STRUCTURAL BIOLOGY

Volume 79 (2023)

Supporting information for article:

Rhizobium etli has two L-asparaginases of low sequence identity but similar structure and catalytic center

Joanna I. Loch, Paulina Worsztynowicz, Joanna Sliwiak, Marta Grzechowiak, Barbara Imiolczyk, Kinga Pokrywka, Mateusz Chwastyk, Miroslaw Gilski and Mariusz Jaskolski

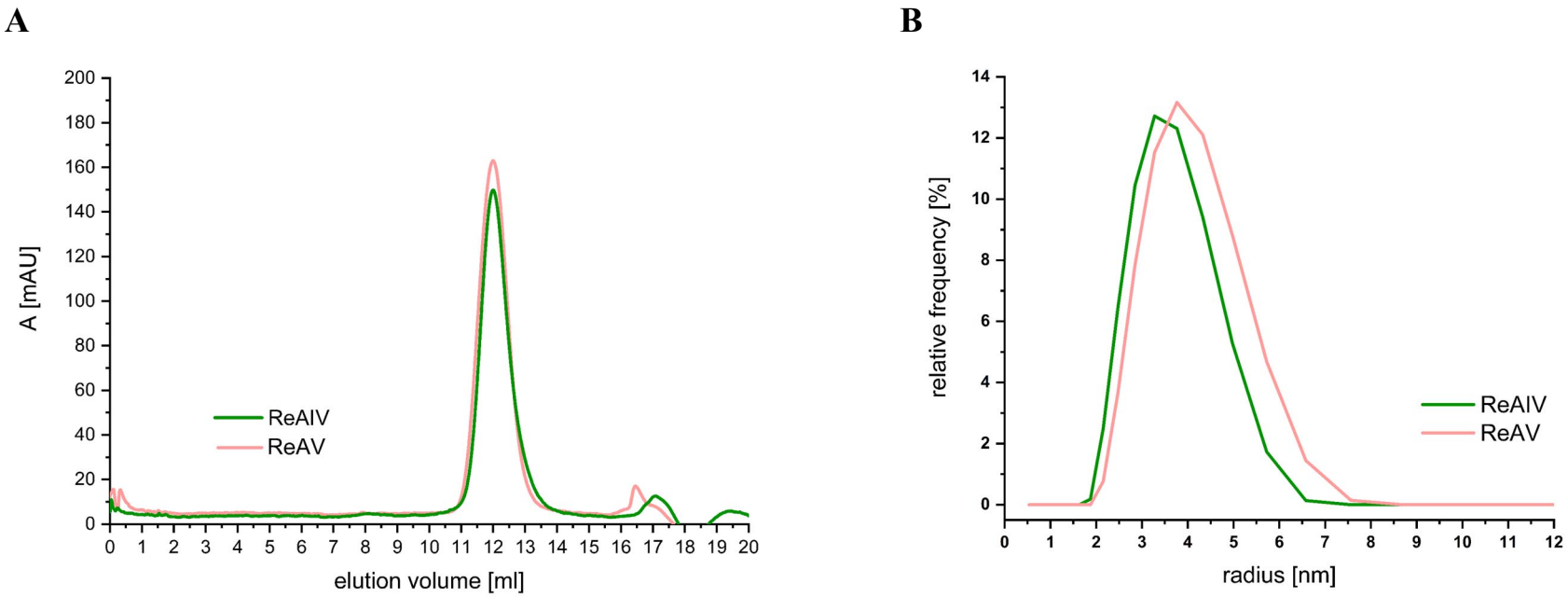

Figure S1 (A) Superposition of gel filtration chromatograms of ReAIV and ReAV obtained using the Superdex 10/300 GL column. (B) nanoDLS Determination of the radius of gyration of ReAIV and ReAV dimers.

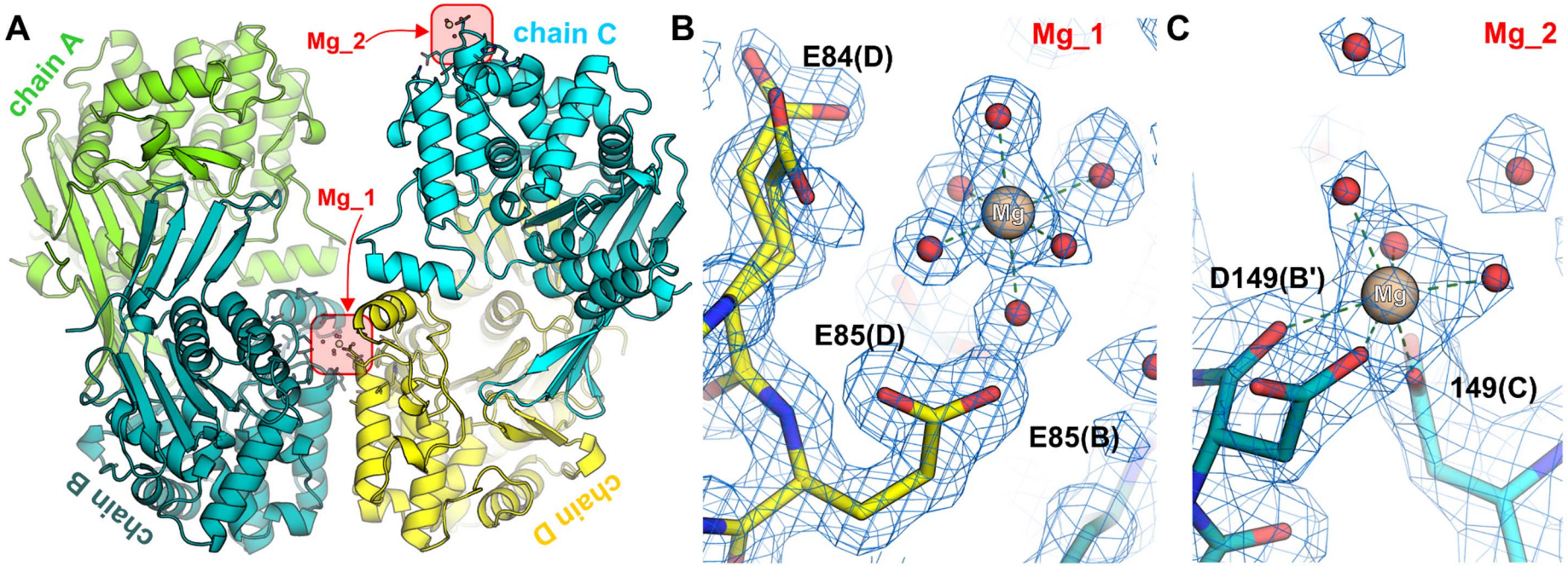

Figure S2 Magnesium coordination sites. (A) Magnesium coordination sites Mg_1 and Mg_2 identified in structure **R4oP-2** (2.00 Å); site Mg_1 was also found in the **R4oP-1** structure (1.35 Å). 2Fo-Fc electron density maps (contour 1.0σ) around the magnesium ions (gray spheres) identified near: (B) Glu85 (structure **R4oP-1)** and (C) near Asp149 (structure **R4oP-2)**. Water molecules are shown as small red spheres.

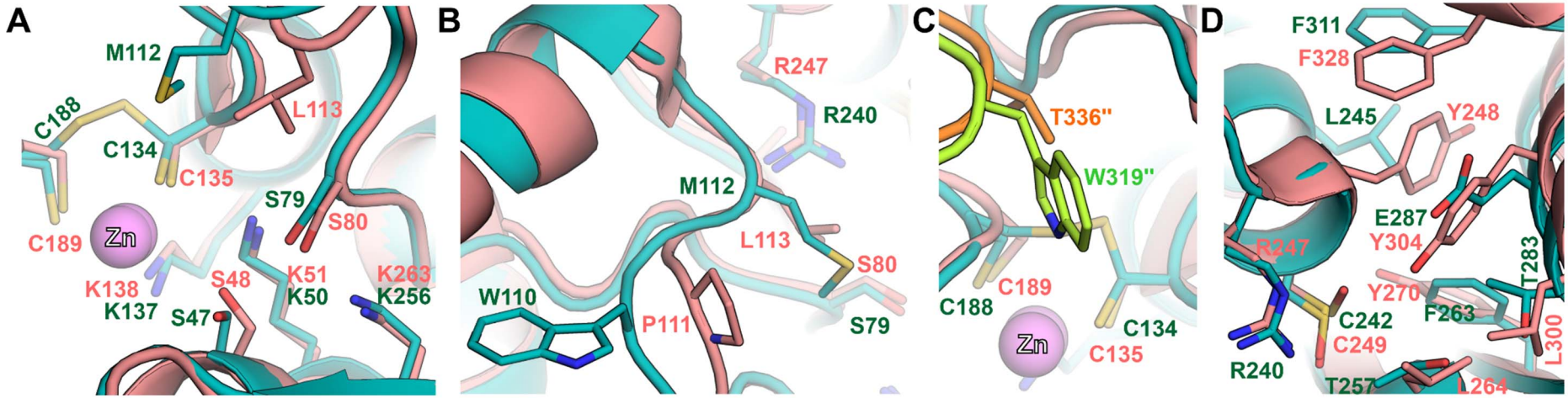

Figure S3 Comparison of the active sites of ReAIV and ReAV. (A) Superposition of the ReAIV (**R4oP-2**, chain A, dark green) and ReAV (PDB ID 7os5, chain A, salmon) active sites. (B) Entrance to the active site of ReAIV (obstructed by Trp110) and ReAV (Pro111). (C) Trp319 from the dimer complementary subunit (", light green) at the entrance to the ReAIV active site; in ReAV (orange) Thr336 is present in the corresponding position. (D) Sequence differences in the close neighborhood of the oxidized Cys242 of ReAIV (dark green) and Cys249 of ReAV (salmon).

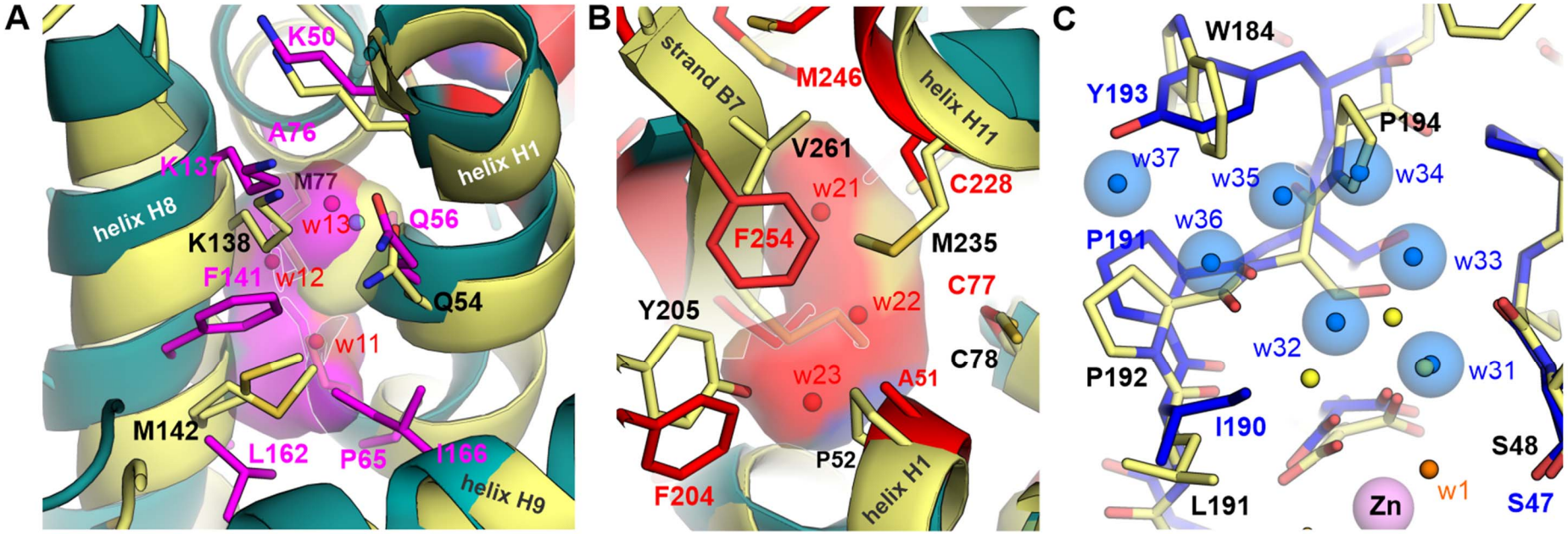

Figure S4 Superposition of ReAIV (dark green, magenta, red, blue, R4tP, chain A) and ReAV (PDB 7os5, yellow). (A) In the region of cavity I in the ReAIV (magenta) molecule, RaAV (yellow) has a small hole with a single water molecule. (B) In the region corresponding to cavity II in ReAIV (red), no cavity is present in ReAV. (C) In the region corresponding to cavity III of ReAIV (blue), ReAV contains only three water molecules (yellow).

Table S1 A gallery of ReAIV crystal structures: summary of structural information.

Structure code	**R4mC-1**		**R4mC-2**		**R4tP**		**R4oP-1**				**R4oP-2**			
pre-incubation with L-Asn	**yes**		no		no		**yes**				**yes**			
chain	A	B	A	B	A	B	A	B	C	D	A	B	C	D
Zn^{2+} occupancy	**0.50**	**0.50**	**0.80**	**0.90**	**1.00**	**1.00**	**0.50**	**0.50**	**0.50**	**0.50**	**0.60**	**0.70**	**0.60**	**0.60**
C134-C188 S-S bond	yes	yes	no	no	no	no	yes	yes	yes	yes	yes	yes	no	no
S47: hydration	no	no	yes	yes	no	no	yes	yes	yes	yes	no	no	no	no
S47: double conformation	yes	yes	no	no	no	no	yes	yes	yes	yes	no	no	yes	no
S79: double conformation	no	no	no	no	no	no	yes	yes	yes	yes	no	no	no	no
D186: double conformation	yes	yes	no	no	no	no	yes	yes	yes	yes	yes	no	yes	yes
C242: oxidation	yes	yes	yes	yes	no	no	yes	yes	yes	yes	yes	yes	yes	yes
C242: double conformation	no	no	yes	yes	no	no	yes	yes	yes	yes	no	yes	yes	yes
Mg^{2+} site	no		no		no		**yes**, single site				**yes**, two sites			

Table S2 Functional states of the ReAIV molecule (A = "conformation A", B = "conformation B", as described in the main text).

functional states	**resting**	**active**	**relaxed**	**intermediate**	**S-S-resting**	**S-S-active**	**S-S-relaxed**
Zn^{2+} present	**yes**	**yes**	**yes**	Zn is leaving	no	no	no
C134-C188 S-S bond	no	no	no	being formed	**yes**	**yes**	**yes**
S47 hydration/polarization	no	**yes**	no	no	no	**yes***	no
S47 in conformation	**A**	**A**	B	-	**A**	**A**	B
S79 in conformation	**A**	**A**	B	-	**A**	**A**	B
D186 in conformation	**A**	**A**	**A**	-	B	B	B

*Speculative, in the presence of substrate.

Table S3 Hydrogen bonds of the water molecules in the **R4mC-1** cavities. The water molecules in each cavity are labeled in bold as in Fig. 8.

Cavity I		**Cavity II**	
w11	(Å)	**w21**	(Å)
w12	2.91	**w22**	2.79
Gln53(O)	2.85	Ala51(N)	2.84
Leu57(N)	3.05	Ala48(O)	2.84
		Lys50(N)	3.36
w12		**w22**	
w11	2.91	**w21**	2.79
w13	2.91	**w23**	2.91
Lys137(O)	2.92	Cys77(O)	2.85
w13		**w23**	
w12	2.91	**w22**	2.91
Ala76(O)	2.68	Val255(O)	2.79
ALA54(N)	3.19		
Lys50(O)	2.94		
Cavity III			
w31		**w35**	
w32	3.47	**w34**	2.86
w1	2.99	**w36**	2.68
Gln53(Oε1)	2.60	Gln53(Nε2)	2.94
Arg46(O)	2.95		
Lys137(Nζ)	2.96		
w32		**w36**	
w31	3.47	**w35**	2.68
w33	2.77	**w37**	3.36
Asp186A(Oδ2)	2.75	Gln163(Oε1)	2.92
Asp186B(Oδ2)	2.85	Pro191(O)	2.82
Pro191(O)	2.80	Gln53(Nε2)	3.49
Gln53(Nε2)	2.97		
w33		**w37**	
w32	2.77	**w36**	3.36

w34	2.78	Gln163(Oε1)	2.77
Arg46(O)	2.74	Gln163(O)	2.86
Arg46(N)	3.45	Tyr193(Oη)	2.63
Thr192(Oγ1)	2.75		
w34			
w33	2.78		
w35	2.86		
Tyr193(N)	2.96		