Supporting information

The architecture of the diaminobutyrate acetyltransferase active site provides mechanistic insight into the biosynthesis of the chemical chaperone ectoine

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Running title: Crystal structure of L-2,4-diaminobutyrate acetyltransferase

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Table S1. Interaction between both monomers in the dimer of (*Pl*)EctA.

Hydrogen bonds			
##	**Monomer A**	**Dist. [Å]**	**Monomer B**
1	A:MET 148[O]	2.70	B:THR 20[OG1]
2	A:LEU 34[O]	3.88	B:SER 36[N]
3	A:ASN 35[OD1]	2.94	B:SER 36[N]
4	A:ASN 35[OD1]	2.78	B:SER 36[OG]
5	A:THR 115[OG1]	2.64	B:TYR 38[OH]
6	A:GLU 113[OE2]	2.64	B:TYR 46[OH]
7	A:ASP 45[O]	3.05	B:ARG 71[NH1]
8	A:ASP 49[OD2]	3.00	B:ARG 71[NH1]
9	A:TYR 46[O]	3.72	B:ARG 71[NH1]
10	A:ASP 45[O]	2.88	B:ARG 71[NH2]
11	A:TYR 38[OH]	2.61	B:THR 115[OG1]
12	A:ASP 45[OD2]	2.65	B:TYR 144[OH]
13	A:THR 20[OG1]	2.69	B:MET 148[O]
14	A:SER 36[N]	3.90	B:LEU 34[O]
15	A:SER 36[N]	2.92	B:ASN 35[OD1]
16	A:SER 36[OG]	2.81	B:ASN 35[OD1]
17	A:TYR 46[OH]	2.67	B:GLU 113[OE2]
18	A:ARG 71[NH1]	2.95	B:ASP 45[O]
19	A:ARG 71[NH1]	3.07	B:ASN 48[OD1]
20	A:ARG 71[NH1]	3.69	B:TYR 46[O]
21	A:ARG 71[NH1]	2.87	B:ASP 49[OD1]
22	A:ARG 71[NH2]	3.78	B:ASP 45[OD1]
23	A:ARG 71[NH2]	2.93	B:ASP 45[O]
24	A:TYR 144[OH]	2.64	B:ASP 45[OD2]
Salt bridges			
##	**Monomer A**	**Dist. [Å]**	**Monomer B**
1	A:ASP 49[OD2]	3.00	B:ARG 71[NH1]
2	A:ARG 71[NH1]	2.87	B:ASP 49[OD1]
3	A:ARG 71[NH2]	3.78	B:ASP 45[OD1]

Table S2. Primers used for the construction of the expression vector carrying the codon-optimized (*Pl*)*ectA* gene for heterologous expression in *E. coli*, and for the generation of the (*Pl*)*ectA* variants.

Primer name	Primer sequence
pLC46_for	CAAGCTCTTCAATGGCAG
pLC46_rev	CAAGCTCTTCACCCAATATC
Q5_EctA_Y38A_F	GAATAGCCCGgcgTGTTATATGCTGCTGG
Q5_EctA_Y38A_R	AGATCCAGGCTACCGGTA
Q5_EctA_D33A_F	CGGTAGCCTGgcgCTGAATAGCC
Q5_EctA_D33A_R	GTATCACGAATCAGTTCCC
Q5_EctA_W79A_F	CCTGTTTGTTgcgCAGGTTGCAGTTG
Q5_EctA_W79A_R	GTTTCCGGATTACGCGGA
Q5_EctA_Q80A_F	GTTTGTTTGGgcgGTTGCAGTTGCAAG
Q5_EctA_Q80A_R	AGGGTTTCCGGATTACGC
Q5_EctA_T115A_F	TATTGAAACCgcgGTTAGCCCGAG
Q5_EctA_T115A_R	AAACGCACACCATGACATG
Q5_EctA_H155A_F	TGGCACCACCgcgGAAGATGAACCG
Q5_EctA_H155A_R	TCTGGAAACATTTCTGCAC
Q5_EctA_E158A_F	CCATGAAGATgcgCCGCTGTTTGTG
Q5_EctA_E158A_R	GTGGTGCCATCTGGAAAC

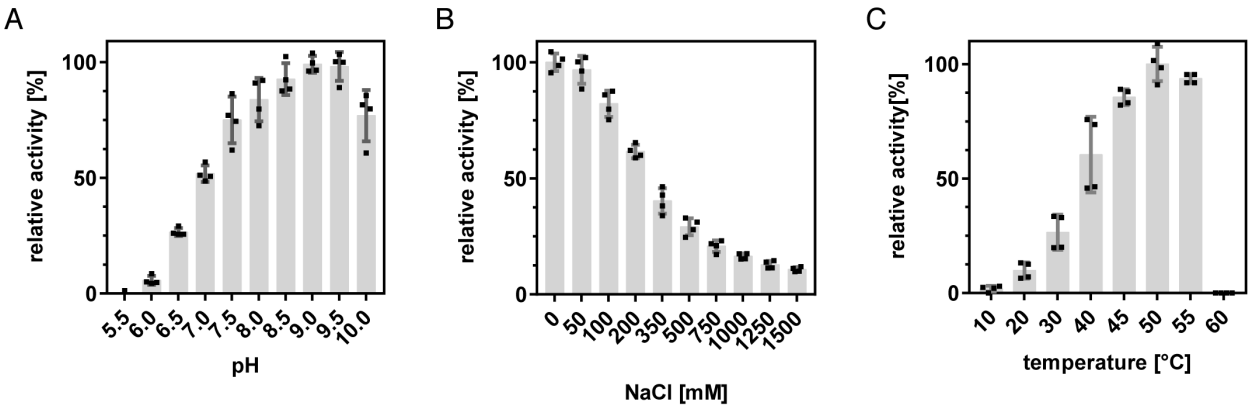

Figure S1. Determination of the optimal reaction conditions of the (*Pl*)EctA enzyme. *A,* The pH-optimum, *B,* the tolerance against NaCl and *C,* the optimal temperature of (*Pl*)EctA were assayed. Activities for Temperatures > 40°C were determined within the first 30 seconds of reaction time, due to temperature dependent loss of activity. The error bars represent the standard deviation calculated from two technical and two biological replicates.

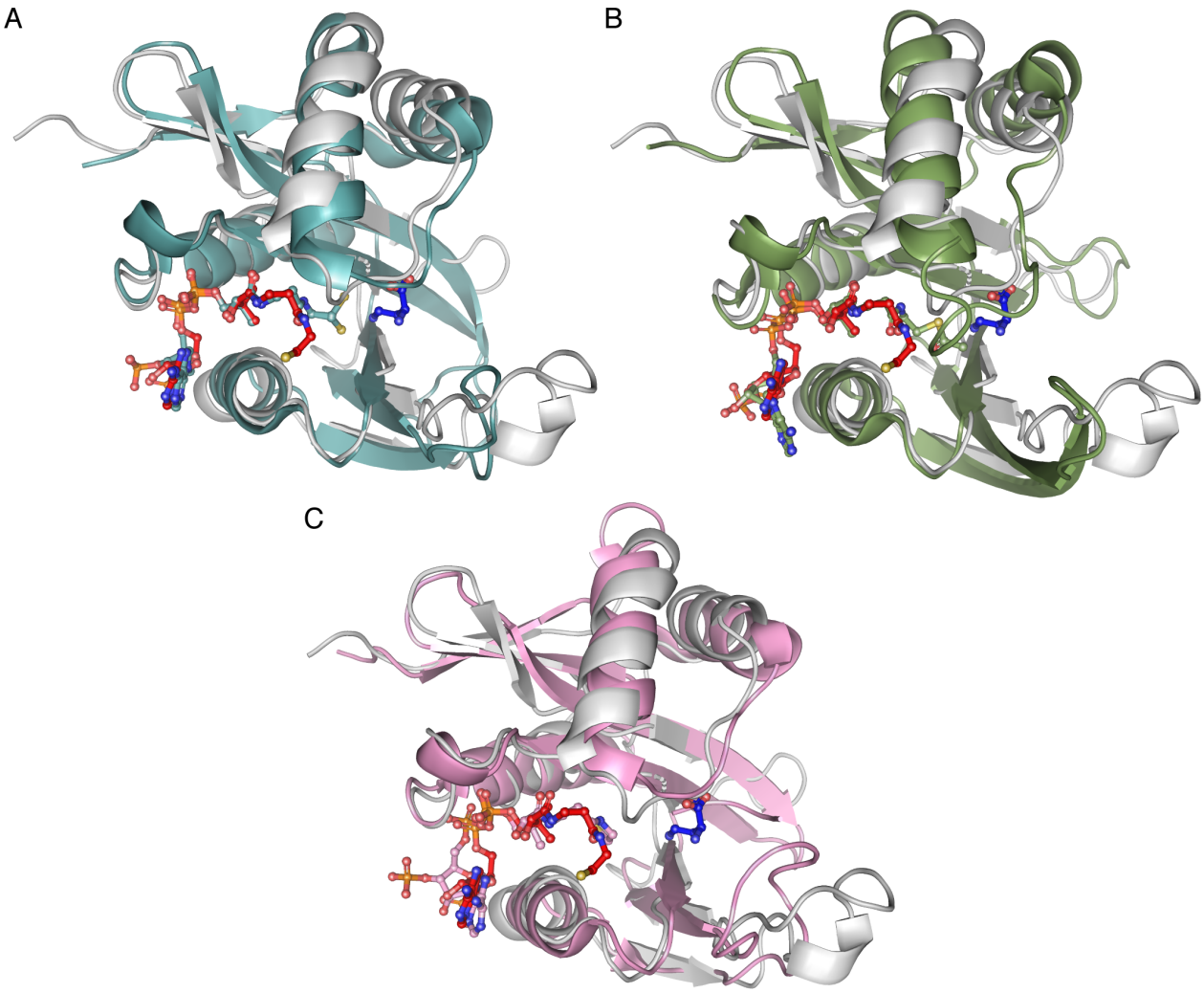

Figure S2. Structural comparison of (*Pl*)EctA with acetyltransferases from three domains of life. Overlay of the (*Pl*)EctA **(**grey) containing CoA (red) and DAB (blue) (PDB entry 6SLL) with *A*, the acetyltransferase ARD1 (blue) from the archaeon *Sulfolobus solfactarius* (PDB entry 2X7B), *B*, a probable acetyltransferase (green) from the bacterium *Agrobacterium tumefaciens* (PDB entry 2GE3), and *C*, the acetyltransfersae NAA50 (pink) from *Homo sapiens* (PDB entry 4X5K).

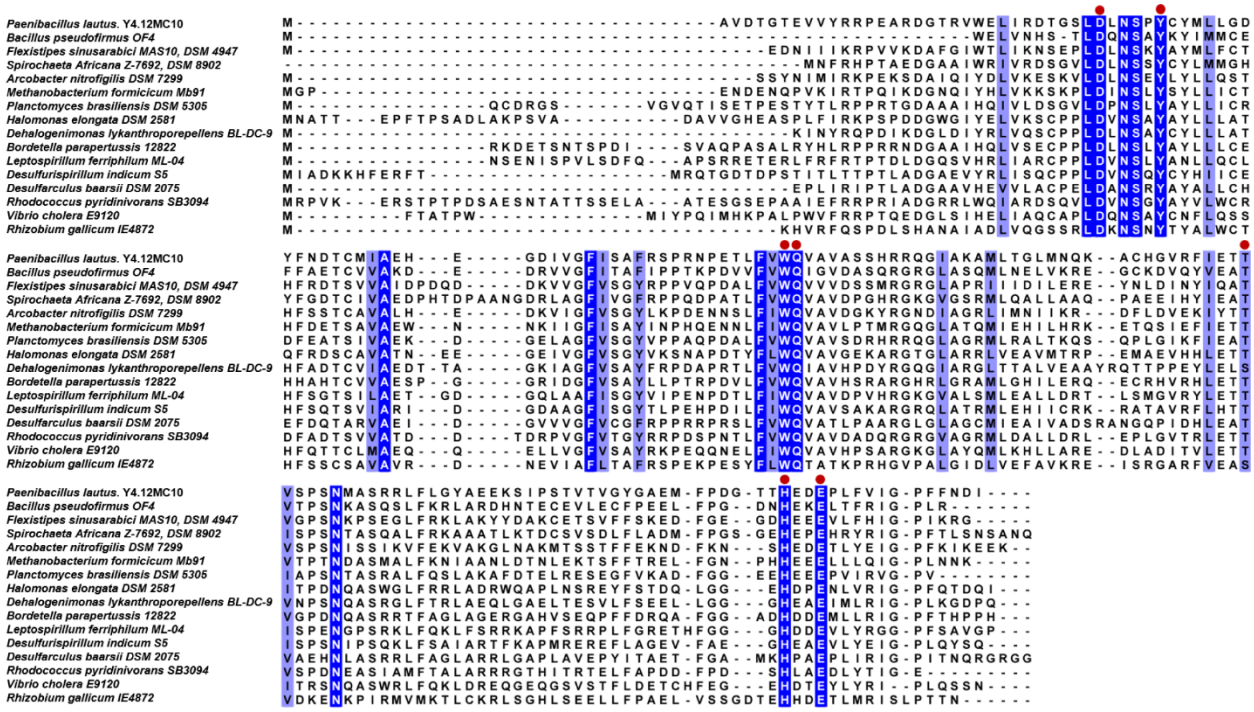

Figure S3. Amino acid sequence alignment of randomly chosen EctA proteins. Strictly conserved amino acids are shaded in blue. Red dots indicate the amino acids involved in binding of the substrate DAB.

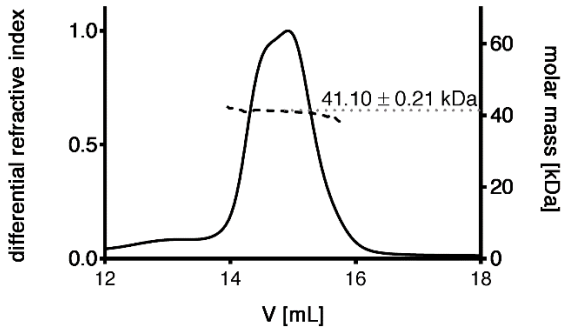

Figure S4. Quaternary assembly of the (*Pl*)EctA Tyr38/Ala mutant. MALS-RI analysis shows that EctATyr38/Ala elutes with an absolute molecular mass (MW) consistent with that of a homodimer.

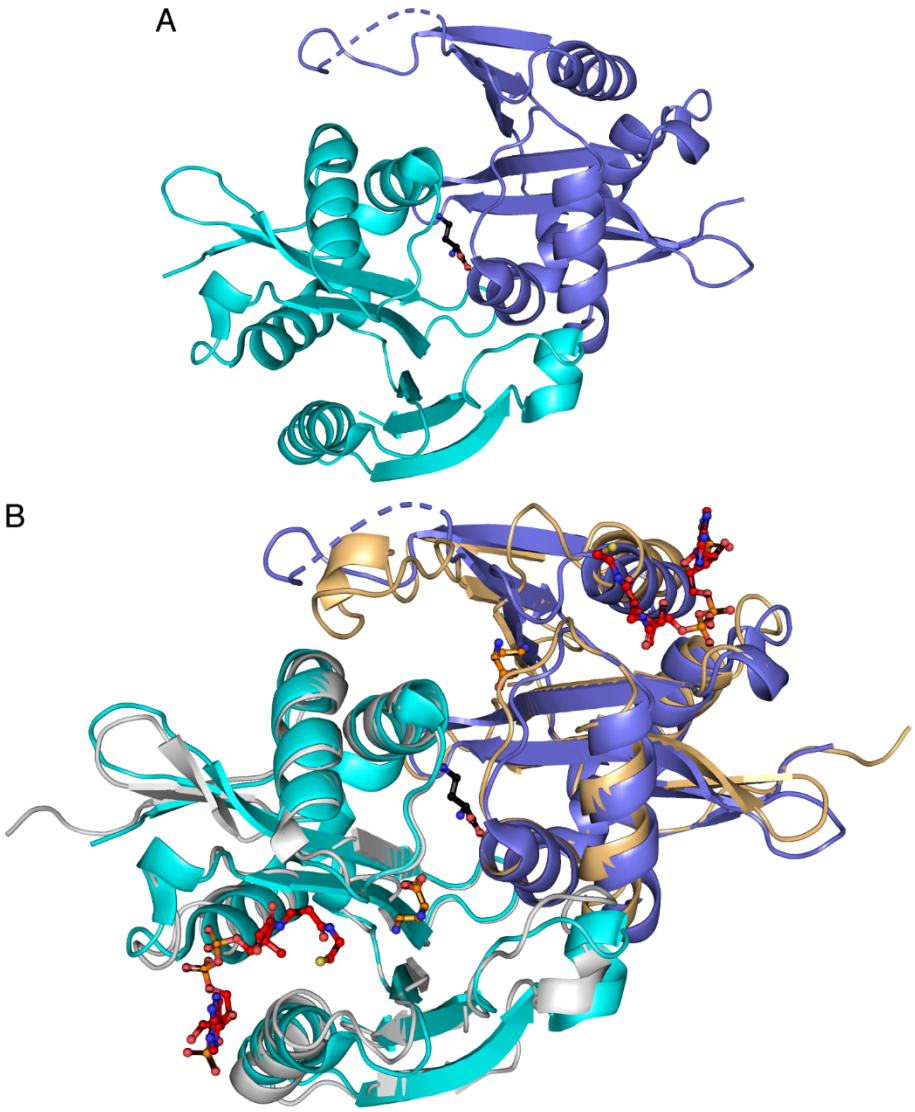

Figure S5. Structural comparison of (*Bp*)EctA (PDB entry: 3D3S) with (*Pl*)EctA:CoA:DAB (PDB entry: 6SLL) with respect to the position of the substrate DAB. *A*, Crystal structure of (*Bp*)EctA, including the DAB molecule (black stick) positioned within the interface of the two monomers (cyan and blue colored). *B,* Overlay of the (*Bp*)EctA structure and (*Pl*)EctA (gray and light orange), illustrating the different positions of the (*Bp*)EctA-DAB (black stick) and the (*Pl*)EctA-DAB (orange stick) relative to the position of CoA (red).