Electronic Supplementary Information

for

A Thermoresponsive Poly(Ionic Liquid) Membrane Enables Concentration of Proteins from Aqueous Media

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1. Materials and Instrumentation

Tetra-*n*-butylphosphonium bromide ([P_{4444}]Br), tri-*n*-butyl-*n*-octylphosphonium bromide ([P_{4448}]Br), sodium 4-styrene sulfonate (Na[SS]), 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone were purchased from Tokyo Chemical Industry Co. Cytochrome *c from equine heart* (Cyt.*c*), myoglobin *from equine heart* (Mb), horseradish peroxidase (HRP), and poly(ethylene glycol) (i.e., PEG) diacrylate (average M_n = 700) were purchased from Sigma-Aldrich. All solvents were purchased from the Kanto Chemical Industry Co. All of the chemicals and solvents were used as received for synthesis. Both [P_{4444}][SS] and [P_{4448}][SS] monomers were prepared according to our reported procedures.[1] UV-visible spectroscopy was performed with JASCO V-660 spectrometer at the Tokyo University of Agriculture and Technology.

2. Fabrication of poly(ionic liquid) membranes (PILMs)

The procedure used to fabricate PILMs was that established at the University of Colorado, Boulder.[2] First, a desired amount of IL monomers, PEG diacrylate cross-linker, and radical photo-initiator with a molar ratio of 100/1/1 were mixed vigorously with vortexing and mild heating until a homogeneous mixture was obtained. Then, the resulting mixture was degassed by placement *in vacuo* and then sandwiched between two quartz plates separated by a 150-μm-thick spacer. The plates were irradiated with 365 nm light (2.3 mW cm^{-2} at the sample surface) for 1 h at ambient temperature. The resulting PEG-diacrylate-cross-linked poly([P_{4444}][SS]$_x$-*co*-[P_{4448}][SS]$_{1-x}$)-type PILMs with x value (i.e., molar fraction of [P_{4444}][SS]) ranging from 0 to 0.4 were then removed from the quartz plates using a razor blade for the subsequent studies.

3. Evaluation of extraction efficiency (EE) of proteins

A number of PEG-diacrylate-cross-linked poly([P_{4444}][SS]$_{0.3}$-*co*-[P_{4448}][SS])-type PILM **1** samples with weights of approximately 50 mg were prepared according to the above-mentioned procedure. The resulting membranes were soaked in 0.1 mol L^{-1} aqueous potassium phosphate buffer (PKB, pH = 7.0) and stored at 5 °C for 24 h to remove any possible uncross-linked polymers and low-molecular-weight molecules, during which time the PKB solution was repeatedly exchanged several times. Each membrane was stored at desired temperature (5 °C, 22 °C, or 30 °C) for 2 h, and then PKB was removed exclusively. Subsequently, 3.0 ml of protein aqueous PKB solution with protein concentration of 1.0 mg mL^{-1} was added to each membrane and stored them for 24 h at the measurement temperature. UV-visible analysis was then carried out on the aqueous phase by using a 0.5-mm-thick demountable quartz cell. The EE (%) values of the proteins in the PILM **1** samples were calculated using the following equation;

$$EE\ (\%) = \frac{(Abs_{before} \times V_{before}) - (Abs_{after} \times V_{after})}{(Abs_{before} \times V_{before})} \times 100$$

where Abs_{before}, Abs_{after}, V_{before}, and V_{after} respectively denote the absorbance and volume of the aqueous phase before and after the protein extraction experiments. Since the volume of the aqueous phase was essentially constant under these experimental conditions, both V_{before} and V_{after} were set as 3.0 mL. A Soret band for each protein (408 nm for Cyt.*c* and Mb, and 402 nm for HRP) was used to evaluate the protein concentration in aqueous phases. For each protein extraction experiment, three separately prepared membranes were used to determine an average EE value for each sample, and standard deviation error bars were calculated from the three experiments.

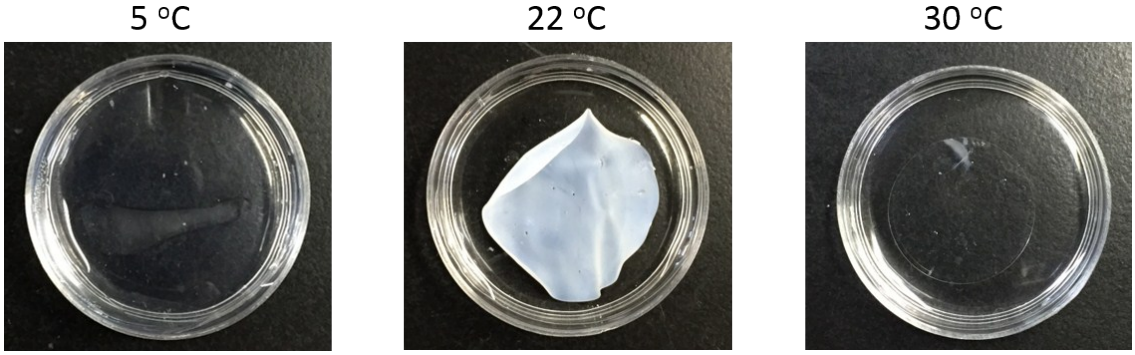

Fig. S1 Visual appearances of PILM **1** sample after soaking in pure water at different temperatures for 24 h.

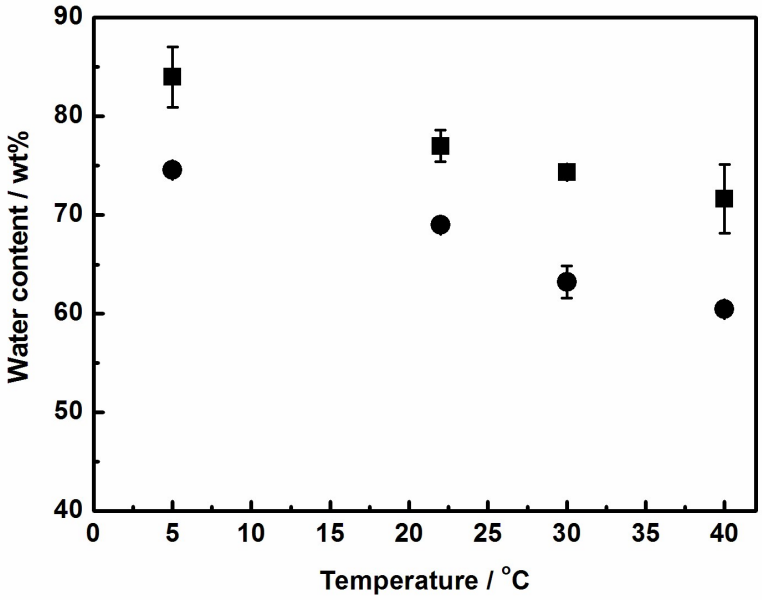

Fig. S2 The water content of PILM **1** as a function of temperature after soaking it in 0.01 (■) or 0.1 mol L^{-1} aq. PKB solution (●) for 24 h.

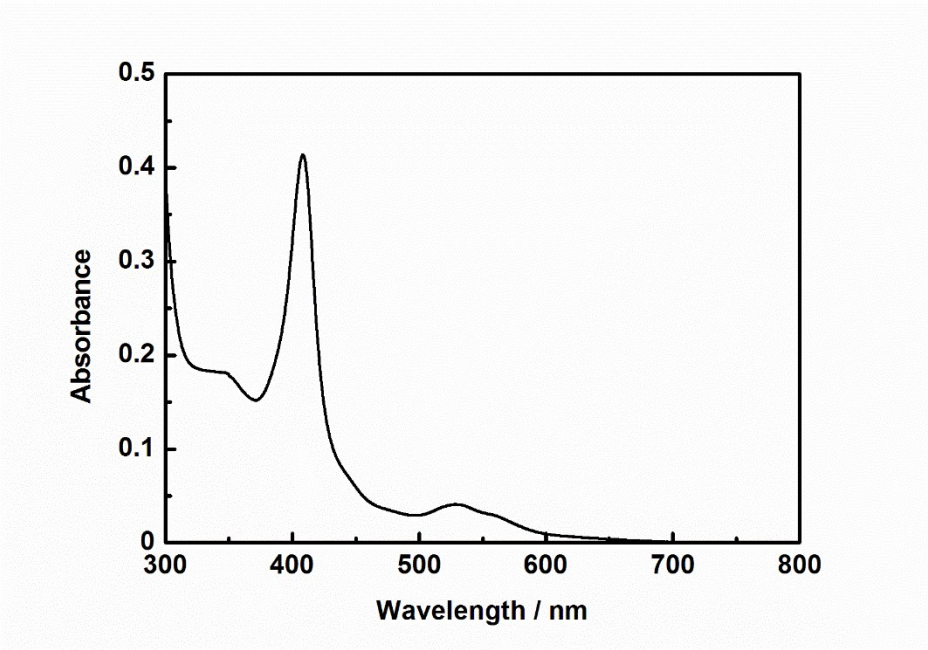

Fig. S3 UV-visible spectrum of PILM **1** sample after soaking the membrane in a Cyt.*c*-containing 0.1 mol L^{-1} aqueous PKB solution. Since the absorbance of Cyt.*c* was too high to measure by UV-visible spectroscopy, the concentration of Cyt.*c* in the aqueous PKB solution was set as 0.1 mg mL^{-1} in this case.

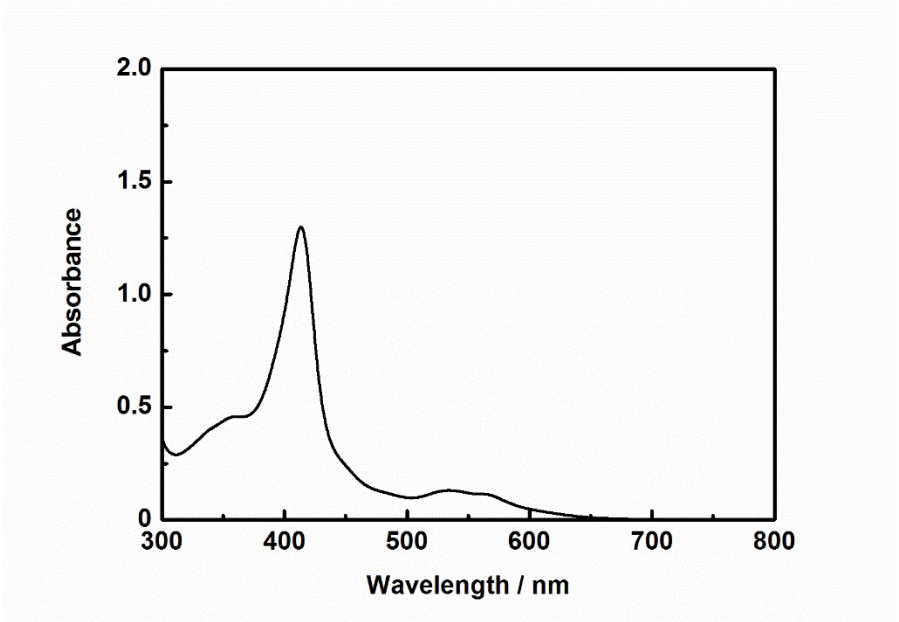

Fig. S4 UV-visible spectrum of a PILM **1** sample after soaking the membrane in a Mb-containing 0.1 mol L^{-1} aqueous PKB solution.

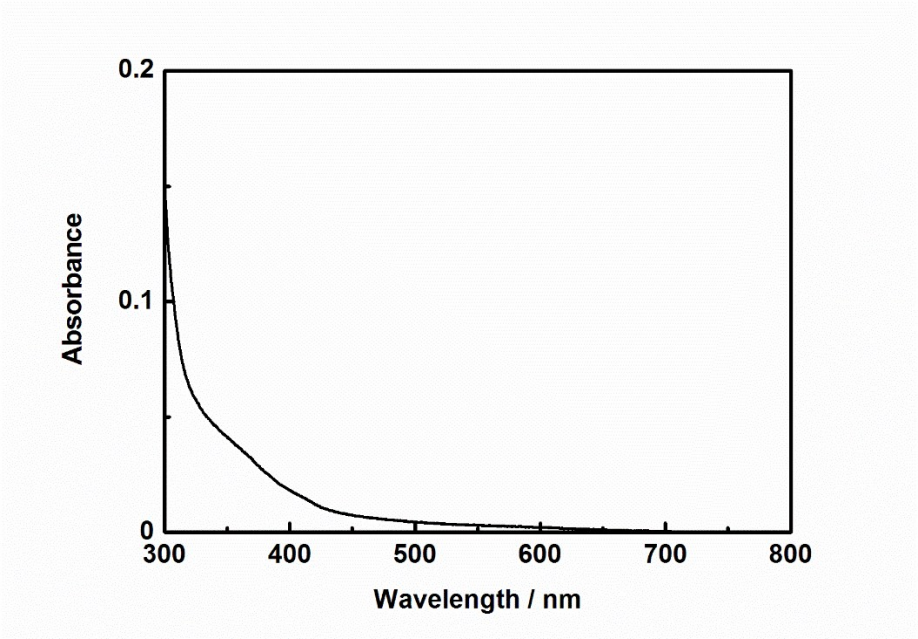

Fig. S5 UV-visible spectrum of a PILM **1** sample after soaking the membrane in an HRP-containing 0.1 mol L^{-1} aqueous PKB solution.

References for the ESI

1 Y. Kohno and H. Ohno, *Aust. J. Chem.*, 2012, **65**, 91.

2 M. G. Cowan, M. Masura, W. M. McDanel, Y. Kohno, D. L. Gin and R. D. Noble, *J. Membr. Sci.*, 2016, **498**, 408.