**Enhanced activity of *Candida antarctica* lipase B in choline amino acid ionic liquids: A combined experimental and computational approaches**

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**Supplementary Information**

**1. NMR characterization of [Ch][AA]**

**[Cho][Gly]**

1H NMR (700 MHz, D2O, TMS): δ = 3.13 (s, 9H, (CH3)3–N), 3.15 (s, 2H, CH2–NH2), 3.46–3.48 (m, 2H, CH2CH2OH), 3.99–4.01 (m, 2H, CH2CH2N)

13C NMR (176 MHz, D2O, TMS): δ = 44.5, 53.8, 55.5, 67.4, 180.5



**Fig. S1.** (A) 1H NMR and (B) 13C NMR spectra for [Cho][Gly]. The peak of the D2O solvent was indicated near 4.79 ppm.

**[Cho][Ala]**

1H NMR (700 MHz, D2O, TMS): δ = 2.26–2.27 (m, 2H, CH2CH2NH2), 2.76–2.78 (m, 2H, CH2CH2NH2), 3.13 (s, 9H, (CH3)3–N), 3.44–3.45 (m, 2H, CH2CH2OH), 3.97–3.99 (m, 2H, CH2CH2N)

13C NMR (176 MHz, D2O, TMS): δ = 37.7, 39.6, 53.8, 55.5, 67.4, 180.7

Chart, histogram

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**Fig. S2.** (A) 1H NMR and (B) 13C NMR spectra for [Cho][Ala]. The peak of the D2O solvent was indicated near 4.79 ppm.

**[Cho][Phe]**

1H NMR (700 MHz, D2O, TMS): δ = 2.80–2.81 (m, 1H, CH2), 2.97–2.98 (m, 1H, CH2), 3.08 (s, 9H, (CH3)3–N), 3.38 (m, 2H, CH2), 3.47 (m, 1H, CH-N), 3.95 (m, 2H, CH2), 7.25-7.35 (m, 5H, C6H5)

13C NMR (176 MHz, D2O, TMS): δ = 40.65, 53.83, 55.52, 57.41, 67.36, 126.72, 128.67, 129.49, 138.28, 181.67

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**Fig. S3.** (A) 1H NMR and (B) 13C NMR spectra for [Cho][Ala]. The peak of the D2O solvent was indicated near 4.79 ppm.

**2. Lipase activity assay**

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**Fig. S4.** Standard curve for lipase activity assay.

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**Fig. S5.** Relative activity of CALB after one hour of incubation in 0.05 M [Cho][Phe] at 25oC and 50oC.

**3. MD simulation of choline-based ILs at 25oC**

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**Fig. S6.** (A) RMSD, (B) RMSF, (C) Rg, and (D) intramolecular hydrogen bond number of CALB as a function of time during 100 ns simulations in water, showing three replicate simulations of the same system, indicated by different colours.

Chart, histogram

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**Fig. S7.** (A) RMSD, (B) RMSF, (C) Rg, and (D) intramolecular hydrogen bond number of CALB as a function of time during 100 ns simulations in [Cho][OH], showing three replicate simulations of the same system, indicated by different colours.

Chart, histogram

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**Fig. S8.** (A) RMSD, (B) RMSF, (C) Rg, and (D) intramolecular hydrogen bond number of CALB as a function of time during 100 ns simulations in [Cho][Gly], showing three replicate simulations of the same system, indicated by different colours.

Chart, histogram

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**Fig. S9.** (A) RMSD, (B) RMSF, (C) Rg, and (D) intramolecular hydrogen bond number of CALB as a function of time during 100 ns simulations in [Cho][Ala], showing three replicate simulations of the same system, indicated by different colours.

Chart

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**Fig. S10.** (A) RMSD, (B) RMSF, (C) Rg, and (D) intramolecular hydrogen bond number of CALB as a function of time during 100 ns simulations in [Cho][Phe], showing three replicate simulations of the same system, indicated by different colours.

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**Fig. S11.** RDF of anions of choline-based ILs around the surface of CALB α5-helix.

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**Fig. S12.** RDF of (A) [Cho]+ and (B) [OH]- that exist within 0.35 nm from the CALB surface as a function of time during 100 ns simulations in [Cho][OH], showing three replicate simulations of the same system, indicated by different colours.

Chart, line chart, histogram

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**Fig. S13.** RDF of (A) [Cho]+ and (B) [Gly]- that exist within 0.35 nm from the CALB 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.

Chart, line chart, histogram

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**Fig. S14.** RDF of (A) [Cho]+ and (B) [Ala]- that exist within 0.35 nm from the CALB surface as a function of time during 100 ns simulations in [Cho][Ala], showing three replicate simulations of the same system, indicated by different colours.

Chart, line chart, histogram

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**Fig. S15.** RDF of (A) [Cho]+ and (B) [Phe]- that exist within 0.35 nm from the CALB surface as a function of time during 100 ns simulations in [Cho][Phe], showing three replicate simulations of the same system, indicated by different colours.

**4. MD simulation of CALB in 0.05 M [Cho][Phe] at 50oC**

**Chart, histogram

Description automatically generated**

**Fig. S16.** (A) RMSD, (B) RMSF, (C) Rg, and (D) intramolecular hydrogen bond number of CALB as a function of time during 100 ns simulations in [Cho][Phe] at 50oC, showing three replicate simulations of the same system, indicated by different colours.

Chart, line chart, histogram

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**Fig. S17.** RDF of (A) [Cho]+ and (B) [Phe]- that exist within 0.35 nm from the CALB surface as a function of time during 100 ns simulations in [Cho][Phe] at 50oC, showing three replicate simulations of the same system, indicated by different colours.

**Table S1.** Average RMSF values of α5-helix residues (Residues 139-150) in CALB-solvent systems.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Residue No. | Averaged RMSF (Å) | | | | |
| Water | [Cho][OH] | [Cho][Gly] | [Cho][Ala] | [Cho][Phe] |
| 139 | 0.86 | 0.81 | 0.82 | 0.71 | 0.80 |
| 140 | 1.00 | 1.10 | 0.97 | 0.82 | 0.84 |
| 141 | 1.61 | 1.66 | 1.47 | 1.35 | 1.42 |
| 142 | 2.21 | 2.44 | 2.14 | 1.95 | 1.80 |
| 143 | 3.10 | 3.25 | 2.83 | 2.50 | 2.21 |
| 144 | 2.76 | 2.37 | 2.29 | 1.86 | 1.75 |
| 145 | 3.10 | 3.11 | 2.60 | 2.51 | 2.38 |
| 146 | 2.73 | 2.50 | 2.09 | 2.03 | 2.09 |
| 147 | 2.30 | 2.20 | 1.90 | 1.80 | 2.06 |
| 148 | 1.34 | 1.49 | 1.41 | 1.29 | 1.58 |
| 149 | 1.31 | 1.40 | 1.39 | 1.23 | 1.39 |
| 150 | 0.77 | 0.83 | 0.79 | 0.78 | 0.89 |