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ELECTRONIC SUPPLEMENTARY INFORMATION

New Journal of Chemistry (2022)

Imidazolium-based ionic liquids with increasing alkyl chain length of cation decrease the stability and fibrillation propensity of lysozyme

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Figure S1

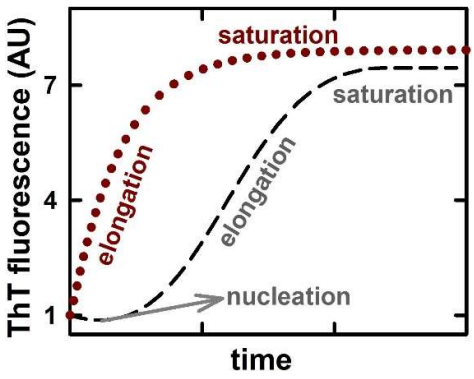

Fig. S1. Simulated kinetic data to show the fibril formation of a protein by following nucleation-dependent (black dashed lines) and nucleation-independent (red dotted lines) pathways, when probed with the fluorescence changes of ThT dye. Different steps involved namely, nucleation (or lag) phase, elongation phase and saturation phase are labelled.

Figure S2

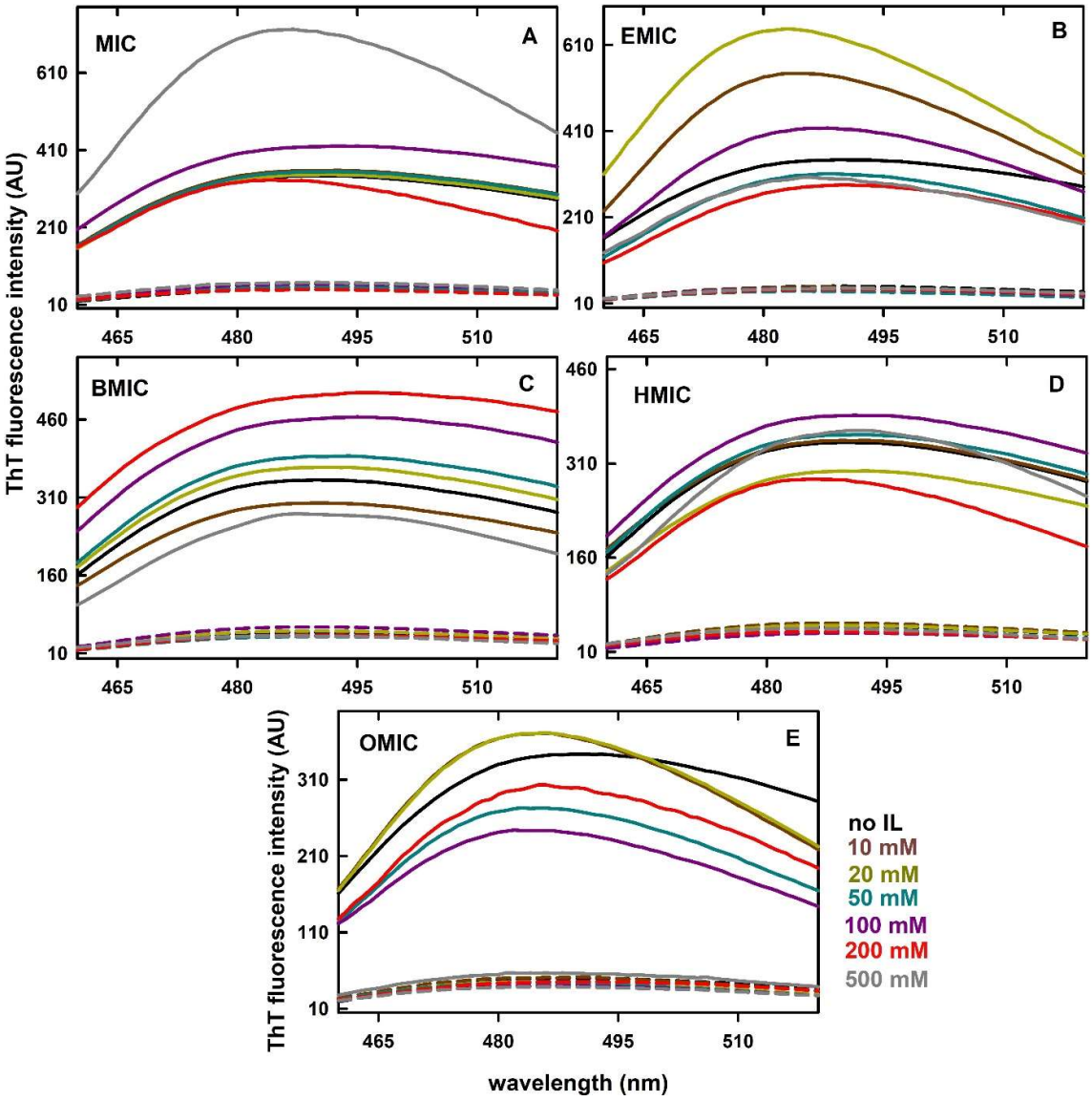

Fig. S2. Representative fluorescence emission spectra of ThT in DTT-reduced lysozyme in varying concentrations of (A) MIC, (B), EMIC, (C) BMIC, (D) HMIC, and (E) OMIC obtained before (dotted lines) and after (solid lines) incubation of the samples at 50 ^{o}C to measure the rate of fibril formation (refer to Fig. 3 in the main text). It may be noted that the fluorescence intensity of ThT was not significantly increased in the presence of 500 mM of OMIC (gray solid line in panel E) due to inhibition of fibril formation.

Figure S3

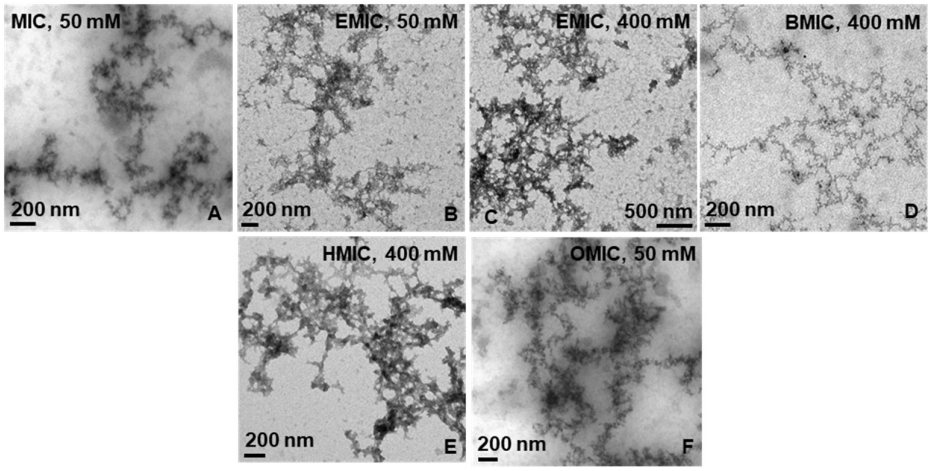

Fig. S3. Representative TEM images of fibrils formed by Lyz in the presence of (A) MIC, (B&C) EMIC (D) BMIC, (E) HMIC, and (E) OMIC.

Figure S4

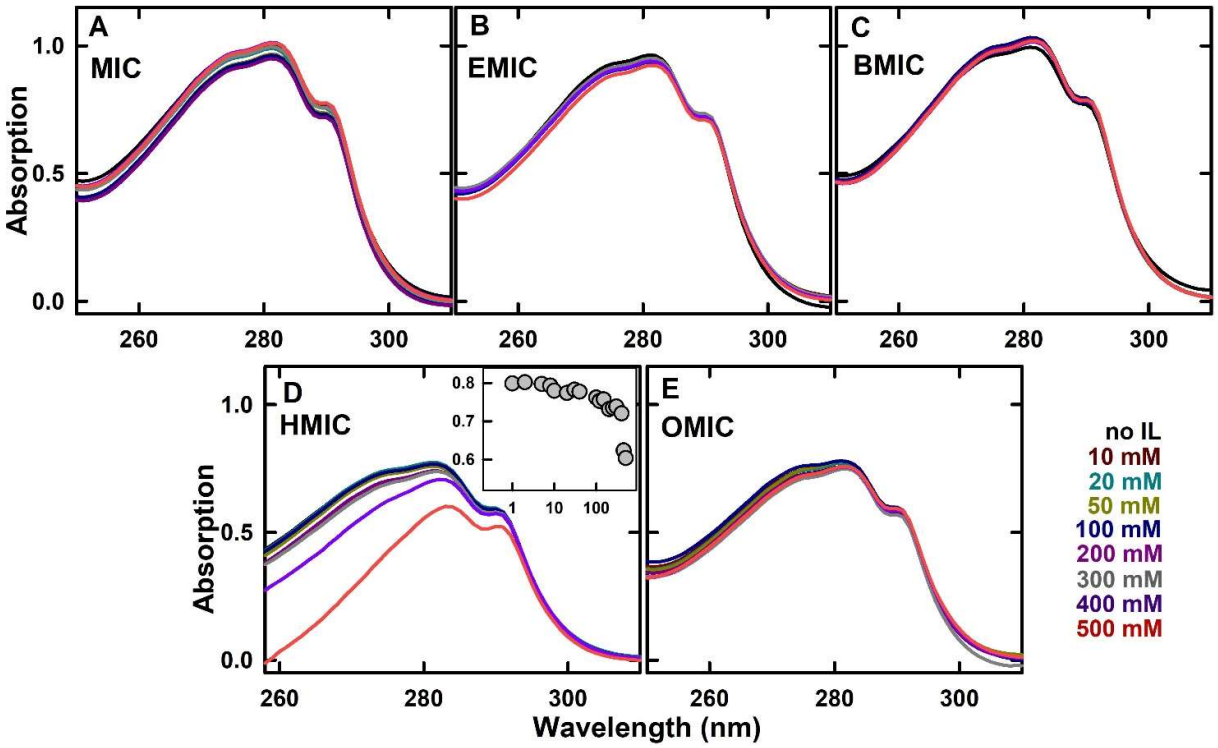

Fig. S4. Absorption spectra of Lyz in the presence of varying concentrations of ILs, (A) MIC, (B) EMIC, (C) BMIC, (D) HMIC, and (E) OMIC. The inset in panel (D) shows the change in absorption of Lyz at 283 nm in the presence of varying concentrations of HMIC.

The colours of the spectra represent different concentrations of ILs as mentioned in the legend.

Figure S5

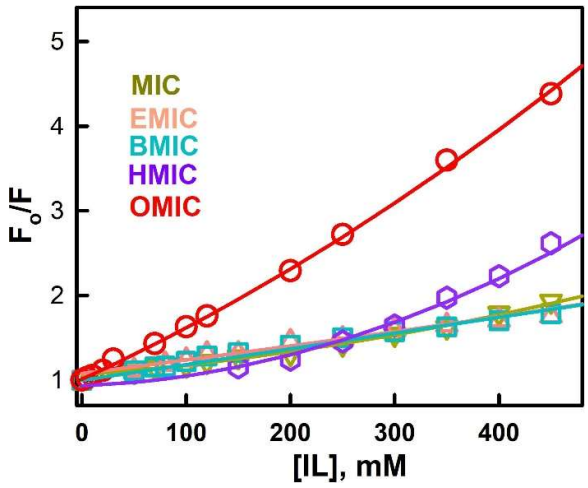

Fig. S5. The Stern-Volmer plot of fluorescence emission of Lyz in varying concentrations of the ILs, MIC (green inverted triangles), EMIC (pink triangles), BMIC (cyan squares), HMIC (purple hexagons), and OMIC (red circles). F_o and F represent the fluorescence emission of Lyz at 340 nm in the absence and the presence of ILs, respectively. The solid lines are merely to display the non-linear trend.

Figure S6

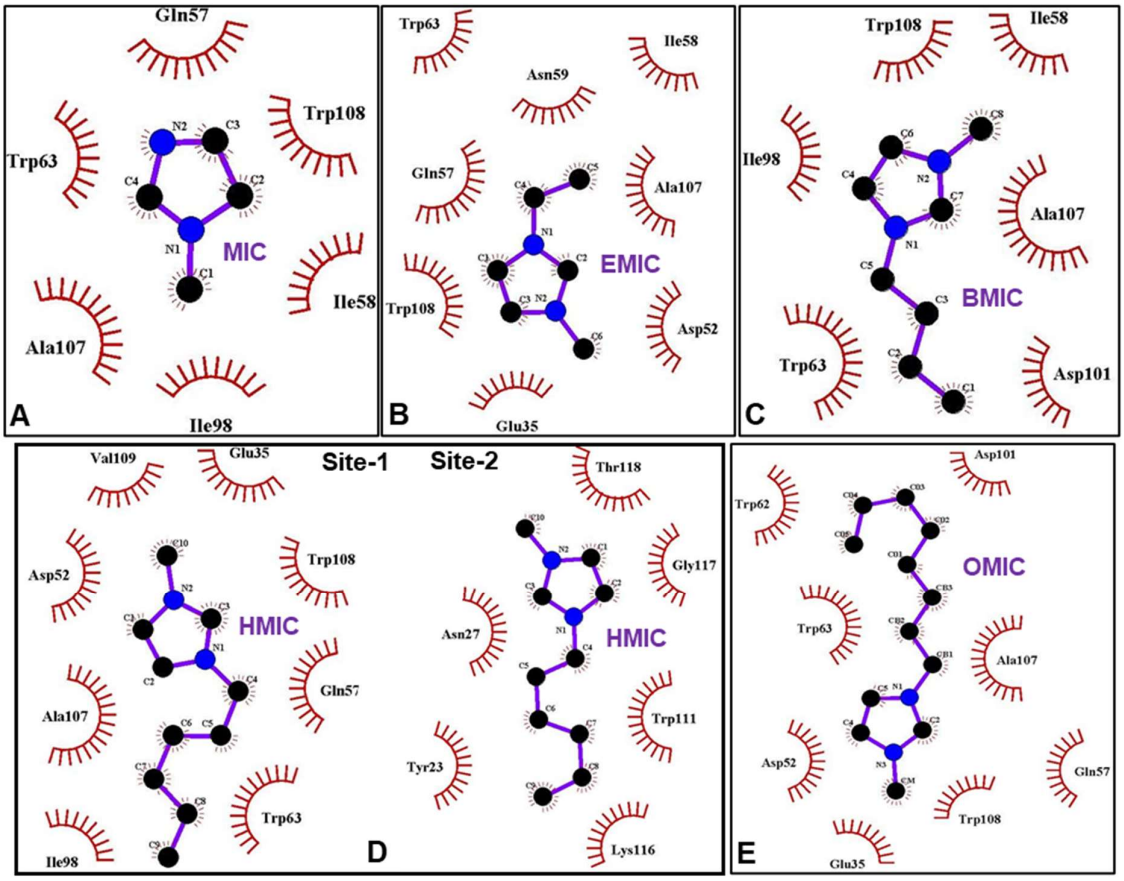

Fig. S6. The residues in the binding pocket of Lyz around the ILs are shown as curved brushes. The ILs are represented as sticks. In the case of HMIC, the two binding sites are shown separately (panel D).

Figure S7

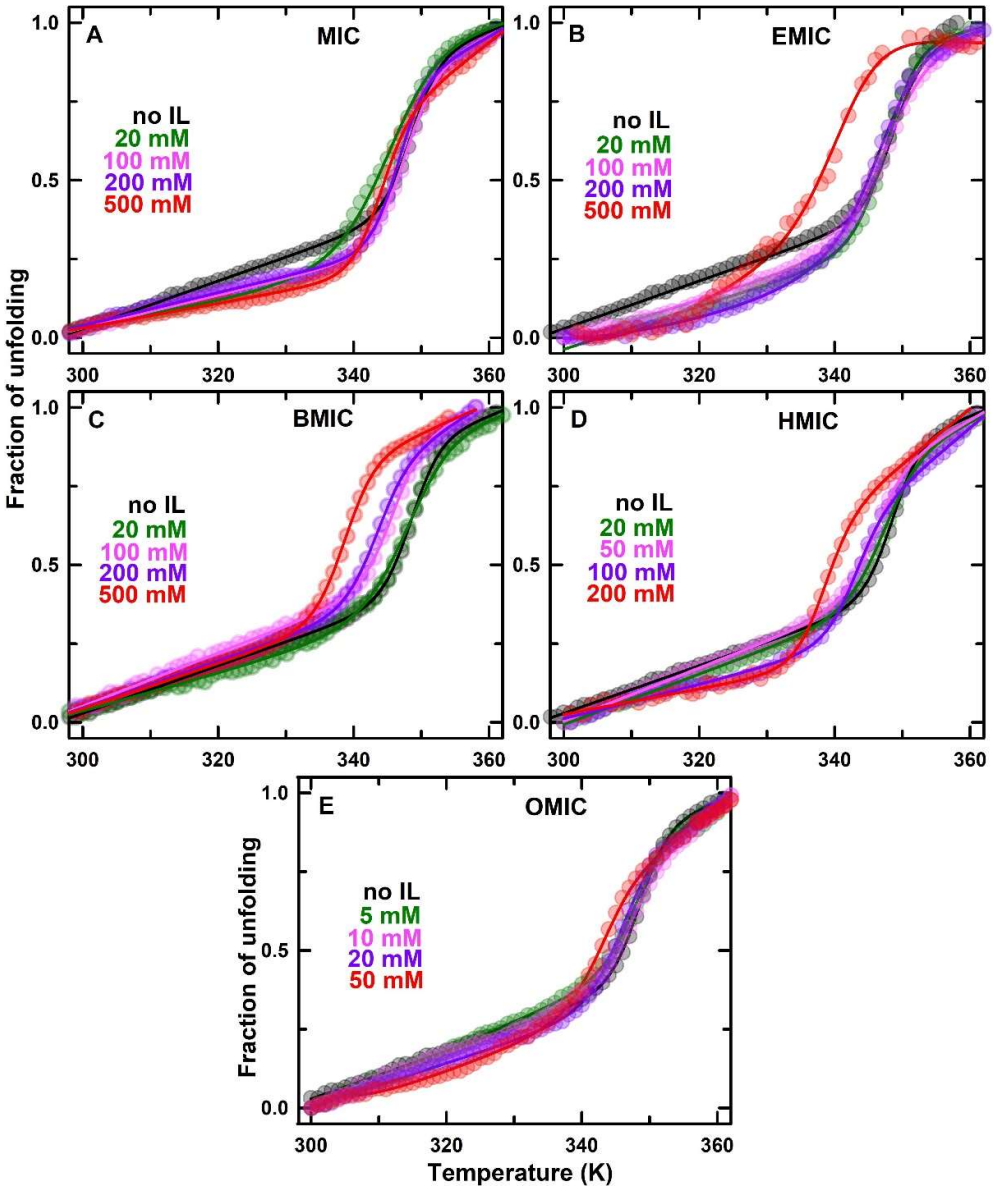

Fig. S7. Representative thermal denaturation transitions of Lyz measured by following the change in absorbance of the protein in the presence of varying concentrations of ILs, (A) MIC, (B) EMIC, (C) BMIC, (D) HMIC, and (E) OMIC. The solid lines represent the data fit using equation (6), for two-state transitions. The colours represent the concentration of ILs as mentioned in the legends of each panel. It may be noted that the thermal denaturation curves showed non-cooperative transitions in HMIC and OMIC at concentrations above 200 and 50 mM, respectively.

Figure S8

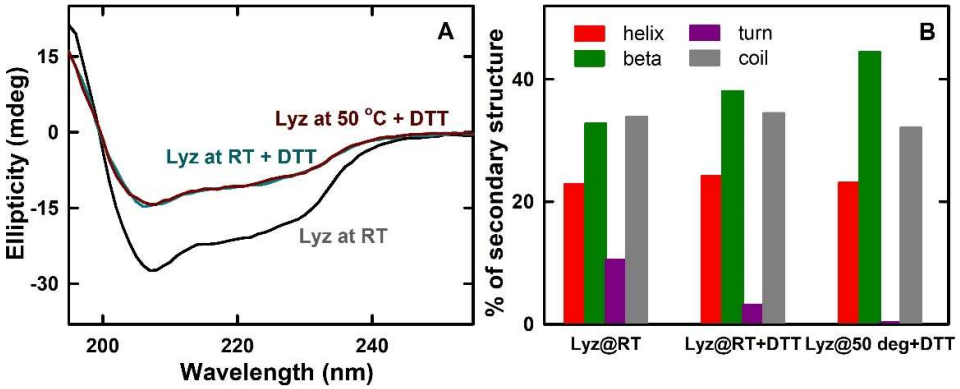

Fig. S8. (A) Far-UV circular dichroism spectra of Lyz at room temperature with (black) and without DTT (cyan), and at fibrillation condition, that is at 50 °C with DTT (red). (B) The secondary structural contents of the protein at these conditions are derived from the spectra using Young's method provided in the instrument, J-1500 circular dichroism spectrophotometer.

Figure S9

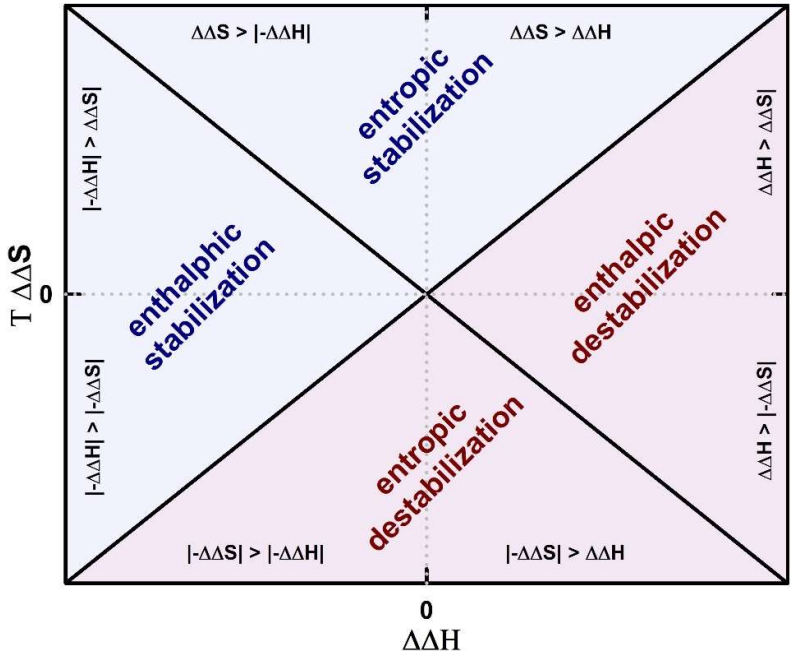

Fig. S9. The enthalpy-entropy plot is divided into four quadrants by diagonal solid lines to represent enthalpically-driven and entropically-driven stabilization and destabilization effects of osmolytes or cosolvents. It is further divided by dotted lines. In each octant, the signs and relative values of enthalpic (ΔΔH) and entropic (ΔΔS) contribution to the stabilization/destabilization are presented (Naidu et al., (2020)[61] and Sukenik et al., (2013)[62])
