

## Supplementary Material

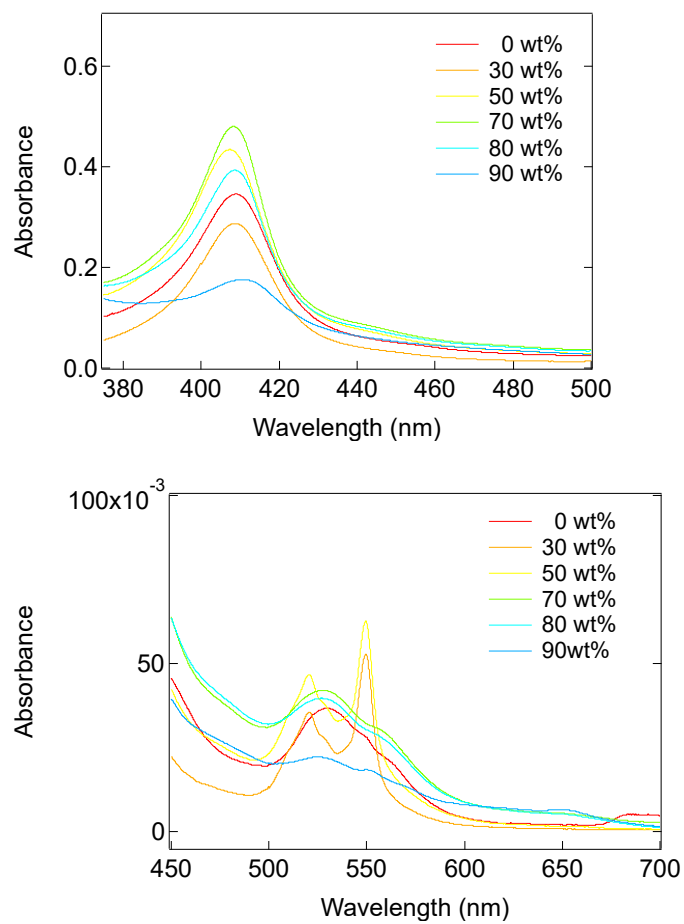
### **A polar liquid zwitterion does not critically destruct cytochrome *c* at high concentration: an initial comparative study with a polar ionic liquid**

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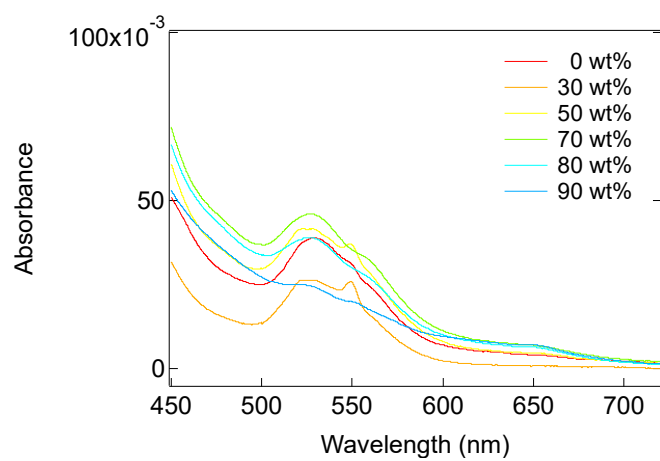
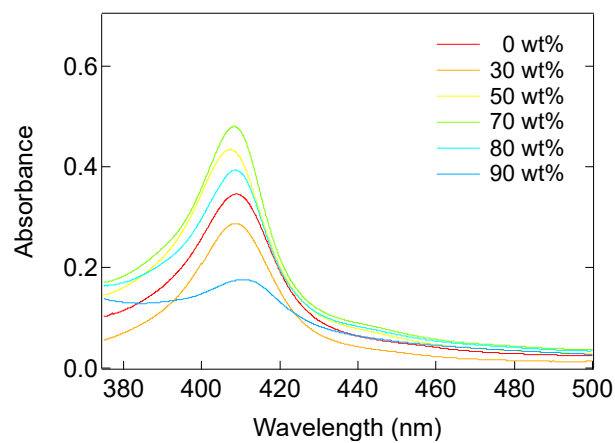
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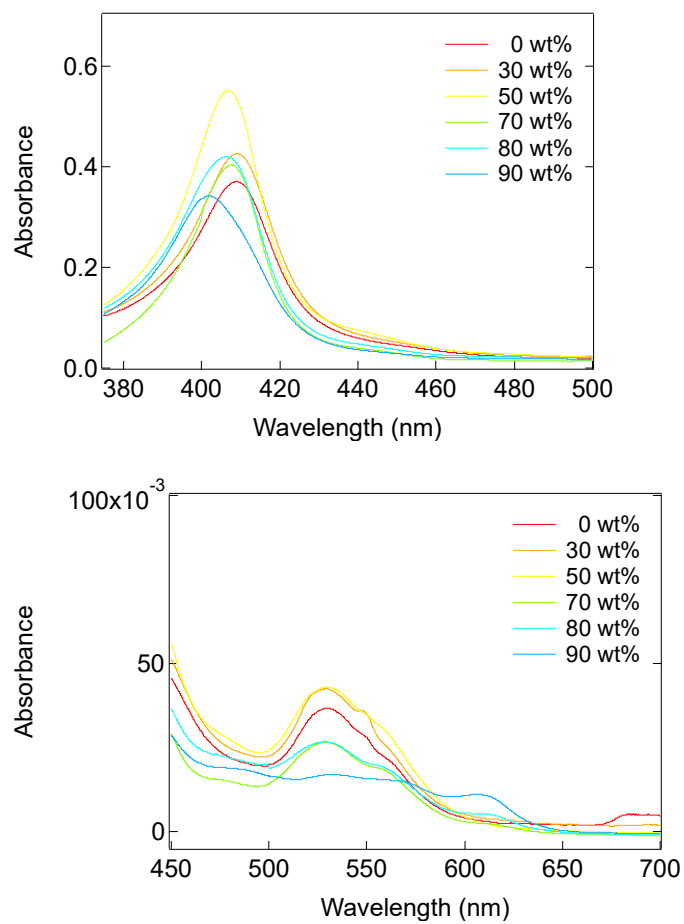
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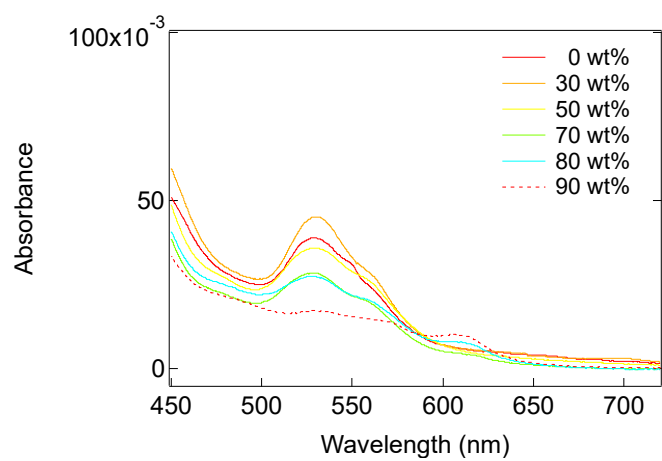
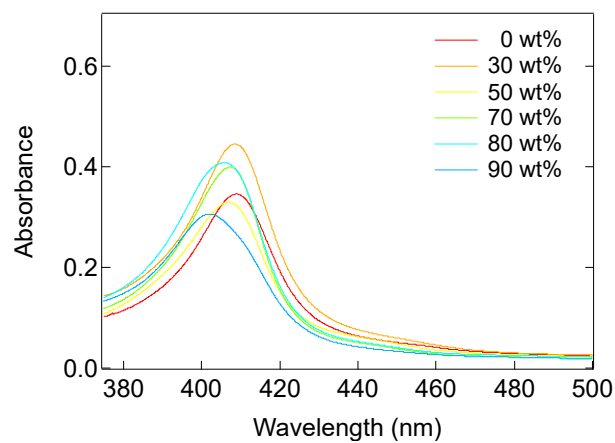
**Figure S1.** Spectra of cyt. *c* in solutions of OE<sub>2</sub>imC<sub>3</sub>C at various concentrations at room temperature.



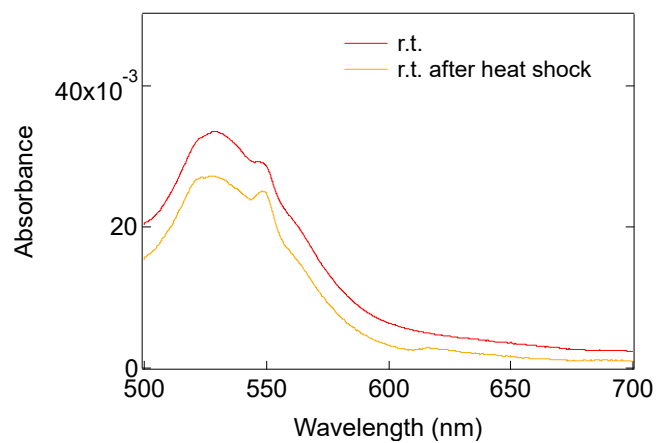
**Figure S2.** Spectra of *cyt. c* in solutions of OE<sub>2</sub>imC<sub>3</sub>C at various concentrations at room temperature after heat shock.



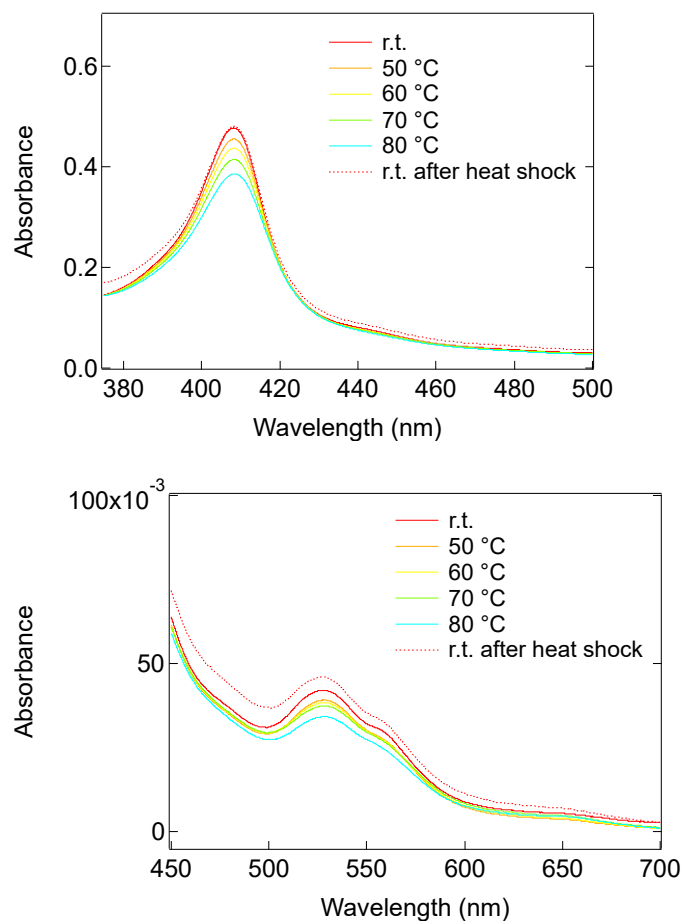
**Figure S3.** Spectra of *cyt. c* in solutions of [C<sub>2</sub>mim]OAc at various concentrations at room temperature.



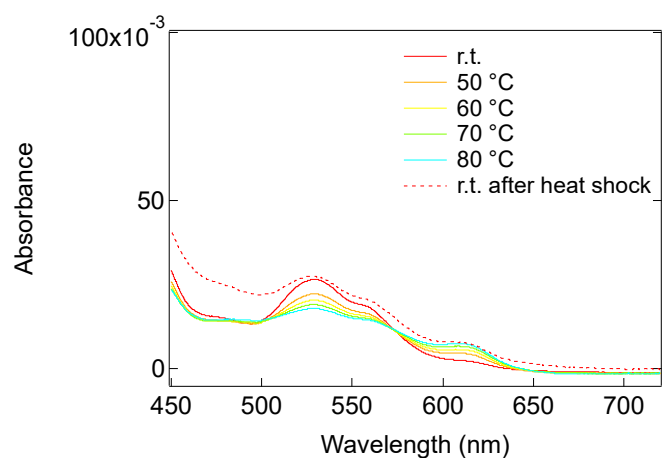
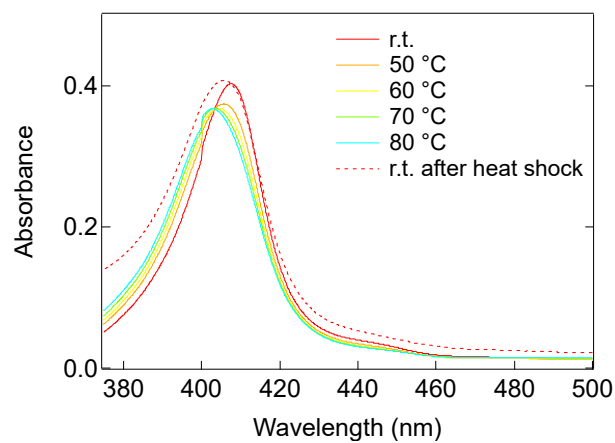
**Figure S4.** Spectra of *cyt. c* in solutions of [C<sub>2</sub>mim]OAc at various concentrations at room temperature after heat shock.



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

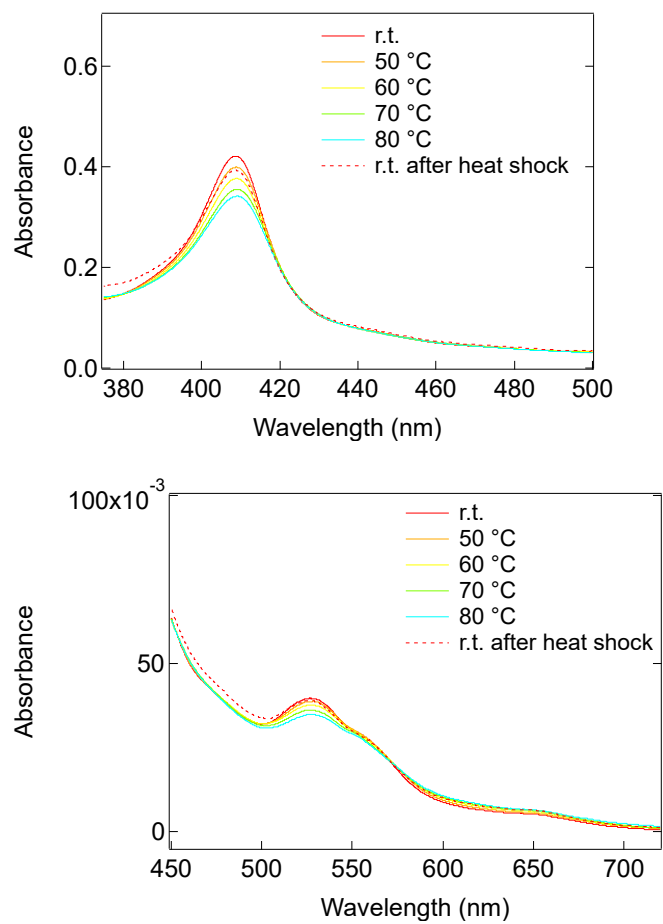


**Figure S6.** Spectra of *cyt. c* in 70 wt% solution of OE<sub>2</sub>imC<sub>3</sub>C at various temperatures.

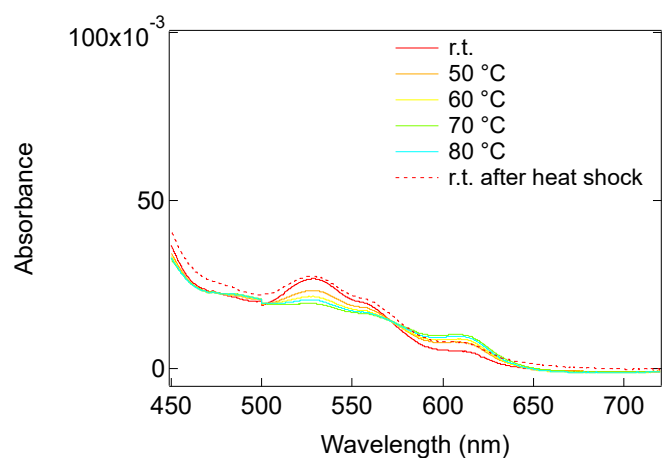
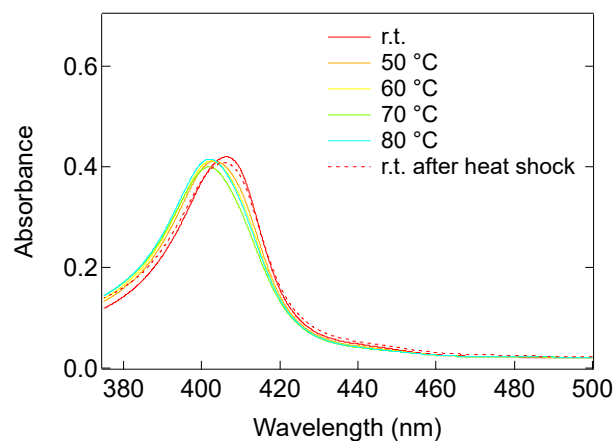


**Figure S7.** Spectra of *cyt. c* in 70 wt% solution of [C<sub>2</sub>mim]OAc at various temperatures.

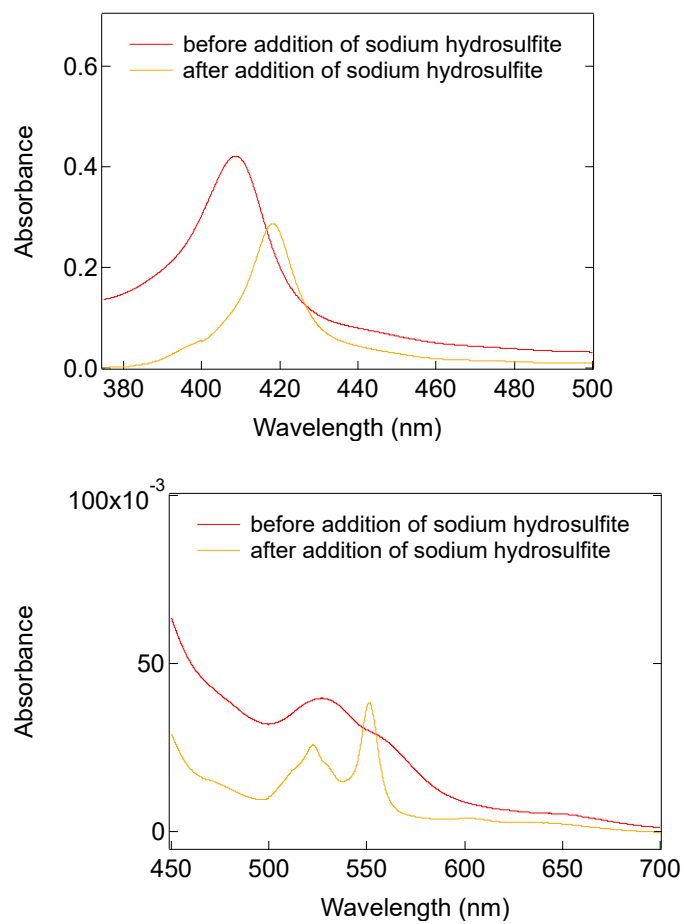




**Figure S8.** Spectra of *cyt. c* in 80 wt% solution of OE<sub>2</sub>imC<sub>3</sub>C at various temperatures.



**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>a</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          | 528.0,<br>549.0 | 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          | – <sup>d</sup>  | – <sup>d</sup>  | – <sup>d</sup> | – <sup>d</sup> | – <sup>d</sup>     | – <sup>d</sup> | – <sup>d</sup>  | – <sup>d</sup>  | – <sup>d</sup> | – <sup>d</sup> | – <sup>d</sup> |
|                                    | ~615 nm <sup>c</sup> | Abs                                   | – <sup>d</sup> | – <sup>d</sup>  | – <sup>d</sup>  | – <sup>d</sup> | – <sup>d</sup> | – <sup>d</sup>     | – <sup>d</sup> | – <sup>d</sup>  | – <sup>d</sup>  | – <sup>d</sup> | – <sup>d</sup> | – <sup>d</sup> |
|                                    | 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           | – <sup>d</sup>  | – <sup>d</sup> | – <sup>d</sup> | – <sup>d</sup>     | – <sup>d</sup> | 0.001           | – <sup>d</sup>  | – <sup>d</sup> | – <sup>d</sup> | – <sup>d</sup> |
|                                    | ~615 nm <sup>c</sup> |                                       | – <sup>d</sup> | – <sup>d</sup>  | – <sup>d</sup>  | 0.001          | 0.002          | 0.004              | – <sup>d</sup> | – <sup>d</sup>  | – <sup>d</sup>  | <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 ( $\lambda_{\max}$ ) 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           | $\lambda_{\max}$                           | 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               | $\lambda_{\max}$                           | 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        |
| [C <sub>2</sub> mim]OAc            | Soret band           | $\lambda_{\max}$                           | 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               | $\lambda_{\max}$                           | 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        |

<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.