

# A polar liquid zwitterion does not critically destruct cytochrome c at high concentration: an initial comparative study with a polar ionic liquid

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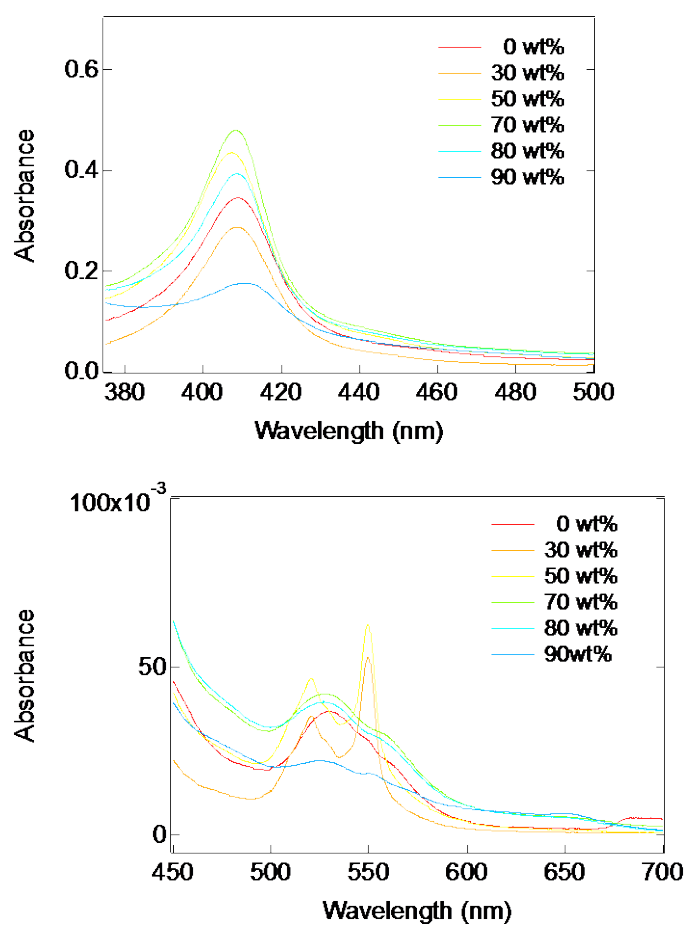
## Supplementary Material

### A polar liquid zwitterion does not critically destruct cytochrome *c* at high concentration: an initial comparative study with a polar ionic liquid

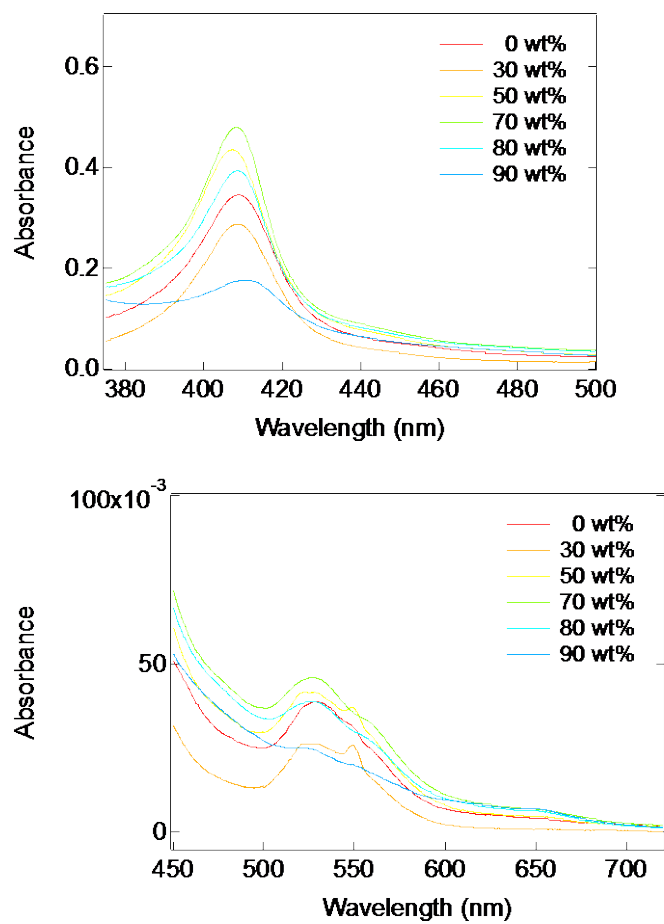
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<sup>A</sup>*Faculty of Biological Science and Technology, Institute of Science and Engineering, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan*

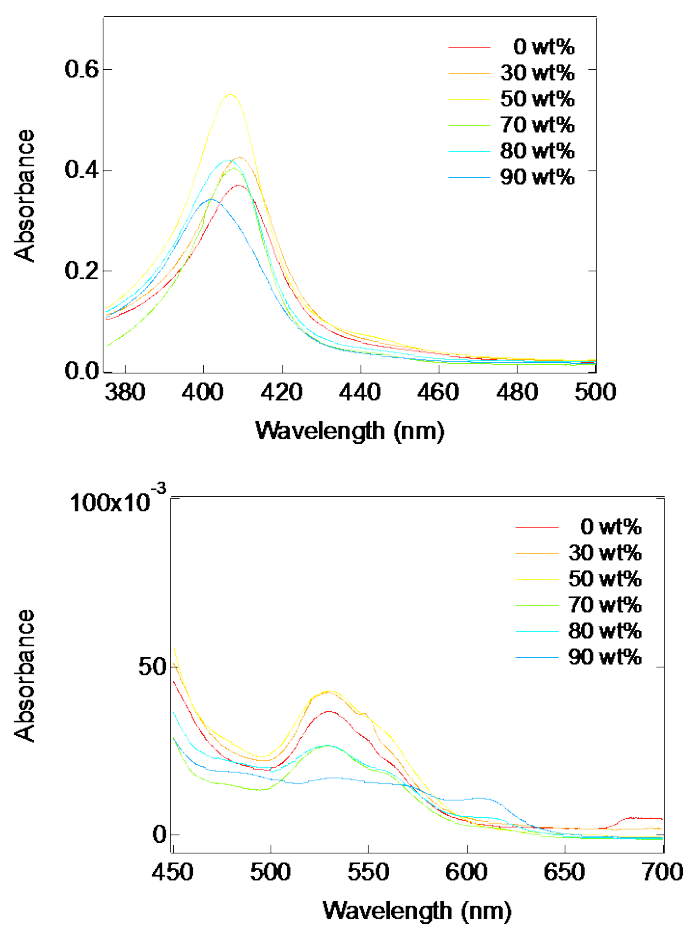
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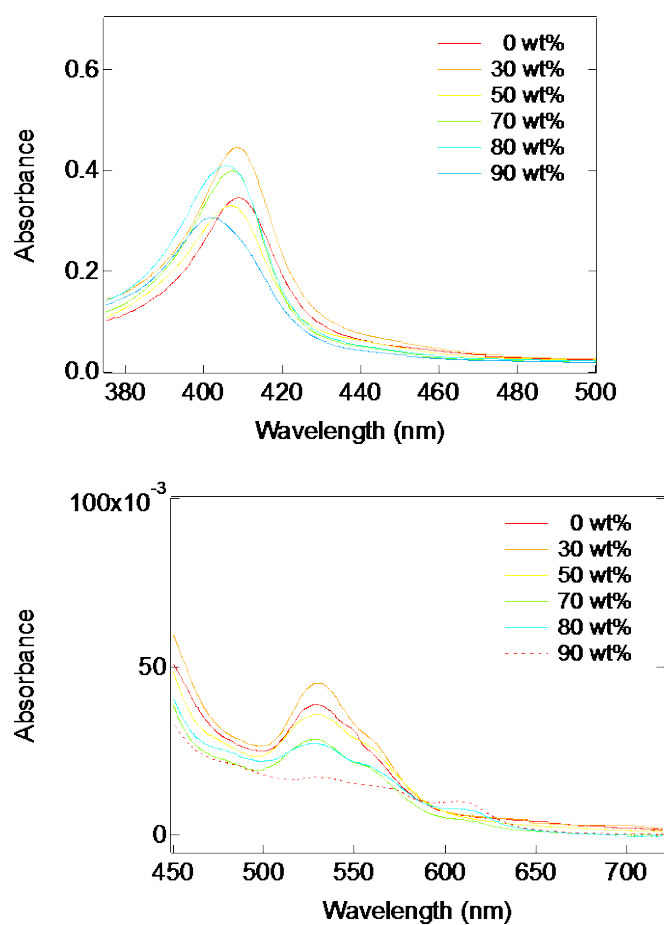
**Figure S1.** Spectra of cyt. *c* in solutions of OE<sub>2</sub>imC<sub>3</sub>C at various concentrations at room temperature.



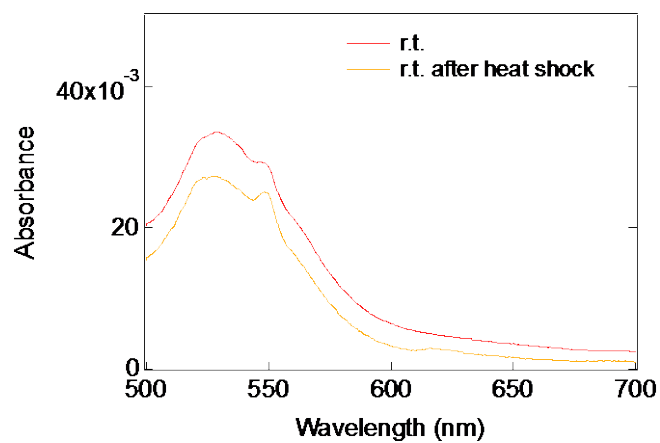
**Figure S2.** Spectra of cyt. *c* in solutions of OE<sub>2</sub>imC<sub>3</sub>C at various concentrations at room temperature after heat shock.



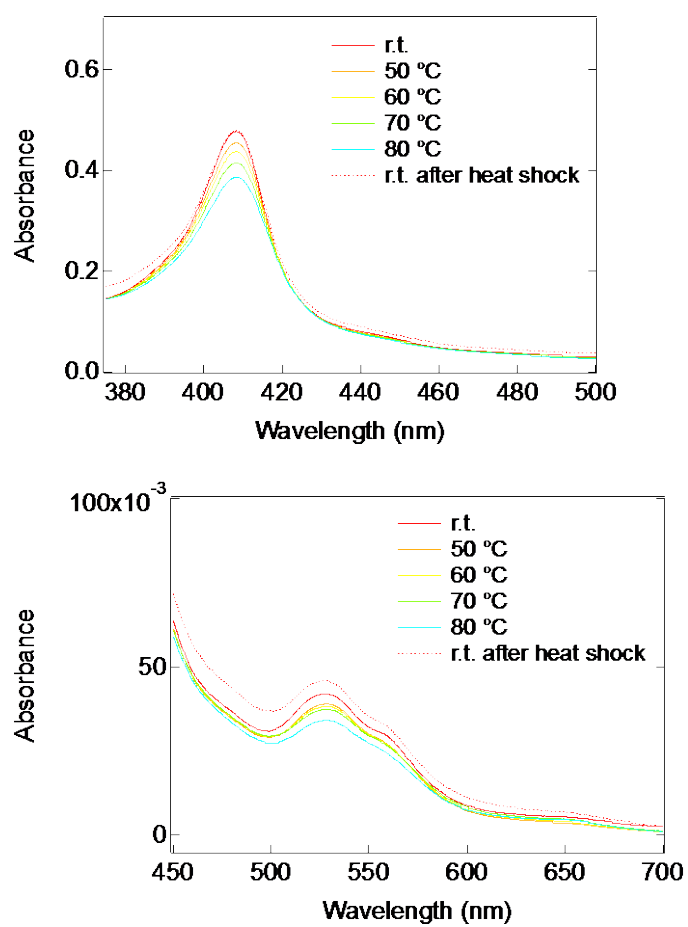
**Figure S3.** Spectra of cyt. *c* in solutions of [C<sub>2</sub>mim]OAc at various concentrations at room temperature.



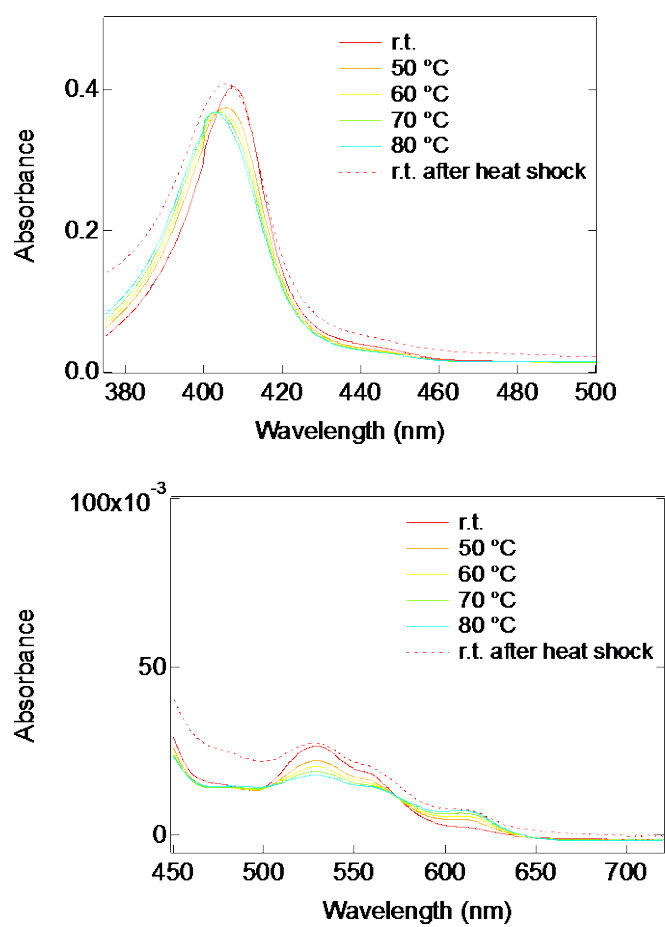
**Figure S4.** Spectra of cyt. *c* in solutions of [C<sub>2</sub>mim]OAc at various concentrations at room temperature after heat shock.



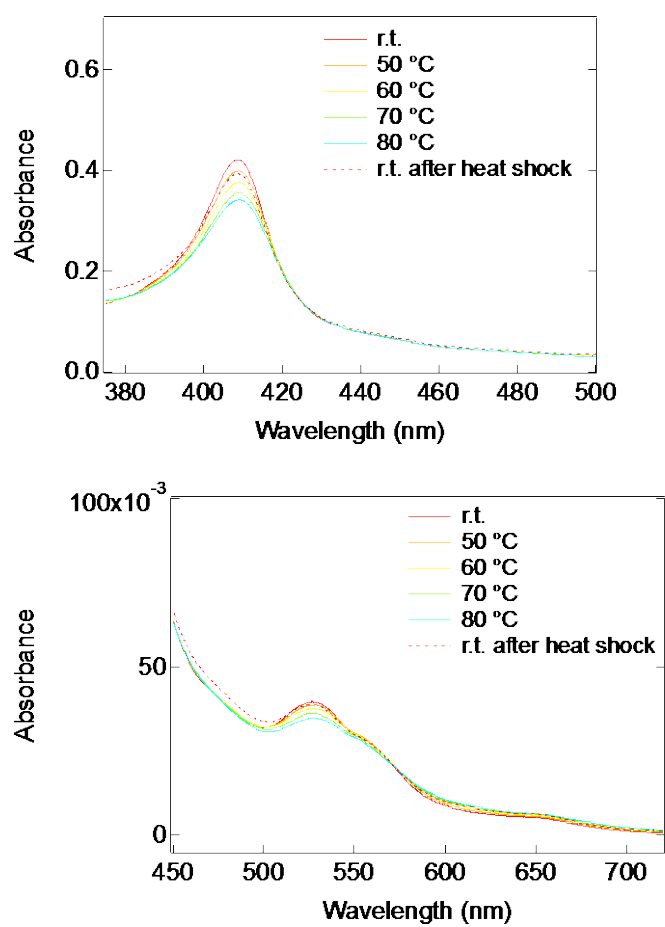
**Figure S5.** Spectrum (around 615 nm) of cyt. *c* in pure water at room temperature before and after heat shock.



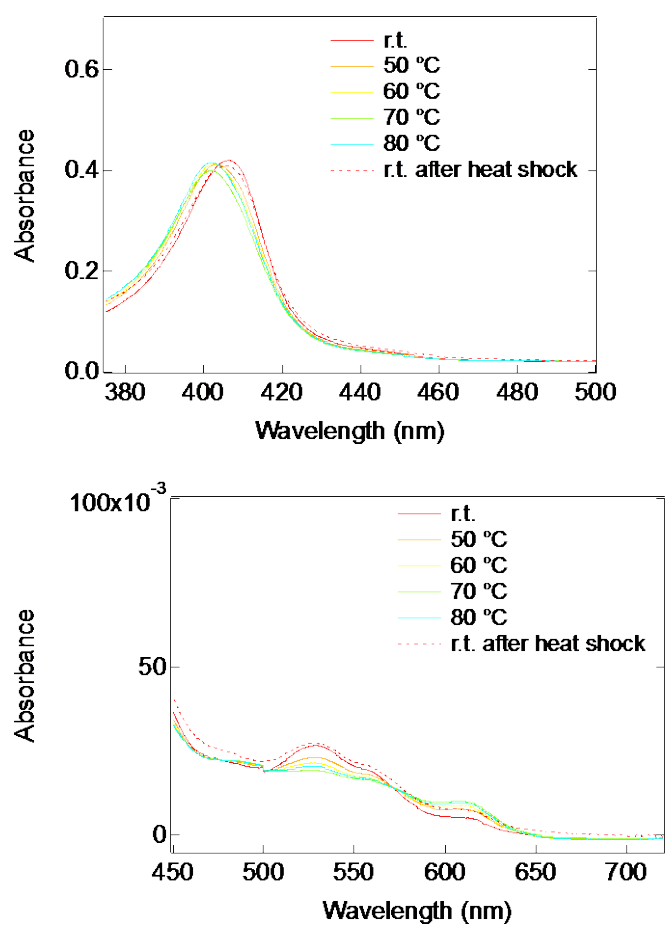
**Figure S6.** Spectra of cyt. *c* in 70 wt% solution of OE<sub>2</sub>imC<sub>3</sub>C at various temperatures.



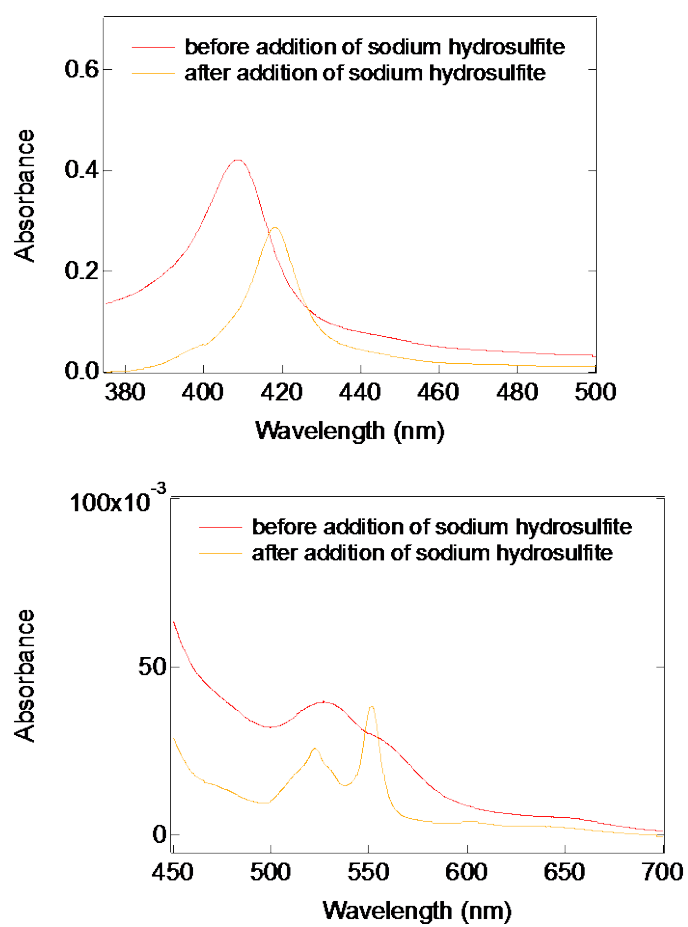
**Figure S7.** Spectra of cyt. *c* in 70 wt% solution of [C<sub>2</sub>mim]OAc at various temperatures.



**Figure S8.** Spectra of cyt. c in 80 wt% solution of OE<sub>2</sub>imC<sub>3</sub>C at various temperatures.



**Figure S9.** Spectra of cyt.c in 80 wt% solution of [C<sub>2</sub>mim]OAc at various temperatures.



**Figure S10.** Spectra of cyt. *c* in 80 wt% solution of OE<sub>2</sub>imC<sub>3</sub>C at room temperature before and after addition of sodium hydrosulfite.

**Table S1.** Wavelength at the maximum absorbance ( $\lambda_{\max}$ ) and the absorbance of the Soret-, Q-, 695 nm-, 615 nm bands of cyt.c in solutions of OE<sub>2</sub>imC<sub>3</sub>C or [C<sub>2</sub>mim]OAc at various concentrations at room temperature before and after heat shock.

|                                    |                      | Temperature & Ion concentration (wt%) |                      |                      |                      |                      |                      |                      |                      |                           |                           |                      |                      |                      |
|------------------------------------|----------------------|---------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|---------------------------|---------------------------|----------------------|----------------------|----------------------|
|                                    |                      | r.t.                                  |                      |                      |                      |                      |                      | r. t. <sup>d</sup>   |                      |                           |                           |                      |                      |                      |
|                                    |                      | 0 % <sup>b</sup>                      | 30 %                 | 50 %                 | 70 %                 | 80 %                 | 90 %                 | 0 % <sup>b</sup>     | 30 %                 | 50 %                      | 70 %                      | 80 %                 | 90 %                 |                      |
| OE <sub>2</sub> imC <sub>3</sub> C | Soret band           | $\lambda_{\max}$                      | 408.9                | 412.6                | 410.4                | 408.4                | 408.7                | 411.2                | 409.0                | 408.7                     | 407.4                     | 408.3                | 408.7                | 410.6                |
|                                    |                      | Abs                                   | 0.370                | 0.318                | 0.424                | 0.476                | 0.419                | 0.159                | 0.345                | 0.285                     | 0.434                     | 0.482                | 0.391                | 0.175                |
|                                    | Q band               | $\lambda_{\max}$                      | 529.0                | 520.7,<br>549.5      | 520.4,<br>549.4      | 527.7                | 527                  | 524.6                | 528.7                | 521.7-<br>528.0,<br>549.0 | 522.7-<br>528.0,<br>548.5 | 526.2                | 526.7                | 522.4                |
|                                    |                      | Abs                                   | 0.037                | 0.036,<br>0.053      | 0.046,<br>0.063      | 0.042                | 0.039                | 0.021                | 0.039                | 0.026,<br>0.026           | 0.041<br>0.037            | 0.046                | 0.039                | 0.024                |
|                                    | 695 nm <sup>c</sup>  | Abs                                   | 0.005                | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i>      | <i>—<sup>d</sup></i>      | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> |
|                                    | ~615 nm <sup>c</sup> | Abs                                   | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i>      | <i>—<sup>d</sup></i>      | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> |
|                                    | Presumed state       |                                       | III                  | IV                   | IV                   | III–3.5              | III–3.5              | III–3.5              | III–3.5              | 3.5                       | 3.5                       | III–3.5              | III–3.5              | III–3.5              |
|                                    | pH                   |                                       | 7.0                  | 7.7                  | 8.2                  | 10.1                 | 10.9                 | 11.5                 | —                    | —                         | —                         | —                    | —                    | —                    |
| [C <sub>2</sub> mim]OAc            | Soret band           | $\lambda_{\max}$                      | 408.9                | 409.0                | 406.8                | 407.6                | 406.3                | 401.9                | 409.0                | 408.5                     | 407.02                    | 407.2                | 406.4                | 401.9                |
|                                    |                      | Abs                                   | 0.370                | 0.425                | 0.550                | 0.402                | 0.420                | 0.340                | 0.345                | 0.444                     | 0.328                     | 0.398                | 0.386                | 0.304                |
|                                    | Q band               | $\lambda_{\max}$                      | 529.0                | 528.7                | 529.3                | 528.5                | 528.3                | 533.2                | 528.7                | 528.9                     | 529.3                     | 527.7                | 526.5                | 529.8                |
|                                    |                      | Abs                                   | 0.037                | 0.042                | 0.043                | 0.027                | 0.027                | 0.017                | 0.039                | 0.045                     | 0.036                     | 0.028                | 0.033                | 0.017                |
|                                    | 695 nm <sup>c</sup>  | Abs                                   | 0.005                | 0.001                | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | 0.001                     | <i>—<sup>d</sup></i>      | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> |
|                                    | ~615 nm <sup>c</sup> |                                       | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i> | 0.001                | 0.002                | 0.004                | <i>—<sup>d</sup></i> | <i>—<sup>d</sup></i>      | <i>—<sup>d</sup></i>      | <0.001               | 0.001                | 0.003                |
|                                    | Presumed state       |                                       | III                  | III (–<br>3.5)       | III–3.5              | III–3.5              | III–3.5              | III–3.5              | III–3.5              | III–3.5                   | III–3.5                   | III–3.5              | III–3.5              | III–3.5              |
|                                    | pH                   |                                       | 7.0                  | 6.7                  | 8.0                  | 9.7                  | 11.0                 | 12.1                 | —                    | —                         | —                         | —                    | —                    | —                    |

<sup>a</sup>Measured at room temperature after heated at 80 °C for 1min. <sup>b</sup>Measured in pH 7.0 phosphate buffer. Other samples

were prepared by dilution of OE<sub>2</sub>imC<sub>3</sub>C or [C<sub>2</sub>mim]OAc with pure water. <sup>c</sup> Calculated the difference from the

estimated baseline. <sup>d</sup>Not detected.

**Table S2.** Wavelength at the maximum absorbance ( $\lambda_{\text{max}}$ ) and the absorbance of the Soret-, Q-, 695 nm-, 615 nm bands of cyt.c in 70 and 80 wt% solutions of OE<sub>2</sub>imC<sub>3</sub>C or [C<sub>2</sub>mim]OAc at various temperatures.

|                                    |                      |                        | Ion concentration (wt%) & Temperature (°C) |                |                |                |                |                    |                |                |                |                |                |                    |
|------------------------------------|----------------------|------------------------|--------------------------------------------|----------------|----------------|----------------|----------------|--------------------|----------------|----------------|----------------|----------------|----------------|--------------------|
|                                    |                      |                        | 70 wt%                                     |                |                |                |                |                    | 80 wt%         |                |                |                |                |                    |
|                                    |                      |                        | r. t.                                      | 50             | 60             | 70             | 80             | r. t. <sup>a</sup> | r. t.          | 50             | 60             | 70             | 80             | r. t. <sup>a</sup> |
| OE <sub>2</sub> imC <sub>3</sub> C | Soret band           | $\lambda_{\text{max}}$ | 408.4                                      | 408.2          | 408.2          | 408.4          | 408.6          | 408.3              | 408.7          | 408.9          | 409.1          | 409.1          | 409.1          | 408.7              |
|                                    |                      | Abs                    | 0.476                                      | 0.455          | 0.437          | 0.414          | 0.386          | 0.482              | 0.419          | 0.398          | 0.376          | 0.354          | 0.340          | 0.391              |
|                                    | Q band               | $\lambda_{\text{max}}$ | 527.7                                      | 528.3          | 527.9          | 528.8          | 528.7          | 526.2              | 527.0          | 528.5          | 528.5          | 527.8          | 528.3          | 526.7              |
|                                    |                      | Abs                    | 0.042                                      | 0.039          | 0.038          | 0.037          | 0.034          | 0.046              | 0.039          | 0.039          | 0.038          | 0.036          | 0.035          | 0.039              |
|                                    | 695 nm <sup>b</sup>  | Abs                    | — <sup>c</sup>                             | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup>     | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup>     |
|                                    | ~615 nm <sup>b</sup> | Abs                    | — <sup>c</sup>                             | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup>     | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup>     |
|                                    | Presumed state       | III–3.5                | III–3.5                                    | III–3.5        | III–3.5        | III–3.5        | III–3.5        | III–3.5            | III–3.5        | III–3.5        | III–3.5        | III–3.5        | III–3.5        | III–3.5            |
| [C <sub>2</sub> mim]OAc            | Soret band           | $\lambda_{\text{max}}$ | 407.6                                      | 405.9          | 404.4          | 403.6          | 403.0          | 407.2              | 406.3          | 403.2          | 402.4          | 401.8          | 402.2          | 406.4              |
|                                    |                      | Abs                    | 0.402                                      | 0.374          | 0.368          | 0.368          | 0.367          | 0.398              | 0.420          | 0.410          | 0.411          | 0.399          | 0.414          | 0.386              |
|                                    | Q band               | $\lambda_{\text{max}}$ | 528.5                                      | 528.4          | 528.4          | 528.4          | 528.1          | 527.7              | 528.3          | 528.6          | 526.1          | 526.5          | 526.4          | 526.5              |
|                                    |                      | Abs                    | 0.027                                      | 0.022          | 0.020          | 0.019          | 0.018          | 0.028              | 0.027          | 0.023          | 0.021          | 0.019          | 0.020          | 0.033              |
|                                    | 695 nm <sup>b</sup>  | Abs                    | — <sup>c</sup>                             | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup>     | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup> | — <sup>c</sup>     |
|                                    | ~615 nm <sup>b</sup> | Abs                    | 0.001                                      | 0.001          | 0.002          | 0.002          | 0.003          | <0.001             | 0.002          | 0.002          | 0.003          | 0.004          | 0.004          | 0.001              |
|                                    | Presumed state       | III–3.5                | III–3.5                                    | III–3.5        | III–3.5        | III–3.5        | III–3.5        | III–3.5            | III–3.5        | III–3.5        | III–3.5        | III–3.5        | III–3.5        | III–3.5            |

<sup>a</sup>Measured at room temperature after heated at 80 °C for 1min. <sup>b</sup>Calculated the difference from the estimated baseline.

<sup>c</sup>Not detected.