Supporting Information

Ionozyme: ionic liquids as solvent and stabilizer for efficient bioactivation of CO_2

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Supplementary Figures

Supplementary Tables

Figure S1. Phylogenetic analysis of the FDHs. Phylogenetic tree of the top one ranking FDH protein sequences in 53 unique PPR groups and the well characterized FDH protein sequences.
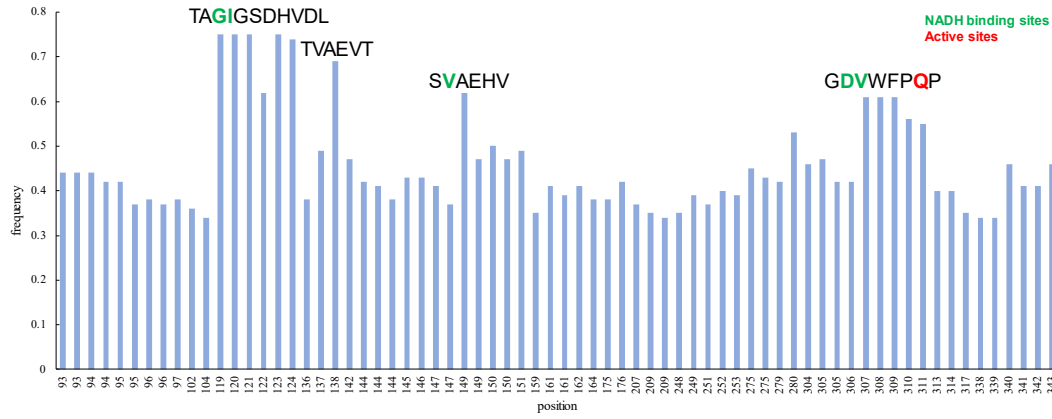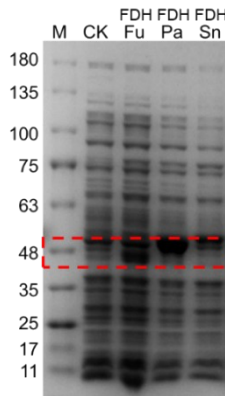
Figure S2. Peptides in the region of NADH binding sites and active site for PPR group 1.


Figure S3. Sequences alignment of the predicted FDHs after the PPR prediction and phylogenetic analysis. The possible NADH


binding sites and active sites are boxed in green and red color, respectively.

Figure S4. SDS-PAGE profile for the selected FDHs. Lane 1 M (marker), lane 2 CK (control check), lane 3 FDHFu from fungal sp. No.11243, lane 4 FDHPa from *Paracoccus* sp., lane 5 FDHSn from *Starkeya novella*.
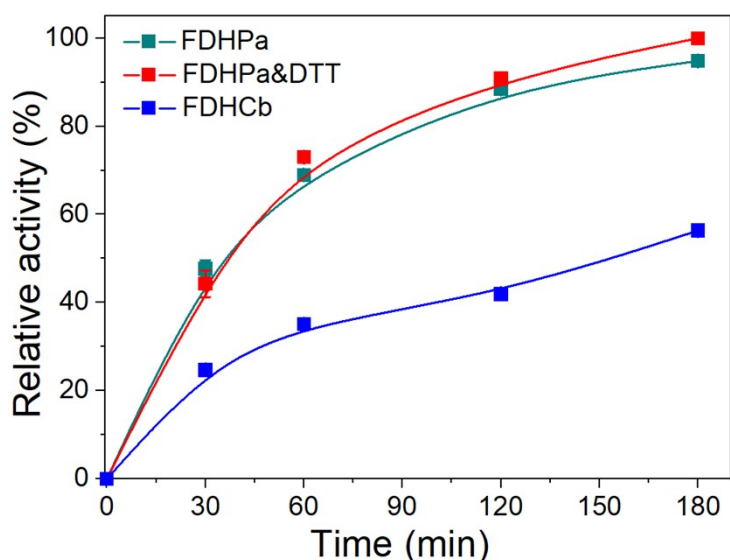
Figure S5. Biocatalytic performance of the recombinant FDHs. Enzyme activities of FDHCb and FDHPa with or without DTT for CO_2 conversion at pH 7.0, 25 °C.
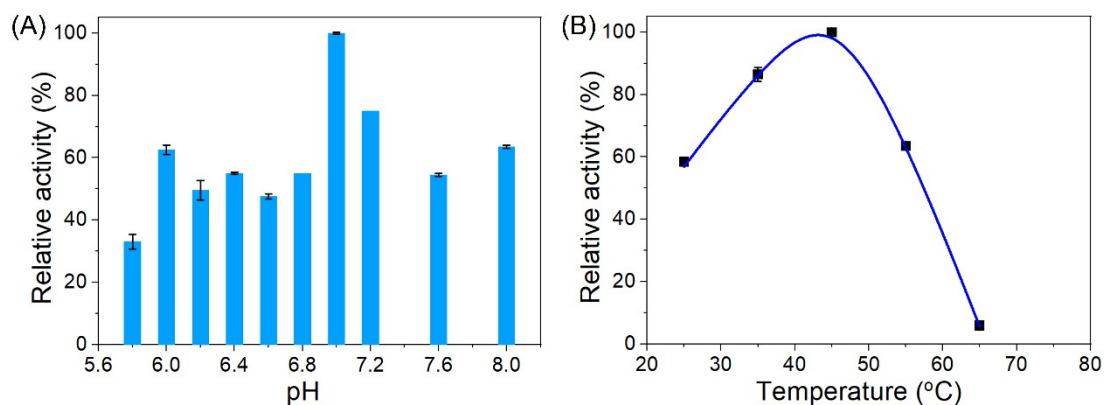

Figure S6. Effect of pH and temperature on enzyme activity of FDHPa. (A) Relative activity as a function of pH. (B) Relative activity observed as a function of temperature. Enzyme assays were carried out in the presence of $NaHCO_3$ (50 mM). The optimum pH for recombinant FDHPa activity was determined by incubating the reaction mixture at 25 °C, while the optimum temperature was at pH 7.0 with PB (100 mM). Activities at the optimal temperature and pH were defined as 100%. Each value represented the mean of triplicate measurements.
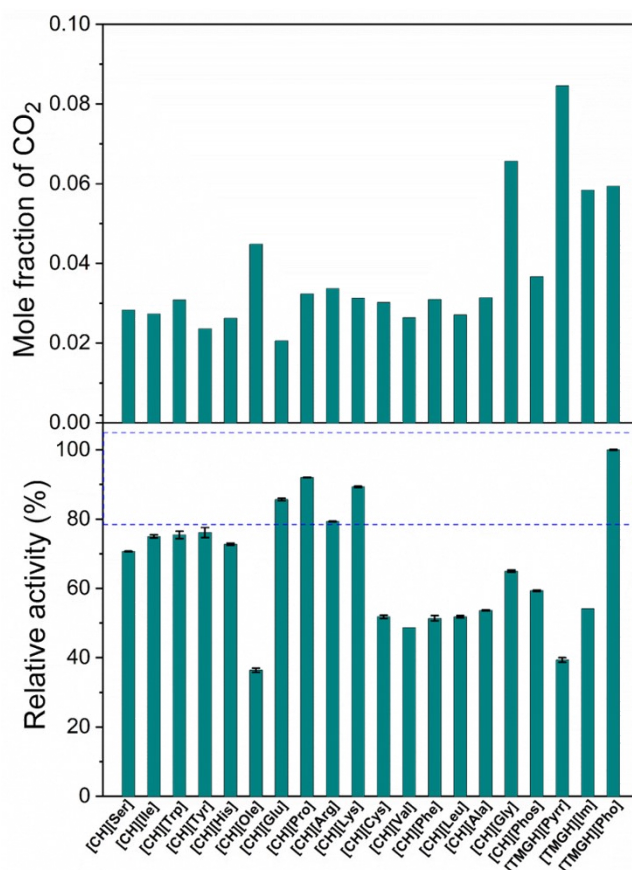
Figure S7. Screening of ILs. (A) Prediction of CO_2 solubility by COSMO-RS model. (B) Relative activity of FDHPa in 20 different kinds of biocompatible ILs (0.05%). Activity in [TNGH][PhO] were defined as 100%. Each value represented the mean of triplicate measurements.
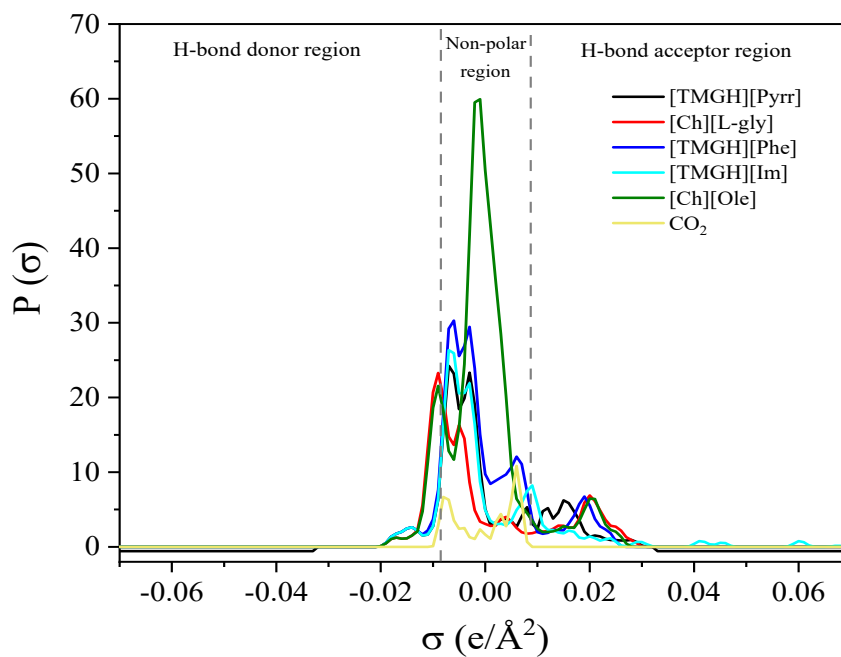

Figure S8. σ-Profiles prediction by COSMO-RS. σ-Profiles of [TMGH][Pyrr] (black line), [CH][Gly] (red line), [TMGH][Phe] (blue line), [TMGH][Im] (Cyan line), [CH][Ole] (Olive line) and CO_2 (yellow line).
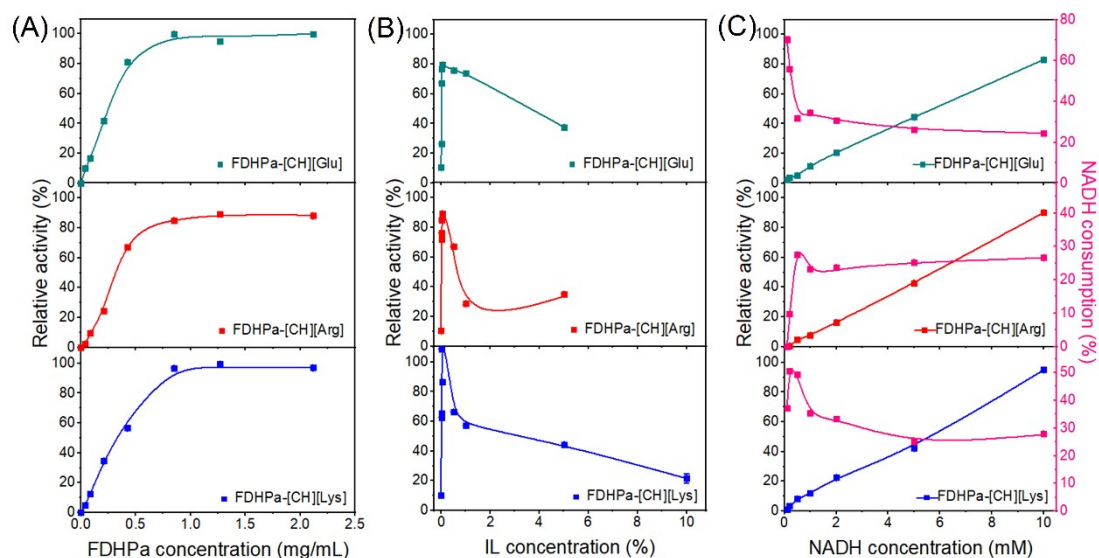
Figure S9. Optimization of reaction conditions for the ionozymes. (A) FDHPa concentration effect. (B) ILs ([CH][Glu], [CH][Arg] and [CH][Lys]) concentration effect. (C) NADH concentration effect on ionozyme activities. Enzyme assays were carried out under standard conditions in the presence of CO_2. Activities at the optimal concentration were defined as 100%. Each value represented the mean of triplicate measurements.
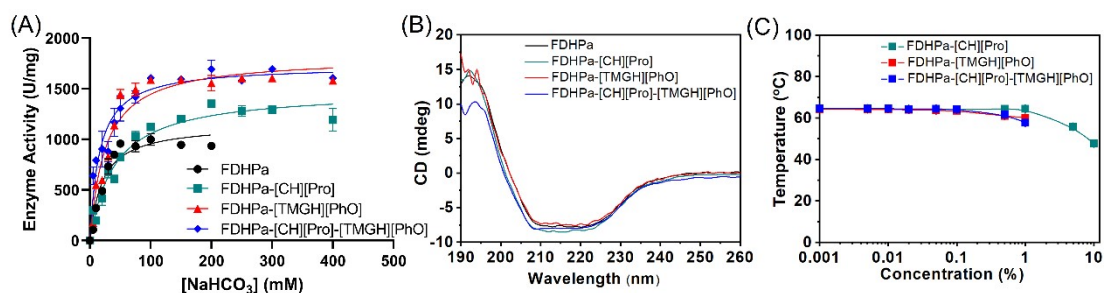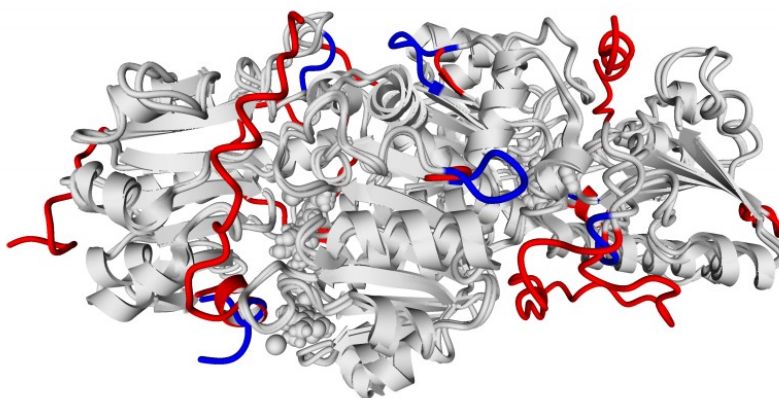

Figure S10. Analysis of determining conditions on catalytic activity of FDHPa and FDHPa-ILs. (A) Kinetic analysis based on Michaelis–Menten equation. (B) Far-UV CD spectra. (C) Thermal stability.
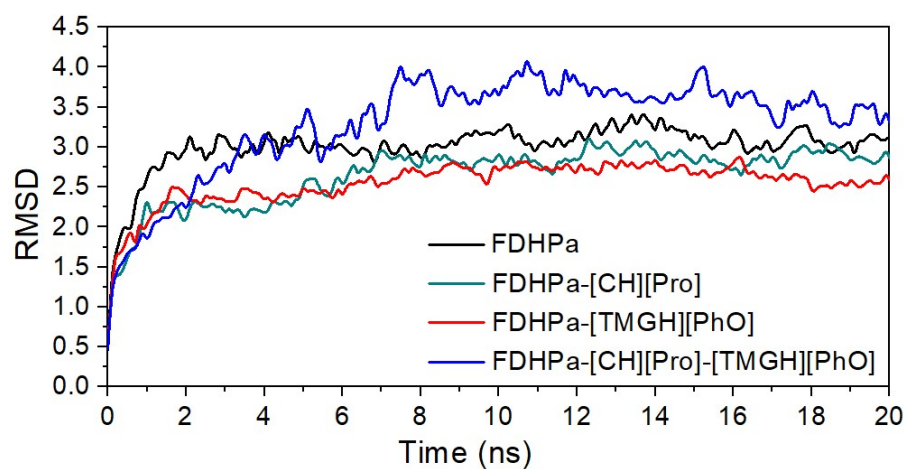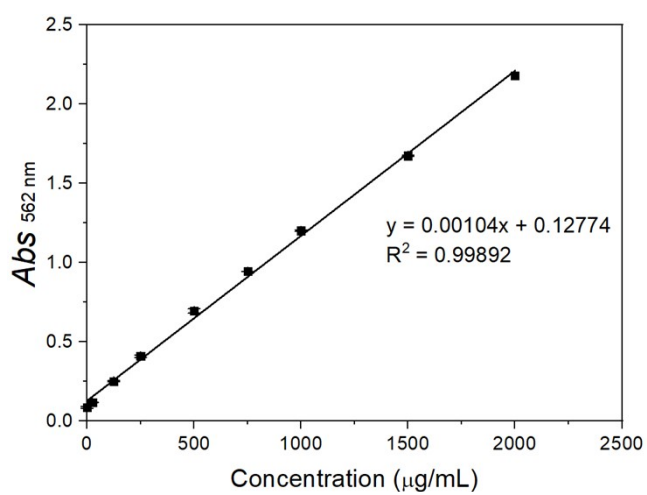

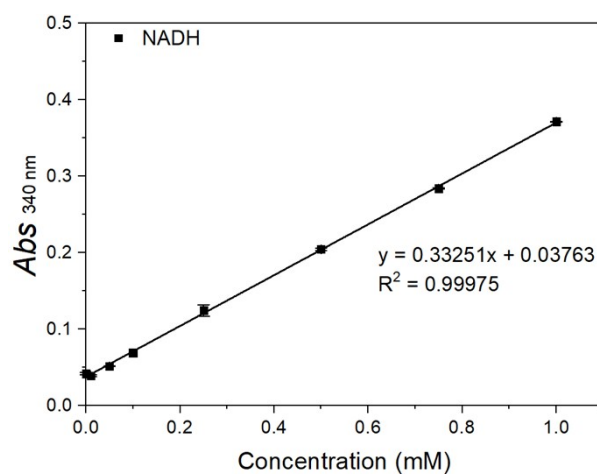**Figure S11.** Root mean square deviation (RMSD) of FDHPa and FDHPa-ILs. All stimulations were carried out at 25 °C.

Figure S12. Structural comparison of FDHPa (red) and FDHCb (blue).

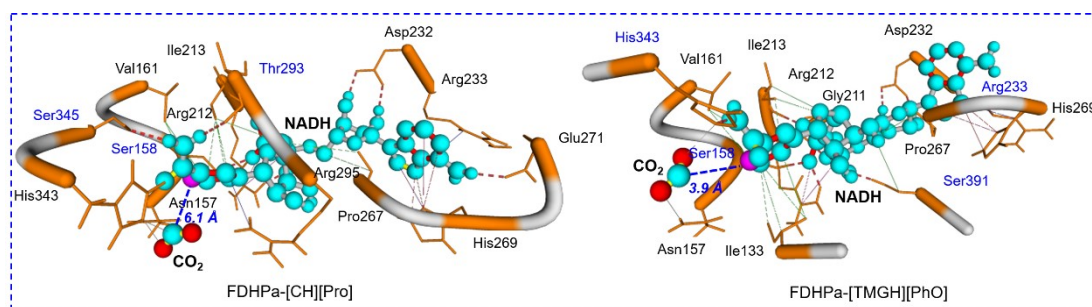$$y = 0.00104x + 0.12774$$
$$R^2 = 0.99892$$

Figure S13. Calibration Curve. Absorbance of FDHPa at 562 nm as a function of concentration.

$$y = 0.33251x + 0.03763$$
$$R^2 = 0.99975$$

Figure S14. Calibration Curve. Absorbance of NADH at 340 nm as a function of concentration.

Figure S15. Proposed hydride transfer process from NADH to CO_2 in ionozymes. The binding models of NADH and CO_2 in the active sites of FDHPa-[CH][Pro] and FDHPa-[TMGH][PhO].

Supplementary Tables

Table S1. Kinetic analysis of ionozymes based on Michaelis–Menten equation.

Ionozymes	K_m (mM)	K_{cat} (s^{-1})	V_{max} (U mg^{-1})	K_{cat}/K_m (s^{-1} mM^{-1})
FDHPa	20.14	0.1839	1148	0.0091
FDHPa-[CH][Pro]	40.61	0.2244	1484	0.0055
FDHPa-[TMGH][PhO]	25.35	0.2724	1812	0.0107
FDHPa-[CH][Pro]-[TMGH][PhO]	15.74	0.2595	1782	0.0165

Table S2. Full name and structure of IL cations.

No.	Full name	Abbreviation	Structure	COSMO sigma surface
1	Choline	CH		
2	Tetramethylgunidinium	TMGH		

Table S3. Full name and structure of IL anions.

No.	Full name	Abbreviation	Structure	COSMO sigma surface
1	L-glycinate	Gly		
2	L-alaninate	Ala		
3	L-valinate	Val		
4	L-leucinate	Leu		
5	L-isoleucinate	Iso		
6	L-phenylalaninate	Phe		
7	L-prolinate	Pro		
8	L-lysinate	Lys		
9	L-argininate	Arg		
10	L-histidinate	His		
11	L-tryptophan	Try		
12	L-serinate	Ser		
13	L-tyrosinate	Tyr		
14	L-cysteinate	Cys		
15	Oleic acid	Ole		
16	Dihydrogen phosphate	H_2PO_4	$H_2PO_4^-$	
17	L-glutamate	Glu		
18	Pyrrole	Pyrr		
19	Imidazole	Im		
20	Phenol	PhO		