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Supporting information for article:

3-Sulfinopropionyl-coenzyme A (3SP-CoA) desulfinase from *Advenella mimigardefordensis* DPN7^{T}: crystal structure and function of a desulfinase with an acyl-CoA dehydrogenase fold

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Table S1 List of primers used for PCR. If primer name begins with "P" the primer was 5'-phosphorylated. Underlined nucleotides differ from the native sequence in order to generate amino acid mutations. All primers were purchased from MWG Biotech AG, Ebersberg, Germany.

oligonucleotide	**sequence (5'-3')**[a]
P_DPN7_R84K_rev	GCGCAAGCTTTAGCCATTTCTTCAATG
P_DPN7_R84K_fwd	CACTATGGGAAAAATAACAGTGGACTCAAATCTG
P_DPN7C122S_fwd	ACCTGCAAGAACAAGGTCAGCCGC
P_DPN7C122S_rev	GATAAGCCGGCGATCAGTATATCAGAGCC
P_DPN7Q246E_fwd	GAAGAGGGCGTAGCCTATCTTAAGCGC
P_DPN7Q246E_rev	GAAGGCGCATTCTGCAATACCCA

Table S2 Overall folding of Acd_{DPN7}.

Secondary structure	**Apo, monomer A**	**Apo, monomer B**	**Holo, monomer A**	**Holo, monomer F**
α-helix A	4-34	5-34	5-34	5-34
α-helix B	37-46	37-47	37-47	37-47
α-helix C	55-58	56-58	56-58	56-58
α-helix D	65-87	65-87	65-88	65-88
α-helix E	93-100	93-100	93-100	93-100
α-helix F	103-115	103-115	103-115	103-115
β-strands 1	120-123	120-123	120-123	120-123
β-strands 2	137-142	137-140	137-140	137-140
β-strands 3	145-156	145-156	145-156	145-156
β-strands 4	163-172	163-172	163-172	163-172
β-strands 5	175-185	175-185	175-185	175-185
β-strands 6	194-198	194-199	193-199	193-199
β-strands 7	209-221	210-221	210-222	210-222
α-helix G	233-272	233-271	237-271	237-271
α-helix H	284-308	283-308	283-308	283-308
α-helix I	315-345	316-346	316-346	316-346
α-helix K	357-380	353-380	353-380	352-380

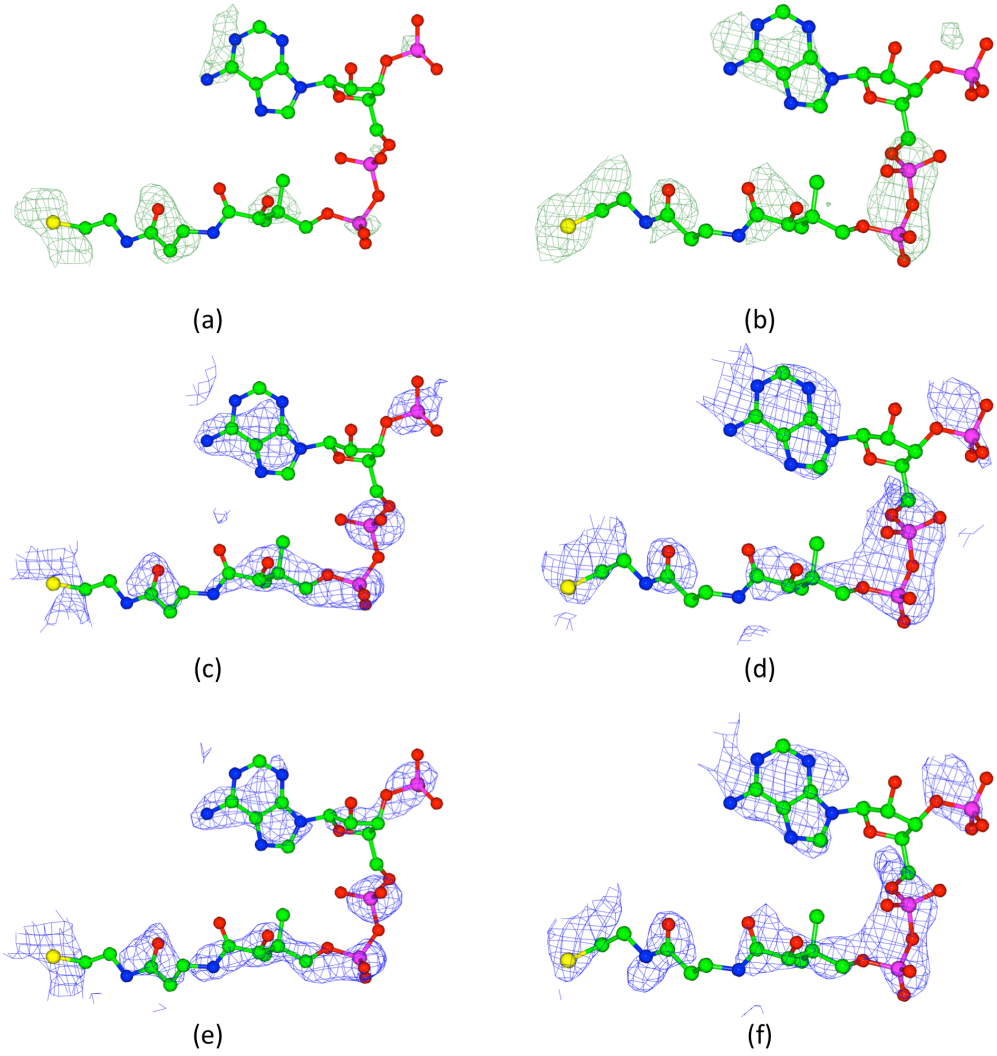

Figure S1 The coenzyme A groups of monomers A (left column) and E (right column) (depicted in ball-and-stick with atoms color coded: carbon = green; oxygen = red; phosphor = magenta; blue = nitrogen; yellow = sulfur) fitted into: a-b) the F*o*-F*c* difference Fourier OMIT map (green mesh; contour level 3.0σ, calculated without the substrate; c-d) the 2F*o*-F*c* difference Fourier map (blue mesh; contour level 1.0σ) calculated with of PHENIX; e-f) the 2F*o*-F*c* difference Fourier map (blue mesh; contour level 2.0σ) calculated using the "feature enhanced" option of PHENIX.

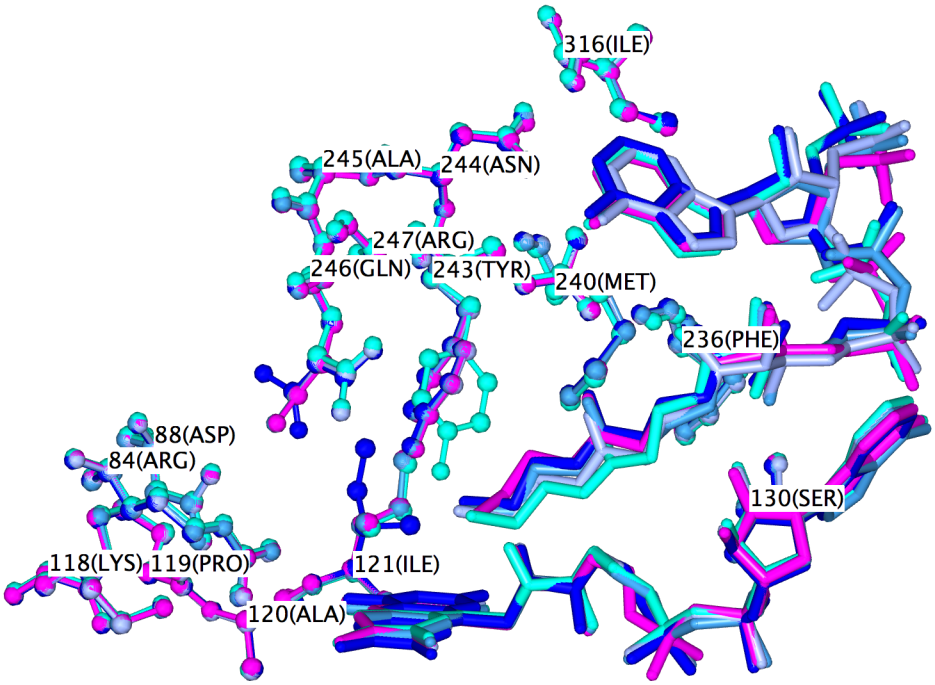

Figure S2 Superimposition of monomer B (color ice blue), D (light blue), E (magenta) and F (blue) to monomer A (cyan) showing the high correlation between position of coenzyme A within the cavity of Acd_{DPN7} 3SP-CoA desulfinase

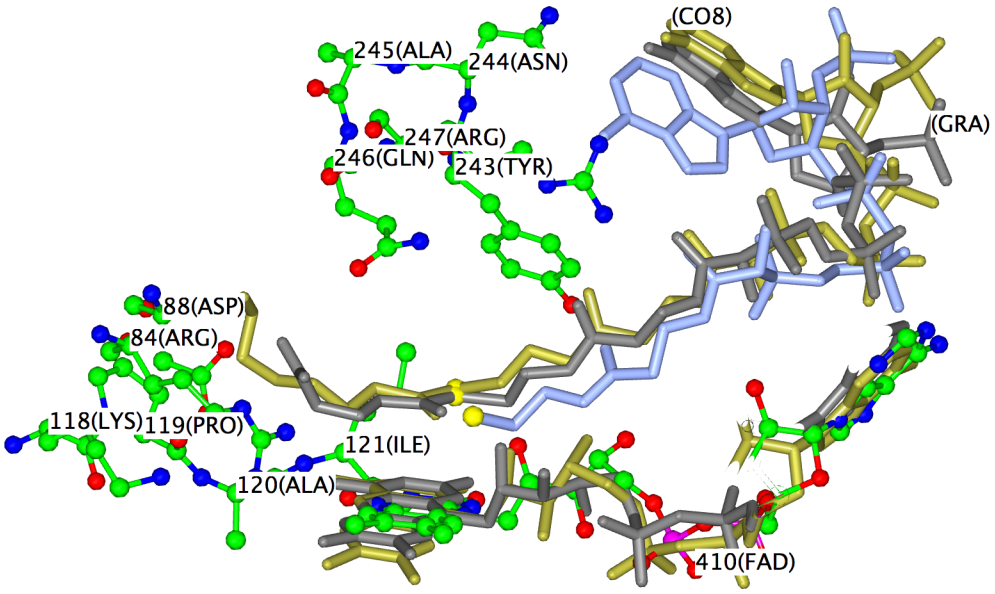

Figure S3 Superimposition of the structures of the glutaryl-CoA dehydrogenase from *Desulfococcus multivorans* in complex with glutaryl-CoA (PDB code: 3mpi (Wischgoll *et al.*, 2010)) (grey) and of the medium chain acyl-CoA dehydrogenase from pig liver mitochondria in complex with octanoyl-CoA (PDB code: 3mde (Kim *et al.*, 1993)) (gold) with the structure of Acd_{DPN7} 3SP-CoA desulfinase monomer A (cyan).

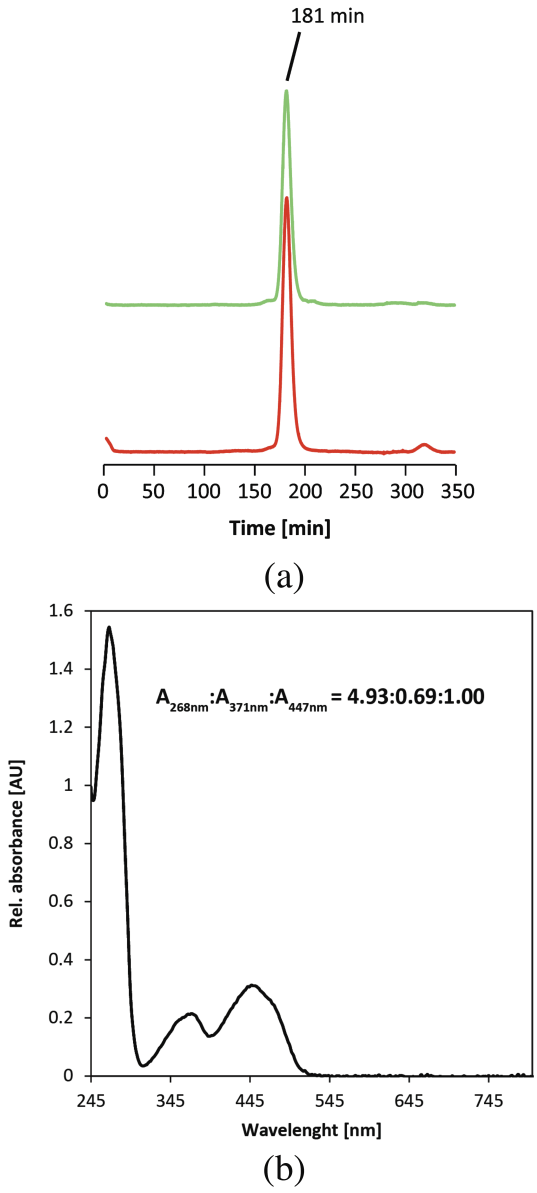

Figure S4 a) Size-exclusion chromatography of wtAcd$_{DPN7}$ and Acd$_{DPN7_R84K}$. It illustrates that Acd$_{DPN7_R84K}$ was purified as an intact tetramer; the retention time of 181 min corresponds to a molecular mass of about 173 kDa (theoretically 179 kDa). b) Absorption spectrum of Acd$_{DPN7_R84K}$. The absorption spectrum of Acd$_{DPN7_R84K}$ is highly similar to the spectrum of Acd$_{DPN7}$ that we published previously (Schürmann et al. 2013). This indicates that all FAD binding sites are saturated.

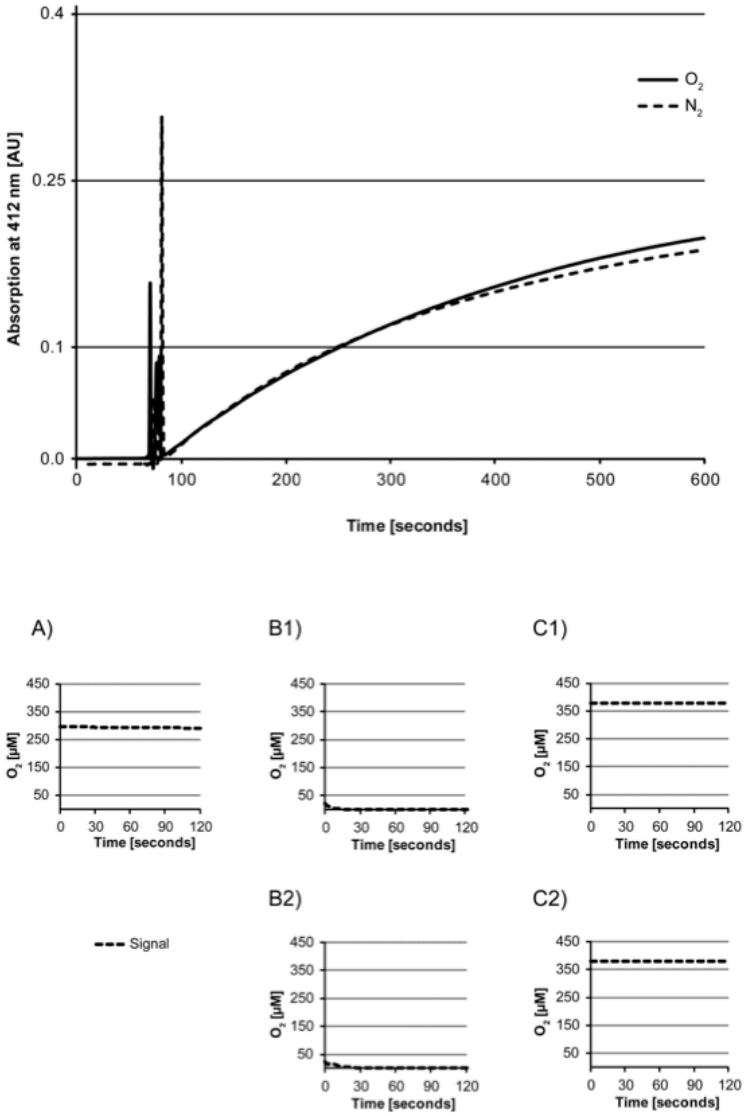

Figure S5 Left panel) Oxygen-independency of Acd_{DPN7}. Purified Acd_{DPN7} was incubated in presence or absence of oxygen (nitrogen saturation) as described in the Materials and Methods section; right panel) A) Assay solution after flushing with compressed air for 2 min. B1) Assay solution after flushing with nitrogen for 5 min. B2) Assay solution after completion of enzyme assay (addition of Acd_{DPN7}). C1) Assay solution after flushing with oxygen for 5 min. C1) Assay solution after completion of enzyme assay (addition of Acd_{DPN7}).

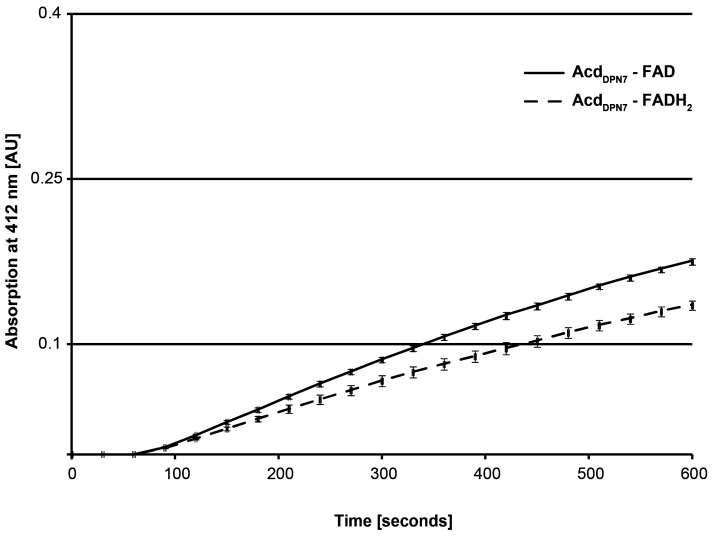

Figure S6 Purified Acd_{DPN7} containing either FAD or $FADH_2$ (after reduction with dithionite) was incubated with 0.1 mM 3SP-CoA in presence of 0.2 mM DTNB in an anaerobic chamber as described in the Materials and Methods section. The increase in absorption was followed at 412 nm.