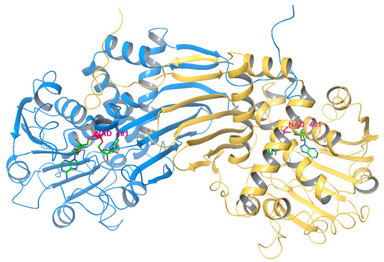
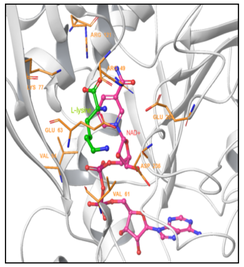
Mechanistic Insights into Lysine Cyclodeaminase Catalysis

Yao Wei[1], Uliano Guerrini[1], Beatrice Rassati[2], Francesca Paradisi[2], Ivano Eberini[1]
[1] Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", DiSFeB, University of Milan
[2] Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern
yao.wei@unimi.it

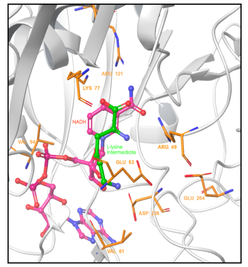
Lysine Cyclodeaminase (LCD) predominantly catalyzes the biosynthesis of piperidine derivatives using NAD^+ as cofactor and exhibits strict substrate size specificity, catalyzing only substrates no larger than L-lysine.



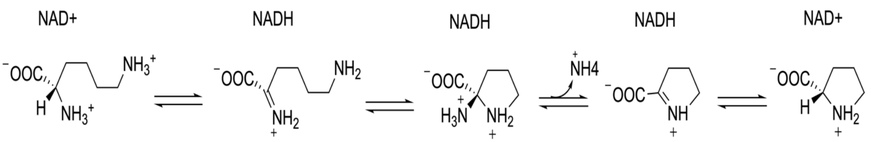
LCD structure (PDB: 5GZJ)



LCD (NAD+) – L-lysine



LCD (NADH) – L-lysine iminium intermediate



The proposed catalytic reaction pathway of LCD from substrate L-lysine to yield L-pipecolic acid

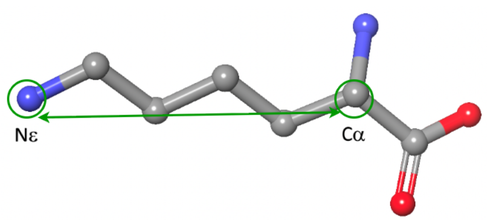
LC-MS analysis for L-lysine or L-lysine ethyl ester in the SpLCD I61V–I94V reaction after 6 H

Compound	Expected mass	Observed mass
L-Lysine ethyl ester	619.3 ($M+H^+$)	619.3
	641.3 ($M+Na^+$)	641.2
L-Lysine	591.3 ($M+H^+$)	n.d.
	613.3 ($M+Na^+$)	n.d.
L-Pipecolic acid ethyl ester	380.2 ($M+H^+$)	n.d.
	402.2 ($M+Na^+$)	n.d.
L-Pipecolic acid	352.2 ($M+H^+$)	352.1
	374.2 ($M+Na^+$)	374.1

Acknowledgement: This work is supported by the European Union's Horizon Europe research and innovation program under the Marie Skłodowska-Curie grant agreement No. 101073546 (MSCA Doctoral Network Metal-containing Radical Enzymes – MetRaZymes), and grants from MIUR - "Progetto Eccellenza 2023 – 2027"

LCD (NAD+) – L-lysine ethyl ester

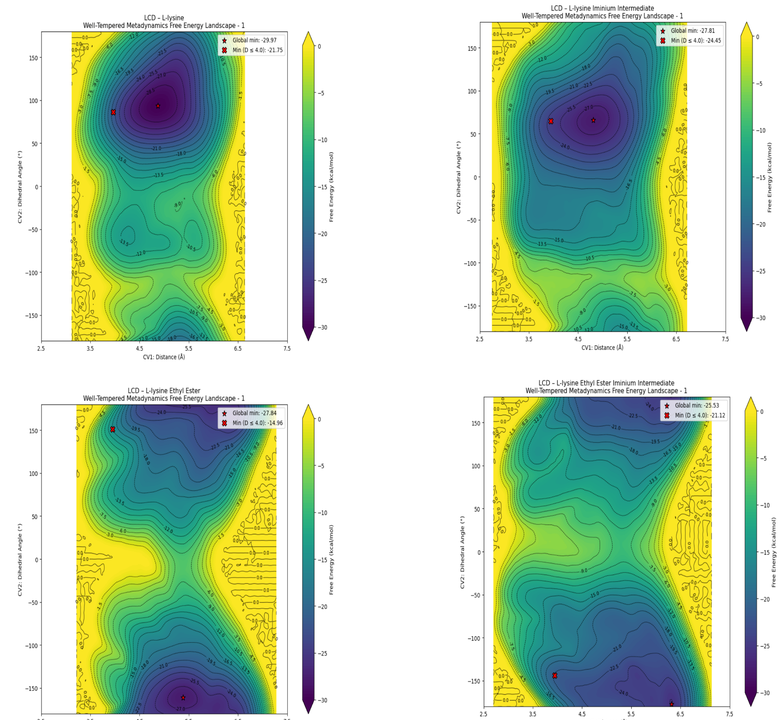
LCD (NADH) – L-lysine ethyl ester iminium intermediate



Well-tempered metadynamics simulations were conducted to explore the substrate cyclization mechanism for the reaction

Nε–Cα distances sampled during Well-Tempered Metadynamics simulations (total frames per simulation = 4000)

Sample	Distance (mean ± std)	Num of frames (≤ 3.5 Å)	Num of frames (≤ 4.0 Å)
L-lysine - 1	4.94 ± 0.52	8	14
L-lysine - 2	4.99 ± 0.50	3	76
L-lysine - 3	5.00 ± 0.48	0	80
L-lysine iminium intermediate - 1	4.75 ± 0.65	108	595
L-lysine iminium intermediate - 2	4.80 ± 0.64	99	519
L-lysine iminium intermediate - 3	4.82 ± 0.58	75	384
L-lysine ethyl ester - 1	5.50 ± 0.67	1	44
L-lysine ethyl ester - 2	5.47 ± 0.70	8	76
L-lysine ethyl ester - 3	5.42 ± 0.71	13	104
L-lysine ethyl ester iminium intermediate - 1	5.26 ± 0.84	86	336
L-lysine ethyl ester iminium intermediate - 2	5.36 ± 0.81	71	257
L-lysine ethyl ester iminium intermediate - 3	5.34 ± 0.81	78	280



Free energy landscapes of LCD well-tempered metadynamics simulations