ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Fluorescence Energy Transfer Efficiency in Labeled Yeast Cytochrome *c*: A Rapid Screen for Ion Biocompatibility in Aqueous Ionic Liquids

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Experimental Section

Chemicals and Reagents. The following materials were obtained in the highest purity available from Sigma-Aldrich (St. Louis, MO) and were used without auxiliary purification: ethyl acetate, dichloromethane, sodium chloride, sodium hydrogen phosphate heptahydrate, sodium dihydrogen phosphate monohydrate, sodium dicyanamide, sodium acetate, sodium methanesulfonate, sodium nitrate, sodium tetrafluoroborate, ethylamine, and nitric acid. Crystalline guanidine hydrochloride (GuHCl) was purchased from MP Biomedicals, LLC (Solon, OH) in ultra pure grade (99.5%; O.D. at 260 nm << 0.03 for a 6.0 M solution). Tetramethylrhodamine-5-iodoacetamide dihydroiodide (5-TMRIA, single isomer) was purchased from Invitrogen Corp. (Carlsbad, CA). 1-Methylimidazole (Aldrich, ≥99%), 1-chlorobutane (Aldrich, 99.5%, anhydrous), and 1-bromobutane (Sigma-Aldrich, ReagentPlus®, ≥99%) were redistilled immediately prior to use. All aqueous solutions were formulated using doubly distilled–deionized water treated to specific resistivity of ≥18.2 MΩ cm via a Barnstead NANOpure II system.

[bmim]Cl and [bmim]Br were synthesized according to recently published methods.[S1] All other ILs, apart from EAN, were prepared commencing with the metathesis of [bmim]Br using the desired anion source, followed by exhaustive liquid–liquid extraction with dichloromethane, as previously described. Ethylammonium nitrate (EAN) was prepared following the work of Shotwell and Flowers.[S2] The following densities, determined at 25 °C using calibrated 2.0 cm^3 glass density bottles, were used to prepare 80% (v/v) IL stocks in phosphate buffered water (PB; 100 mM Na_2HPO_4, pH 7.50, passed through a 0.2-μm nylon filter) for use in TMR(C102)-cyt *c* unfolding titrations: [bmim]Cl (1.092 g cm^{-3}); [bmim]Br (1.303 g cm^{-3}); [bmim][dca] (1.127 g cm^{-3}); [bmim][BF_4] (1.177 g cm^{-3}); [bmim][ac] (1.286 g cm^{-3}); [bmim][NO_3] (1.251 g cm^{-3});

[bmim][ms] (1.292 g cm^{-3}); EAN (1.179 g cm^{-3}). Exact GuHCl concentrations were determined from an empirical relationship between [GuHCl] and refractive index[S3] (Milton Roy Abbe-3L refractometer).

Preparation of TMR(C102)-cyt *c* Adduct. Iso-1 cytochrome *c* from *Saccharomyces cerevisiae* was purchased from Sigma. The samples featured an *Rz* value ($Rz = A_{410}/A_{280}$) greater than 4.5 and were thus used without further purification. The C102 sulfhydryl group of *S. cerevisiae* iso-1 cyt *c* was derivatized with the thiol-reactive fluorophore 5-TMRIA in the dark to curtail any deleterious photochemical side reactions, following methods reported previously.[S4–S6] As an added post-dialysis step, TMR(C102)-cyt *c* was passed through Sephadex G-25 twice to remove any non-covalently bound 5-TMRIA. After purification, TMR(C102)-cyt *c* was oxidized with $K_3Fe(CN)_6$, followed by exhaustive dialysis into PB.

Fluorescence Spectroscopy. Steady-state fluorescence measurements were performed by using a PTI QuantaMaster Model C-60/2000 L-format scanning spectrofluorometer equipped with a 75 W xenon lamp and single-grating monochromators. Peak maxima determinations were based on emission spectral fits to log-normal functions for wavelengths spanning the TMR probe emission spectrum (560–600 nm), a method resulting in very low uncertainty based on standard error estimates ($\sigma < 0.05$).

For unfolding studies, TMR(C102)-cyt *c* in PB was kept at 22 °C by a Peltier temperature element. Samples were titrated with aqueous [bmim][X] with constant slow stirring (50 rpm). To ensure equilibration was achieved, samples were allowed to equilibrate for at least 20 min after each addition before readings were taken with a shutter blocking the excitation beam. A magnetic stirrer and an 8-mm-long stirring bar provided the convective transport during this equilibration period. All reported spectra have been corrected for detector response and are

background subtracted using appropriate solvent blanks with dilution duly accounted for. The excitation wavelength was set to 545 nm and the excitation and emission spectral bandpasses were both maintained at 2 nm. Integrated fluorescence intensities for TMR(C102)-cyt *c* were calculated between the limits of 560 and 620 nm. The initial TMR(C102)-cyt *c* concentration used in all studies was 5 μM (prior to dilution with IL stocks).

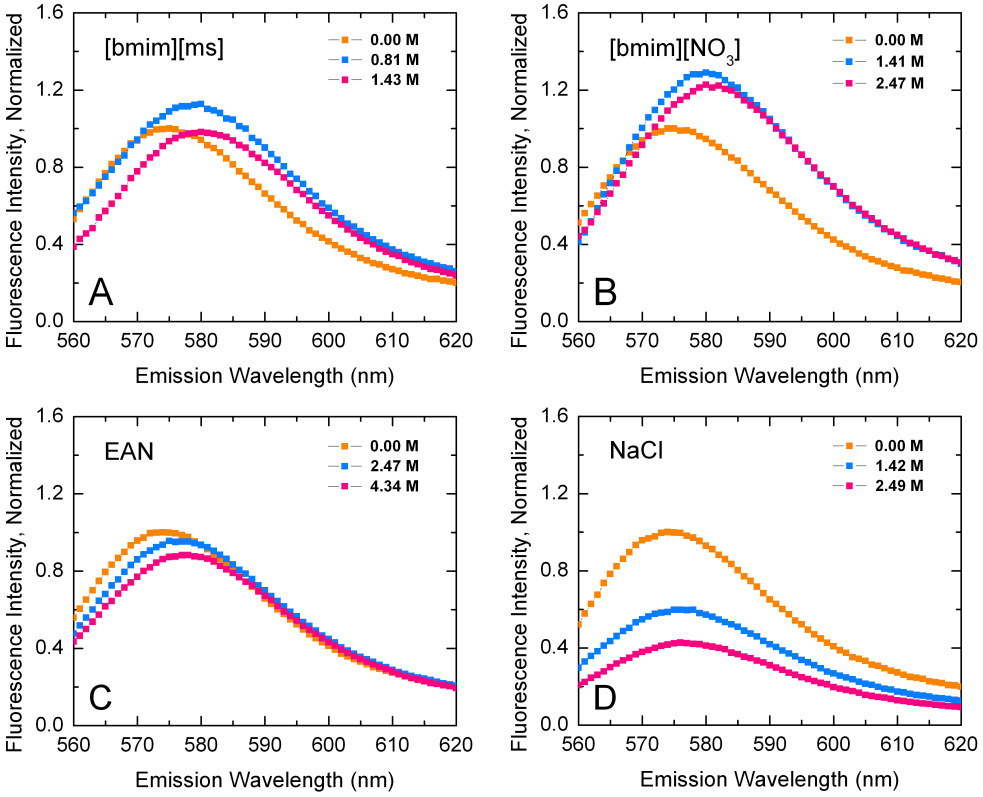

Fig. S1 Corrected emission spectra of TMR(C102)-cyt *c* in PB (100 mM, pH 7.50) containing (A) [bmim][ms], (B) [bmim][NO_3], (C) EAN, and (D) NaCl at the indicated molar levels.

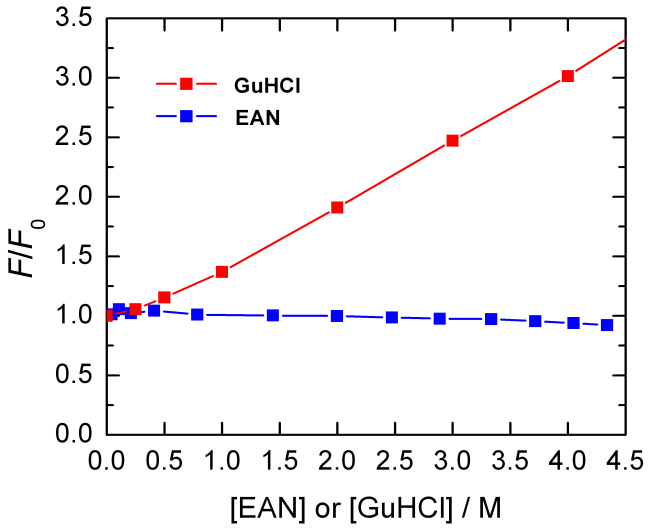

Fig. S2. Relative fluorescence ratio (F/F_0) for TMR(C102)-cyt *c* versus [EAN] in PB (100 mM, pH 7.50) over a higher co-solvent range (to 40 vol%) than presented in Fig. 3A. Parallel GuHCl results are provided for ready contrast.

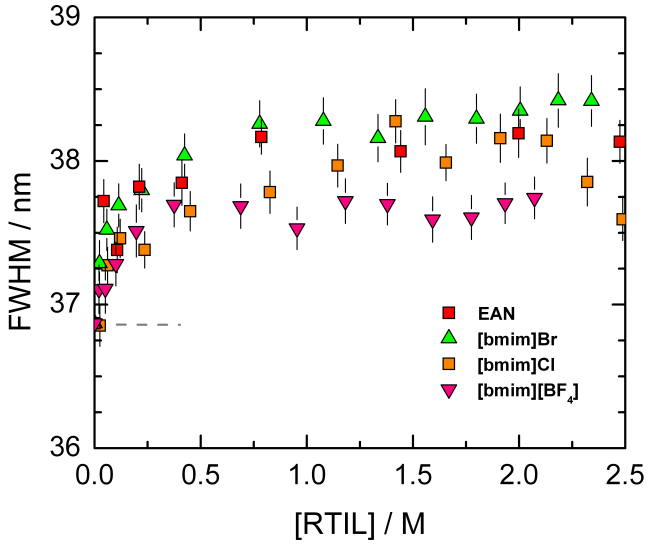

Fig. S3 TMR(C102)-cyt *c* emission spectral full widths at half-maxima (FWHM) for selected aqueous IL systems whose related F/F_0 and peak maxima are shown in Fig. 3.

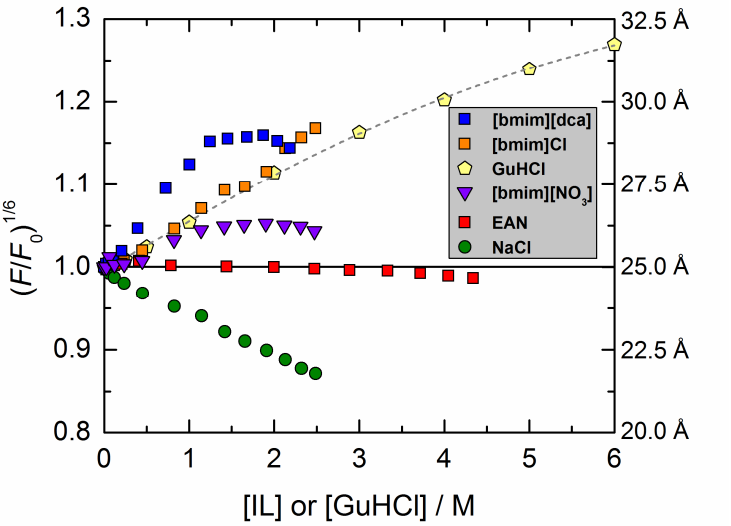

Fig. S4 $(F/F_0)^{1/6}$ values for representative IL systems as a rough estimate of the fractional change in donor–acceptor distance relative to the mean distance in the folded protein, $(D–A)_0$. Note that $(D–A)_0 \approx 25$ Å for TMR(C102)-cyt *c* with a 5 Å FWHM.[S5,S6]

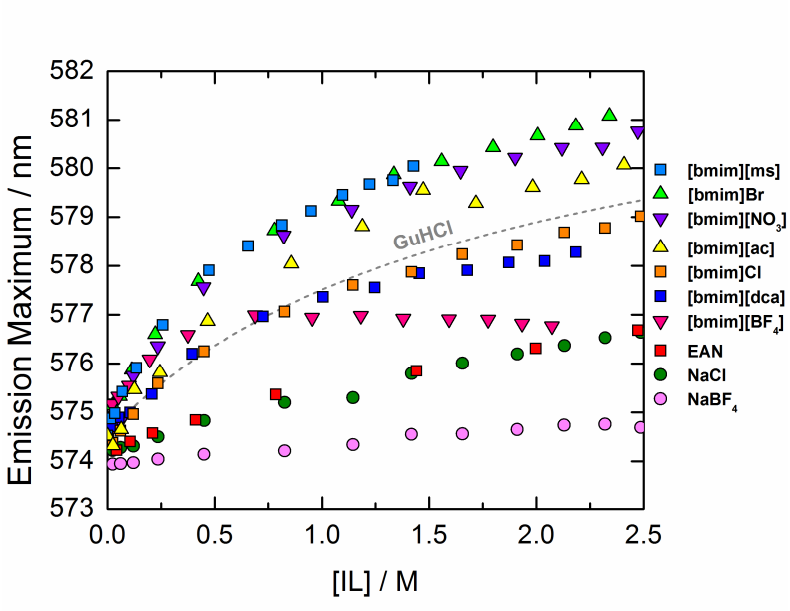

Fig. S5 Emission wavelength maxima for TMR(C102)-cyt *c* as a function of IL (or inorganic salt) concentration in PB. Results for GuHCl are provided for comparison. Emission maxima for TMR(C102)-cyt *c* were obtained from log-normal fits to the emission spectral data. For completeness, these data are provided, however, shifts in the emission maxima indicate localized microenvironmental changes surrounding the TMR label which are a complex convolution of solvent exposure and solvent polarity, complicated by the possibility of preferential solvation. The lowest degree of emission spectral shift was observed for NaCl, $NaBF_4$, and EAN where the protein retaining near-native configuration. This indicates that the nature of the solvent immediately surrounding the TMR label retains similarity to that in PB. Interestingly, it was in the moderately destabilizing IL systems that the largest degree of shift occurred. While the underlying mechanism is the subject of future scrutiny, a plausible explanation for the observed behavior must involve protein surface coordination with $[bmim]^+$ with varying influence from counter anions.

References

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