Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# Water and a protic ionic liquid acted as refolding additives for chemically denatured enzymes

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**Experimental Details** Page 4-5

**Fig. 1S** The conformational of urea-unfolded enzyme was refolded by 100 µl of water in 2 mg/ml concentration of S Con A at 25 $^0$C. Normalized profiles of fluorescence spectra analysis of the S Con A (2mg/ml) in buffer (black), in 1 M urea (red), in 2 M urea (dark cyan), in 3 M urea (dark yellow) and water in 1 M urea (blue), in 2 M urea (magenta) and in 3 M urea (orange) .

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**Fig. 2S** The conformational of urea-unfolded enzyme was refolded by 100 µl of water in 2 mg/ml concentration of S Con A at 25 $^0$C. Normalized profiles of fluorescence spectra analysis of the S Con A in buffer (black), in 4 M urea (red), in 5 M urea (dark cyan) and water in 4 M urea (blue) and in 5 M urea (magenta)

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**Fig. 3S** The conformational of urea-unfolded enzyme was refolded by 100 µl of water in 2 mg/ml concentration of CT at 25 $^0$C. Normalized profiles of fluorescence spectra analysis of the CT (2mg/ml) in buffer (black), in 1 M urea (red), in 3 M urea (dark cyan) and water in 1 M urea (blue) and in 3 M urea (magenta)

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**Fig. 4S** The conformational of urea-unfolded enzyme was refolded by 100 µl of water in 2 mg/ml concentration of S Con A at 25 $^0$C. Normalized profiles of maximum fluorescence intensity of S Con A with time in buffer (black), in 3 M urea (red), in 4 M urea (dark cyan),

in 5 M urea (dark yellow) and water in 3 M urea (blue), in 4 M urea (magenta) and in 5 M urea (orange).

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**Fig. 5S** The conformational of urea-unfolded enzyme was refolded by 100 µl of water in 2 mg/ml concentration of CT at 25 $^{0}$C. Normalized profiles of maximum fluorescence intensity of CT with time in buffer (black), in 1 M urea (red), in 2 M urea (dark cyan), in 3 M urea (dark yellow), in 4 M urea (green) and water in 1 M urea (blue), in 2 M urea (magenta), in 3 M urea (orange) and in 4 M urea (pink).

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**Fig. 6S** The conformational of urea-unfolded enzyme was refolded by 100 µl of water in 2 mg/ml concentration of S Con A at 25 $^{0}$C. Far-UV CD spectra analysis of the S Con A in buffer (black), in 3 M urea (red), in 4 M urea (dark cyan), in 5 M urea (dark yellow) and water in 3 M urea (blue), in 4 M urea (magenta) and in 5 M urea (orange)

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**Fig. 7S** The conformational of urea-unfolded enzyme was refolded by 100 µl of water in 2 mg/ml concentration of S Con A at 25 $^{0}$C. Near-UV CD spectra analysis of the S Con A in buffer (black), in 3 M urea (red), in 4 M urea (dark cyan), in 5 M urea (dark yellow) and water in 3 M urea (blue), in 4 M urea (magenta) and in 5 M urea (orange).

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**Fig. 8S** The conformational of urea-unfolded enzyme was refolded by 100 µl of water in 2 mg/ml concentration of CT at 25 $^{0}$C. Near-UV CD spectra analysis of the CT in buffer (black), 1 M urea (red) and water in 1 M urea (blue).

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**Fig. 9S** The conformational of urea-unfolded enzyme was refolded by 100 µl of TEAP in 2 mg/ml concentration of S Con A at 25 $^{0}$C. Normalized profiles of maximum fluorescence intensity of S Con A with time in TEAP (black) and TEAP in 2 M urea (red), in 3 M urea (blue), in 4 M urea (dark cyan) and in 5 M urea (magenta).

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**Fig. 10S** The conformational of urea-unfolded enzyme was refolded by 100 µl of TEAP in 2 mg/ml concentration of CT at 25 $^{0}$C. Normalized profiles of maximum fluorescence intensity of CT with time in TEAP (black) and TEAP in 3 M urea (red), in 4 M urea (blue) and in 5 M urea (dark cyan).

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**Fig. 11S** The conformational of urea-unfolded enzyme was refolded by 100 µl of TEAP in 2 mg/ml concentration of S Con A at 25 $^{0}$C. Far-UV CD spectra analysis of the S Con A in TEAP (black), in 3 M urea (red), in 5 M urea (dark cyan) and TEAP in 3 M urea (blue) and in 5 M urea (magenta).

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# Experimental

## Methods

α-Chymotrypsin (CT) from bovine pancreas type II, essentially salt free (molecular weight: 25 kDa) and succinylated con A (S Con A) (molecular weight: 55,000 Daltons) were used as received from Sigma–Aldrich (USA). All other chemicals used for the preparation of TEAP were purchased from different commercial sources. Buffer solution was prepared using distilled and deionized water with a resistivity of 18.3 Ω. cm. The stock solution of 2 mg/ ml was prepared in 10 mM of sodium acetate buffer at pH 4 is prepared for S Con A and 2 mg/ ml was prepared in 10 mM of Tris-HCl buffer pH 8.20 solutions is prepared for CT . All mixture samples were prepared gravimetrically using a Mettler Toledo balance with a precision of $\pm 10^{-4}$ g.

## NMR studies

Spectra were recorded on a Bruker-Biospin 500 instrument. The spectra are the result of 256 scans, 1 s (delay time) at 303.4 K, and were processed with Bruker Topspin version 2.1. The concentration of proteins in all solutions used for NMR spectroscopy was 40 mg/mL prepared. About 10% of $^{2}H_2O$ was added to provide an internal-field-frequency lock signal. A small amount of buffer was added (50 mM) to stabilize the pH.

## Circular dichroism spectroscopy

CD spectroscopic studies were performed using a PiStar-180 spectrophotometer (Applied Photophysics, UK) equipped with a Peltier system for temperature control. CD calibration was performed using (1S)-(+)-10-camphorsulfonic acid (Aldrich, Milwaukee, WI), which exhibits a 34.5 M/cm molar extinction coefficient at 285 nm, and 2.36 M/cm molar ellipticity (θ) at 295 nm. The sample was pre-equilibrated at the desired temperature for 15 min and the scan speed was fixed for adaptative sampling (error F 0.01) with a response time of 1 s and 1 nm bandwidth. The tertiary structures of CT were monitored by using near-UV (240–300

nm), (1.0 cm path length cuvette). The CT concentration was 2 mg/ml and each spectrum was collected by averaging six spectra. Each sample spectrum was obtained by subtracting appropriate blank media without CT from the experimental enzyme spectrum.

**Fluorescence spectroscopy**

Steady-state fluorescence measurements were conducted with a Cary Eclipse spectrofluorimeter (Varian optical spectroscopy instruments, Mulgrave, Victoria, Australia) equipped with thermostated cell holders and temperature was kept constant by a circulating water bath using a peltier device attached to the sample holder of the fluorimeter. The excitation wavelength was set at 295 nm in order to calculate the contribution of the tryptophan residues to the overall fluorescence emission. The experiments were performed at 25 $^{0}$C by using a 1cm sealed cell and both excitation and emission slit width were set at 5 nm, corrected for background signal and in 2 mg/ml concentration of enzymes. Both the change in fluorescence intensity and the shift in fluorescence maximum wavelength were recorded to monitor the unfolding transition.

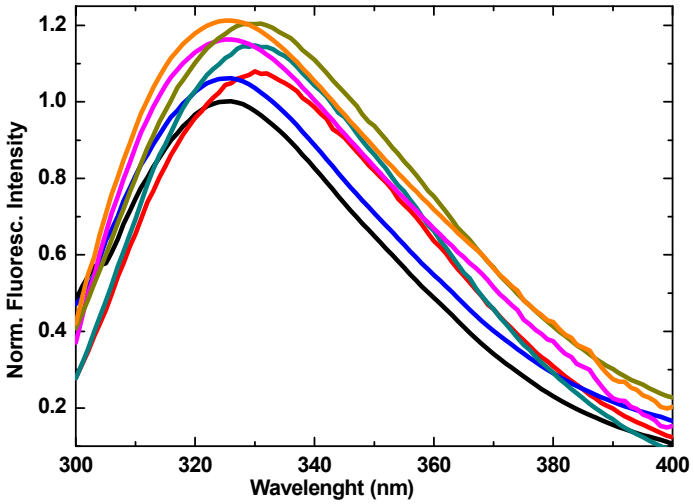



**Fig. 1S**

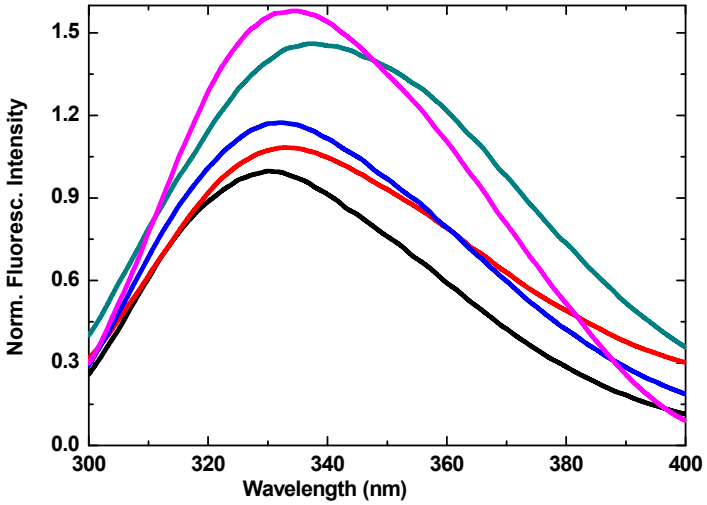



**Fig. 2S**

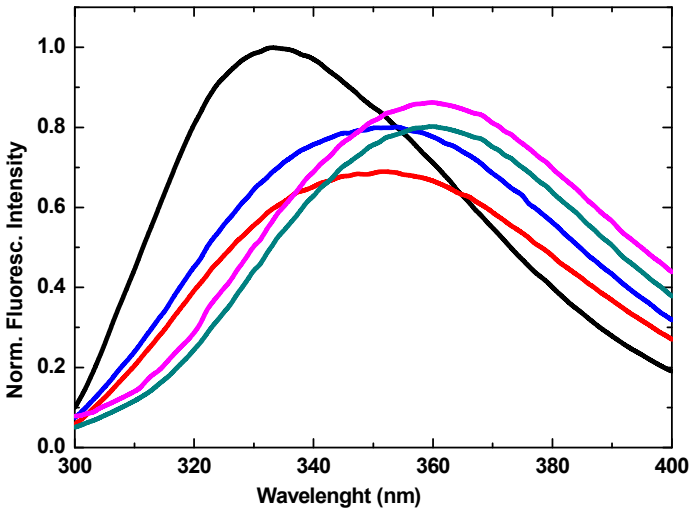
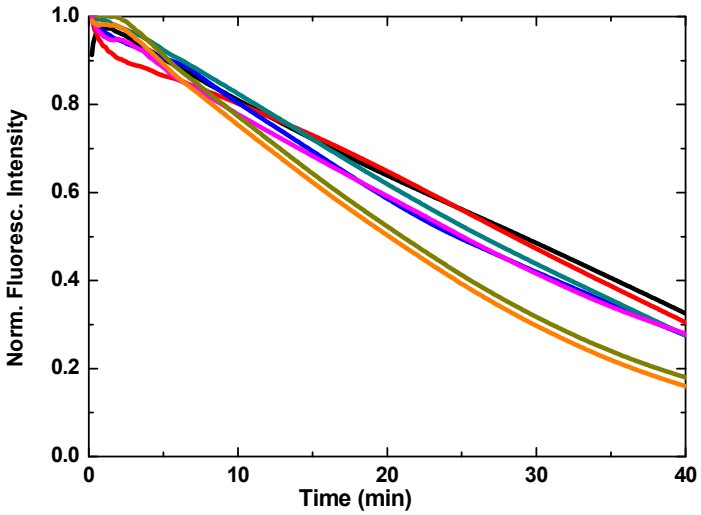



**Fig. 3S**

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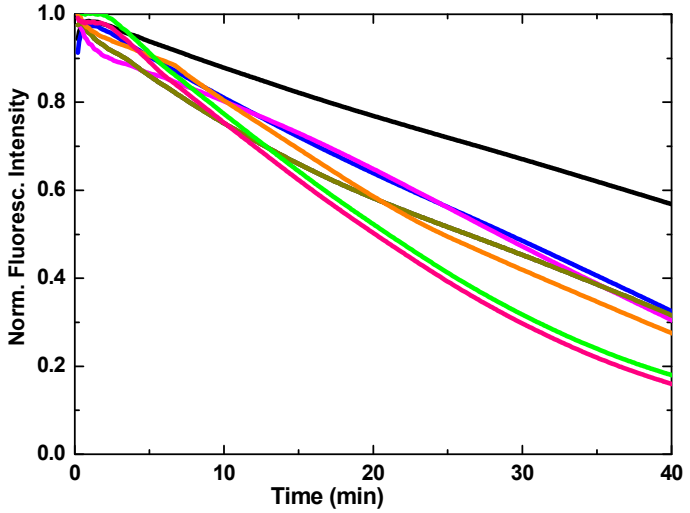


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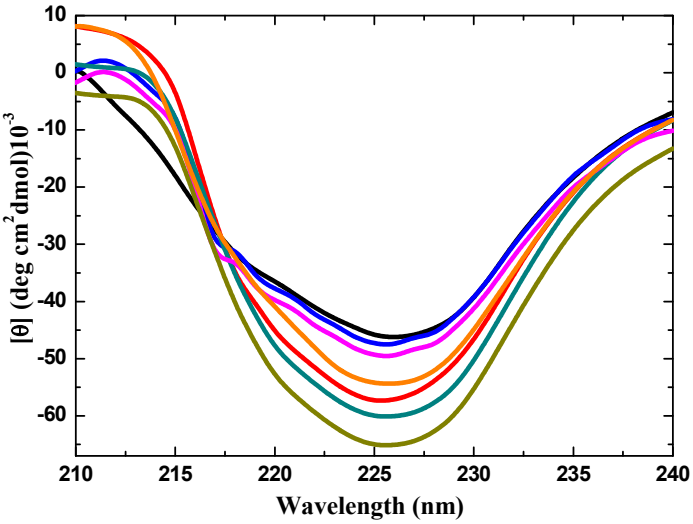


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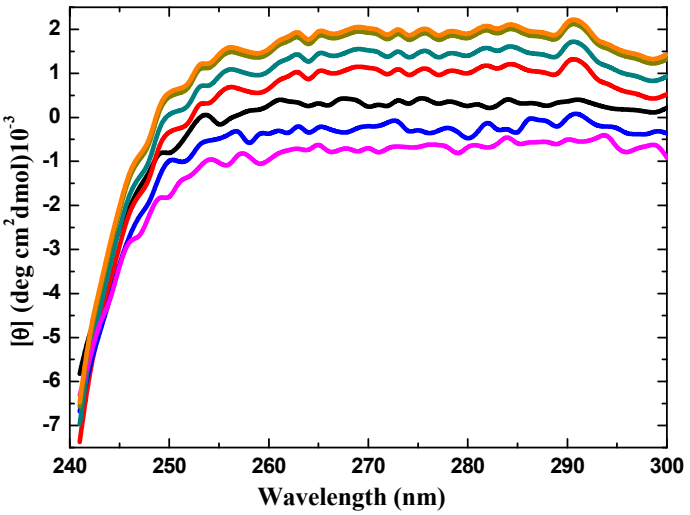


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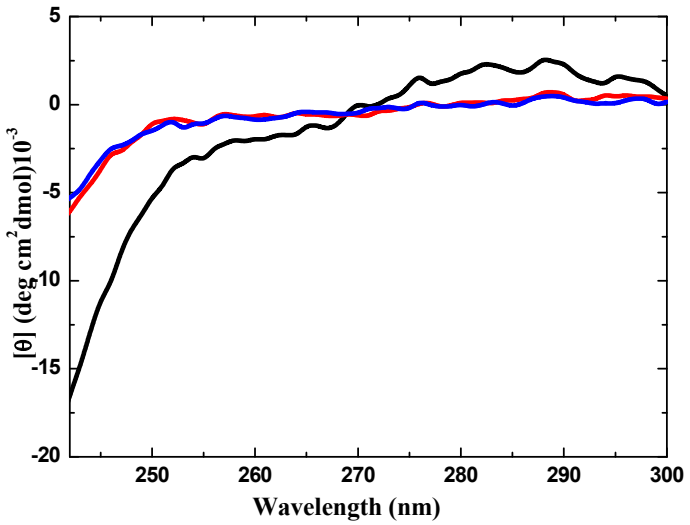


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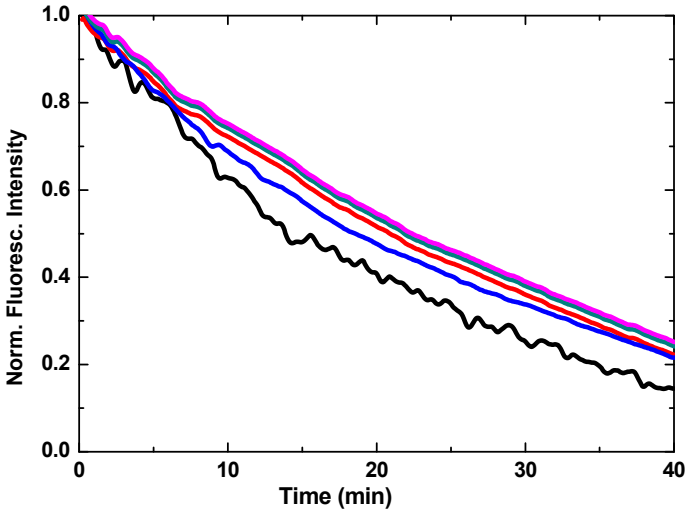


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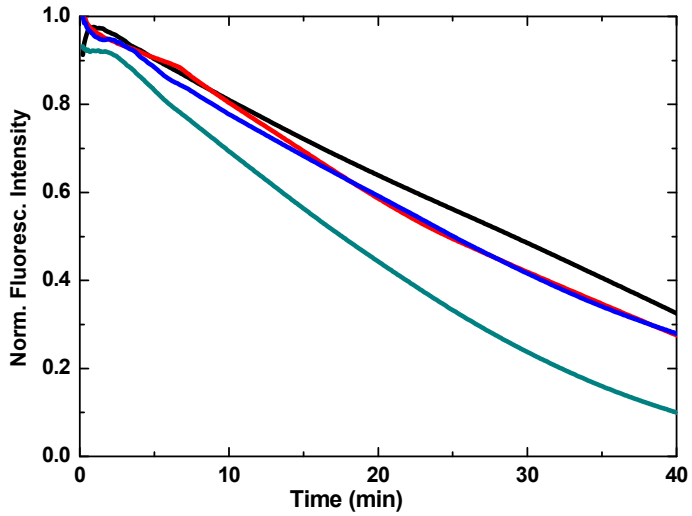


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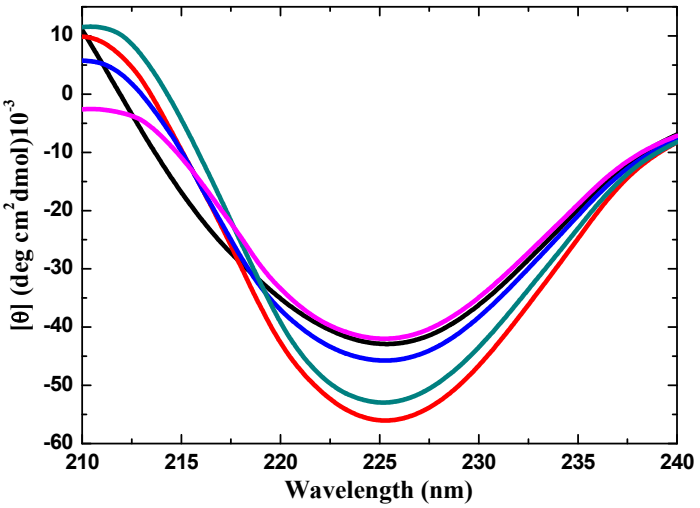


**Fig. 11S**