

Supporting Information

Title: Hydrated Ionic Liquids as Liquid Chaperon for Refolding of Aggregated Recombinant Protein Expressed in Escherichia coli

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Materials and Methods.

CcCel6A preparation

Preparation of *Escherichia coli* BL21 (DE3) (Merck, Darmsadt, Germany) transformant harboring the pET21a vector containing the *cccel6a* gene were described previously.[1] The transformant was grown in 100 mL of LB medium at 37°C until it had reached an optical density of 0.2 at 600 nm, and then expression was induced with 0.2 mM isopropyl- β -D-thiogalactopyranoside for 24h at 18°C or 1 week at 4°C. The cells were harvested and then resuspended in 5 ml of 20 mM Tris-HCl buffer (pH 7.4) followed by sonication (Tomy Seiko Co., Ltd., Tokyo, Japan) for 10 min. The soluble fraction and the insoluble material were separated by centrifugation at 10,000 \times g. The obtained supernatant was applied to a HisTrap FF column (GE Healthcare UK Ltd., Buckinghamshire, England), equilibrated with Tris-HCl buffer (pH7.4) containing 500 mM sodium chloride and 20 mM imidazole. The column was washed with the same buffer, and the recombinant protein were subsequently eluted by using 20-500 mM imidazole linear gradient in Tris-HCl buffer (pH7.4) containing 500 mM sodium chloride. The fraction was dialyzed against 20 mM Tris-HCl buffer (pH7.4) and the molecular masses of purified proteins were estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis.^[1] Specific details are

provided in the Supporting Information. Water-soluble and aggregated (inclusion body) CcCel6A were collected after cultivation of *E. coli* at 4°C for 7 days and at 18°C for 1 day, respectively. The expression level of the aggregated sample was much greater than that of the soluble sample despite the shorter culture time. The soluble sample was used after sonication followed by purification with a His-trap and DEAE column. Cultured *E. coli* producing aggregated proteins were treated with ultrasonic fragmentation, several washing steps, and centrifugation, and white precipitation was observed.

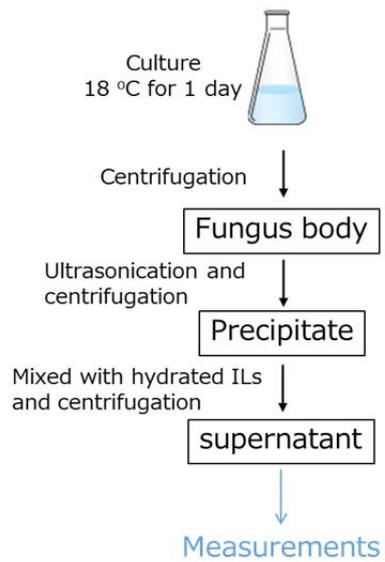
Fluorescence measurement of CcCel6A in hydrated ILs

Excitation light was used at 280 nm. Fluorescence measurement is commonly used for the analysis of the folding state of proteins because the fluorescence spectrum around 300 to 350 nm is sensitive to the surrounding tryptophan residues.^[2] When the tryptophan in the protein is denatured and exposed to a highly polar solvent such as water, the spectral maximum is shifted to a longer wavelength (red-shifted). In general, proteins show a spectral shift to 350 nm in the presence of GdmHCl, which is one of the typical denaturants used to induce unfolding and dissolution, even in aggregated proteins.^[3] On the other hand, proteins are blue-shifted when tryptophan is present in a

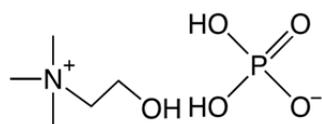
low-polarity surrounding, e.g., the proteins are strongly aggregated and tryptophan is located more internally.

References

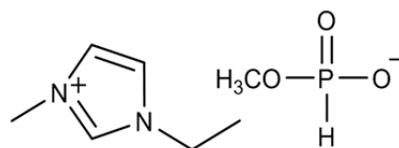
- [1] M. Tamura, T. Miyazaki, Y. Tanaka, M. Yoshida, A. Nishikawa, T. Tonozuka, *Febs J.* **2012**, *279*, 1871-1882; M. Yoshida, K. Sato, S. Kaneko, K. Fukuda, *Bioscience Biotechnology and Biochemistry* **2009**, *73*, 67-73.
- [2] M. R. Eftink, *Methods of Biochemical Analysis* **1991**, *35*, 127-205.
- [3] J. T. Vivian, P. R. Callis, *Biophysical Journal* **2001**, *80*, 2093-2109.



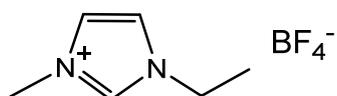
Scheme S1. Procedure of the experiment.



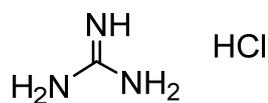
cholinium dihydrogen phosphate ([ch][dhp])



1-ethyl-3-methylimidazolium
methylphosphate ([C₂mim][MeO(H)PO₂])



1-ethyl-3-methylimidazolium
tetrafluoroborate ([C₂mim]BF₄)



guanidine hydrochloride (GdnHCl)

Figure S1. Structure of ILs and organic salt.

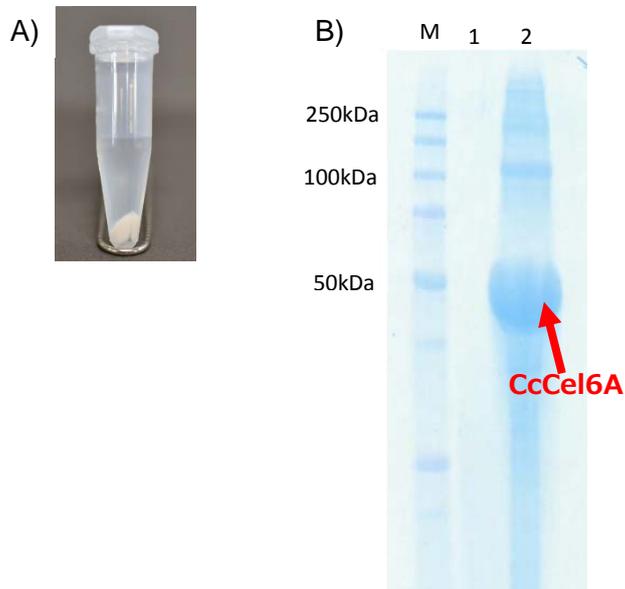


Figure S2. A) Photograph of the white precipitate observed after sonication of CcCel6A expressed in *E. coli* after centrifugation. B) SDS-PAGE after ultrasonication of *E. coli* expressed CcCel6A (52.2 kDa) followed by centrifugation. Lane M, molecular weight standards; lane 1, supernatant; lane 2, precipitate.

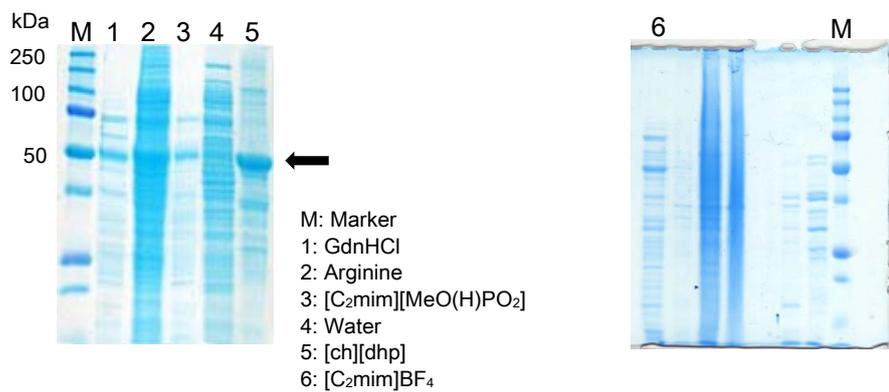


Figure S3. SDS-PAGE of supernatant after mixing the precipitate in hydrated ILs followed by centrifugation.

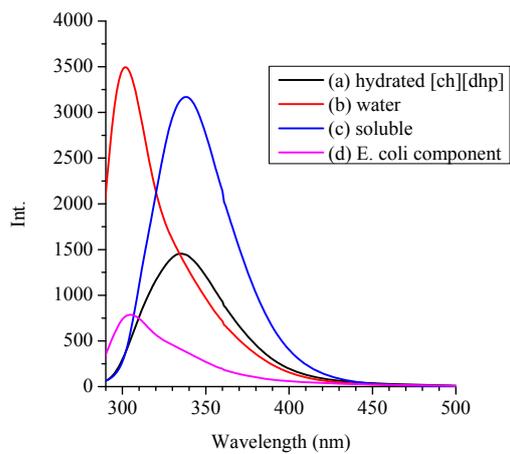


Figure S4. Fluorescence spectra of supernatant after mixing the precipitate contained CcCel6A expression in (a) hydrated [ch][dhp], (b) water. (c) soluble CcCel6A (native) in buffer, (d) supernatant after mixing white precipitate without CcCel6A expression (E. coli component only) in hydrated [ch][dhp].

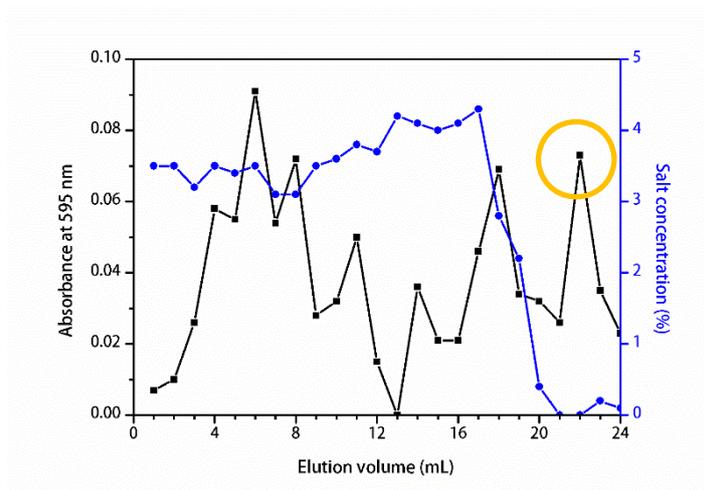
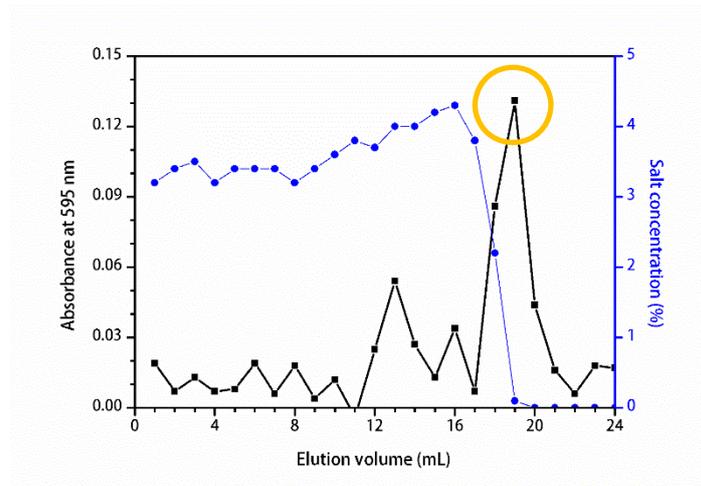


Figure S5. Protein and salt concentration curve measured by protein assay and ionic conductivity during phenyl column.

Upper: native CcCel6A was mixed in hydrated [ch][dhp]. Lower: supernatant after mixing of aggregated CcCel6A in

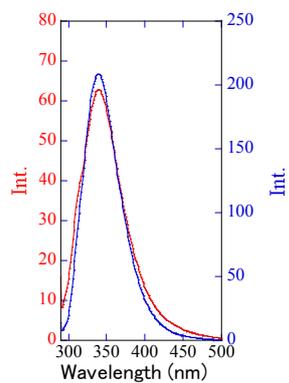


Figure S6. Fluorescence spectra of the eluted solution after desalination procedure. Blue: native CcCel6A dissolved in [ch][dhp], Red: aggregated CcCel6A dissolved in [ch][dhp].

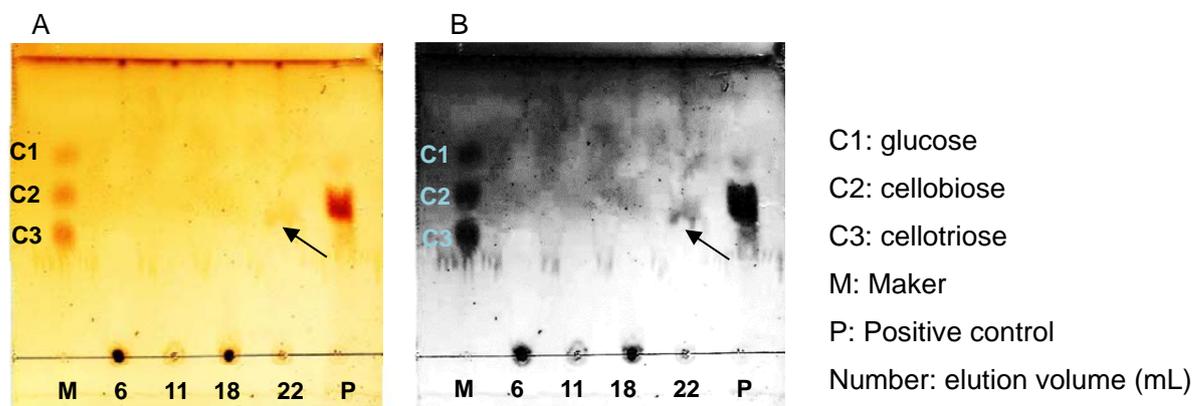


Figure S7. TLC results after mixing cellulose in each eluted fraction. (A) Colour image, (B) Colour split image (blue)