Enhancing Protein Stability under Stress: Osmolyte-based DES as a Robust Stabilizing Medium for Lysozyme under Heat and Cold Shock

Anja Damjanović, Marijan Logarušić, Lidija-Marija Tumir, Thanos Andreou, Marina Cvjetko Bubalo, Ivana Radojčić Redovniković

SUPPORTING INFORMATION

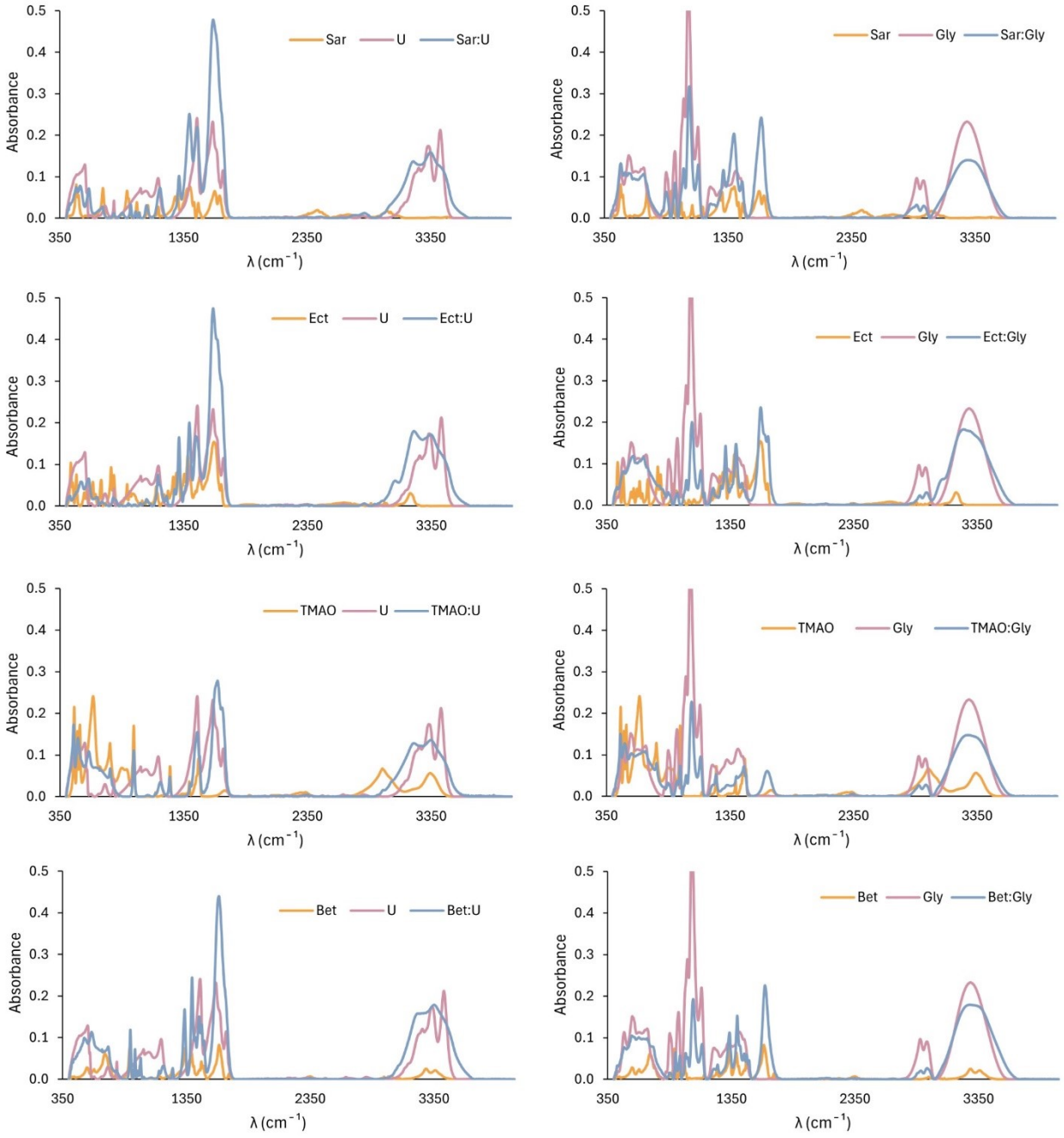

Figure S1. FT-IR spectra of sarcoine-, ectoine-, TMAO-, and betaine-based DES.

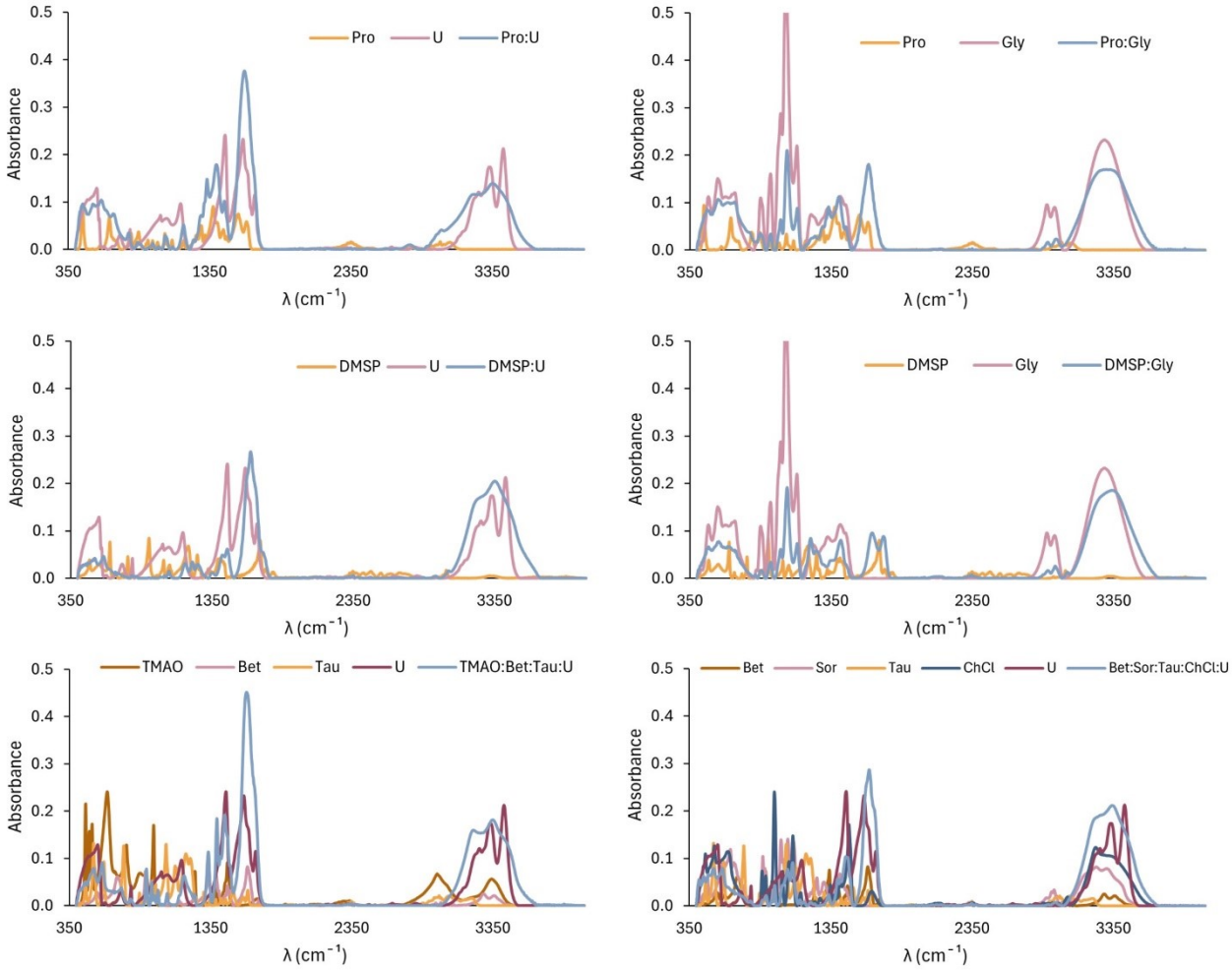

Figure S2. FT-IR spectra of proline- and DMSP-based DES, together with spectra of multicomponent bioinspired DES TMAO:Bet:Tau:U and Bet:Sor:Tau:GPC:U.

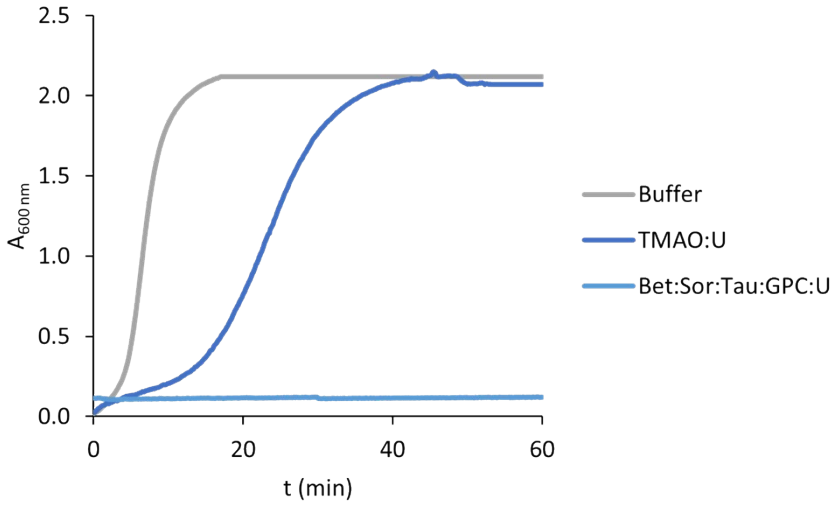

Figure S3. Time-dependent optical density (λ= 600 nm) of lysozyme solution (c_{Lys} = 5 mg ml^{-1}) at 80°C in 50 mM potassium phosphate buffer solution (pH 6.4), TMAO:U (40% of water, w/w), and bioinspired multicomponent DES Bet:Sor:Tau:GPC:U (40% of water, w/w).

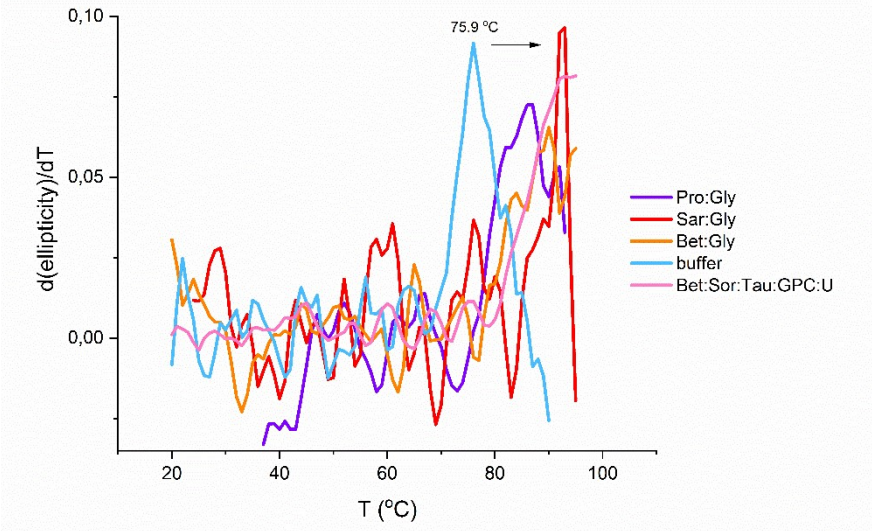

Figure S4. First derivations of melting curves of thermal CD scans of lysozyme in 50 mM potassium phosphate buffer (pH 6.4) and DESs containing 40% of water, w/w (c_{Lys} = 0.3-0.4 mg ml^{-1}; λ=227±5 nm).

Table S1. Thermal melting (Tm) values for lysozyme in 50 mM potassium phosphate buffer (pH 6.4) and DES containing 40% of water, w/w (c_{Lys} = 0.3-0.4 mg ml^{-1}; λ=227±5 nm) calculated from CD scans (Δ=227±5 nm).

	T_m / °C	ΔT_m / °C
Buffer	75.9	-
Pro:Gly	85.5	9.6
Sar:Gly	>87	>11.1
Bet:Gly	88.9	13
Bet:Sor:Tau:Gpc:U	91.5	15.6

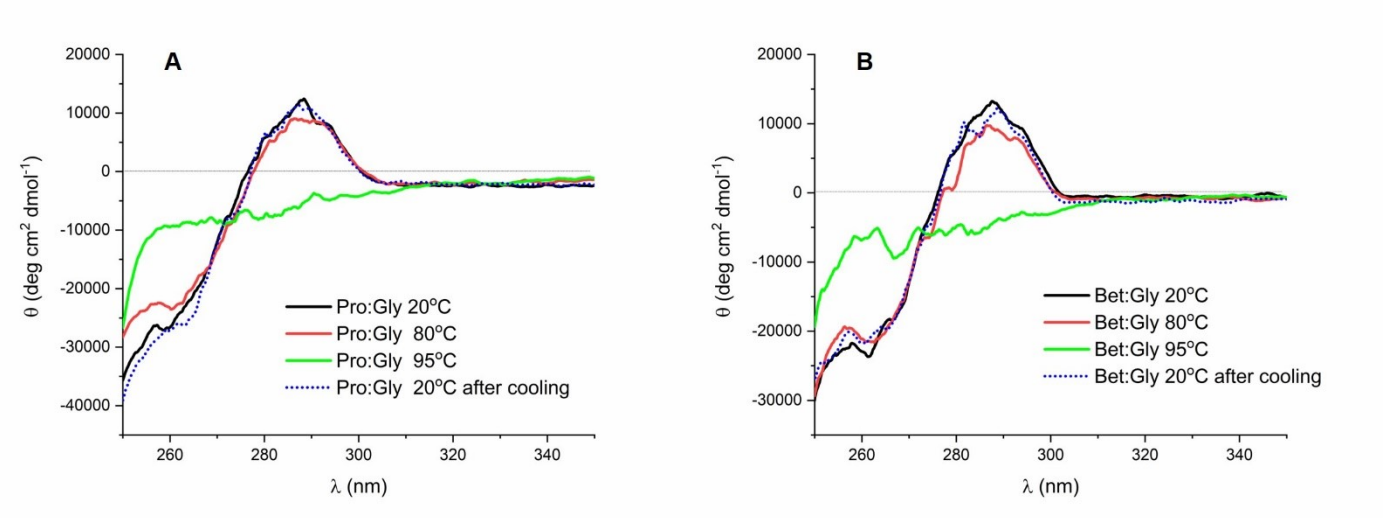

Figure S5. Near UV CD spectra of lysozyme (c_{Lys} = 0.2 mg ml^{-1}) dissolved in (A) Pro:Gly (40% of water, w/w) and (B) Bet:Gly (40% of water, w/w) before thermal treatment (black line), at 80°C (red line), 95°C (green line), and 20°C after cooling (blue dotted line).

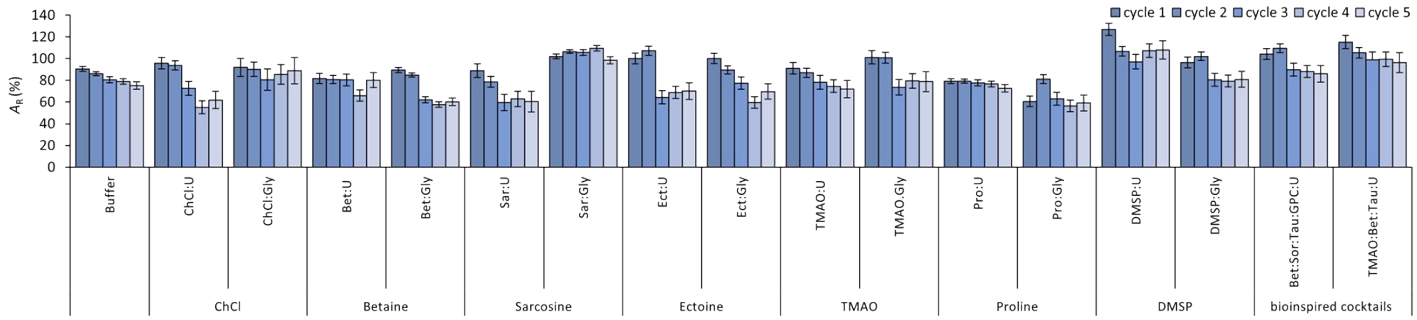

Figure S6. Residual lysozyme activity (A_R) after incubation in DES (40% of water, w/w) and 50 mM potassium phosphate buffer (pH 6.4) after each freeze/thaw cycle at -20°C (c_{Lys} = 5 mg ml^{-1}). The residual lysozyme activity (A_R) was calculated from the initial reaction rate obtained by the enzyme after incubation, compared to the one obtained without previous exposure.

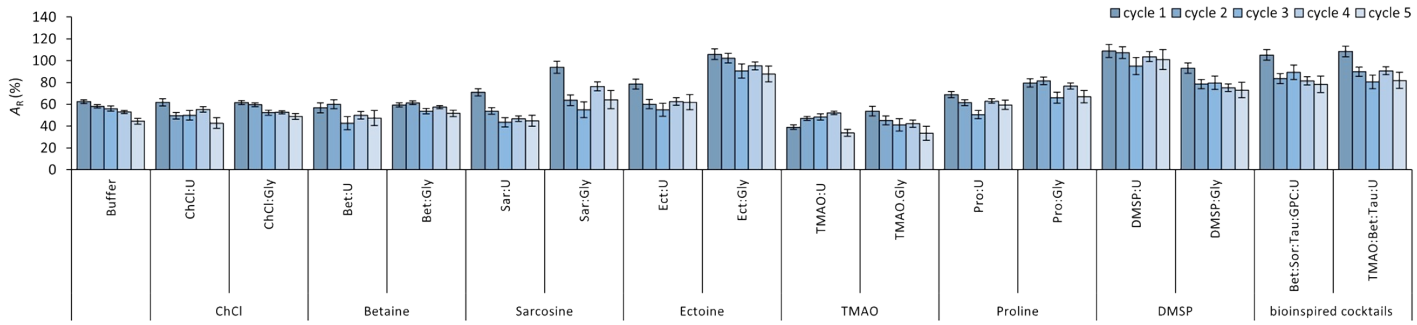

Figure S7. Residual lysozyme activity (A_R) after incubation in DES (40% of water, w/w) and 50 mM potassium phosphate buffer (pH 6.4) after each freeze/thaw cycle at -80°C (c_{Lys} = 5 mg ml^{-1}). The residual lysozyme activity (A_R) was calculated from the initial reaction rate obtained by the enzyme after incubation, compared to the one obtained without previous exposure.

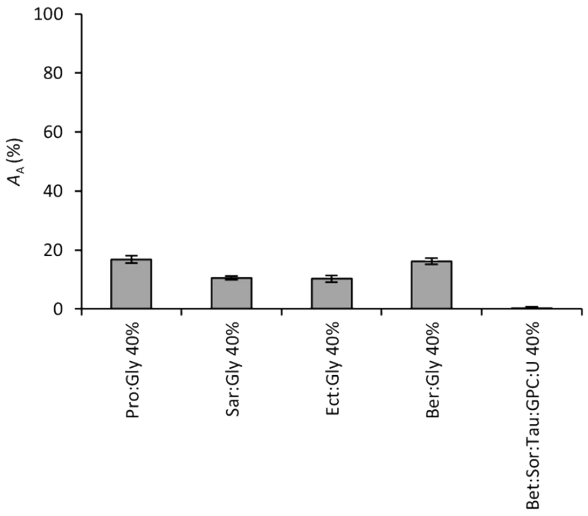

Figure S8. Relative lysozyme activity (A_A) in DES (40% of water, w/w; c_{Lys} = 5 mg ml^{-1}). The relative lysozyme activity (A_A) was calculated from the initial reaction rate obtained by the enzyme after incubation, compared to the one obtained without previous exposure.

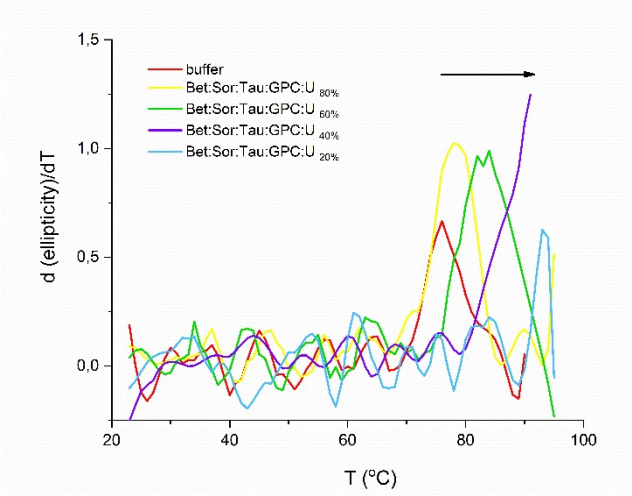

Figure S9. First derivations of melting curves of thermal CD scans of lysozyme in 50 mM potassium phosphate buffer solution (pH 6.4) and Bet:Sor:Tau:GPC:U at different water shares (20, 40, 60 and 80%, w/w) (c_{Lys} = 0.4 mg ml^{-1}; λ=225 nm).

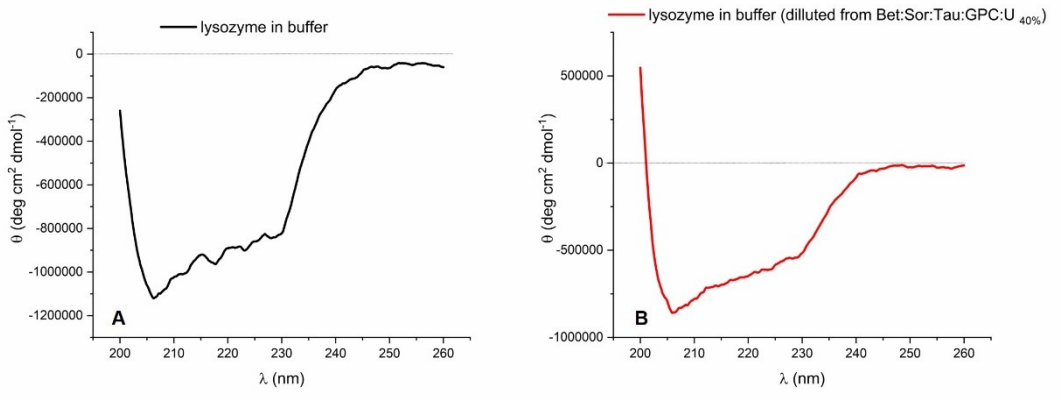

Figure S10. (A) Far UV CD spectra of lysozyme dissolved in 50 mM potassium phosphate buffer (pH 6.4) (c_{Lys} = 0.2 mg ml^{-1}) and hydrated lysozyme (dilution of lysozyme solution pre-incubated in Bet:Sor:Tau:GPC:U (40% of water, w/w)) into the buffer, resulting in a final concentration of Bet:Sor:Tau:GPC:U in the buffer of 0.5% (v/v).