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Supporting information for article:

Crystallographic binding studies of rat peroxisomal multifunctional enzyme type 1 with 3-ketodecanoyl-CoA: capturing active and inactive states of its hydratase and dehydrogenase catalytic sites

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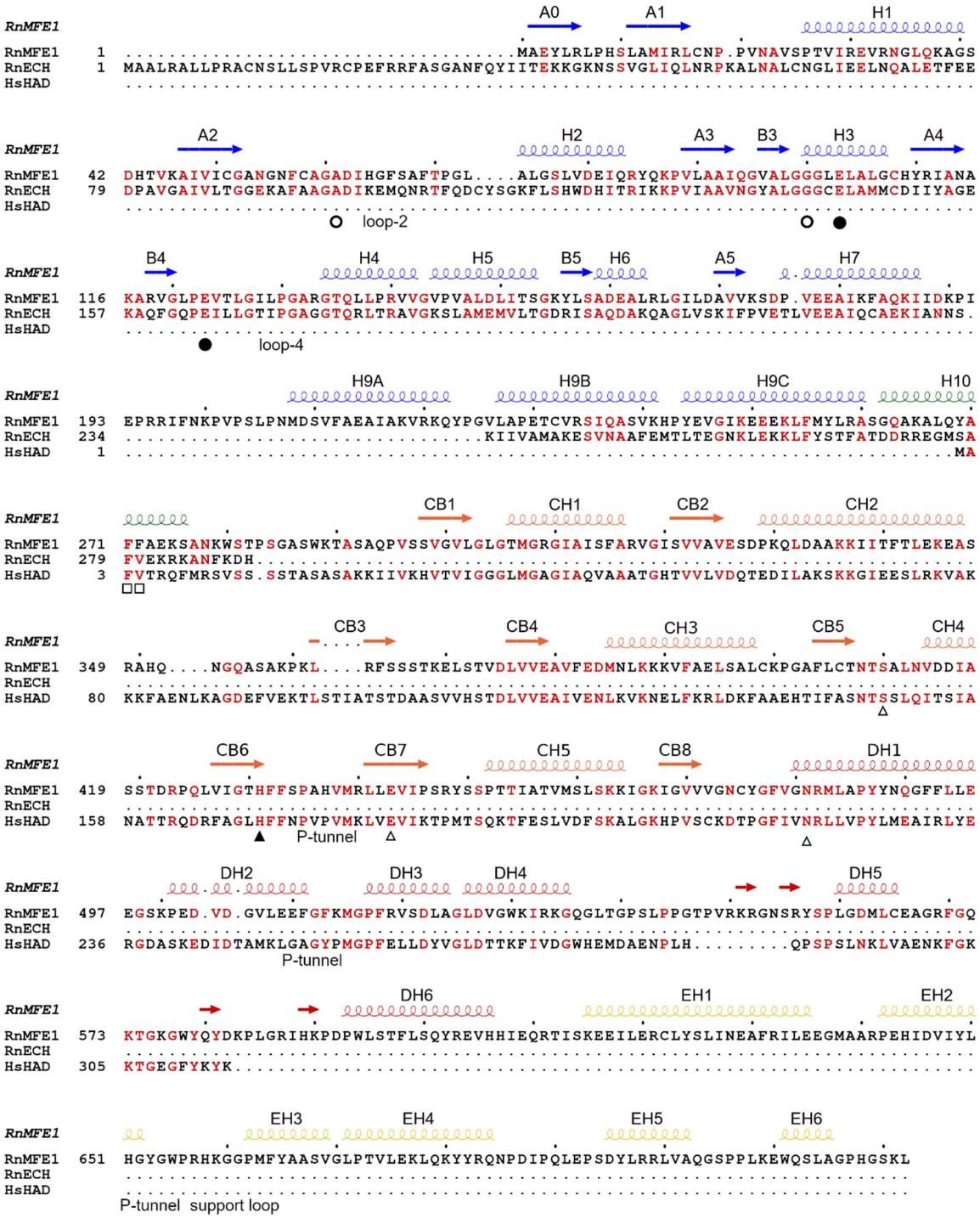

Figure S1 Sequence alignment of the RnMFE1 sequence with the sequences of the corresponding monofunctional homologues, RnECH and HsHAD. The top line provides the secondary structure information of the RnMFE1 structure. The color coding of the secondary structure elements follows the division in 5 domains of the MFE1 structure, as shown also in Fig. 4. ● labels the ECH catalytic residues, o identifies the oxyanion hole residues of the ECH active site, ▲identifies the HAD catalytic residue, Δ labels other HAD active site residues. □ highlights Phe271 and Phe272 of the linker helix of RnMFE1.

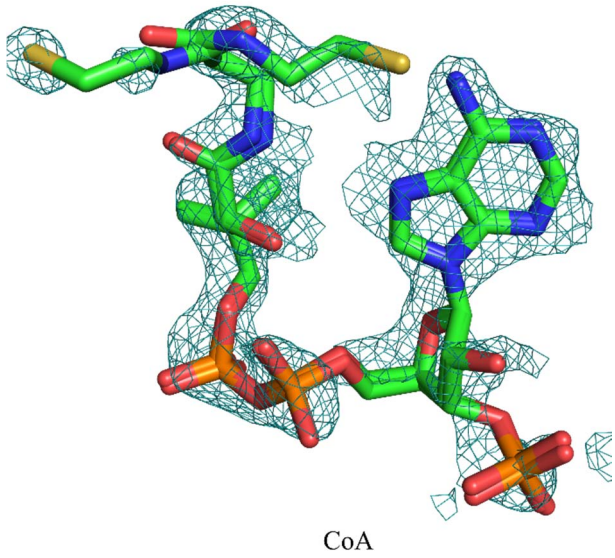

Figure S2 Superposition of bound ligand and its 2Fo-Fc omit map. CoA bound at the ECH active site of the single-molecule structure (contour level of the map is 0.9sigma). The cysteamine part of the CoA molecule has been built in two conformations.

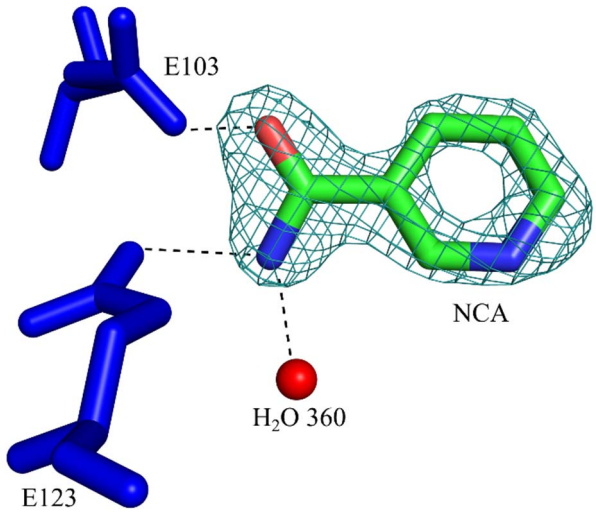

Figure S3 Superposition of bound ligand and its 2Fo-Fc omit map. Nicotinamide (NCA) bound to the ECH active site of the single-molecule structure (contour level of the map is 1.7sigma). The water (red sphere, H_2O 360) is bound in the oxyanion hole.

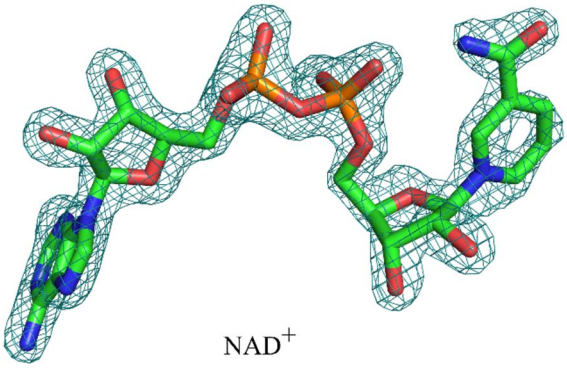

Figure S4 Superposition of bound ligand and its 2Fo-Fc omit map. NAD^+ bound to the HAD active site of the single-molecule structure (contour level of the map is 1.5sigma).

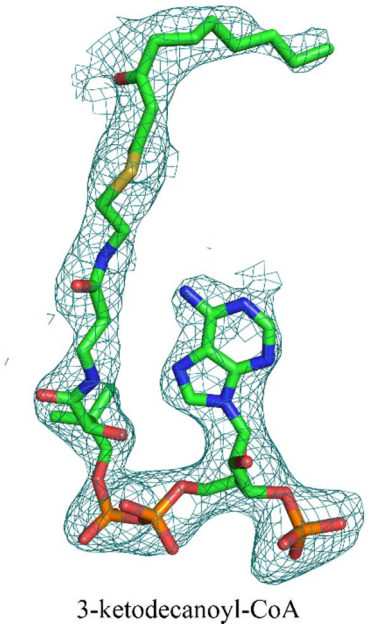

Figure S5 Superposition of bound ligand and its 2Fo-Fc omit map. 3-ketodecanoyl-CoA bound to the ECH active site of molecule A of the 3keto-1mM-NAD^+ structure (contour level of the map is 1.0sigma).

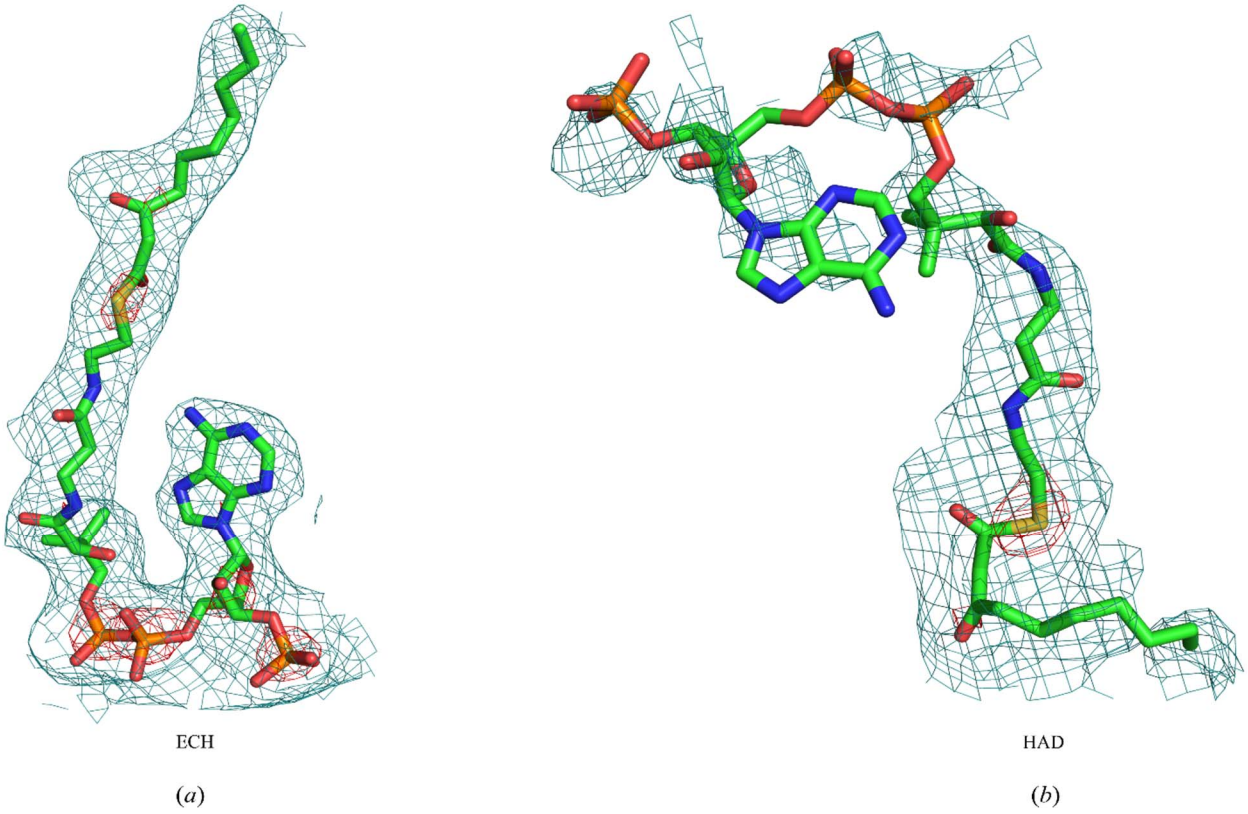

Figure S6 Superposition of bound ligand and its PHENIX polder omit map. (a) 3-ketodecanoyl-CoA bound to the ECH active site of molecule B of the 3keto-1mM-NAD^+ structure, contoured ar 3sigma (blue) and 10sigma (red). (b) 3-ketodecanoyl-CoA bound to the HAD active site of molecule A of the 3keto-3mM-NAD^+ structure, contoured at 3sigma (blue) and 7sigma (red).

Video S1. Conformational flexibility of MFE1. In this animation the four structures of molecule A are shown, superimposed using the Cα-atoms of the residues of domain D, as described in Table 4. Green: A-6Z5O (single-molecule). Cyan: B-5MGB. Yellow: A-5MGB. Orange: A-6Z5F (3keto-3mM-NAD^+).

Table S1 Crystallization and crystal treatment conditions.

Structure	single-molecule	HAD-3keto	3keto-1mM-NAD^+	3keto-3mM-NAD^+
Protein buffer (1 µL)	8 mg/mL dissolved in 10 mM PIPES, pH 6.5, and 50 mM NaCl, that was supplemented with 2 mM CoA and incubated for 30 minutes at room temperature.	8 mg/mL dissolved in 10 mM PIPES, pH 6.5, and 50 mM NaCl, that was supplemented with 2 mM CoA and incubated for 30 minutes at room temperature.	8 mg/mL dissolved in 10 mM PIPES, pH 6.5, and 50 mM NaCl, that was supplemented with 2 mM CoA and incubated for 30 minutes at room temperature.	8 mg/mL dissolved in 10 mM PIPES, pH 6.5, and 50 mM NaCl, that was supplemented with 2 mM CoA and incubated for 30 minutes at room temperature.
Well solution (1 µL)	75 mM MES, pH 6.0, 125 mM ammonium sulfate, 16% w/v PEG4000.	100mM MES pH6.0, 150 mM ammonium sulfate, 15% w/v PEG4000.	125 mM MES, pH 6.0, 175 mM ammonium sulfate, 17% w/v PEG4000.	100 mM MES, pH 6.0, 150 mM ammonium sulfate, 15% w/v PEG4000.
Crystal soaking solution (in 1 µL drop)	The crystal was transferred in a drop of 1 µL well solution, supplemented with 2 mM NAD^+ and 20% glycerol for 10 minutes.	The crystal was washed in a drop of well solution overnight and subsequently soaked overnight in a drop of well solution supplemented with 0.2 mM *2E*-decenoyl-CoA.	The crystal was washed in a drop of 1 µL well solution overnight and subsequently soaked overnight in a drop of well solution supplemented with 1 mM 3-ketodecanoyl-CoA and 2 mM NAD^+	The crystal was washed in a drop of 1 µL well solution overnight and subsequently soaked overnight in a drop of well solution supplemented with 3 mM 3-ketodecanoyl-CoA and 2 mM NAD^+
Cryocooling	The crystal was subsequently cryocooled in liquid nitrogen.	The crystal was quickly moved through a drop of well solution supplemented with 15% glycerol, 0.2 mM *2E*-decenoyl-CoA, 0.2 mM NAD^+ and cryocooled in liquid nitrogen.	The crystal was quickly moved through a drop of well solution, supplemented with 1 mM 3-ketodecanoyl-CoA, 2 mM NAD^+ and 20% glycerol and cryocooled in liquid nitrogen.	The crystal was quickly moved through a drop of well solution, supplemented with 3mM 3-ketodecanoyl-CoA, 2 mM NAD^+ and 20% glycerol and cryocooled in liquid nitrogen.
PDB entry	6Z5O	5OMO	6Z5V	6Z5F

Table S2 Cα-Cα distances in the ECH active site of RnMFE1 and RnECH.

Structure	PDB entry	N-terminal end of the catalytic helix, helix H3	In the middle of the linker-helix, helix H10	Distance (Å)
RnMFE1, unliganded	3ZW8	Gly100	Phe271	A: 17.5 B: 17.1
RnMFE1, AcAc-CoA in ECH active site and NAD^+ in HAD active site	5MGB	Gly100	Phe271	A: 17.3 B: 16.6
RnMFE1, single-molecule	6Z5O	Gly100	Phe271	A: 17.1
RnMFE1 HAD-3keto	5OMO	Gly100	Phe271	A: 17.2 B: 16.5
RnMFE1, 3keto-1mM-NAD^+	6Z5V	Gly100	Phe271	A: 17.4 B: 16.7
RnMFE1, 3keto-3mM-NAD^+	6Z5F	Gly100	Phe271	A: 17.3 B: 17.1
RnECH, unliganded (subunit D) and liganded with AcAc-CoA (subunits A, B, C, E, F)	1DUB	Gly141	Phe279	A: 15.6 B: 15.6 C: 15.6 D: 15.4 E: 15.6 F: 15.6

Table S3 Cα-Cα distances in the HAD active site of RnMFE1 and HsHAD.

Structure	PDB entry	At the N-terminus of the pyrophosphate binding helix	C-terminal end of helix DH3	Distance (Å)
RnMFE1, unliganded	3ZW8	Thr306	Ala524	A: 10.9 B: 13.6
RnMFE1, AcAc-CoA in ECH active site and NAD^+ in HAD active site	5MGB	Thr306	Ala524	A: 10.2 B: 12.8
RnMFE1, single-molecule	6Z5O	Thr306	Ala524	A: 12.4
RnMFE1, HAD-3keto	5OMO	Thr306	Ala524	A: 9.6 B: 13.7
RnMFE1, 3keto-1mM-NAD^+	6Z5V	Thr306	Ala524	A:10.3 B:13.6
RnMFE1, 3keto-3mM-NAD^+	6Z5F	Thr306	Ala524	A: 9.8 B: 13.2
RnMFE1, BCDE-construct, unliganded	1ZCJ	Thr306	Ala524	A: 12.8
HsHAD, liganded with AcAc-CoA and NAD^+	1F0Y	Leu25	Val253	A: 8.3
HsHAD, lliganded with 3S-hydroxybutanoyl-CoA	1F12	Leu25	Val253	A: 10.8
HsHAD, unliganded	1F14	Leu25	Val253	A: 12.0