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Supplementary material

Enantioselectivity of D-amino acid oxidase in presence of Ionic liquids

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Methods and Materials

CE analysis

The capillary electrophoresis apparatus Beckmann MDQ-30638 47 used in this work was equipped with a UV detector and an uncoated fused-sillica capillary of 60 cm (effective length 47 cm) × 75 μm. Detection at 210 nm was performed. The new fused-capillary was washed with 0.1 M NaOH and water for 30 min in order. Prior to sample introduction, the capillary was flushed with 0.1 M NaOH, water and running electrolyte for 2 min, respectively. The electrolyte was composed of borax buffer solution (pH 9.2, 20 mM), 30 mM β-CD, 25 mM SDS and 17% IPA. Sample solutions were introduced at the cathodic end at a pressure of 20 KV. Separations were performed in 20 mM borax buffer (pH 9.2). To detect the racamization of L-Ala, 25 μL 0.25 mM L-Ala was added to 1 mL borax buffer (20 mM, pH=9.0) containing different amounts of ILs. After the addition, the mixtures were acclimatized for 30 min. Then the solution was diluted into one forth and was divided into two parts. One of them was for the CE analysis after derivatization. D-Ala of the same concentration was added to the other part and was for the CE analysis after derivatization. Derivatization of the amino acids was conducted according to the previous literature modified as follows[1]. Briefly, 200 μL of 10 mM FMOC was thus added to 200 μL of amino acids in 20 mM borate buffer (pH 9.0). This mixture was kept for 2 min, and then extracted with 0.5 mL pentane to remove excess of reagent. Measurement of transformation of other L-amino acids was tested under the same condition.

Reference

1. H. Wan, P. E. Andersson, A. Engstrom and L. G. Blomberg, *Journal of Chromatography A*, 1995, **704**, 179-193.

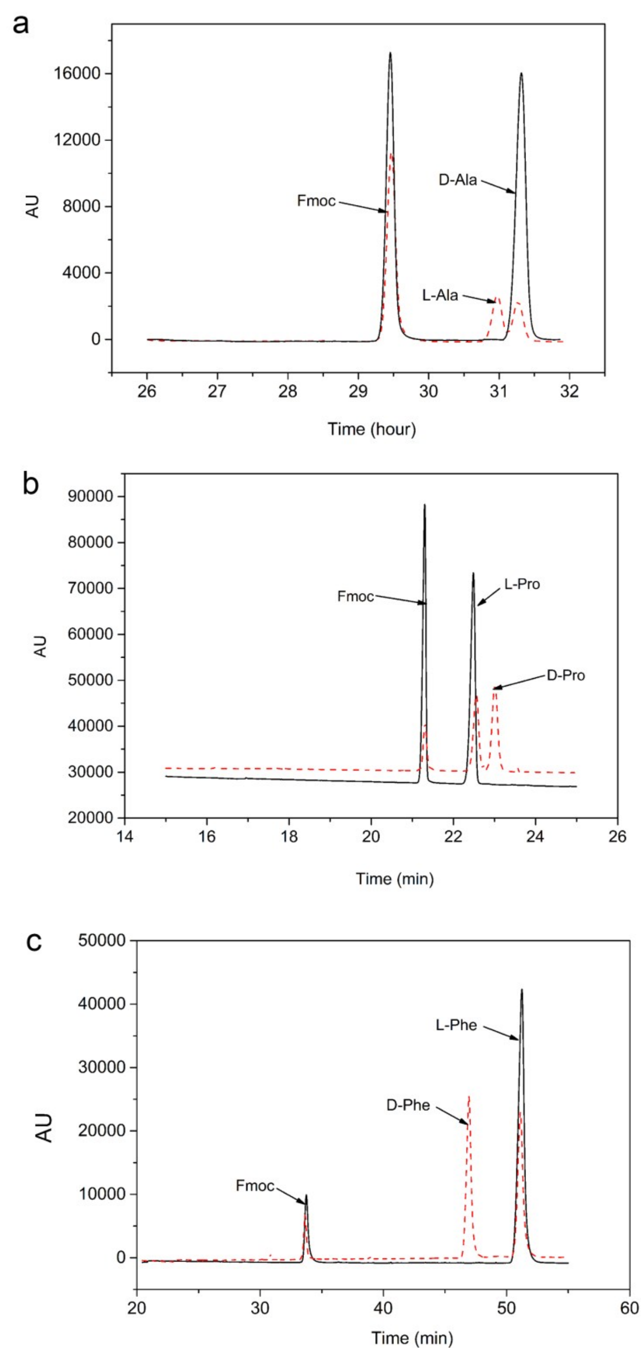

Fig. S1 CE analysis of L-amino acids in presence of 40% (v/v) [MIM][COO]. (a) L-Ala, (b) L-Pro, (c) L-Phe.

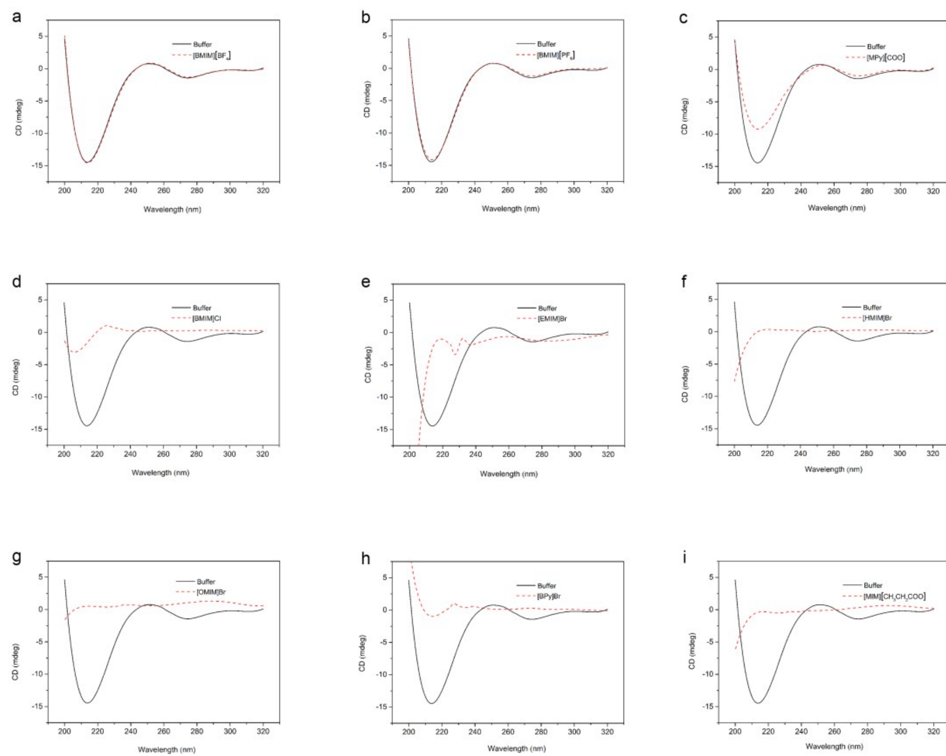

Fig. S2 CD spectrum of DAAO in presence of ILs.

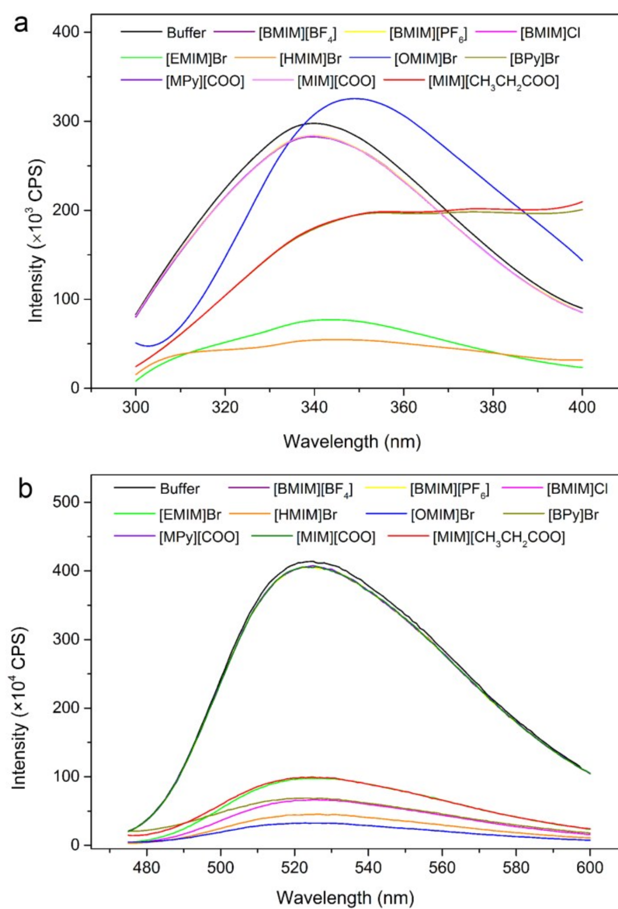

Fig. S3 Fluorescence spectra of DAAO in the presence of ILs. Fluorescence spectra of Trp (a) and flavin (b) of DAAO in borate saline buffer (50 mM) in the absence and presence of 40% IL (v/v) after incubation for 30 min. The excitation wavelength of Trp and flavin was 280 and 450 nm, respectively.

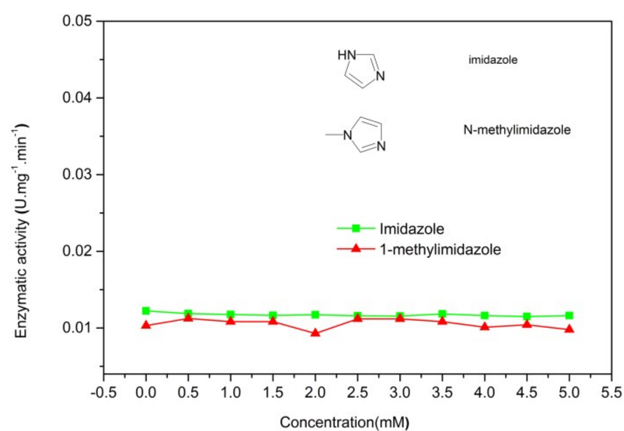

Fig. S4 Enzyme activity of DAAO with L-Ala as substrate in presence of various contrations of imidazole and N-methylimidazole. Reaction mixtures containing borate saline buffer (20 mM), imidazole and N-methylimidazole in a range of concentrations, 228 U/L DAAO, 300 U/L POD, 0.275 nM 4-AAP, 5.5 mM DHBS, 0.1 mM FAD and 25 mM L-Ala were incubated for 30 min at 25 ^{o}C.

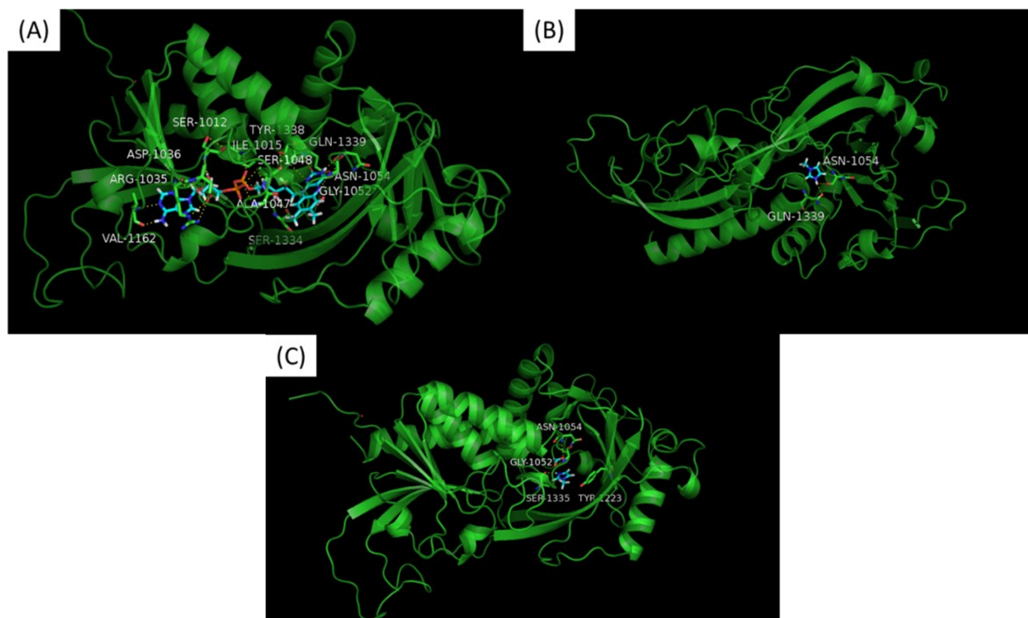

Fig. S5 Structures of D-amino acid oxidase (labeled in green) combined with FAD (A), [MIM] (B) and [MIM] [COO] (C) (labeled in cyan). The hydrogen bond interaction (labelled in yellow dot line) between protein and ligands was analyzed.