Investigation of laccase activity in cholinium-based ionic liquids using experimental and molecular dynamics techniques

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Supplementary Information

1. NMR characterization of [Ch][AA]

[Cho][Gly]

^1H NMR (300 MHz, D_2O, TMS): δ = 3.03 (s, 9H, $(CH_3)_3$–N), 3.14 (s, 2H, CH_2–NH_2), 3.32–3.34 (m, 2H, CH_2CH_2OH), 3.85–3.87 (m, 2H, CH_2CH_2N)

^{13}C NMR (300 MHz, D_2O, TMS): δ = 45.4, 53.8, 55.5, 67.3, 178.9

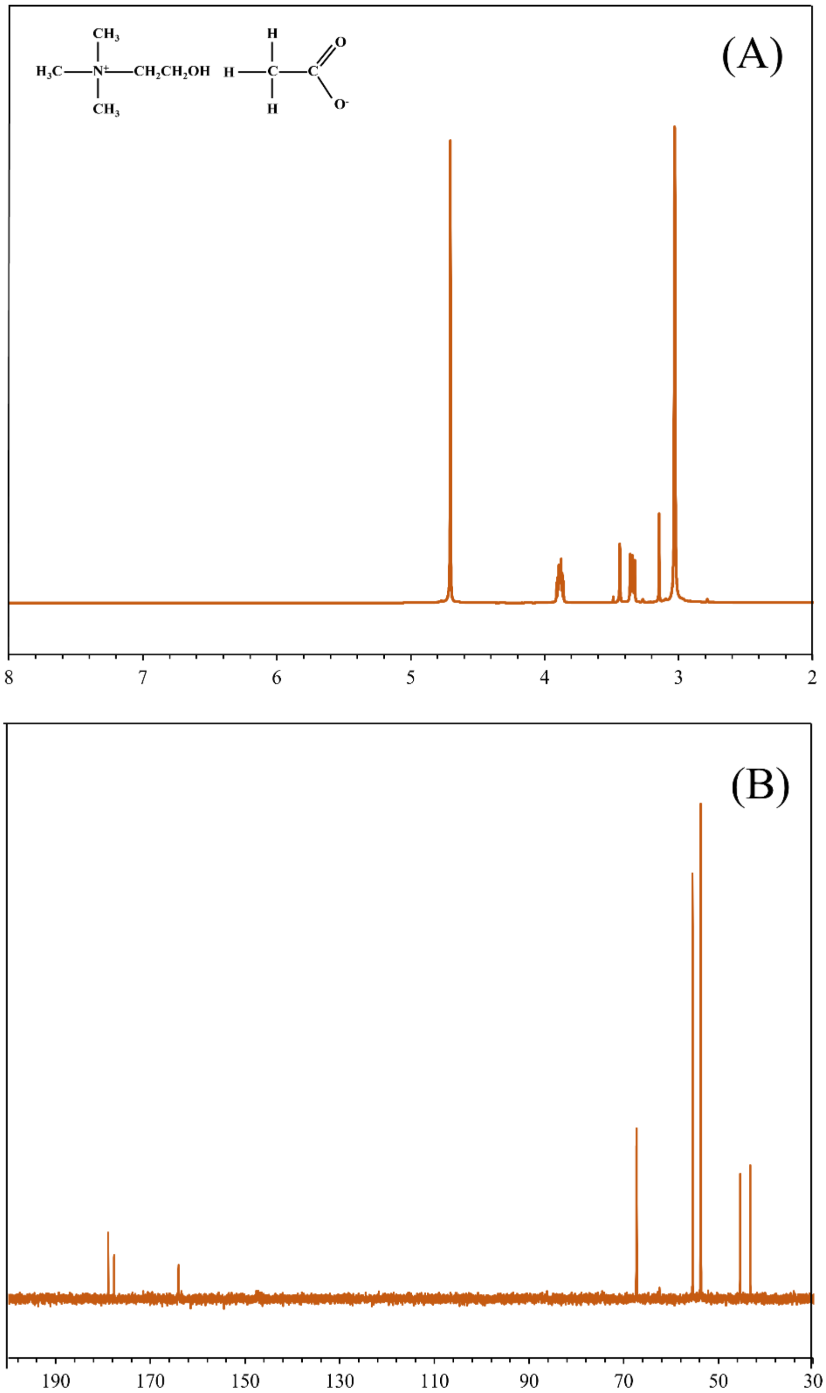

Fig. S1. (A) ^1H NMR and (B) ^{13}C NMR spectra for [Cho][Gly]. The peak of the D_2O solvent was indicated near 4.7 ppm.

[Cho][Phe]

^{1}H NMR (300 MHz, D_2O, TMS): δ = 2.71–2.72 (m, 1H, CH_2), 2.87–2.88 (m, 1H, CH_2), 3.03 (s, 9H, $(CH_3)_3$–N), 3.33 (m, 2H, CH_2), 3.41 (m, 1H, CH-N), 3.88 (m, 2H, CH_2), 7.14-7.26 (m, 5H, C_6H_5)

^{13}C NMR (300 MHz, D_2O, TMS): δ = 40.0, 53.81, 55.5, 57.15, 67.3, 126.73, 128.6, 129.36, 137.72, 181.03

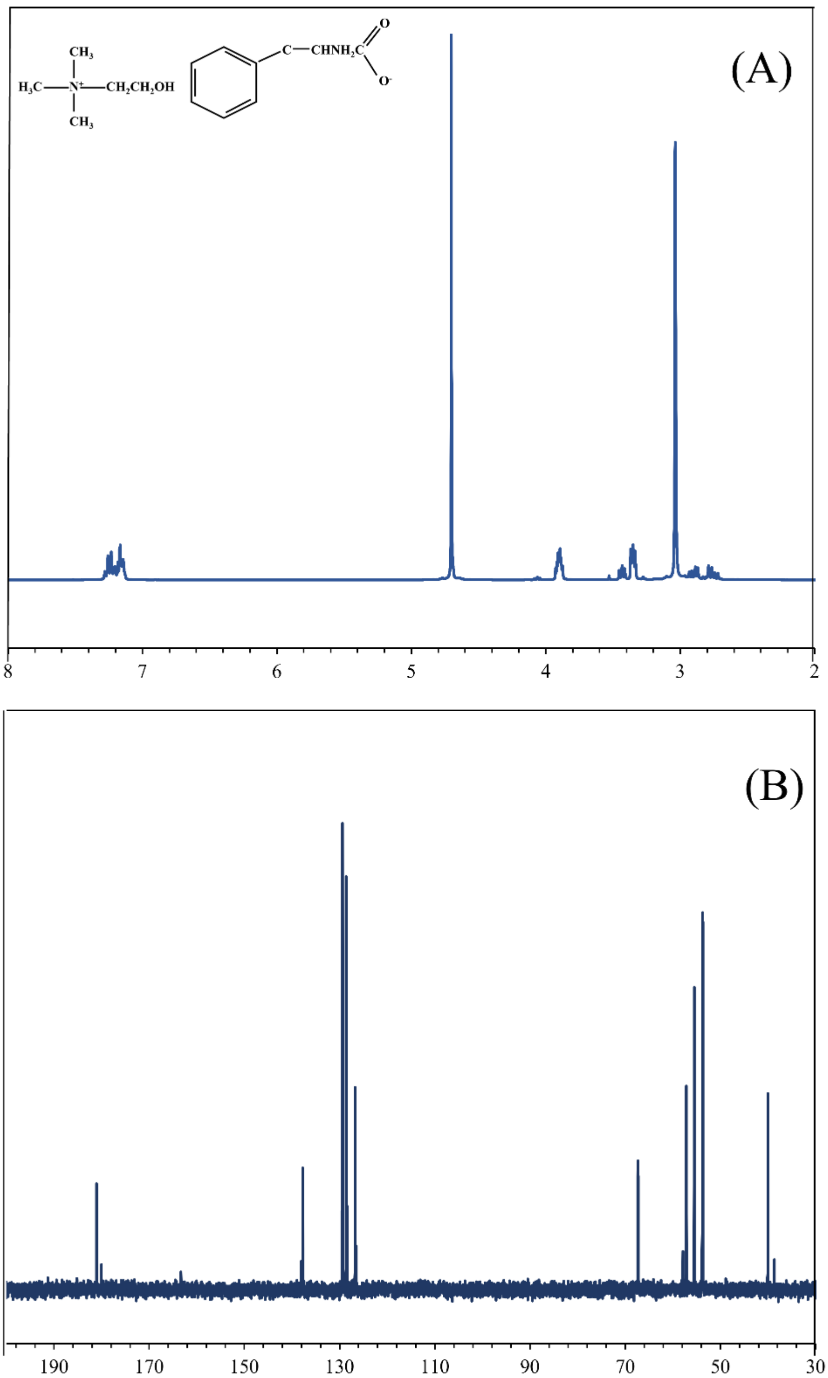

Fig. S2. (A) ^{1}H NMR and (B) ^{13}C NMR spectra for [Cho][Phe]. The peak of the D_2O solvent was indicated near 4.7 ppm.

2. MD simulation of laccase from *T. versicolor* in water and ILs at 25ºC

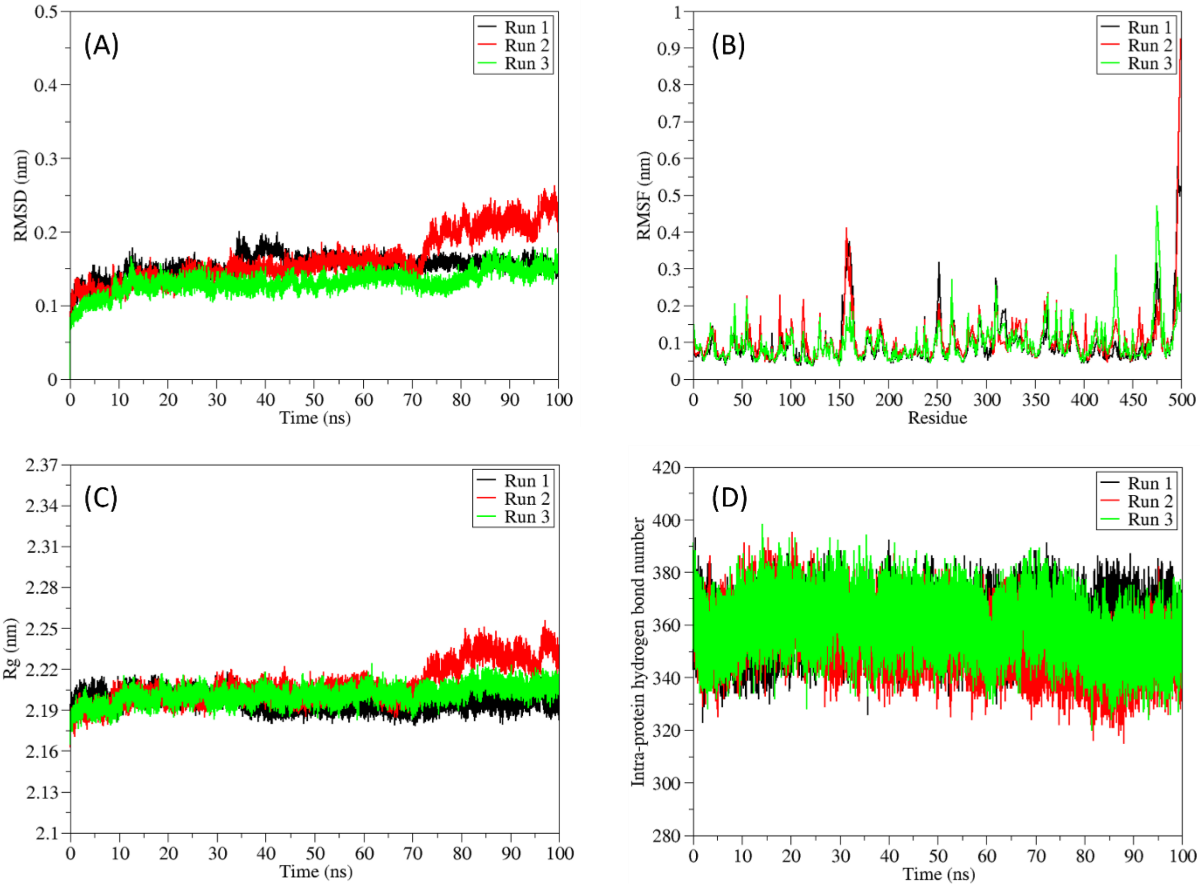

Fig. S3. (A) RMSD, (B) RMSF, (C) Rg, and (D) intramolecular hydrogen bond number of laccase from *T. versicolor* as a function of time during 100 ns simulations in water, showing three replicate simulations of the same system, indicated by different colours.

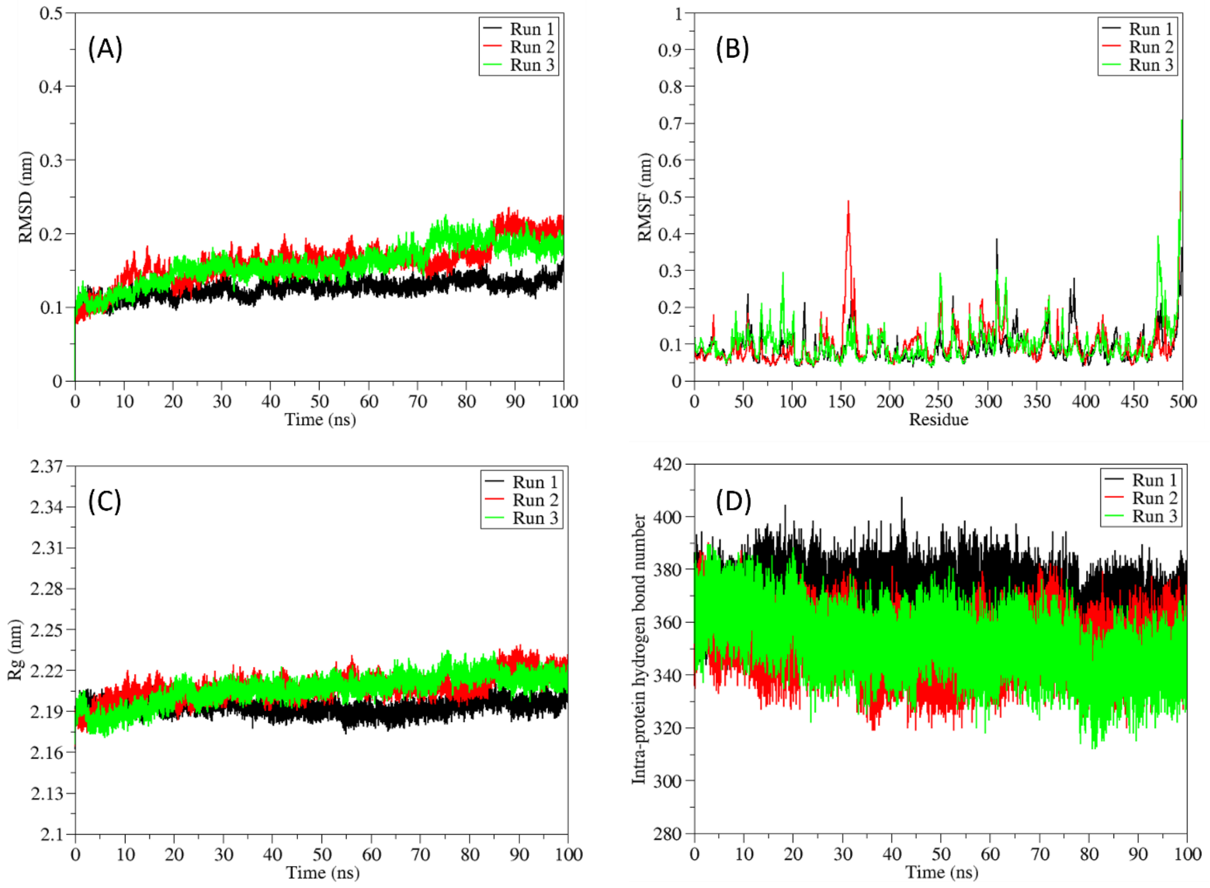

Fig. S4. (A) RMSD, (B) RMSF, (C) Rg, and (D) intramolecular hydrogen bond number of laccase from *T. versicolor* as a function of time during 100 ns simulations in [Cho][DHC], showing three replicate simulations of the same system, indicated by different colours.

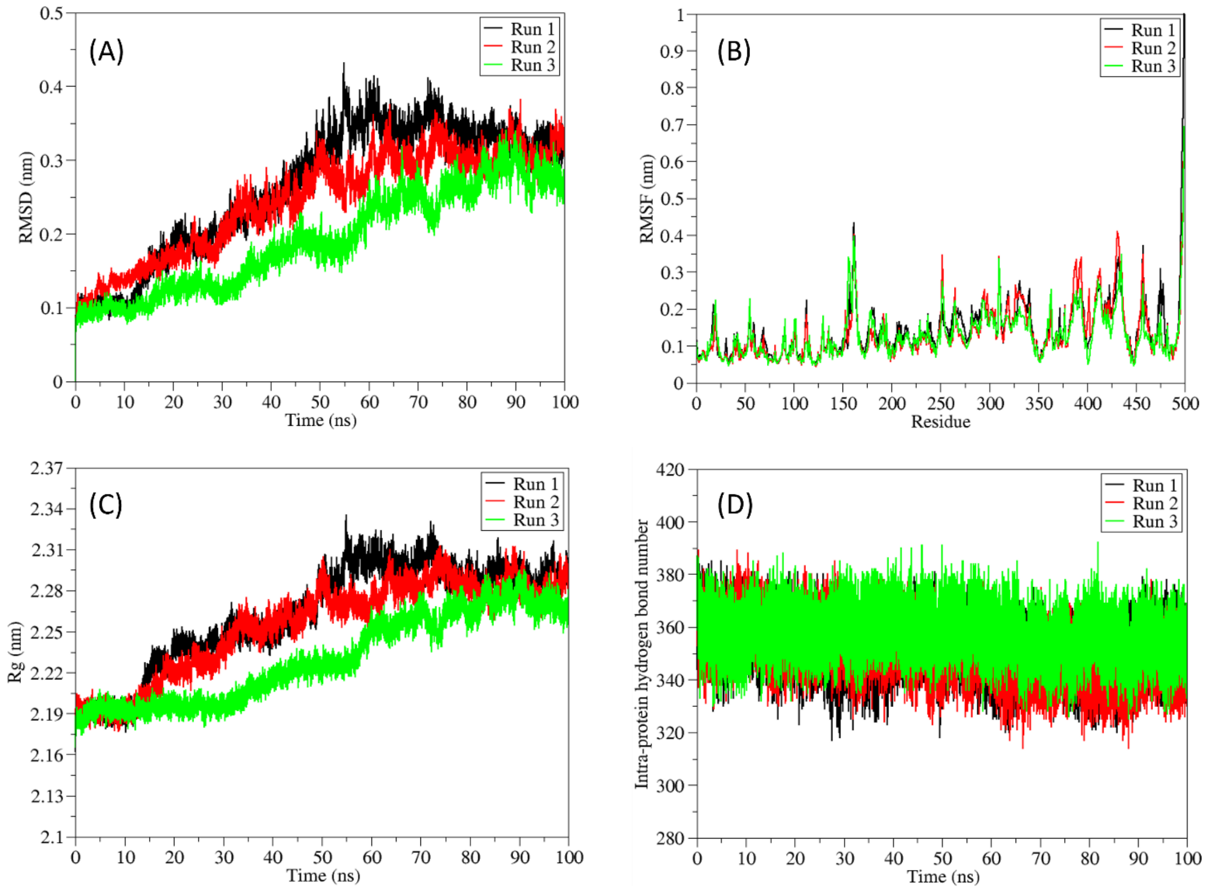

Fig. S5. (A) RMSD, (B) RMSF, (C) Rg, and (D) intramolecular hydrogen bond number of laccase from *T. versicolor* as a function of time during 100 ns simulations in [Cho][Gly], showing three replicate simulations of the same system, indicated by different colours.

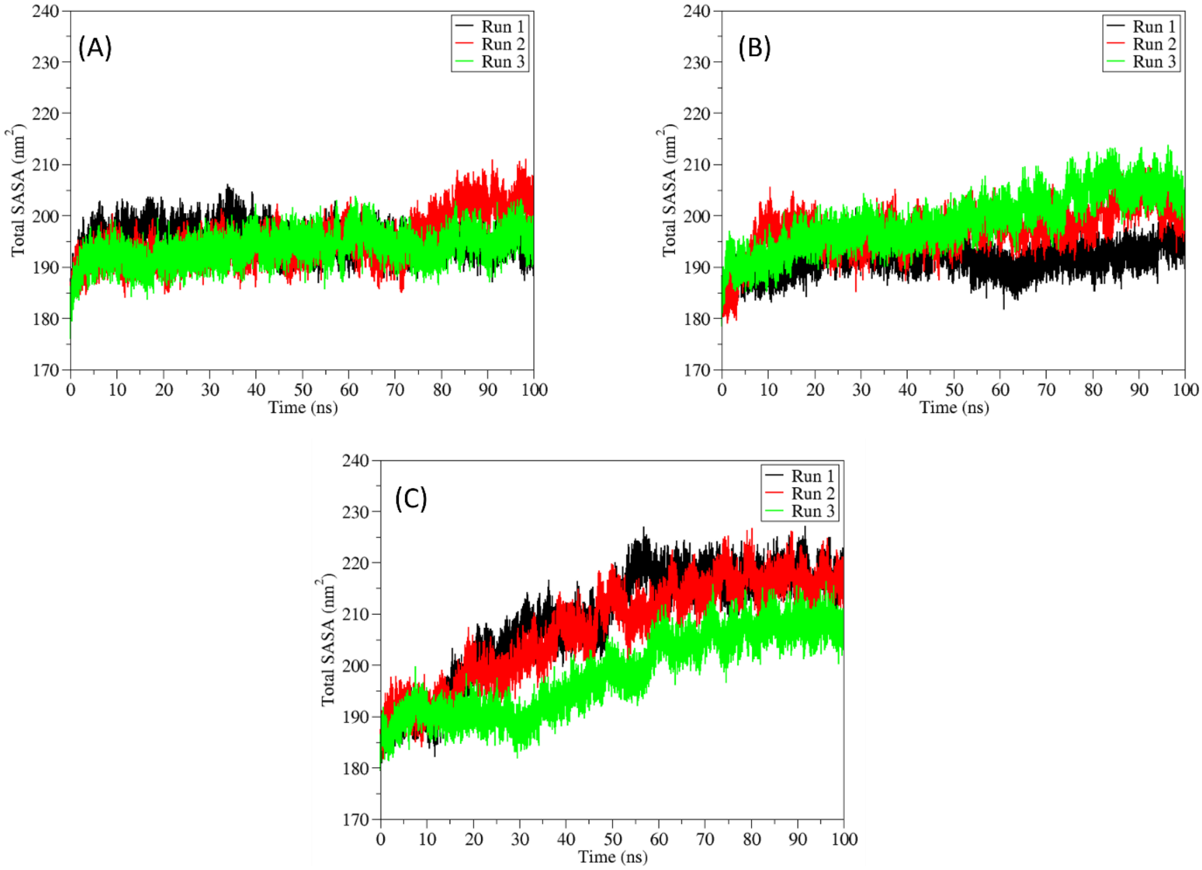

Fig. S6. SASA of laccase from *T. versicolor* in (A) water, (B) [Cho][DHC], and (C) [Cho][Gly] systems, as a function of time during 100 ns simulations, showing three replicate simulations of the same system, indicated by different colours.

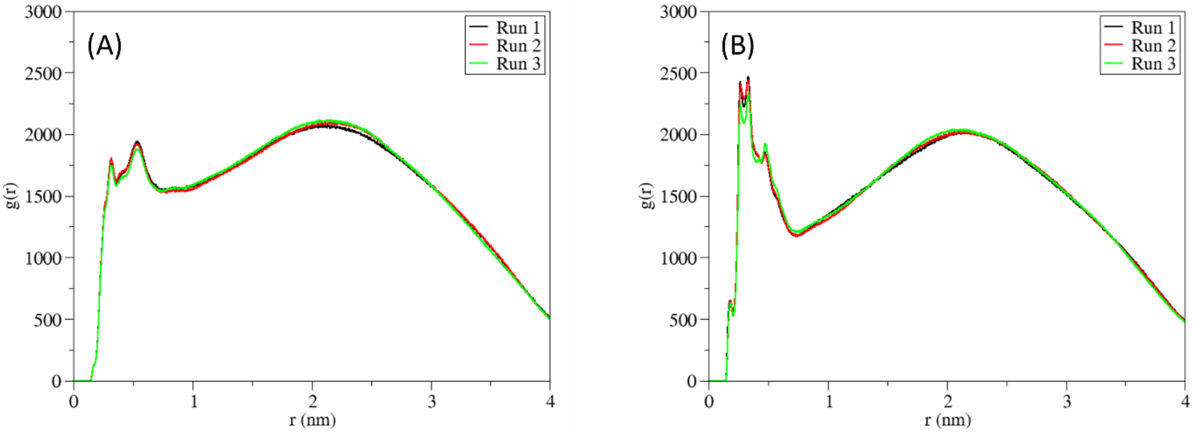

Fig. S7. RDF of (A) $[Cho]^+$ and (B) $[DHC]^-$ that exist within 0.35 nm from the laccase surface as a function of time during 100 ns simulations in [Cho][DHC], showing three replicate simulations of the same system, indicated by different colours.

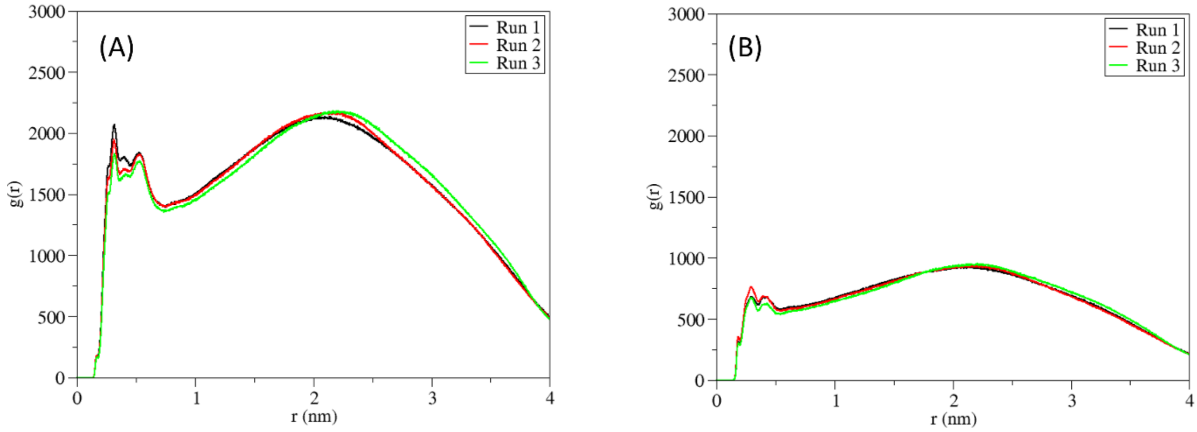

Fig. S8. RDF of (A) $[Cho]^+$ and (B) $[Gly]^-$ that exist within 0.35 nm from the laccase surface as a function of time during 100 ns simulations in [Cho][Gly], showing three replicate simulations of the same system, indicated by different colours.

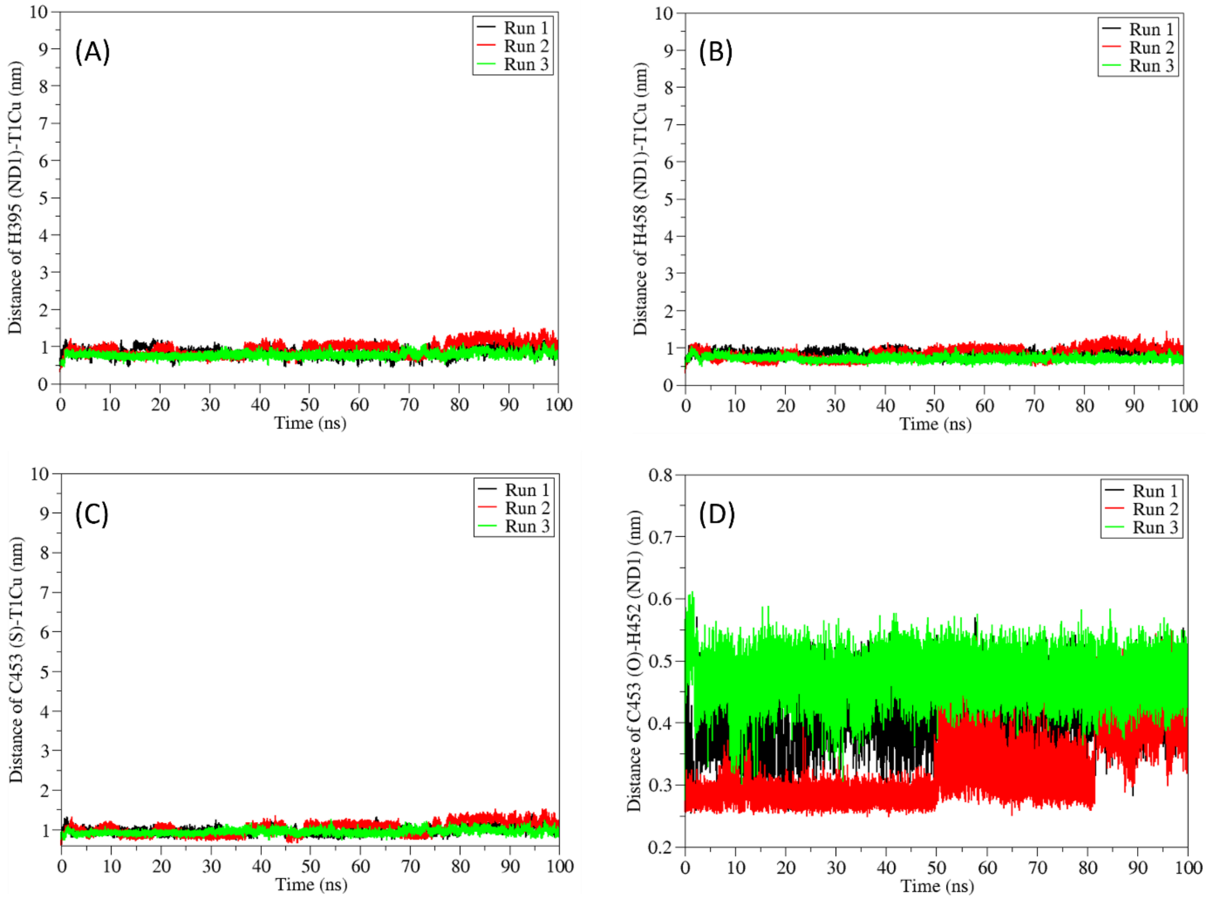

Fig. S9. Distances of (A) H395 (ND1)-T1Cu, (B) H458 (ND1)-T1Cu, (C) C453 (S)-T1Cu, and (D) C453 (O)-H452 (ND1) of laccase over the course of 100 ns simulations at 25℃ in water, showing three replicate simulations of the same system, indicated by different colours.

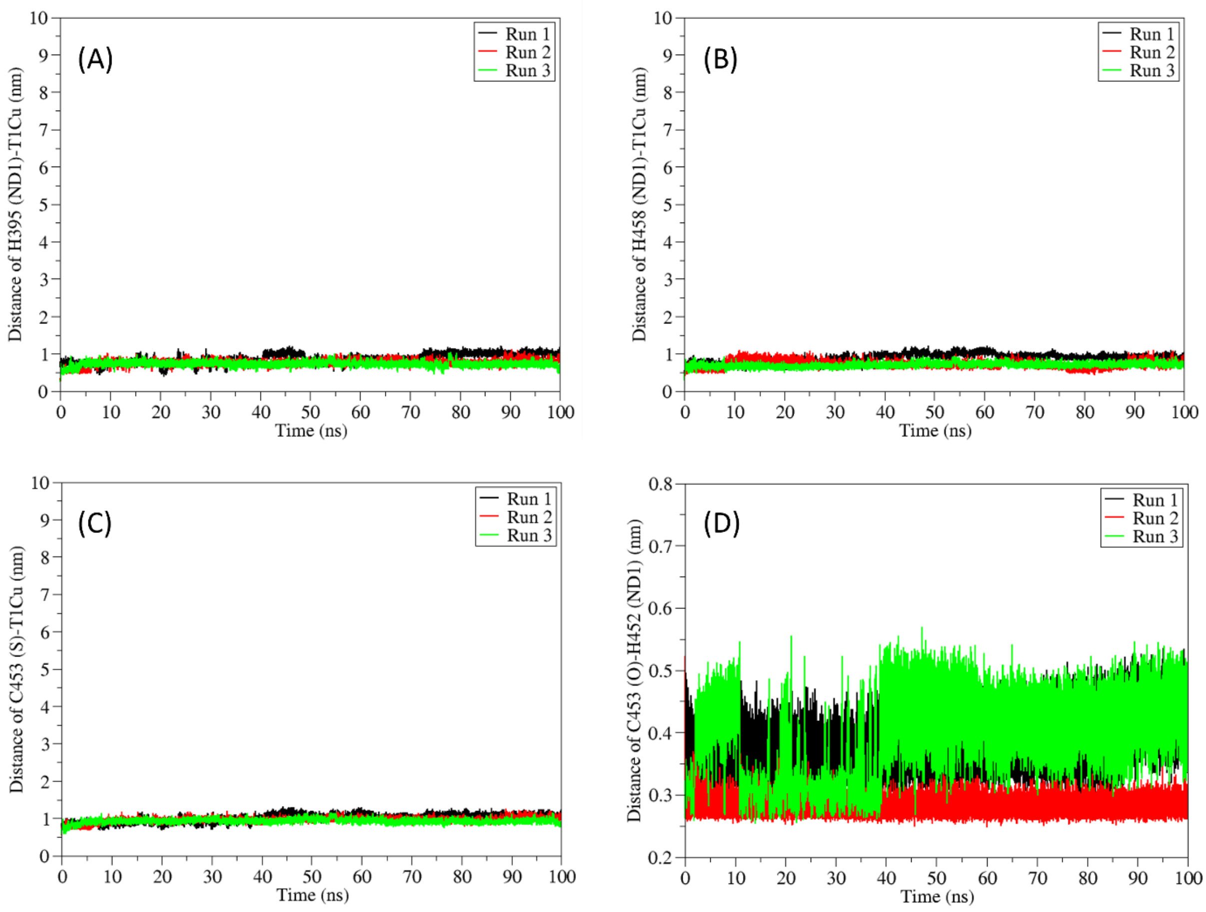

Fig. S10. Distances of (A) H395 (ND1)-T1Cu, (B) H458 (ND1)-T1Cu, (C) C453 (S)-T1Cu, and (D) C453 (O)-H452 (ND1) of laccase over the course of 100 ns simulations at 25℃ in [Cho][DHC], showing three replicate simulations of the same system, indicated by different colours.

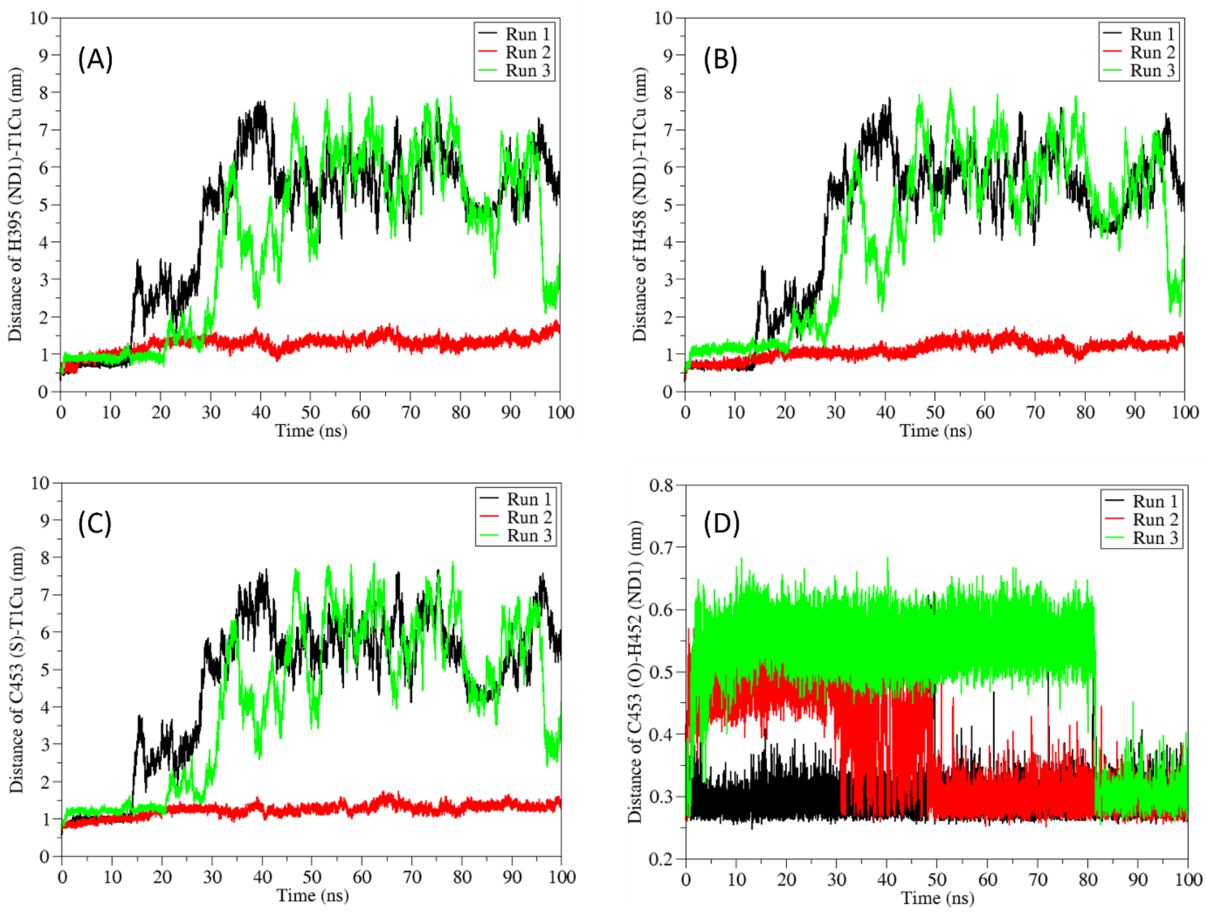

Fig. S11. Distances of (A) H395 (ND1)-T1Cu, (B) H458 (ND1)-T1Cu, (C) C453 (S)-T1Cu, and (D) C453 (O)-H452 (ND1) of laccase over the course of 100 ns simulations at 25℃ in [Cho][Gly], showing three replicate simulations of the same system, indicated by different colours.