

## Supplementary Data

### Structural and biochemical analyses of the butanol dehydrogenase from *Fusobacterium nucleatum*

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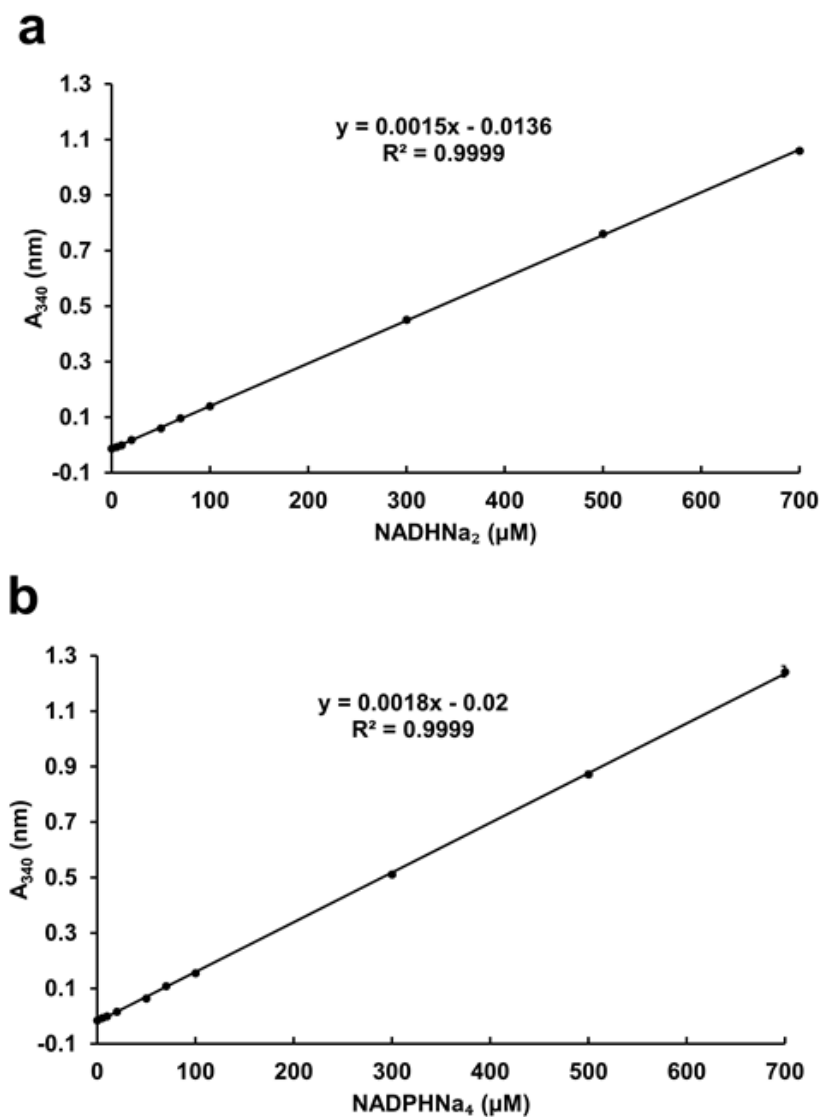
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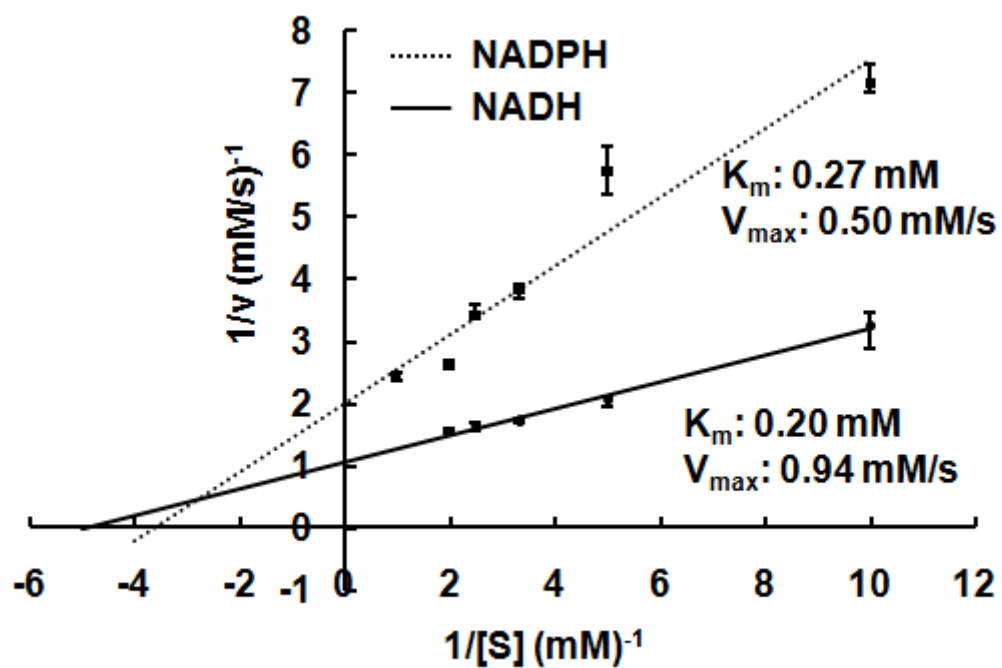
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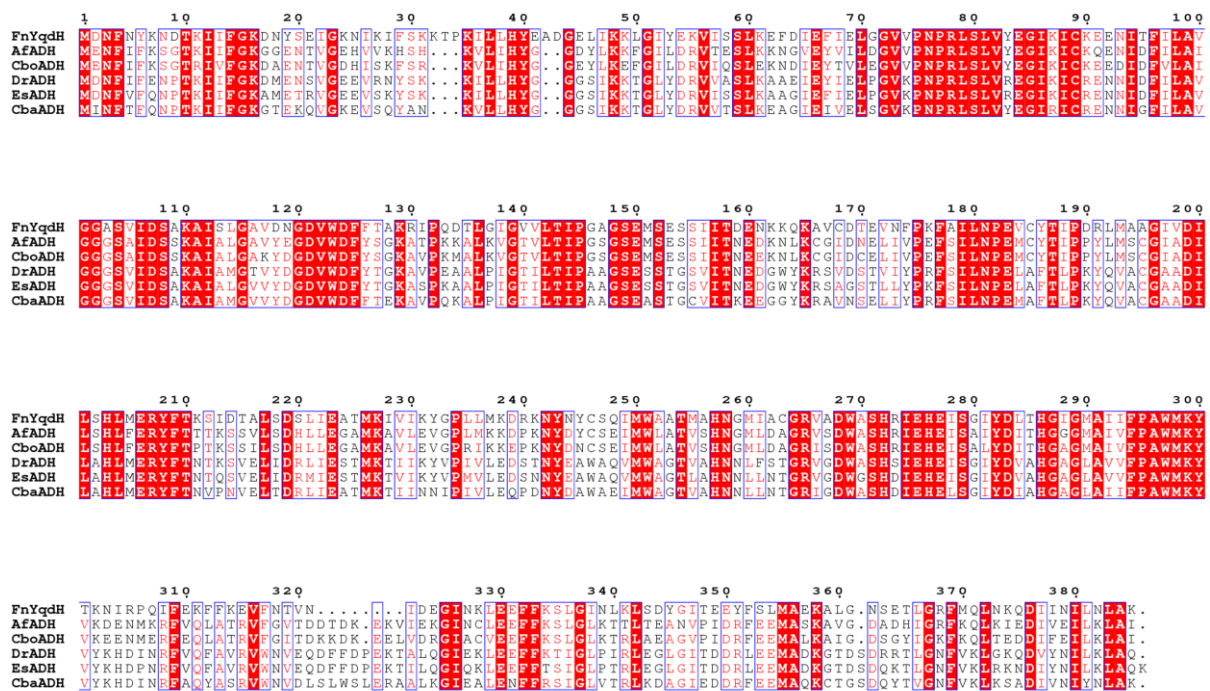
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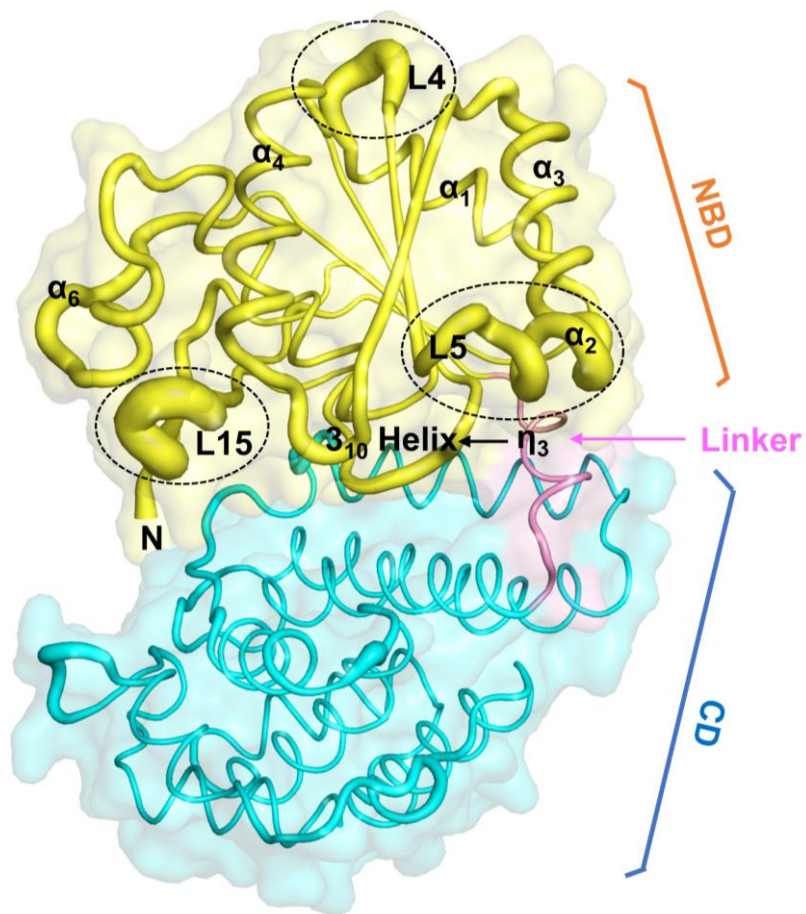
**Figure S1.** Calibration working curve constructed from the average slopes of (a) NADH, and (b) NADPH standards (0-700 μM), obtained from the three-fold assay repetition.



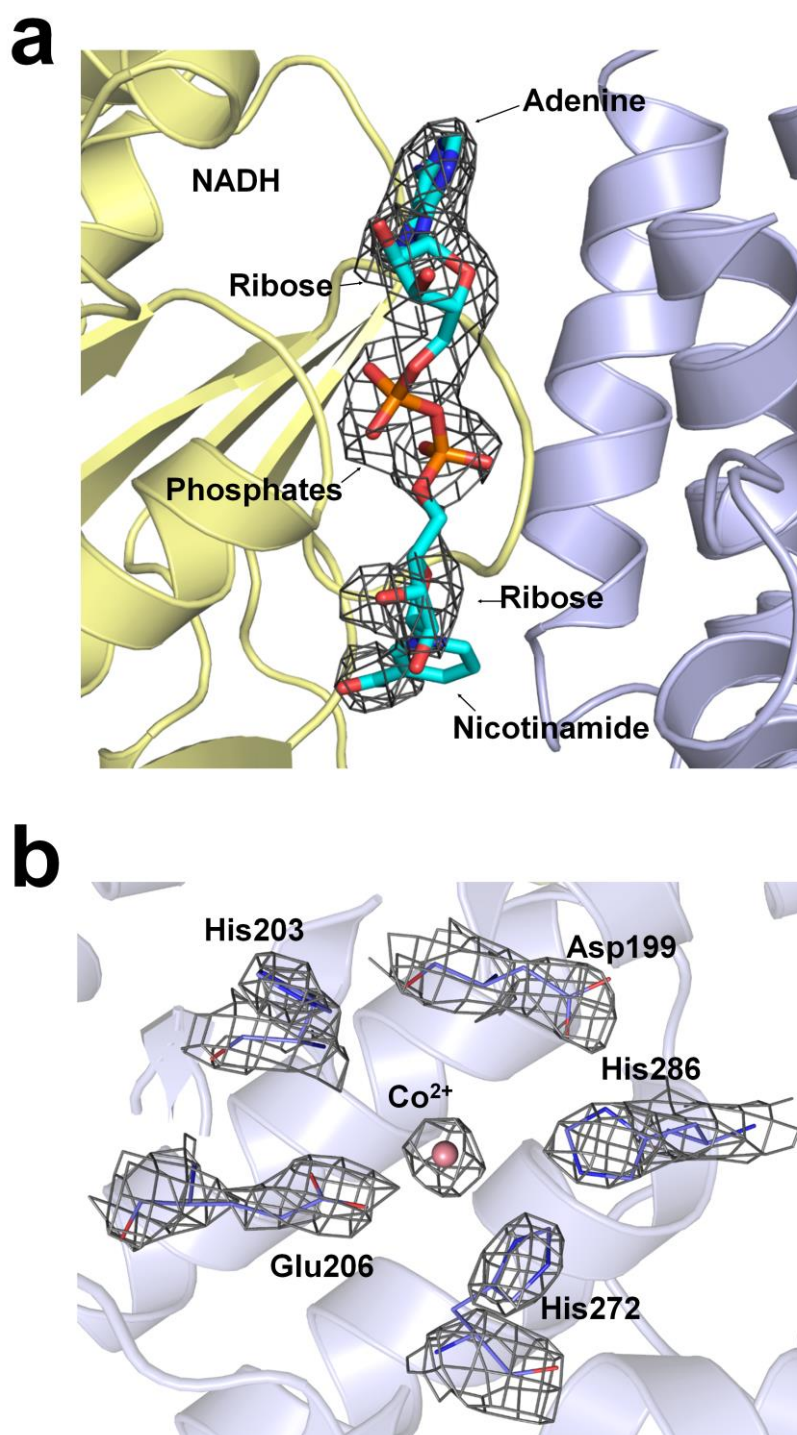
**Figure S2.** The double reciprocal plot was used to calculate  $V_{max}$  and  $K_m$  of the enzyme in the presence of NADH and NADPH. Data represent average  $\pm$  SD of three different samples.



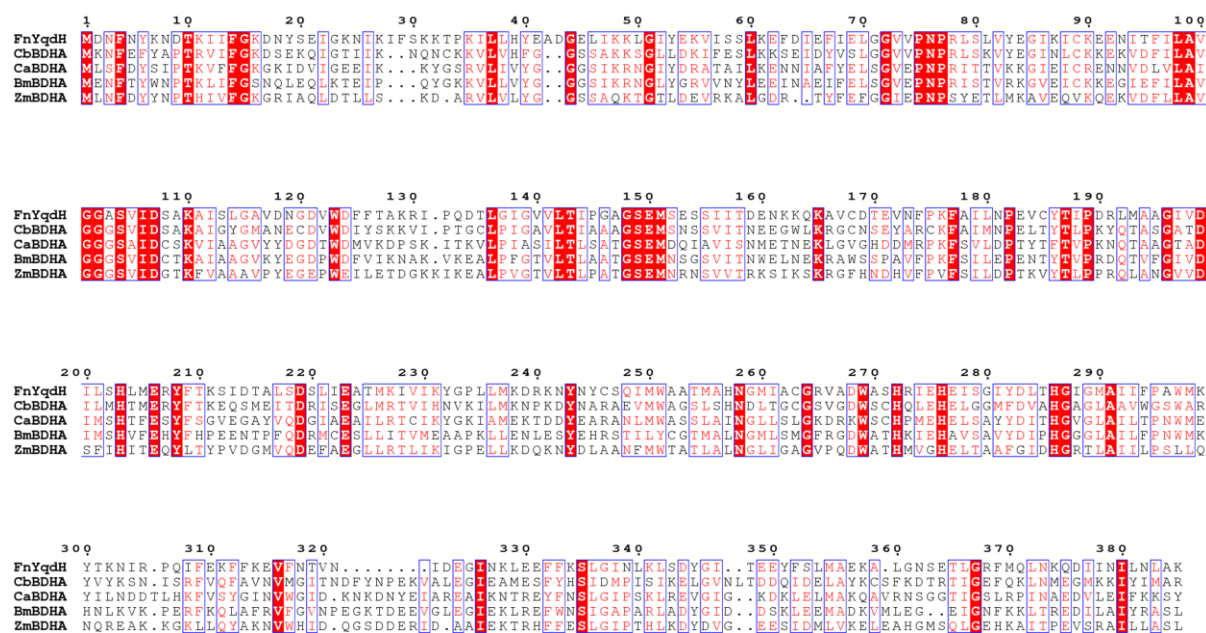
**Figure S3.** Sequence alignment of FnYqdH and homologous proteins. Sequence alignment of FnYqdH, AfADH (NCBI Reference Sequence: WP\_066501503.1), CboADH (GenBank: QGU94363.1), DrADH (NCBI Reference Sequence: WSLF01000004), EsADH (GenBank: NLK98329.1) and CbaADH (GenBank: NLY43117.1)



**Figure S4.** Analysis of the flexible region of FnYqdh. B-factor representation of FnYqdh;  $\alpha_2$ , L4, L5, and L15 show relatively high flexibility.

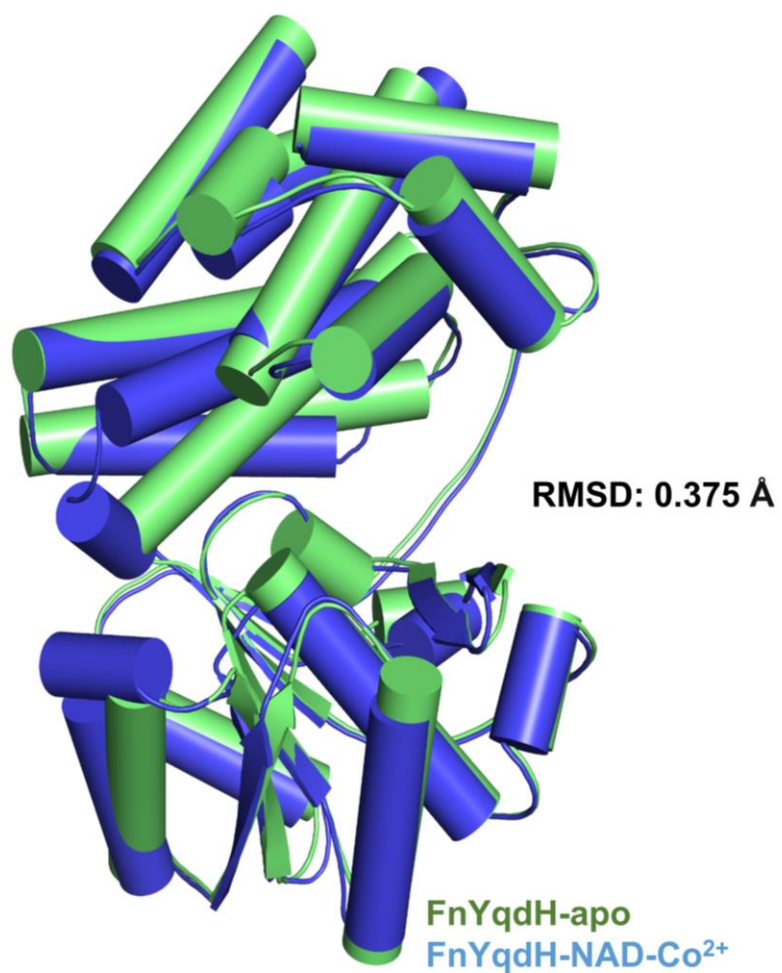


**Figure S5.** The electron density map of NADH and  $\text{Co}^{2+}$ . The electron density map of (a) NADH and (b)  $\text{Co}^{2+}$  are shown as grey mesh.



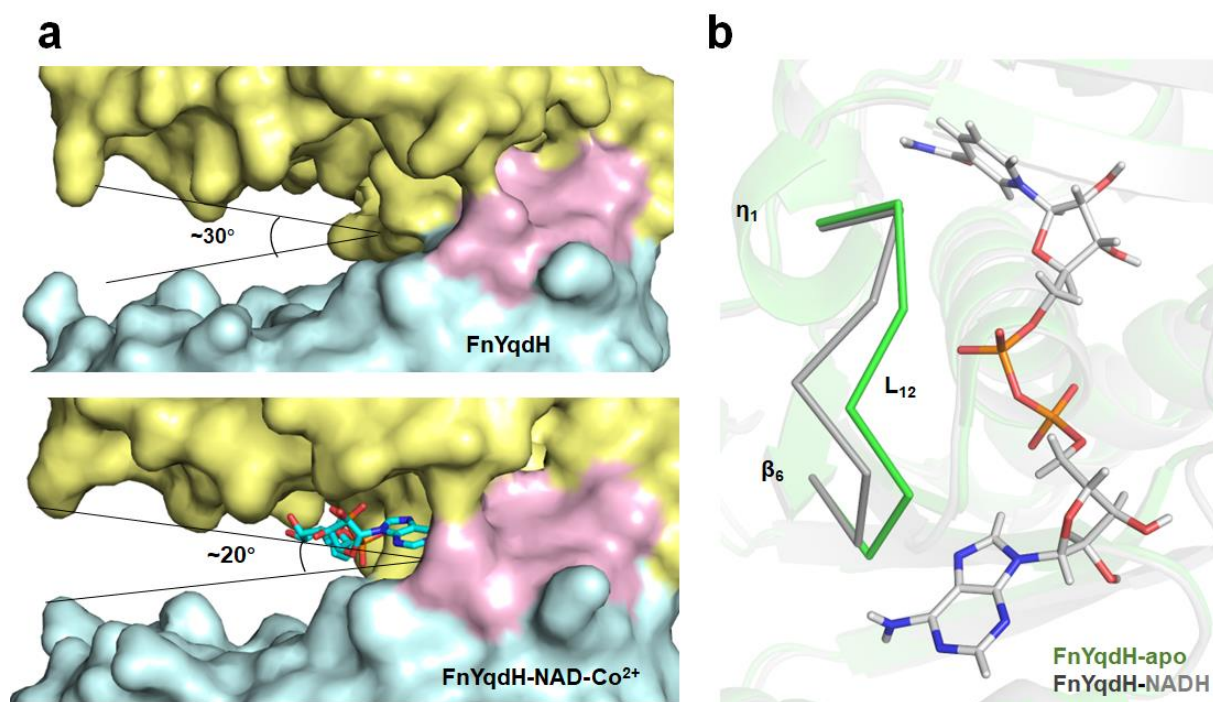
**Figure S6.** Sequence alignment of FnYqdH with BHD from *C. beijerinckii* (CbBDH, Accession No.: WP\_077844128.1), *C. acetobutylicum* (CaBDHA, WP\_010966572.1), *Bacillus megaterium* (BmBDHA, WP\_013085305), and *Zymomonas mobilis* (ZmBDHA, WP\_011241511.1).



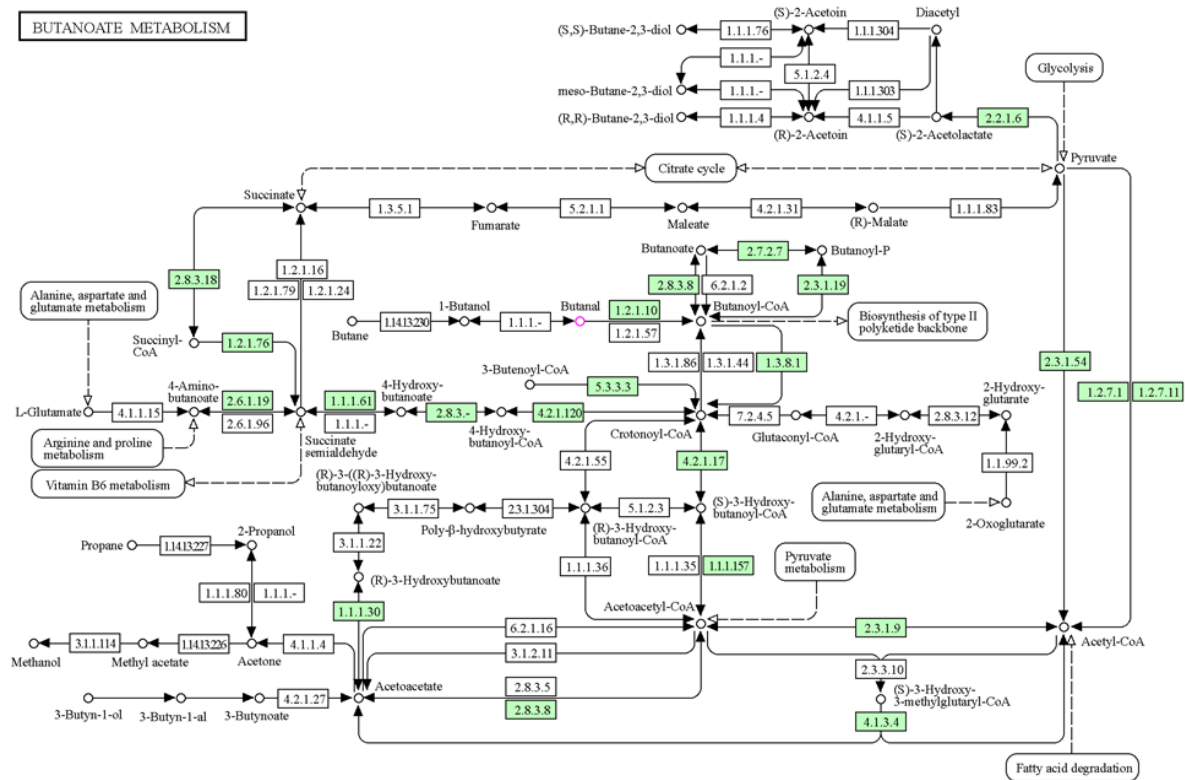


**Figure S7.** Overall fold of FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup>. Comparison of the structures of the FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup>. The crystal structures of the FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup> are colored green and blue, respectively.

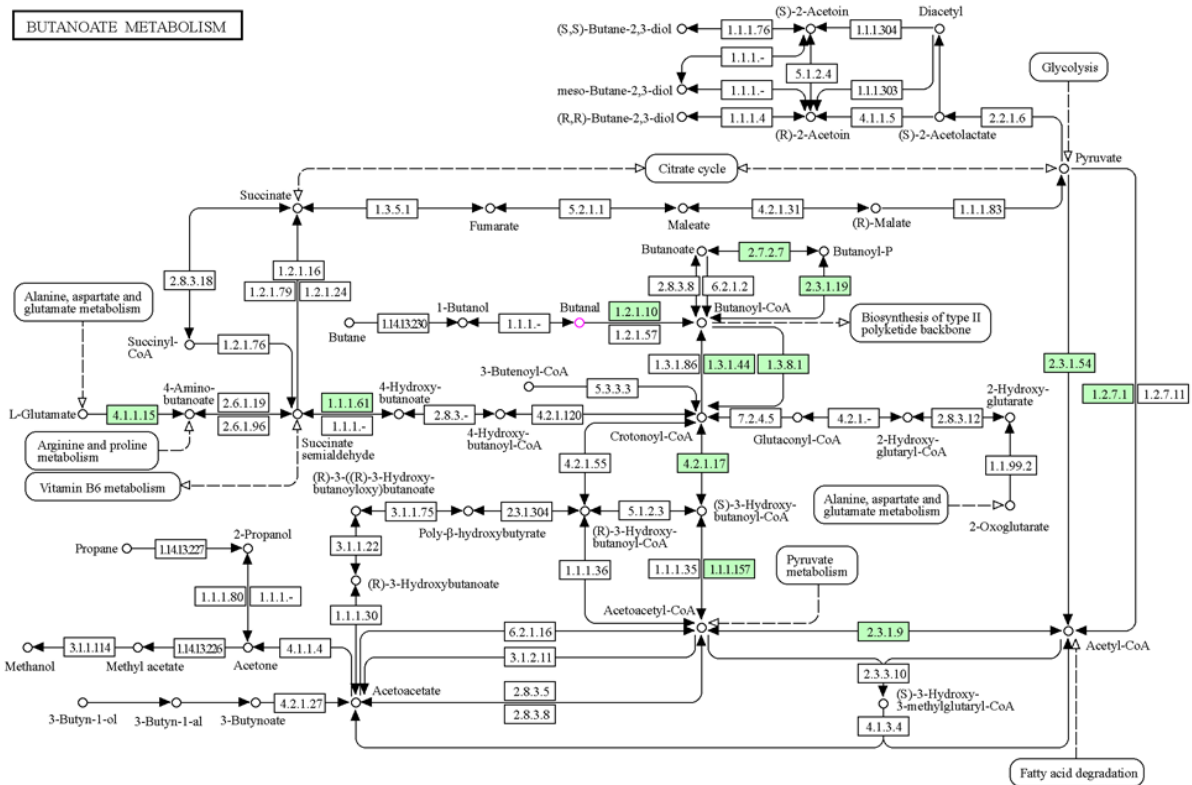




**Figure S8.** Comparison of FnYqdh-apo and FnYqdh-NAD-Co<sup>2+</sup>. (a) Surface comparison of FnYqdh-apo and FnYqdh-NAD-Co<sup>2+</sup>. (b) Cartoon compilation of FnYqdh-apo and FnYqdh-NAD-Co<sup>2+</sup>. FnYqdh-apo and FnYqdh-NAD-Co<sup>2+</sup> are colored green and grey, respectively.



**Figure S9.** Butanoate metabolism of *Clostridioides difficile*.



**Figure S10.** Butanoate metabolism of *Clostridium perfringens*.

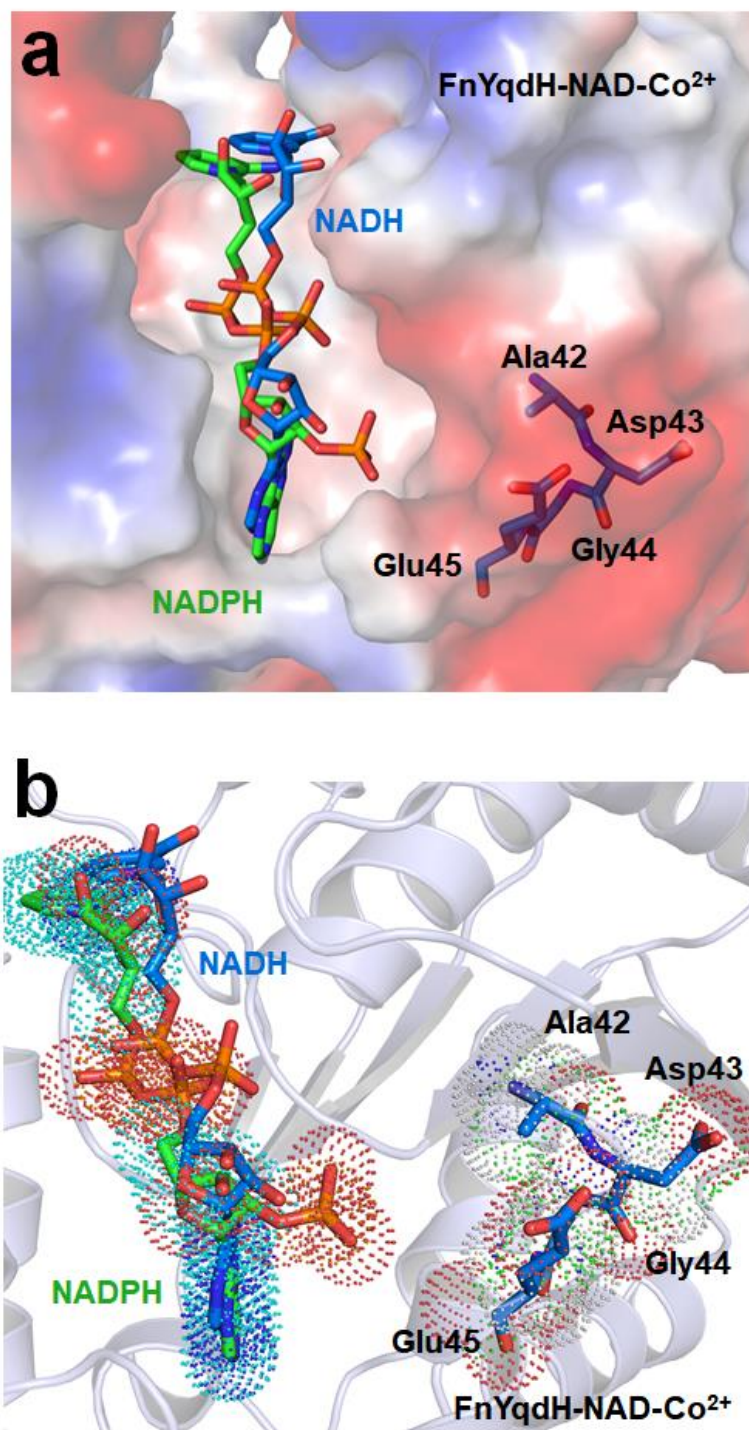
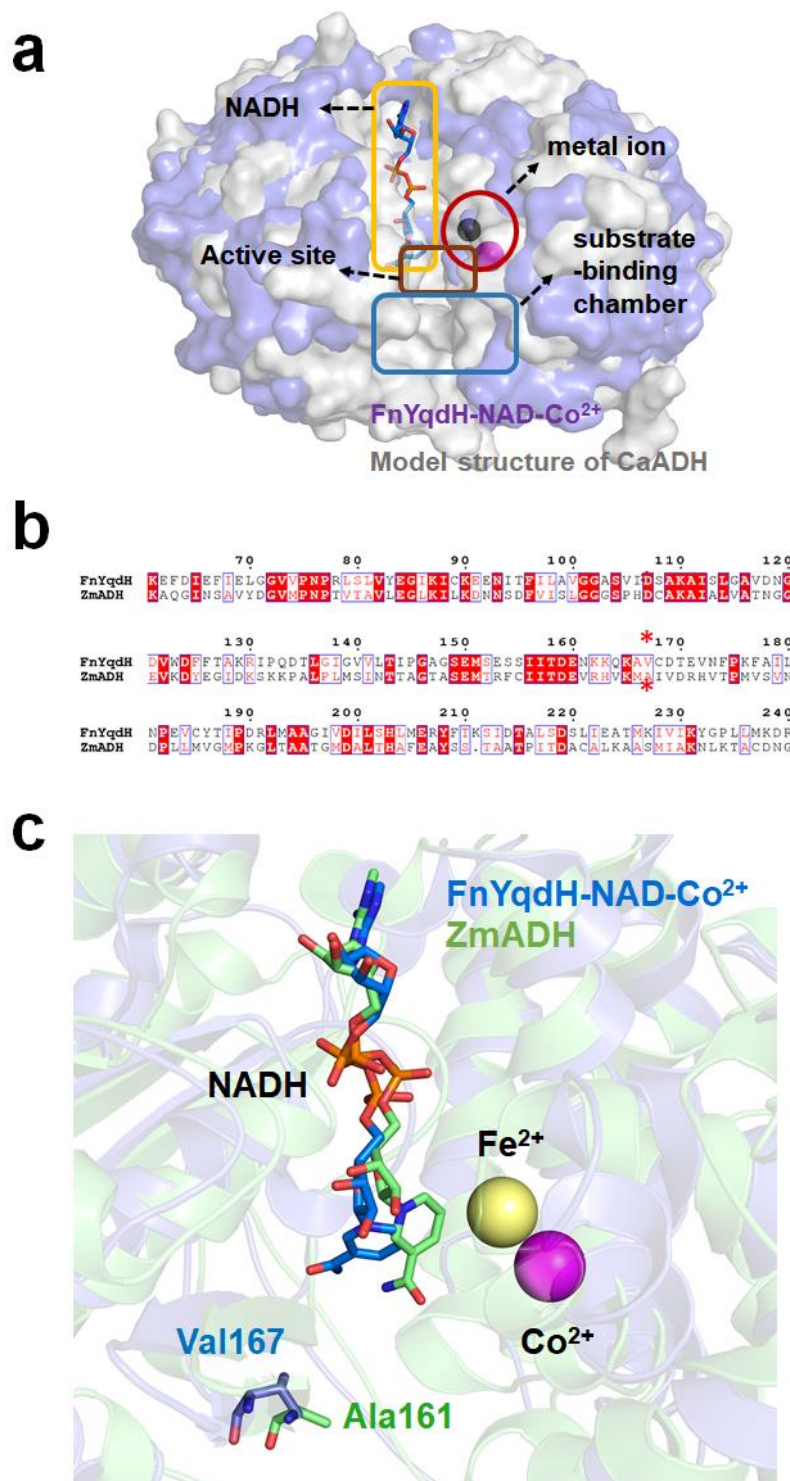


Figure S11. (a) The surface structure of the NAD(P)H-binding pocket of FnYqdh-NAD-Co<sup>2+</sup>, NADH is colored blue and NADPH is colored green. (b) Spatial distribution of NAD(P)H-binding pocket and NAD(P)H.



**Figure S12.** Comparison of FnYqdh-NAD-Co<sup>2+</sup> and other structures. (a) Schematic representation of FnYqdh-NAD-Co<sup>2+</sup> (colored blue) and CaADH (model structure, colored grey). (b) Partial sequence alignment of FnYqdh and ZmADH. The substrate-binding site of ZmBDH is indicated by a red \*. (c) Comparison of FnYqdh-NAD-Co<sup>2+</sup> (colored marine [blue]) and ZmADH (colored green).



**Table S1. Sequence of Primers.**

Primers	Sequence
F-FnYqdH-WT	GGG <u>CCATGG</u> ATATGGACAATTTTAATTATAAAAATGATAC
R-FnYqdH-WT	GGG <u>CTCGAG</u> TTATTTTGCTAAATTTAAGATA
F-FnYqdH-E41A	CTCTTGCATTAT <u>GCA</u> GCAGATGGAGAA
R-FnYqdH-E41A	TTCTCCATCTGC <u>TGC</u> ATAATGCAAGAG
F-FnYqdH-G44A	ATGAGGCAGAT <u>GCA</u> GAATTAATAA
R-FnYqdH-G44A	TTATTAATTC <u>TGC</u> ATCTGCCTCAT
F-FnYqdH-L46A	GCAGATGGAGAA <u>GCA</u> ATAAAAAAACTT
R-FnYqdH-L46A	AAGTTTTTTTAT <u>TGC</u> TTCTCCATCTGC
F-FnYqdH-S104A	GTTGGTGGAGCA <u>GCA</u> AGTTATTGACTCA
R-FnYqdH-S104A	TGAGTCAATAAC <u>TGC</u> TGCTCCACCAAC
F-FnYqdH-D107A	GCAAGTGTTATT <u>GCA</u> TCAGCTAAAGCA
R-FnYqdH-D107A	TGCTTTAGCTGA <u>TGC</u> AATAACACTTGC
F-FnYqdH-T143A	GGTTGTTTTA <u>GCA</u> ATTCCAGGAGCT
R-FnYqdH-T143A	AGCTCCTGGAAT <u>TGC</u> TAAAACAACC
F-FnYqdH-S152A	GGTTCTGAAATG <u>GCA</u> GAAAGCTCTATT
R-FnYqdH-S152A	AATAGAGCTTTC <u>TGC</u> CATTTCAGAACC
F-FnYqdH-K165A	AATAAGAAACAA <u>GCA</u> GCTGTTTGTGAT
R-FnYqdH-K165A	ATCACAAACAGC <u>TGC</u> TTGTTTCTTATT
F-FnYqdH-T187A	GAAGTTTGTTAT <u>GCA</u> ATTCCTGATAGA
R-FnYqdH-T187A	TCTATCAGGAAT <u>TGC</u> ATAACAACTTC
F-FnYqdH-D199A	GCTGGAATTGTA <u>GCA</u> ATTTTATCACAT
R-FnYqdH-D199A	ATGTGATAAAAT <u>TGC</u> TACAATTCCAGC
F-FnYqdH-H203A	GATATTTTATCA <u>GCA</u> TTAATGGAAAGA
R-FnYqdH-H203A	TCTTTCCATTAAT <u>TGC</u> TGATAAAATATC
F-FnYqdH-E206A	TCACATTTAATG <u>GCA</u> AGATATTTTACA
R-FnYqdH-E206A	TGTAAATATCT <u>TGCC</u> CATTAAATGTGA
F-FnYqdH-H272A	GATTGGGCTTCC <u>GCA</u> AGAATTGAACAT
R-FnYqdH-H272A	ATGTTCAATTCT <u>TGC</u> GGAAGCCCAATC
F-FnYqdH-H286A	TATGATTTGACT <u>GCA</u> GGTATTGGTATG
R-FnYqdH-H286A	CATACCAATACC <u>TGC</u> AGTCAAATCATA

The *Nco*I and *Xho*I restriction sites are underlined. The mutant sites are marked red color with underlined.