

Supporting Information

Engineering bi-functional enzyme complex of formate dehydrogenase and leucine dehydrogenase by peptide linker mediated fusion for accelerating cofactor regeneration

Yonghui Zhang¹

Yali Wang¹

Shizhen Wang^{1*}

Baishan Fang^{1,2,3*}

¹Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China

²The Key Lab for Synthetic Biotechnology of Xiamen City, Xiamen University, Xiamen, Fujian, 361005, P. R. China



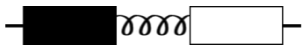




³The Key Laboratory for Chemical Biology of Fujian Province, Xiamen University, Xiamen, Fujian, 361005, China

Correspondence:

Shizhen Wang (szwang@xmu.edu.cn). Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China.

Baishan Fang (fbs@xmu.edu.cn). Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China.

Table S1. The primers for introducing peptide linker in the construction of fusion enzymes

Fusion enzyme*	Primer 2 sequence	Amino acid sequence
F-DL-L 	AGGCCTATGGCAAACA CGATAA AAAGATGACATTGGAAATCTTC GAATAT	
F-R1-L 	GCCTATGGCAAACACGATAAAA AGGAAGCTGCTGCTAAAATGAC ATTGGAAATCTTCGA-3	(EAAAK) ₁
F-R2-L 	AGGCCTATGGCAAACA CGATAA AAAGGAAGCTGCTGCTAAA GAAGCTGCTGCTAAAATGACAT TGGAAATCTTCGAATAT	(EAAAK) ₂
F-R3-L 	GGCCTATGGCAAACACGATAAAA AAGGAAGCTGCTGCTAAAGAA GCTGCTGCTAAA GAAGCTGCTG CTAAAATGACATTGGAAATCTTC G	(EAAAK) ₃
F-S1-L 	AGGCCTATGGCAAACA CGATAA AAAGGGTGGTGGTGGTTCTATG ACATTGGAAATCTTCGAATAT	(GGGS) ₁
F-S2-L 	AGGCCTATGGCAAACA CGATAA AAAGGGTGGTGGTGGTTCTGGT GGTGGTGGTTCTATGACATTGG AAATCTTCGAATAT	(GGGS) ₂
F-S3-L 	TATGGCAAACACGATAAAAAGG GTGGTGGTGGTTCTGGTGGTGG TGGTTCTGGTGGTGGTGGTTCT ATGACATTGGAAATCTTCGAAT	(GGGS) ₃

*: The black box represents FDH sequence and the open box represents LeuDH sequence. The inserted peptide linker was shown as helix and curve between two boxes, indicating sequence of rigid peptide linker and flexible peptide linker respectively.

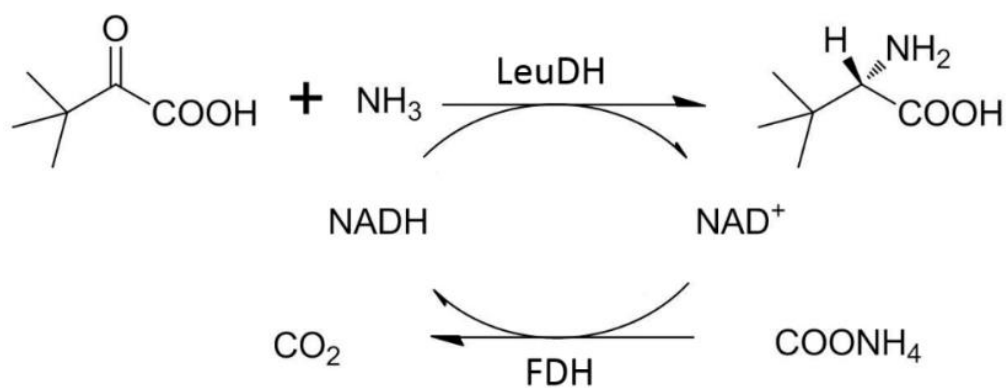


Figure S1. The enzymatic reaction of FDH and LeuDH system for L-tle production and cofactor regeneration.

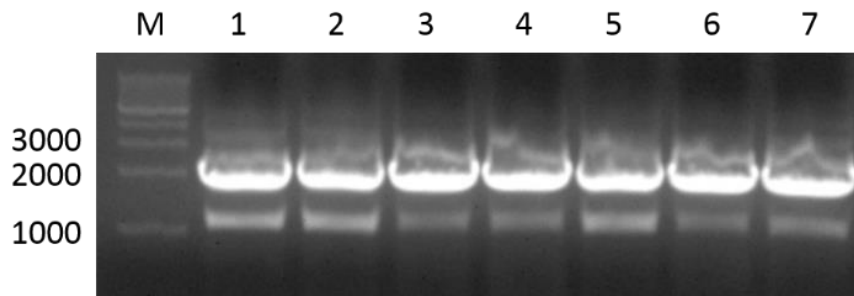


Figure S2. Construction of FDH-LeuDH fusion gene. Lane M: DNA marker; Lane 1 - 7 were loaded with FDH-DL-LeuDH, FDH-S1-LeuDH, FDH-S2-LeuDH, FDH-S3-LeuDH, FDH-R1-LeuDH, FDH-R2-LeuDH and FDH-R3-LeuDH.

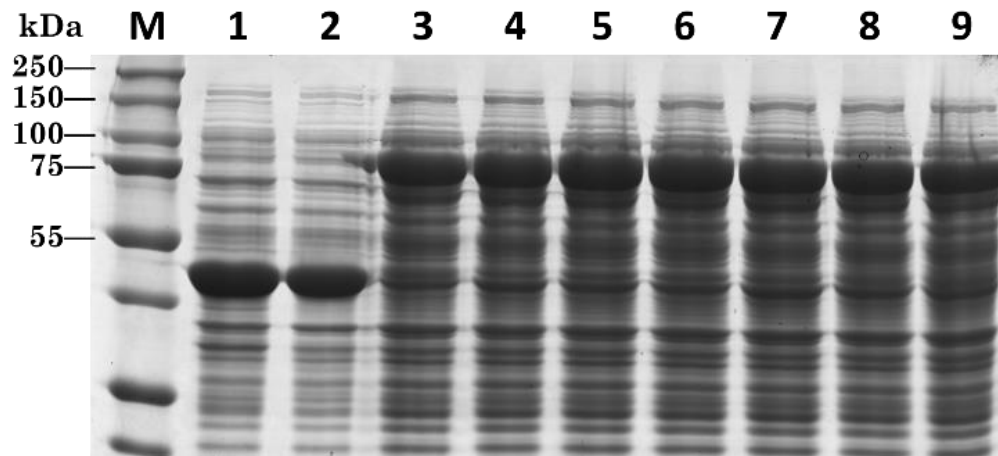


Figure S3. The SDS-PAGE profiles of the whole cell proteins of the parental enzyme and the fusion FDH-LeuDH complex expressed in *E.coli* BL21(DE3). Lane M: protein marker; Lane 1: FDH; Lane 2: LeuDH; Lane 3: FDH-DL-LeuDH; Lane 4: FDH-S1-LeuDH; Lane 5: FDH-S2-LeuDH; Lane 6: FDH-S3-LeuDH; Lane 7: FDH-R1-LeuDH; Lane 8: FDH-R2-LeuDH; Lane 9: FDH-R3-LeuDH.

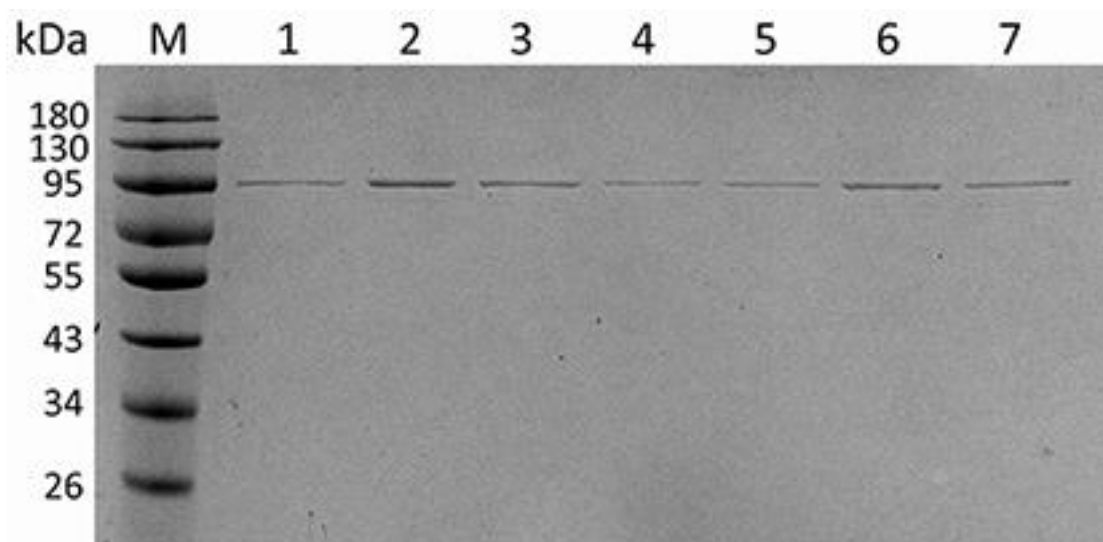


Figure S4. SDS-PAGE analyses of the purified fusion enzyme mediated by different peptide linker. Lane M: protein marker; Lane 1: F-DL-L; Lane 2: F-S1-L; Lane 3: F-S2-L; Lane 4: F-S3-L; Lane 5: F-R1-L; Lane 6: F-R2-L; Lane 7: F-R3-L.