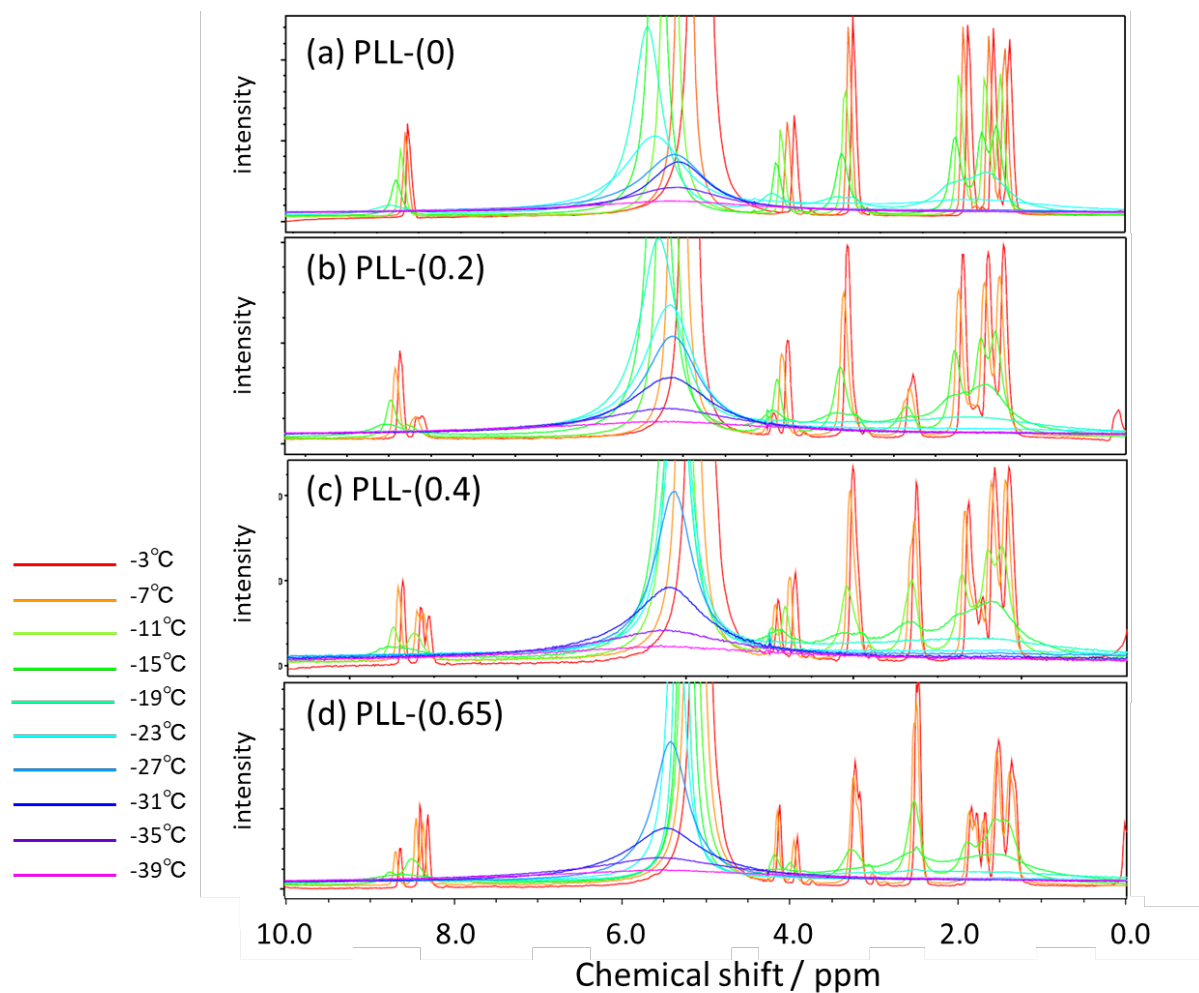
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50 **Supplementary Fig. 7 Effect of osmotic pressure on the proton signal of PLL-(0.65) as measured by**
 51 **solid-state ^1H NMR spectroscopy. (a) At 1000, (b) at 600, and (c) at 130 mOsm.**

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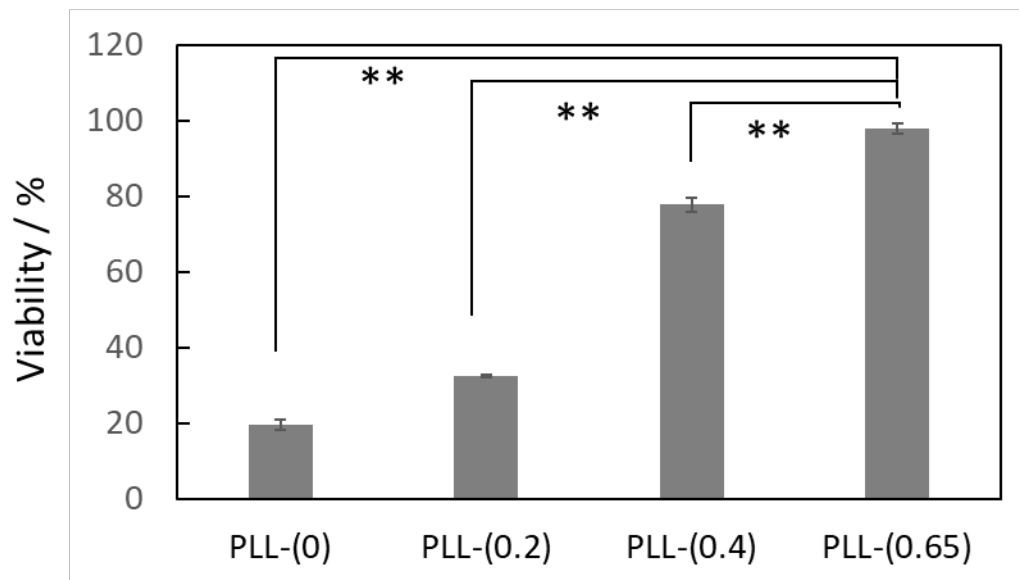
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Supplementary Fig. 8 Investigating the behavior of the solute protons at a range of temperatures. Solid-state ^1H NMR analysis of: (a) 7.5% PLL-(0), (b) 7.5% PLL-(0.2), (c) 7.5% PLL-(0.4), and (d) 7.5% PLL-(0.65).

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Supplementary Fig. 9 Viabilities after cryopreservation with 7.5% solutions of various COOH-PLLs. ** p<0.01