Supplementary Information

Engineering of lysine cyclodeaminase conformational dynamics for relieving substrate and product inhibitions in the biosynthesis of L-pipecolic acid

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Table S1 Summary of molecular chaperone plasmids information.

Plasmid	Chaperone	Promoter	Inducer	Resistant Marker
pG-KJE8	dnaK-dnaJ-grpE-groES-groEL	araB Pzt-1	L-Arabinose Tetracycline	Cm
pGro7	groES-groEL	araB	L-Arabinose	Cm
pKJE7	dnaK-dnaJ-grpE	araB	L-Arabinose	Cm
pTf16	tig	araB	L-Arabinose	Cm

Figure S1 SDS-PAGE results of co-expression *Sp*LCD and different molecular chaperons.

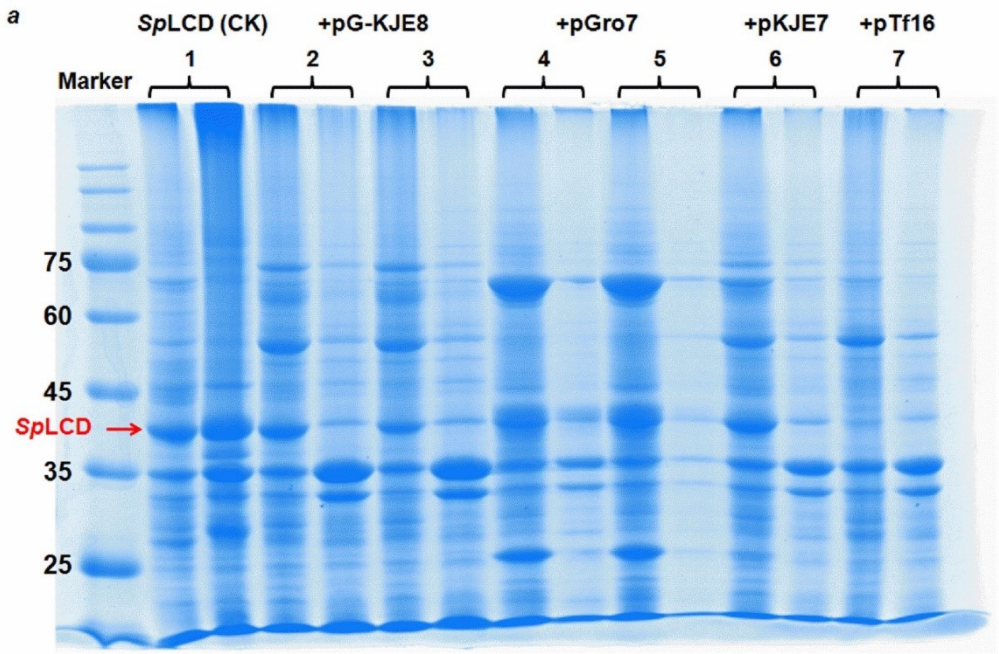

Figure S1. SDS PAGE analysis of the heterologous expression of *Sp*LCD and co-expression of *Sp*LCD with four different molecular chaperons, supernatant on the left and the precipitates on the right. The chaperons were induced with minimum amount of either arabinose or tetracycline at OD_{600}=0.2 and the *Sp*LCD was induced with 0.2 mM IPTG at OD_{600}=0.6. After the induction of *Sp*LCD, the incubation temperature was decreased to 18 ℃.

Figure S2 The RMSD and RMSF of 100ns simulation for LCD-NAD$^+$-lysine and LCD-NAD$^+$-pipecolic acid.

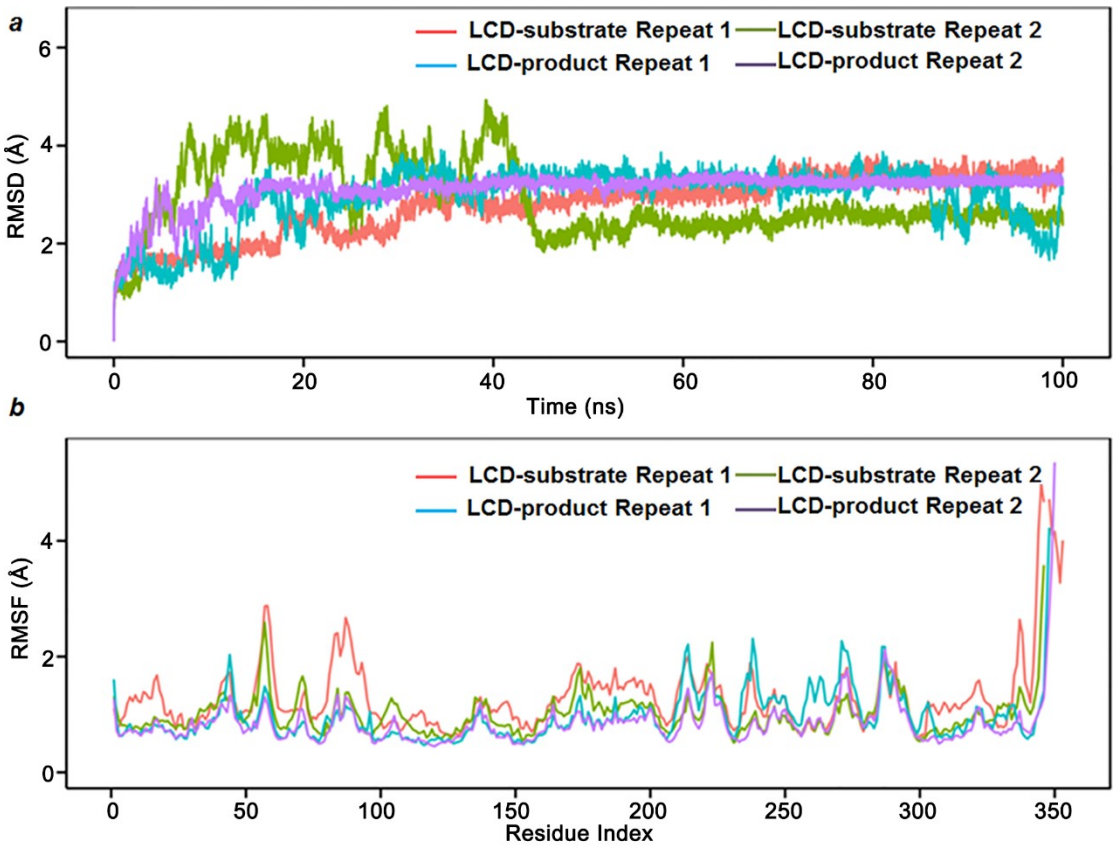

Figure S2. The RMSD (a) and RMSF (b) of 100nm simulation for LCD-NAD$^+$ with substrate lysine or product L-pipecolic acid. The RMSD and RMSF are both calculated by C-alpha.

Figure S3 Detailed substrate delivery process 1.

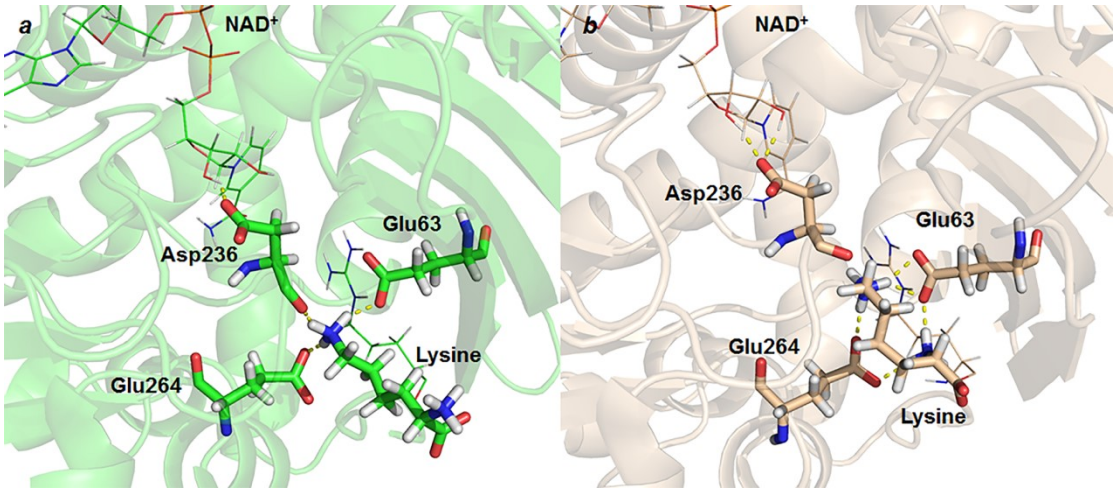

Figure S3. H-bond network of the substrate lysine was formed between Glu63 and Glu264 while Asp236 forms a stable H-bond with the cofactor NAD^+ Later, the lysine is pulled down by Glu264 along the substrate-access channel 1 (MD timeline: Fig. S2a. 4.52 ns, Fig. S2b. 72.76 ns).

Figure S4 Detailed substrate delivery process 2

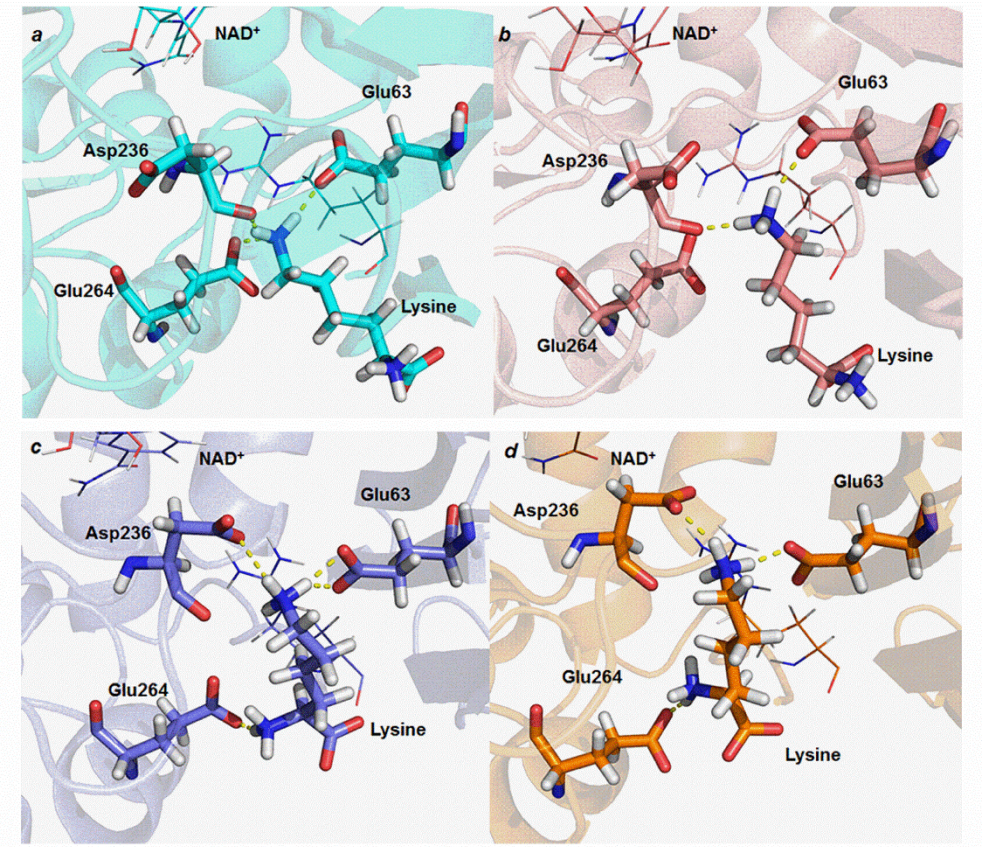

Figure S4. In comparison with the location in substrate delivery process 1, the carboxylate moiety of Asp236 rotated and formed a stable H-bond with lysine, thereby helping to anchor the substrate among Asp236, Glu63, and Glu264. Once the lysine was captured, the delivery occurred through the synergic action of these three residues. (correspond MD timeline: Fig. S3a. 5.76 ns, S3b. 10.27ns, S3c. 40.16, ns S3d. 80.44ns)

Figure S5 Structures and surfaces of the inactive product exits.

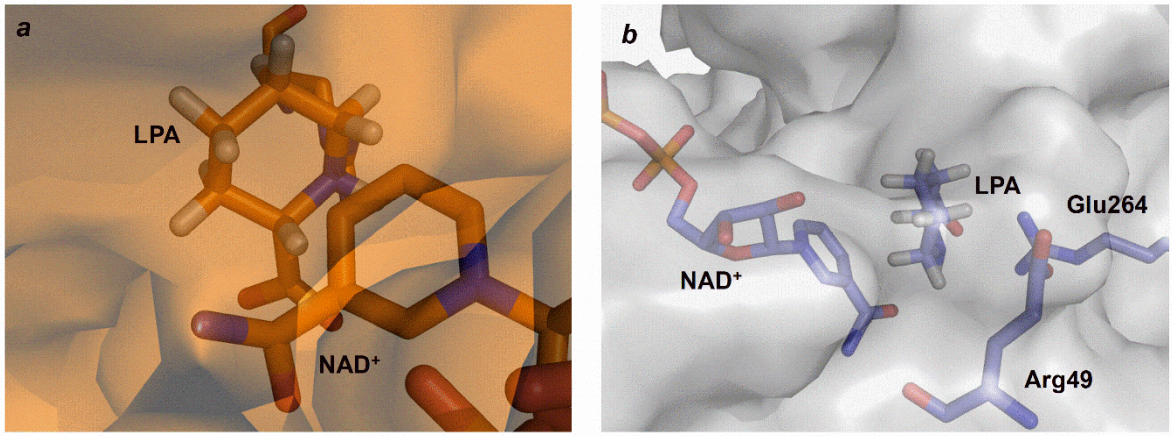

Figure S5. Only one exit is active during the enzymatic process at any particular time, when exit 1 is open in substrate delivery state 1, exit 2 is blocked by the nicotinamide nucleoside moiety of NAD^+. Similarly, when exit 2 is open in substrate delivery state 2, the location of Glu264 blocks product release in exit 1.

Figure S6. Visualization of the substrate delivery tunnel 2 of Val61-*Sp*LCD

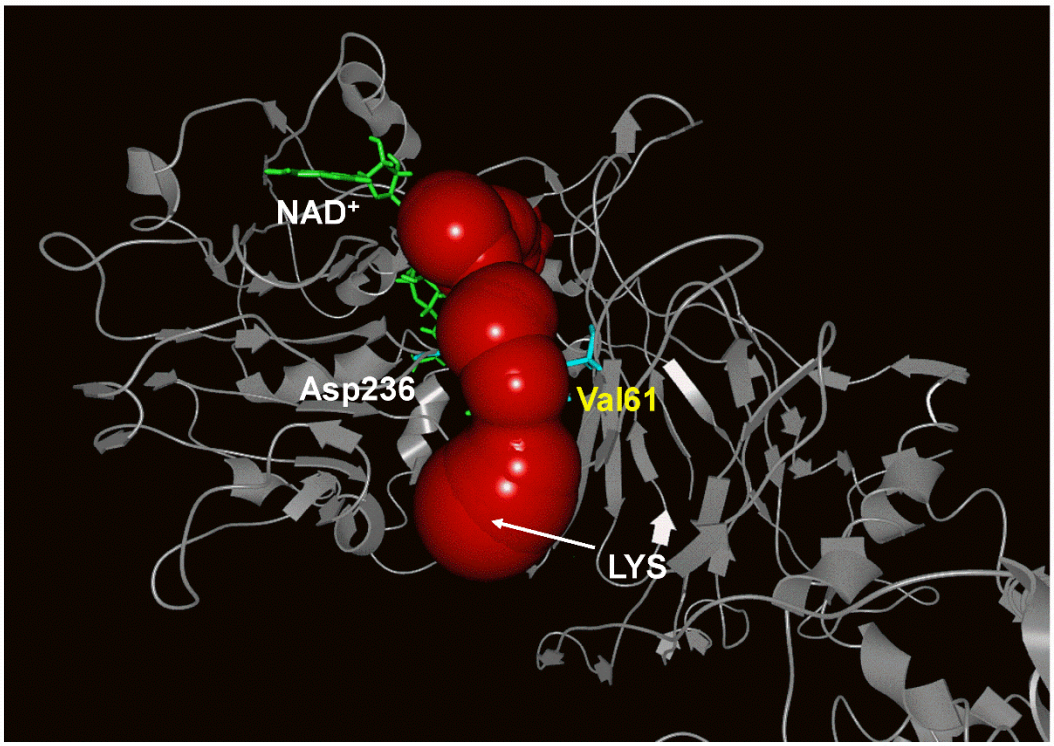

Figure S6. Visualization of substrate delivery tunnel 2 of variant Val61-*Sp*LCD via CAVERdock. The residues are presented in cyan, LYS and NAD^+ are presented in green. The bottleneck (Asp236-Ile61) of substrate delivery tunnel 2 of *Sp*LCD broadened critically owing to the substitution of Val in 61 position (radius from 1.6 Å to 2.3 Å).

Supporting Information Available: Experimental procedures including information on molecular chaperon plasmids, RMSFs and RMSDs of MD simulation systems, detailed substrate delivery motions and product release motions, visualization of substrate tunnel 2 of variant Val61-*Sp*LCD.

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