

Supplementary information

Crystal structure of L-2-keto-3-deoxyfuconate 4-dehydrogenase reveals a unique binding mode as a α -furanosyl hemiketal of substrates

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Table S1. Primers used in the present study

Primer	Sequence
Cloning of the L-KDFDH gene from <i>H. huttiense</i> into pQE-80L ¹	
P1 (BamHI)	5'-catggaTCCGCATCCACTGGCCGCCTCGCCG-3'
P2 (HindIII)	5'-attaagctTTAGTTGGACCACCCACCATCAATC-3'
Cloning of the L-KDFDH gene from <i>P. ehimensis</i> into pQE-80L ¹	
P3 (BamHI)	5'-catggatccAGATTGCACAATAAGGTAACGCTCATTAC-3'
P4 (HindIII)	5'-attaagctTTATGCGTTTTTGCCGAAGACGACG-3'
Cloning of the L-KDFDH gene from <i>P. mikurensis</i> into pQE-80L ¹	
P5 (BglII)	5'-catagatctGAAGTCGACGCGCTGGCCCATCTGAC-3'
P6 (HindIII)	5'-attaagctTCAGGAGCGGCCGCGCTCCAACCC-3'
Cloning of the L-KDFDH gene from <i>S. mizutaii</i> into pQE-80L ¹	
P7 (BglII)	5'-catagatctAGGCTAAAAGATAAAGTAGCGATTGTG-3'
P8 (HindIII)	5'-attaagcttTAGTTATTAGCTTAACAAATCCTCCG-3'
Cloning of the HpaI gene from <i>E. coli</i> into pQE-80L ¹	
P11 (BglII)	5'-catagatctGAAAACAGTTTTAAAGCGGCGCTGAAAG-3'
P12 (HindIII)	5'-attaagctTAATACACGCCGGGCTTCACGGCGG-3'
Site-directed mutagenesis in the L-KDFDH gene from <i>H. huttiense</i> ²	
P13 (V89A-F)	5'-ggctacgcagcggcggcaacattctggag-3'
P14 (V89A-R)	5'-ggccgctgcgtagcccgcgagttgaagag-3'
P15 (R148A-F)	5'-gccaatgcctttgcctacggtgcctccaag-3'
P16 (R148A-R)	5'-ggcaaaggcattggccacgccccttcacgct-3'
P17 (T183A-F)	5'-ccgggcgccattgaatcgccctcgctgaac-3'
P18 (T183A-R)	5'-ttcaatggcgcccgggcagatcgcatcgca-3'
P19 (L189A-F)	5'-ccctcggcggaaccagcgcacgacgcaa-3'
P20 (L189A-R)	5'-ctggttcgcccagggcgattcaatggtgcc-3'
P21 (R192A-F)	5'-aaccaggccatcagcagcgaagccaaggag-3'
P22 (R192A-R)	5'-gctgatggcctggttcagcgagggcgattc-3'
P23 (F211A-F)	5'-gccgcccgcgtggcagccagcccatgggc-3'
P24 (F211A-R)	5'-tgccacggcggcggcgcggacttcgtcttc-3'
P25 (R214A-F)	5'-gtggcagcccagcccatgggcccgcacggc-3'
P26 (R214A-R)	5'-gggctgggtgccacgaaggcggcgaggac-3'
P27 (Q215A-F)	5'-gcacgcgcgcccatgggcccgcacggcaag-3'
P28 (Q215A-R)	5'-catgggcgcgcggtgccacgaaggcggcgcg-3'
P29 (W252A-F)	5'-ggtggggcggtccaactaaagcttaattagc-3'
P30 (W252A-R)	5'-ggtggacgccccaccatcaatcatgtggat-3'
P31 (W252F-F)	5'-ggtgggtttctccaactaaagcttaattagc-3'
P32 (W252F-R)	5'-ggtggagaaccaccatcaatcatgtggat-3'
P33 (W252M-F)	5'-ggtgggatgtccaactaaagcttaattagc-3'
P34 (W252M-R)	5'-Gttggacatcccaccatcaatcatgtggat-3'
P35 (W252R-F)	5'-ggtgggcggtccaactaaagcttaattagc-3'
P36 (W252R-R)	5'-ggtggaccgcccaccatcaatcatgtggat-3'
P37 (T183R-F)	5'-ccgggcaggattgaatcgccctcgctgaac-3'
P38 (T183R-R)	5'-ttcaatcctgcccgggcagatcgcatcgca-3'
P39 (I42R-R)	5'-accgatcgcagcaagacgcacatctggaggag-3'
P40 (I42R-R)	5'-cttgctgcgatcggtagcgatcacgcgcgc-3'
P41 (D41A/I42R-F)	5'-gctaccgctcgcagcaagacgcacatctggag-3'
P42 (D41A/I42R-R)	5'-cttgctgcgagcggtagcgatcacgcgcgc-3'
P43 (D41S/I42R-F)	5'-gctaccagtcgcagcaagacgcacatctggag-3'

P44 (D41S/I42R-R)	5'- <u>cttgctg</u> cgactggtagcgatcacgcgcgc-3'
Site-directed mutagenesis in the L-KDFDH gene from <i>S. mizutaii</i> ²	
P45 (R183T-F)	5'-ccagcaacagtgcatacgccttttgttgat-3'
P46 (R183T-R)	5'-atgcactg <u>ttg</u> ctggtgggagatggaatttga-3'
P47 (F192R-F)	5'-gatggacgc <u>cat</u> cgccaaaaactatccaggt-3'
P48 (F192R-R)	5'-ggcgatg <u>cgt</u> ccatcaacaaaaggcgtatg-3'
P49 (T211R-F)	5'-tctaaacgc <u>cca</u> acctatcggacggatggca-3'
P50 (T211R-R)	5'-aggttggcg <u>ttt</u> tagaaaagtttttcaaaca-3'
¹ Lower case letters indicate additional bases for introducing the underlined digestion sites of restriction enzymes in parentheses.	
² F and R indicate forward and reverse primers, respectively. Underlining indicates mutated regions.	

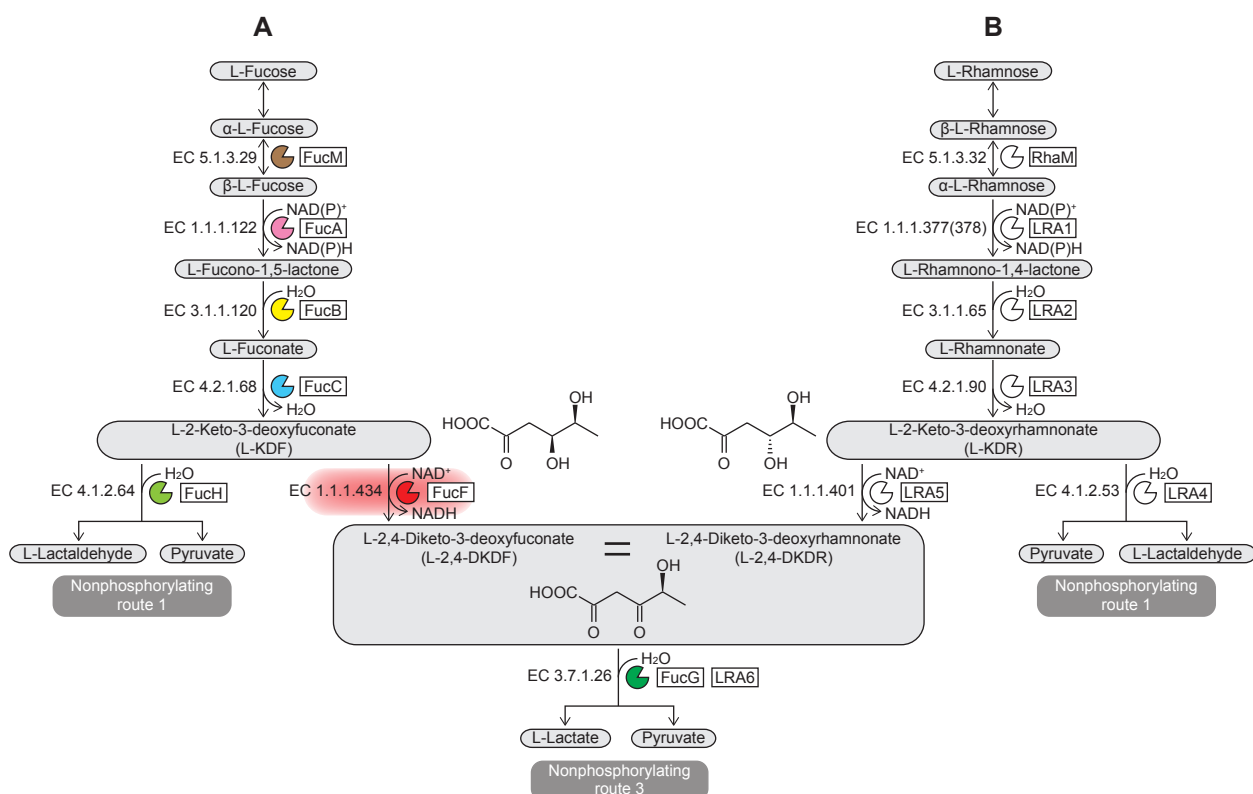


Figure S1. Nonphosphorylating pathways of L-fucose (A) and L-rhamnose (B) metabolism.

L-Fucose and L-rhamnose are commonly converted to the L-2-keto-3-deoxysugar acid intermediates, L-2-keto-3-deoxyfuconate fuconate (L-KDF) and L-2-keto-3-deoxyrhamnonate (L-KDR), respectively, by the consecutive actions of mutarotase, dehydrogenase, lactonase, and dehydratase. One of the metabolic fates of L-KDF and L-KDR is the aldol-cleavage reaction by different aldolases (FucH and LRA4), by which pyruvate and L-lactaldehyde are produced (route 1). In alternative route 3, L-LDF and L-KDR intermediates lose chirality at C4 by the different dehydrogenases (FucF and LRA5), by which the same intermediate as L-2,4-diketo-3-deoxyrhamnonate (L-2,4-diketo-3-deoxyrhamnonate) is produced. Metabolic products are commonly hydrolyzed to pyruvate and L-lactate by hydrolases (FucG or LRA6), which are phylogenetically close each other.

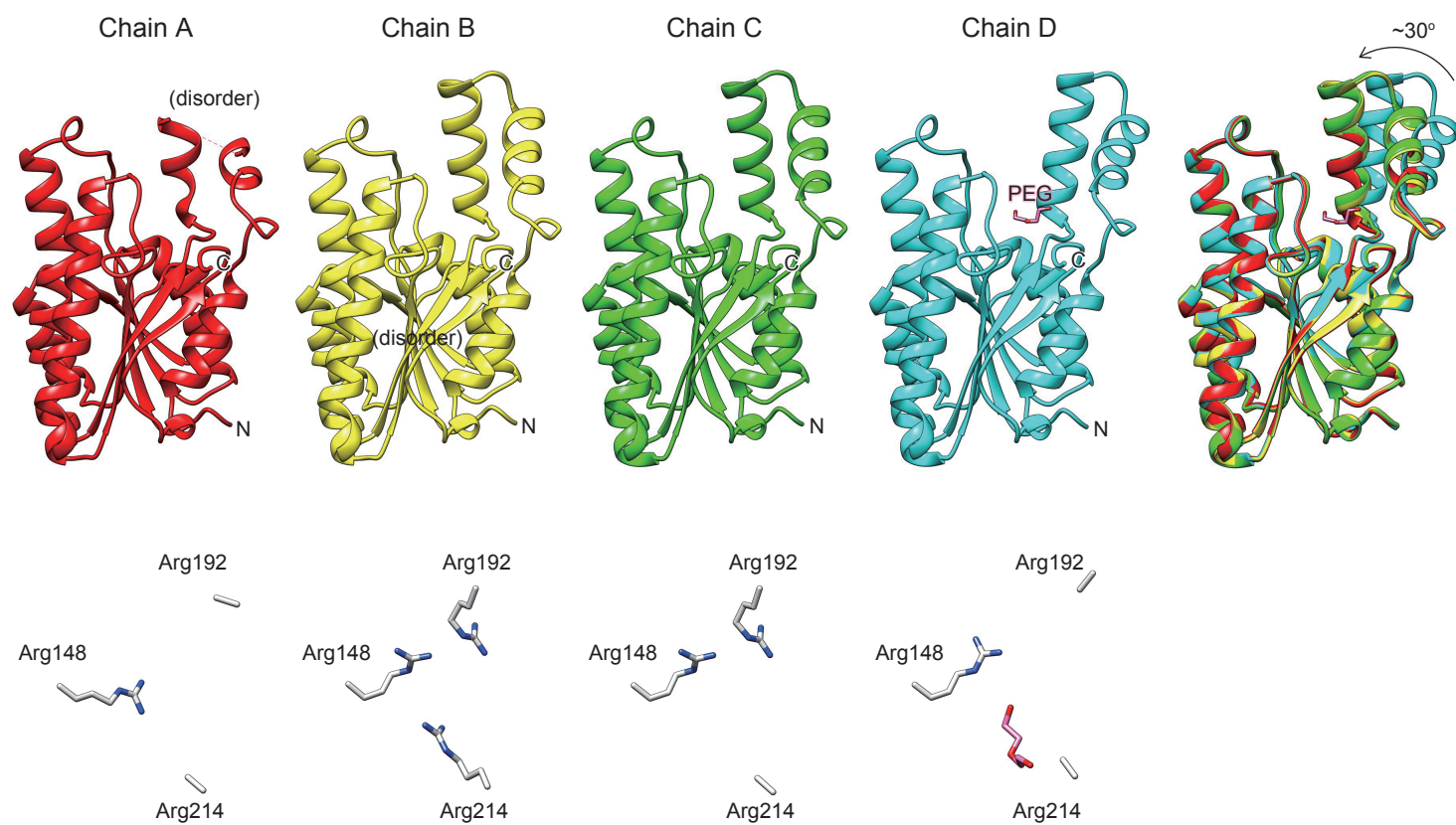


Figure S2. Comparison of the overall structure (upper panel) and three arginine ligands (lower panel) between chains A~D in the ligand-free form of L-KDFFDH from *H. huttiense*.

Chain C was used as the apo-form in the text. The r.m.s.d. values of chain C with chains A, B, and D are 0.469, 0.286, and 0.406 Å, respectively.

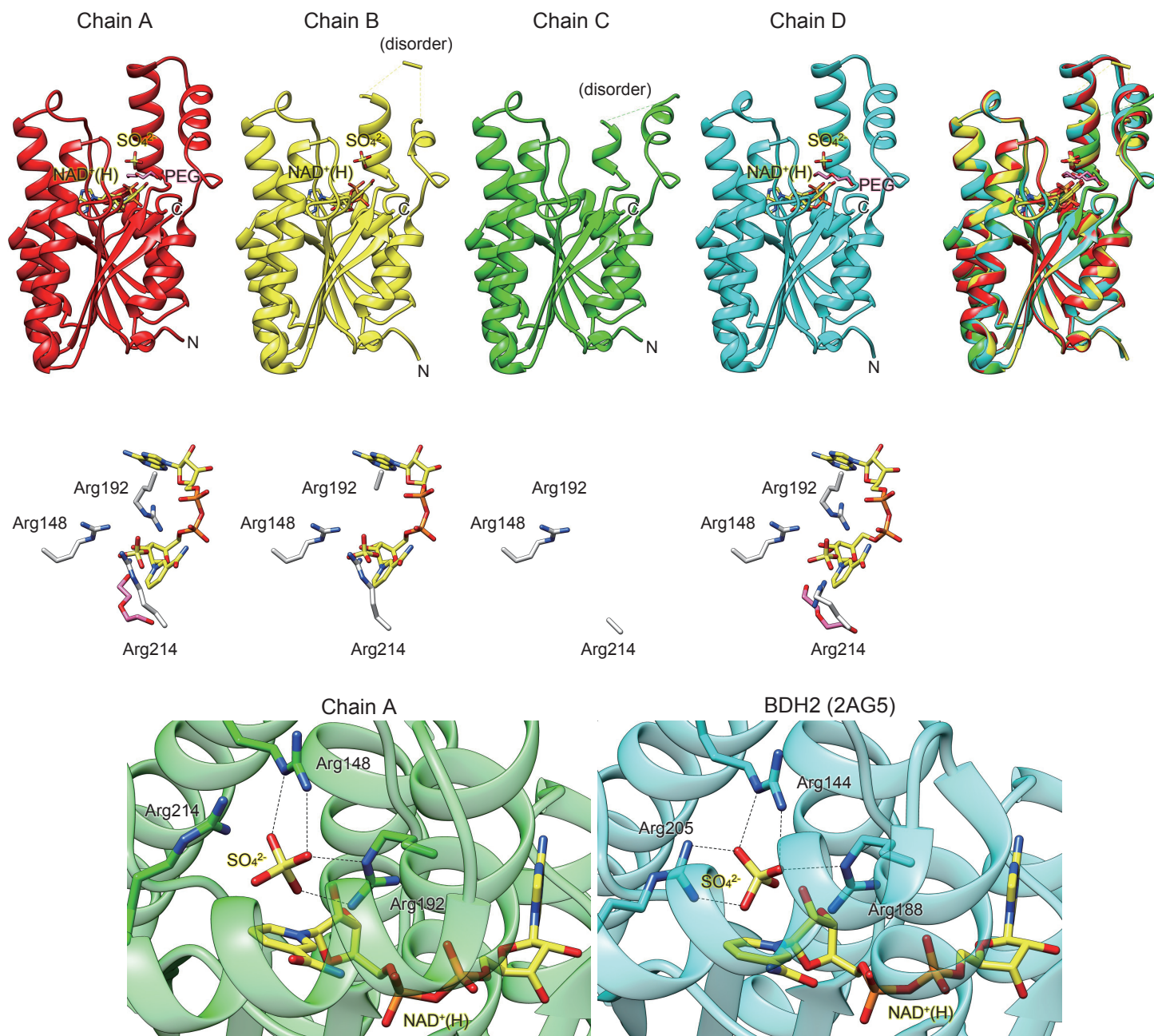


Figure S3. Comparison of the overall structure (upper panel) and three arginine ligands (lower panel) between chains A~D in the sulfate ion and NAD⁺(H) partially bound form of L-KDFDH from *H. huttiense*.

The r.m.s.d. values of chain A with chains B, C, and D are 0.217, 0.435, and 0.185 Å, respectively. Lower panels indicate comparable interaction mode of sulfate ion and NAD⁺(H) between L-KDFDH (left) and BDH2 from human (right).

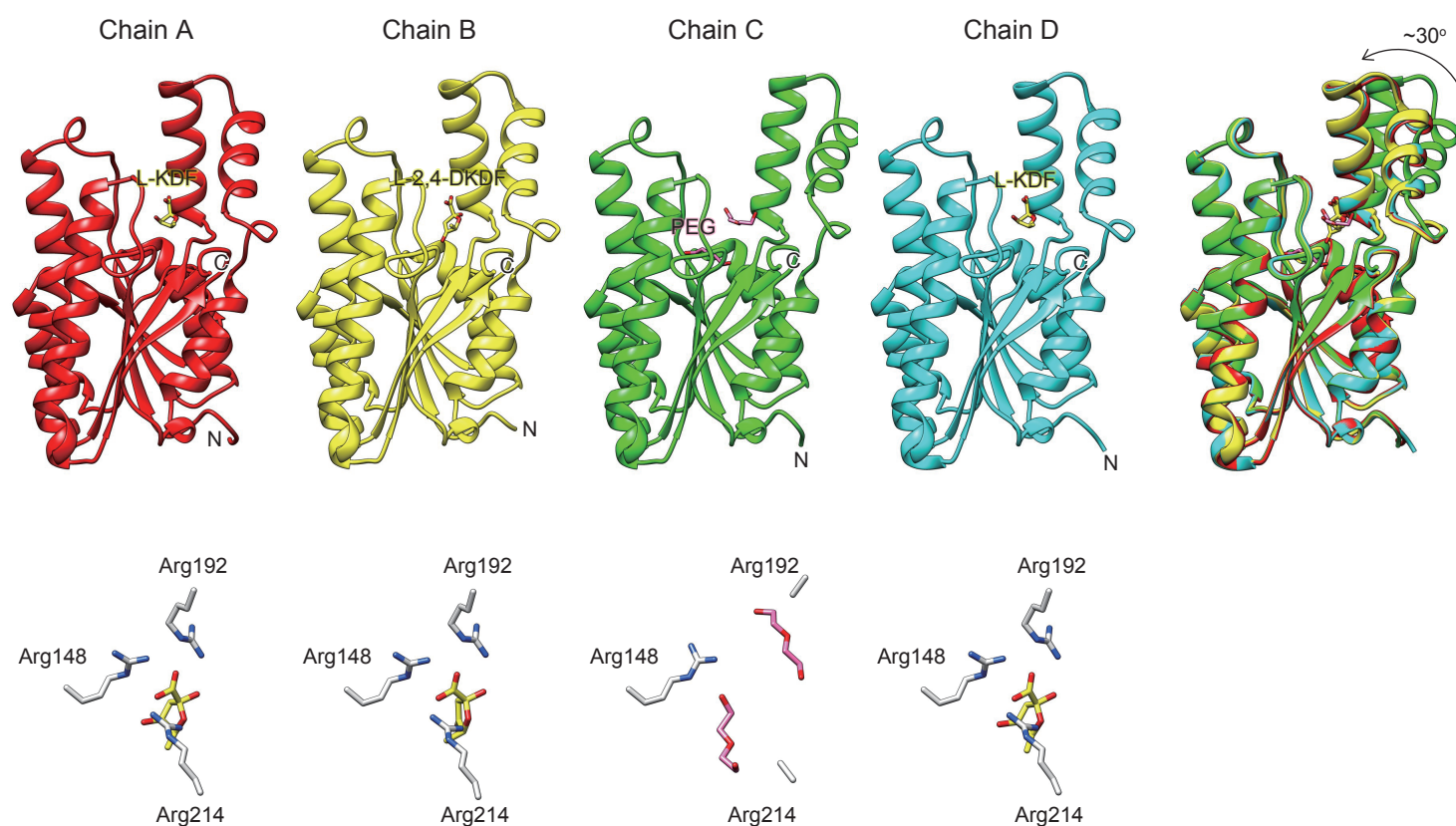


Figure S4. Comparison of the overall structure (upper panel) and three arginine ligands (lower panel) between chains A~D in L-KDF and the L-2,4-LDKDF bound form of L-KDFDH from *H. huttiense*. Chains A and B were used as the L-KDF or L-2,4-DKDF bound forms, respectively, in the text. The r.m.s.d. values of chain A with chains B, C, and D are 0.377, 0.336, and 0.673 Å, respectively.

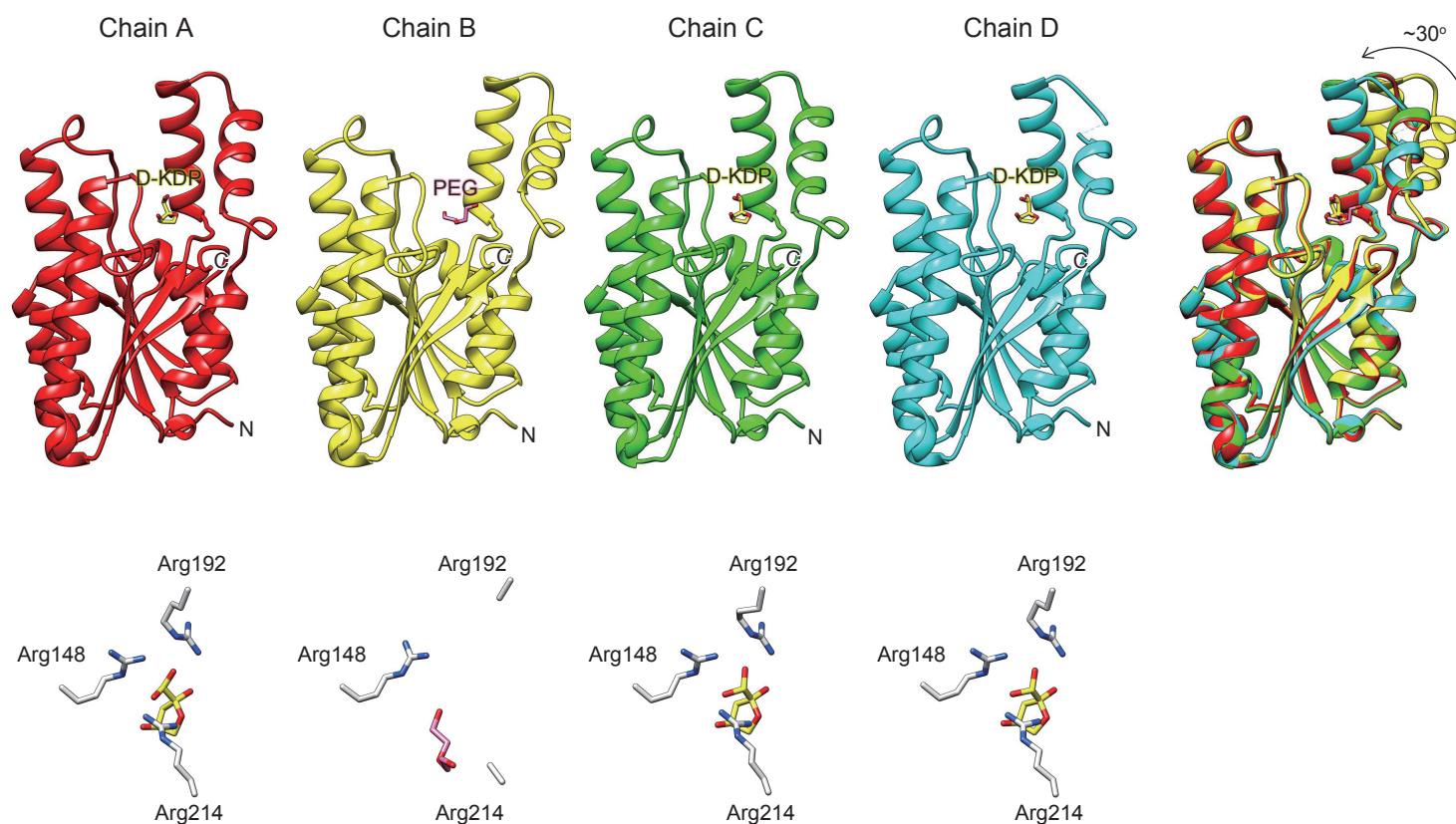


Figure S5. Comparison of the overall structure (upper panel) and three arginine ligands (lower panel) between chains A~D in the D-KDP bound form of L-KDFDH from *H. huttiense*.

Chain C was used as the D-KDP bound form in the text. The r.m.s.d. values of chain C with chains A, B, and D are 0.846, 1.005, and 0.863 Å, respectively.

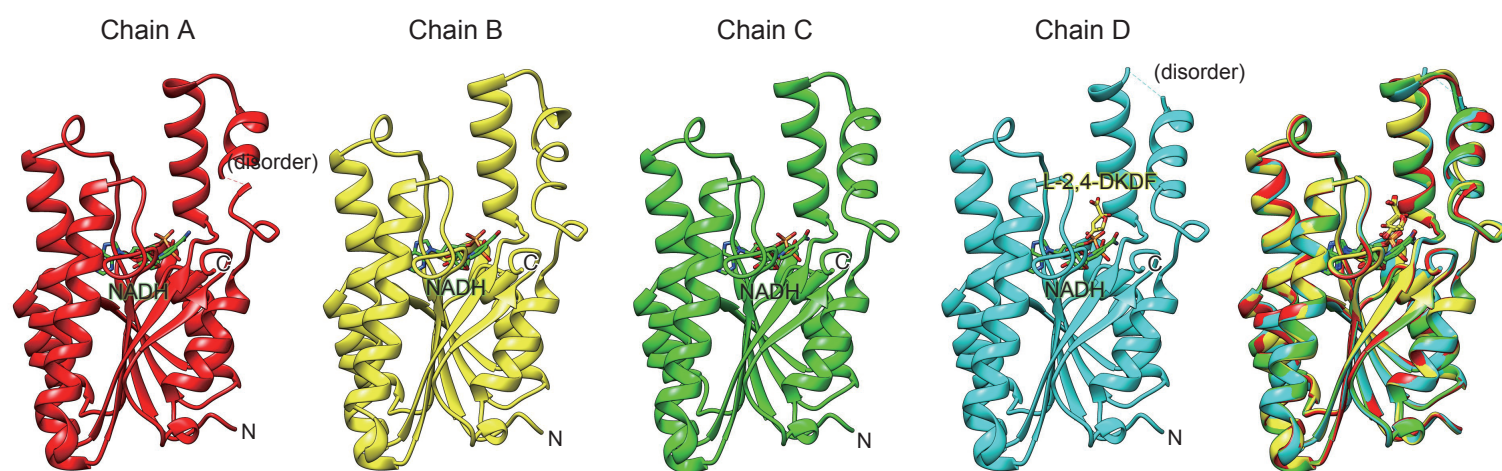


Figure S6. Comparison of the overall structure (upper panel) between chains A~D in NADH and the L-2,4-LDKDF bound form of L-KDFDH from *H. huttiense*.

Chain D was used as the L-2,4-DKDF and NADH bound form in the text. The r.m.s.d. values of chain D with chains A, B, and C are 0.348, 0.801, and 0.345 Å, respectively.

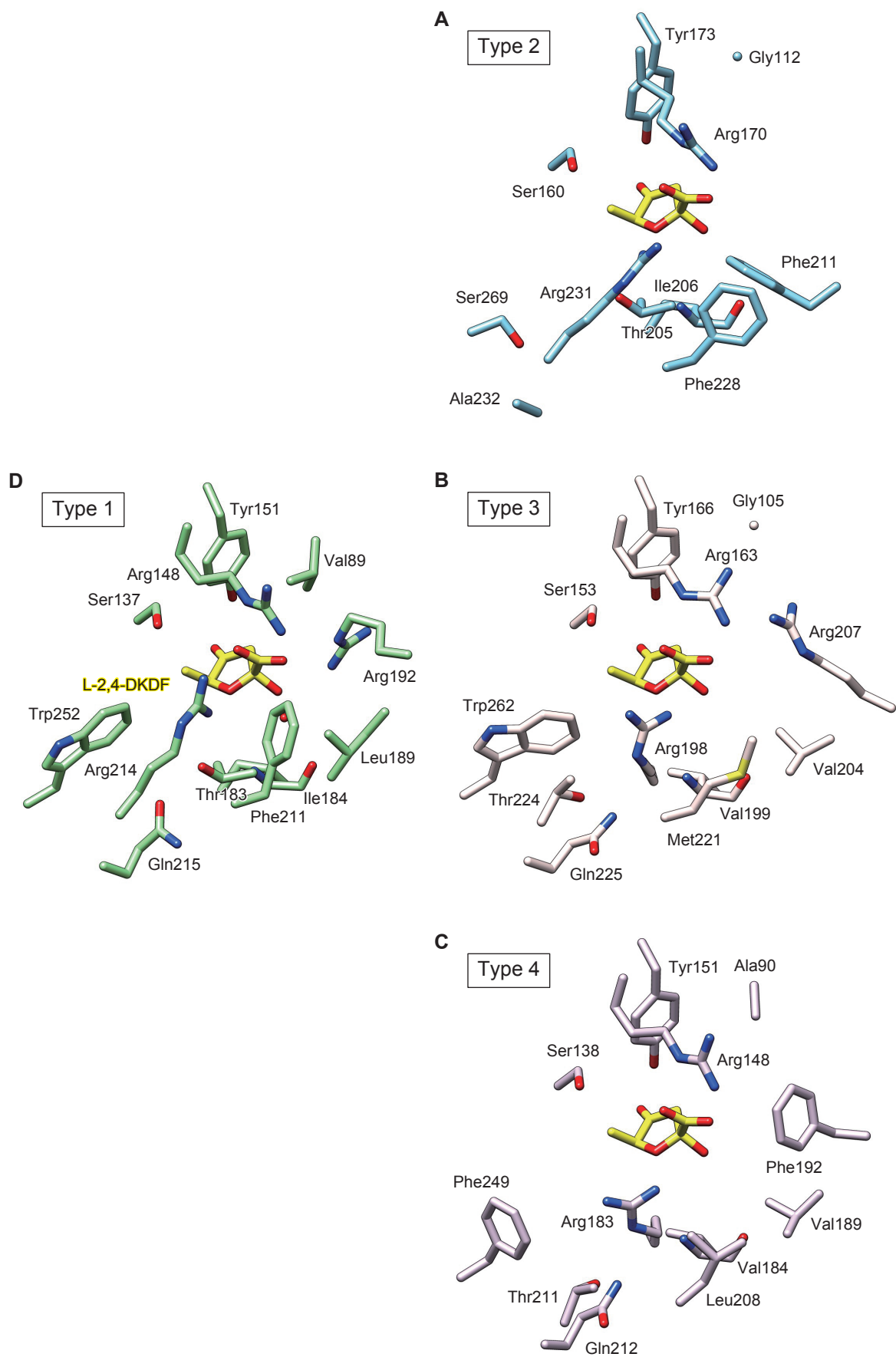


Figure S7. Superposition on structures of type 2 (A), type 3 (B), and type 4 (C) (putative) L-KDFDH on the L-2,4-DKDF bound form of L-KDFDH from *H. huttiense* (D).

(A) A SDR protein from *Rhizobium phaseoli* (4DQX). Models of PSMK_RS04265 (B) and SMI01S_RS12625 (C) constructed using *AlphaFold2*.

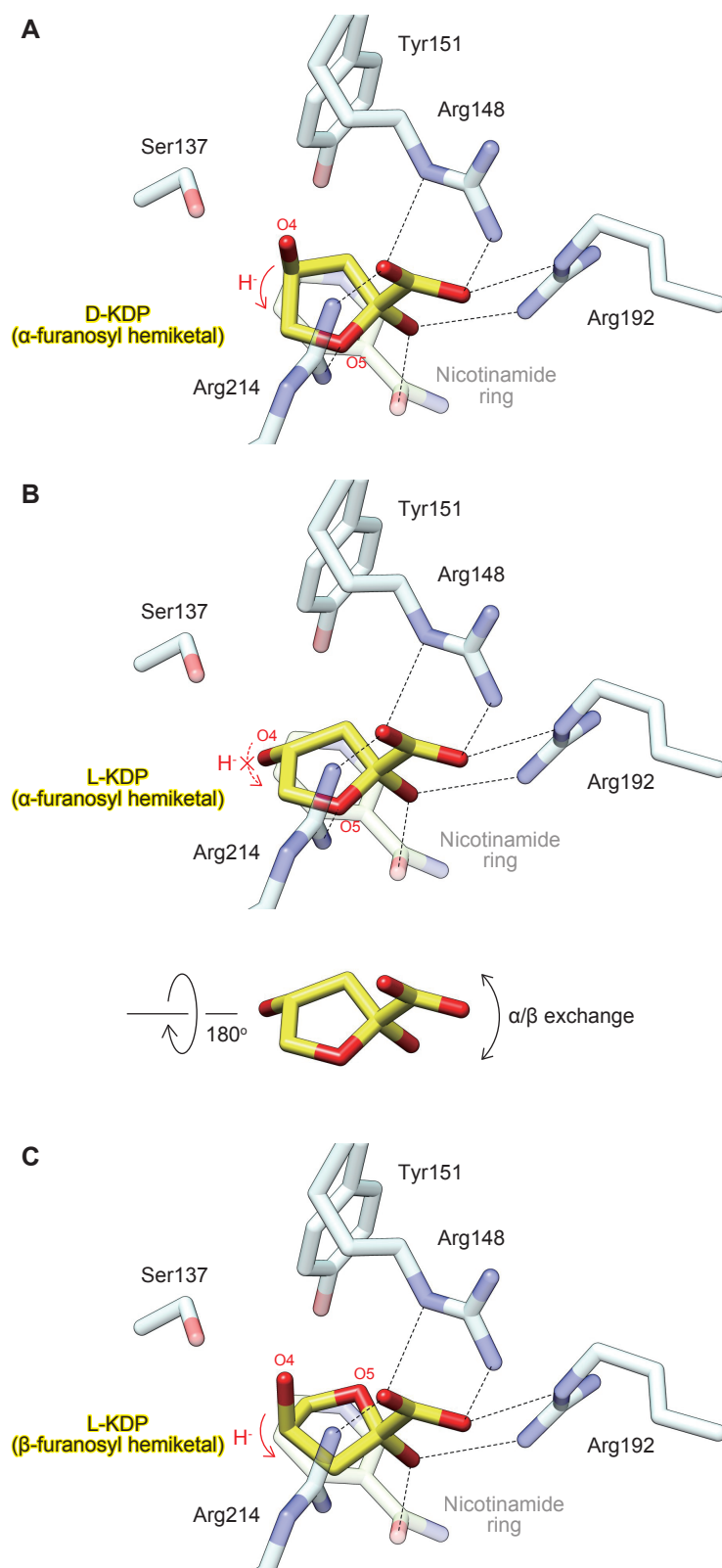


Figure S8. Hypothetical binding mode of L-KDP.

(A) Binding of α -furanosyl hemiketal of D-KDP in the crystal structure of the D-KDP bound form. (B) Model of fitting α -furanosyl hemiketal of L-KDP in the electron density map of D-KDP. The transfer of a hydride ion (H^-) to NAD^+ is impossible. (C) Model of fitting the β -furanosyl hemiketal of L-KDP in the electron density map of D-KDP.

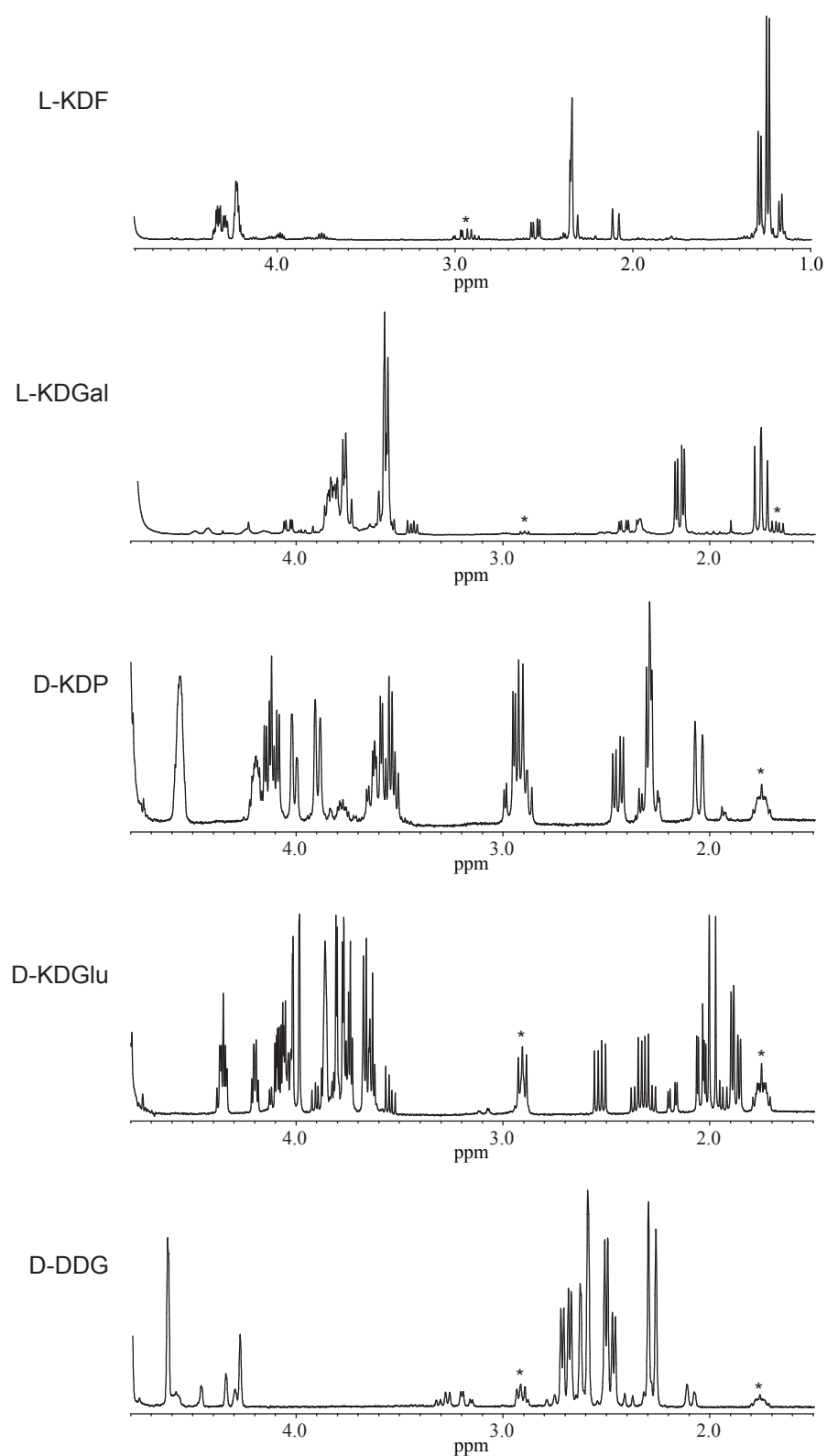


Figure S9. ^1H NMR spectra of 2-keto-3-deoxysugar acid substrates for L-KDFDH.
Asterisks indicate peaks derived from an internal standard.