Supplementary data for:

The assembly of monomeric human L-lactate dehydrogenase into catalytically active homotetramer is hindered by long-chain dicarboxylates

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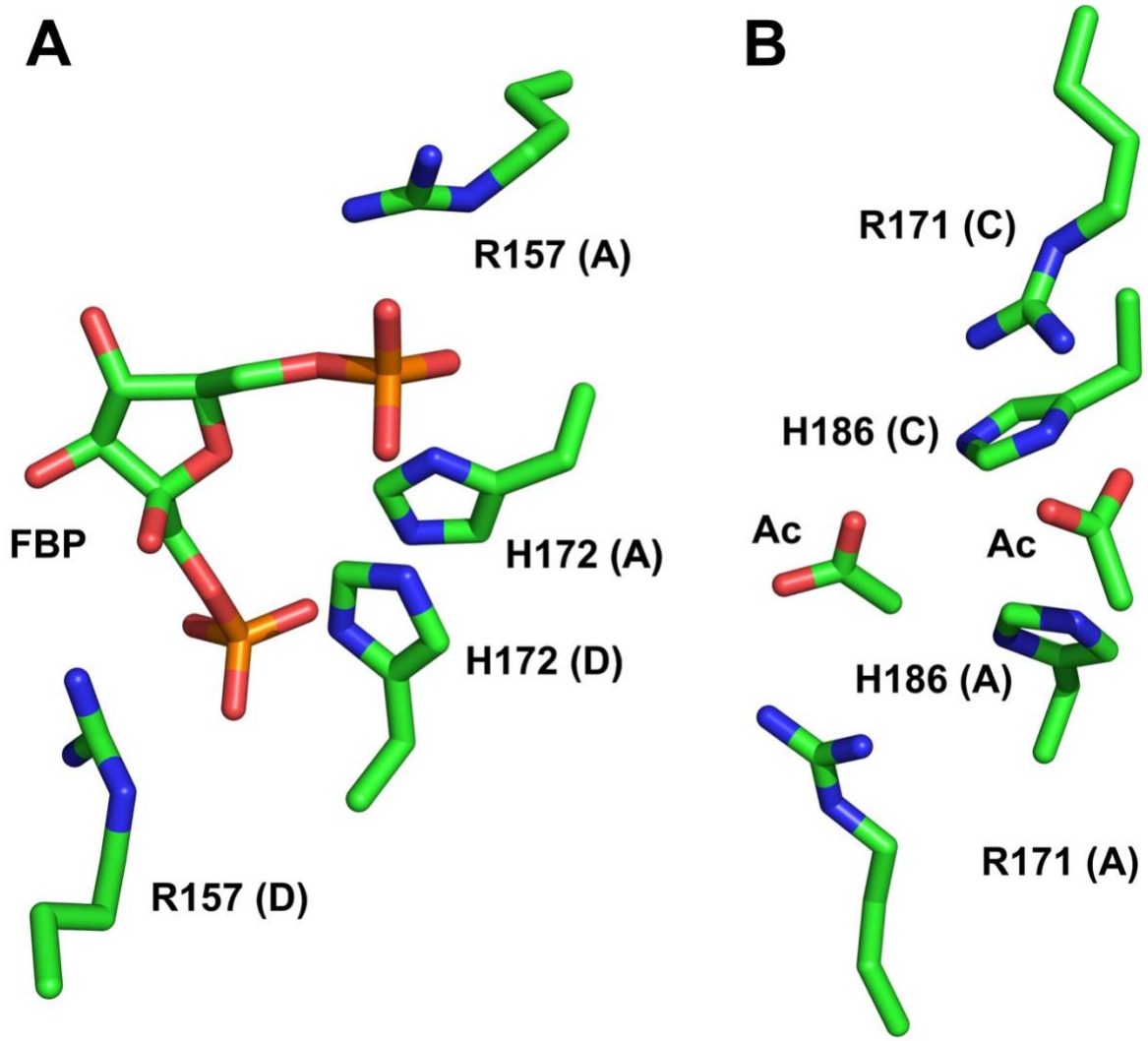
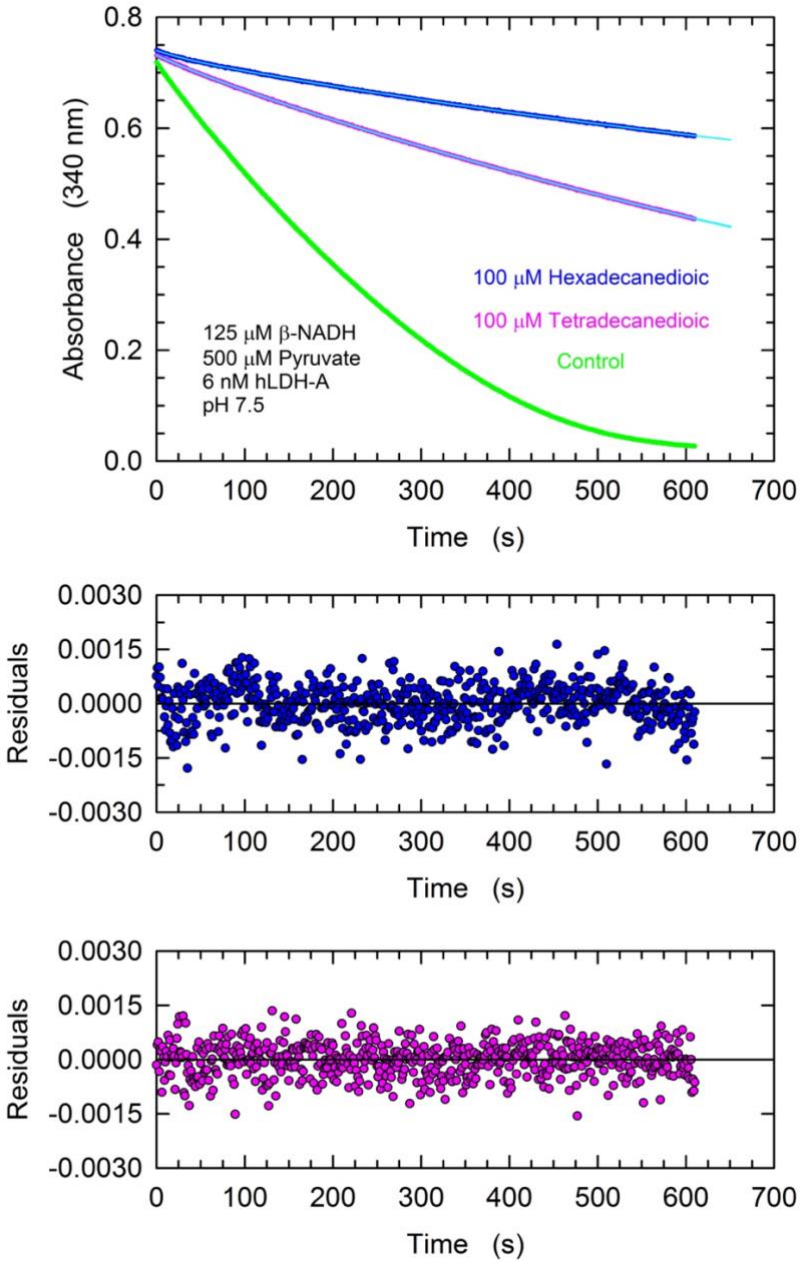


Supplementary Figure S1. Structural detail of the interface between subunit couples of lactate dehydrogenases.
(A) Arginines and histidines belonging to subunits A and D of *Geobacillus stearothermophilus* LDH (PDB file 2LDB) interacting with fructose 1,6-bisphosphate, denoted as FBP. (B) Arginines and histidines from subunits C and A of human LDH-5 (PDB file 1i10) engaged in electrostatic interactions with a couple of acetate (Ac) ions.

Geobacillus stearothermophilus LDH (PDB 2LDB)	
Atoms	**Distance (Å)**
Cα(H172, subunit C)-Phosphate(P_1)	5.1
Cα(R157, subunit C)-Phosphate(P_1)	9.3
Cα(H172, subunit B)-Phosphate(P_2)	5.5
Cα(R157, subunit B)-Phosphate(P_2)	9.7
Cα(H172, subunit A)-Phosphate(P_2)	5.5
Cα(R157, subunit A)-Phosphate(P_2)	9.7
Cα(H172, subunit D)-Phosphate(P_1)	5.1
Cα(R157, subunit D)-Phosphate(P_1)	9.3
Homo sapiens LDH-A (PDB 1i10)	
Atoms	**Distance (Å)**
Cα(H186, subunit C)-Acetate(C_2)	5.9
Cα(R171, subunit C)-Acetate(C_2)	9.9
Cα(H186, subunit A)-Acetate(C_2)	6.0
Cα(R171, subunit A)-Acetate(C_2)	9.7
Cα(H186, subunit D)-Acetate(C_2)	6.0
Cα(R171, subunit D)-Acetate(C_2)	9.8
Cα(H186, subunit B)-Acetate(C_2)	5.9
Cα(R171, subunit B)-Acetate(C_2)	9.8

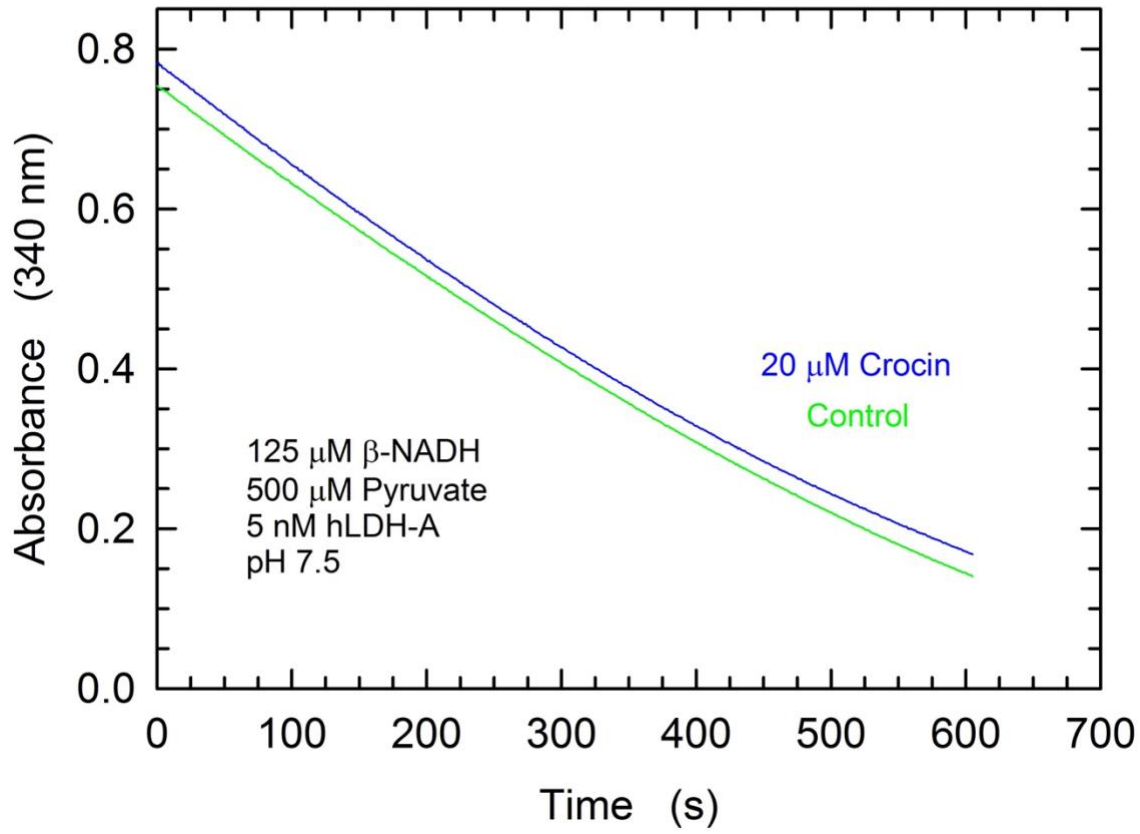
Supplementary Table ST1.
Distance from the α carbon of arginines and histidines to the phosphates of fructose 1,6-bisphosphate or to the C_2 carbons of acetate ions residing at the interface between the subunits of lactate dehydrogenase from *Geobacillus stearothermophilus* and *Homo sapiens*.



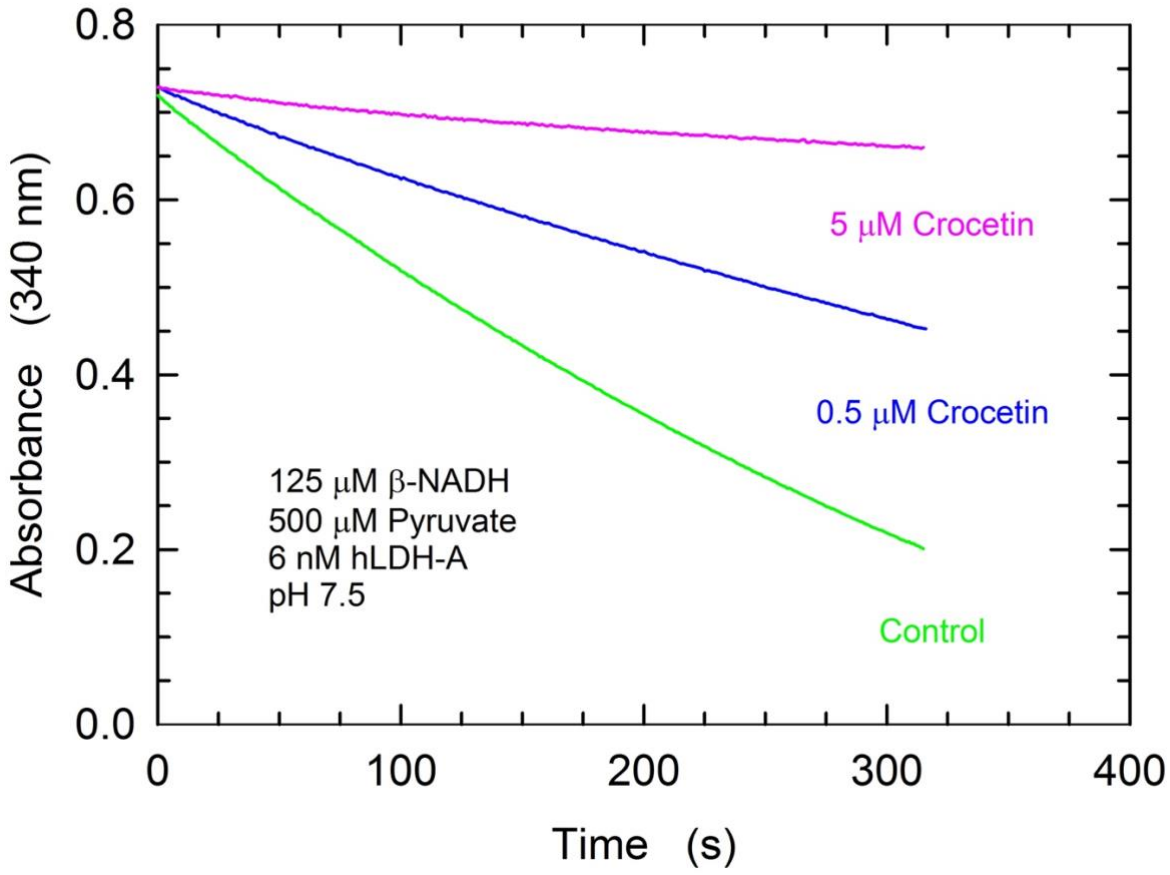
Supplementary Figure S2. Kinetics of β-NADH oxidation coupled to the reduction of pyruvate to L-lactate.
The time-course of the Absorbance decrease at 340 nm was determined in the presence of 125 μM β-NADH, 500 μM pyruvate, 6 nM monomeric hLDH-A (in 10 mM Tris-HCl, pH 7.5). Assays were performed in the absence (green line) or in the presence of 100 μM tetradecanedioic or hexadecanedioic acid (magenta and blue line, respectively). The cyan lines represent the best fit of a double-exponential equation to the experimental observations.

	Tetradecanedioic acid	Hexadecanedioic acid
k_{obs} 1 (s^{-1})	$(7.0\pm0.1)\bullet10^{-4}$	$(8.0\pm0.1)\bullet10^{-4}$
Amplitude 1 (Abs)	0.722±0.009	0.377±0.005
Amplitude 1 (μM)	116±1	61±1
k_{obs} 2 (s^{-1})	0.017±0.001	0.028±0.002
Amplitude 2 (Abs)	0.0101±0.0004	0.0085±0.0002
Amplitude 2 (μM)	1.62±0.06	1.37±0.03

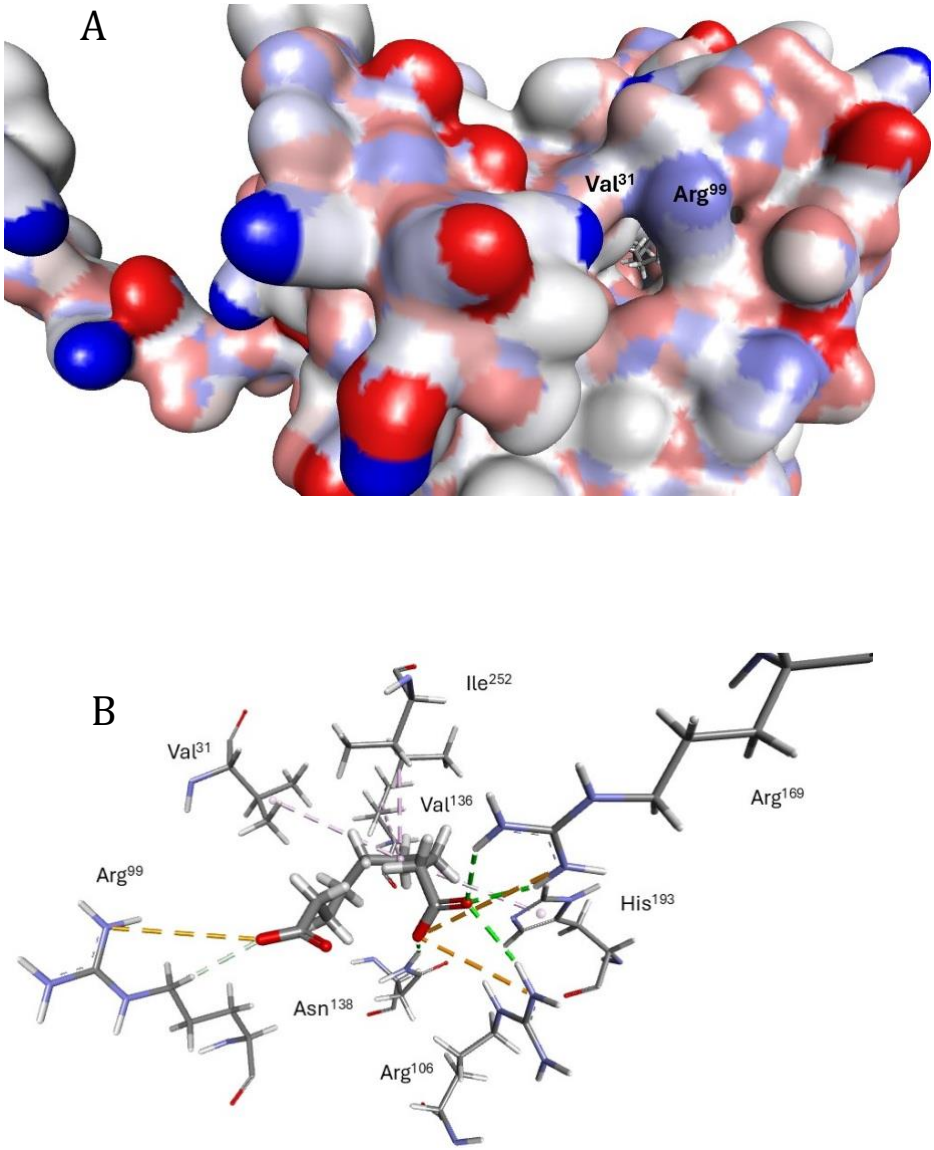
Supplementary Table ST2.
Kinetic rate constants and amplitudes determined by fitting a double-exponential equation to the experimental observations obtained by performing activity assays in the presence of 125 μM β-NADH, 500 μM pyruvate, 6 nM monomeric hLDH-A (in 10 mM Tris-HCl, pH 7.5), and 100 μM tetradecanedioic or hexadecanedioic acid. The corresponding data are shown in Supplementary Figure S2.



Supplementary Figure S3. Catalytic activity of monomeric hLDH-A in the absence or in the presence of crocin.
The time-course of the Absorbance decrease at 340 nm was determined in the presence of 125 μM β-NADH, 500 μM pyruvate, 5 nM monomeric hLDH-A (in 10 mM Tris-HCl, pH 7.5). Assays were performed in the absence (green line) or in the presence of 20 μM crocin (blue line).



Supplementary Figure S4. Inhibition by crocetin of monomeric hLDH-A.
The time-course of the Absorbance decrease at 340 nm was determined in the presence of 125 μM β-NADH, 500 μM pyruvate, 6 nM monomeric hLDH-A (in 10 mM Tris-HCl, pH 7.5). Assays were performed in the absence (green line) or in the presence of 0.5 or 5 μM crocetin (blue and magenta line, respectively).



Supplementary Figure S5. Binding mode of azelaic acid to chain B of hLDH-5.
Best-scoring pose (-4.726 kcal/mol) calculated for azelaic acid by molecular docking analysis with Swissdock, using the tertiary structure of chain B of hLDH-5 (PDB ID: 1i10). The structures were represented using the Discovery Studio Visualizer 2025 application; ligands are rendered in bold sticks and colored by atoms. (A) The protein is represented by its solid solvent-accessible surface, colored by the atomic interpolated charge. (B) Interactions of azelaic acid with chain B of hLDH-5. Receptor's residues are shown with thin sticks. The interactions are reported as dotted lines using the following colors: H-bonds, green; pi-alkyl, grey; pi-pi, pink; salt bridge, yellow.