Supporting information

Structural characterization of a PCP–R di-domain from an archaeal nonribosomal peptide synthetase reveals novel interdomain interactions

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Running title: Structure of an NRPS PCP-R di-domain

Keywords: Nonribosomal peptide synthetase, reductase domain, peptide carrier protein (PCP) domain, short chain dehydrogenase/reductase (SDR), X-ray crystallography, structure-function, protein-protein interaction, archaea, peptide biosynthesis

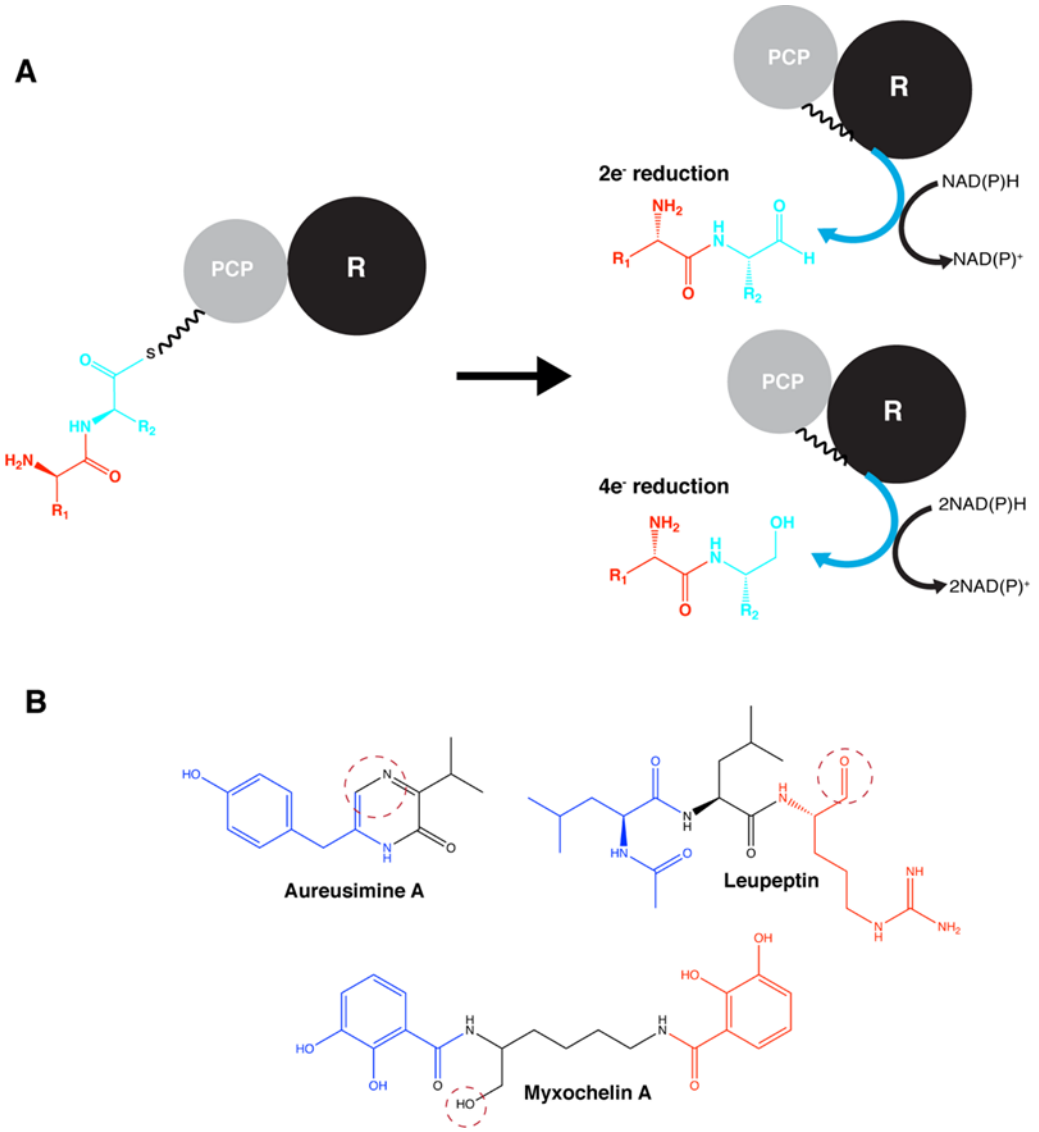

Figure S1 A) Schematic representation elucidating the reductive release of a dipeptide by an NRPS reductase domain resulting in an aldehyde or an alcohol group containing product. PCP – PCP domain, R – Reductase domain. B) Examples of NRPS peptides resulting from termination by reductase domains. The functional groups and bonds created by the action of the reductase domains are circled in red.

Table S1 Comparison of modified 351PCPR structure with other reductase domain structures. Sequence identity from Clustal Omega (51) and RMS deviation values (RMSD) from structural alignment based on secondary structure matching (SSM) (30) from CCP4 suite are shown.

Structure	Resolution	N-terminal sub-domain		C-terminal sub-domain	
		RMSD	Identity	RMSD	Identity
CAR-PCPR	2.34 Å	2.35 Å	21.5%	2.66 Å	22.4%
AusA-R	2.81 Å	1.98 Å	30.2%	2.70 Å	24.1%
Mtb-R	2.3 Å	2.04 Å	25%	2.73 Å	16.1%
MxaA-R	1.89 Å	1.63 Å	28.6%	2.34 Å	10.4%

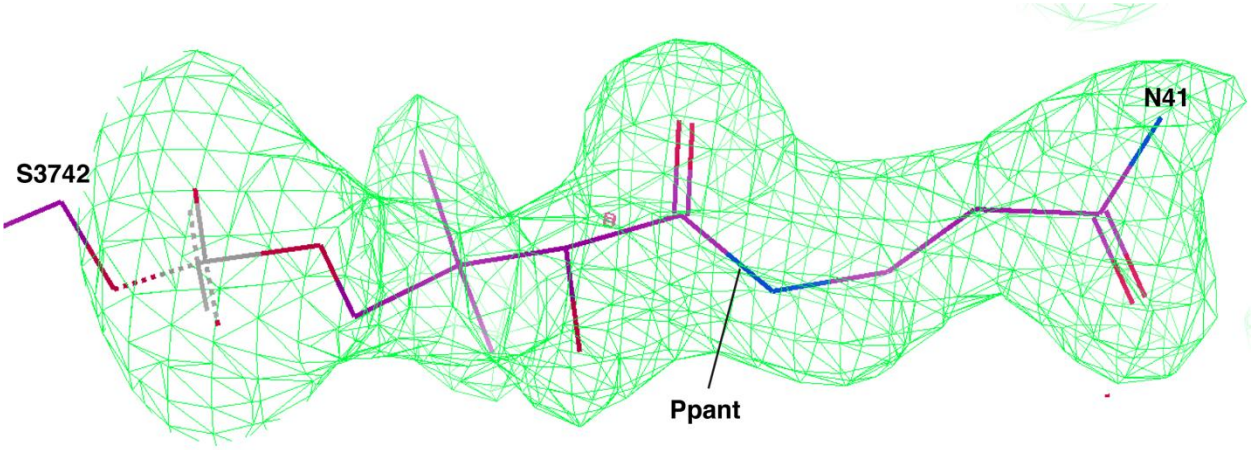

Figure S2 Ppant built in the electron density extending from S3742. No electron density can be seen after the N41 atom of ppant. The F_o-F_c omit map was created using the program phenix.polder (52) from the Phenix package. The omit map is contoured at 5.0 σ.

Figure S3 Sequence alignment of NRPS and extended SDRs by PROMALS 3D (53). Sequence alignment of various NRPS and extended SDRs in the Interface HTH region shows that the interface HTH motif is present in all NRPSs and CARs but is generally absent in extended SDRs. The interface HTH motif region is highlighted by a red outline.

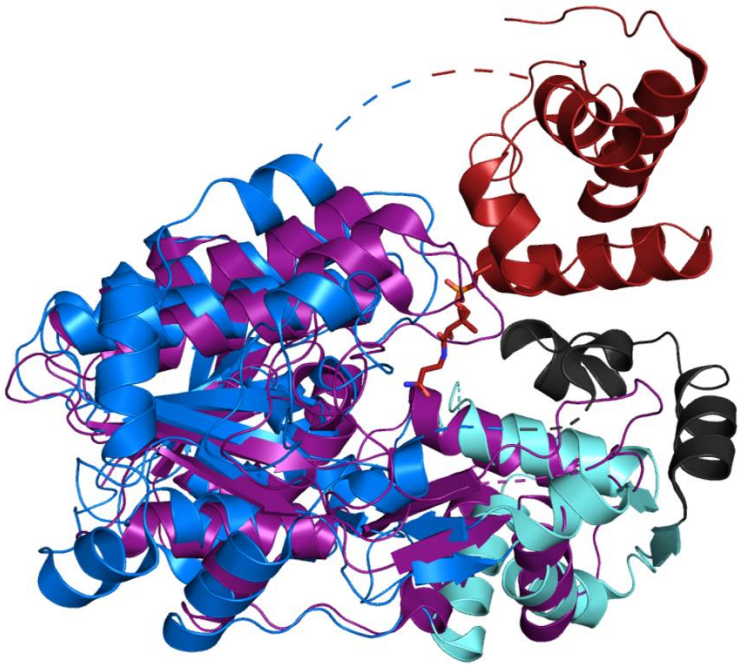

Figure S4 Structural alignment of the modified 351-PCPR (blue,cyan,red,black) with an extended SDR (purple, Kavalactone reductase 1, 6NBR (54) shows the absence of the interface-HTH motif is absent in the latter. The interface-HTH motif of 351-PCP-R is shown in black.

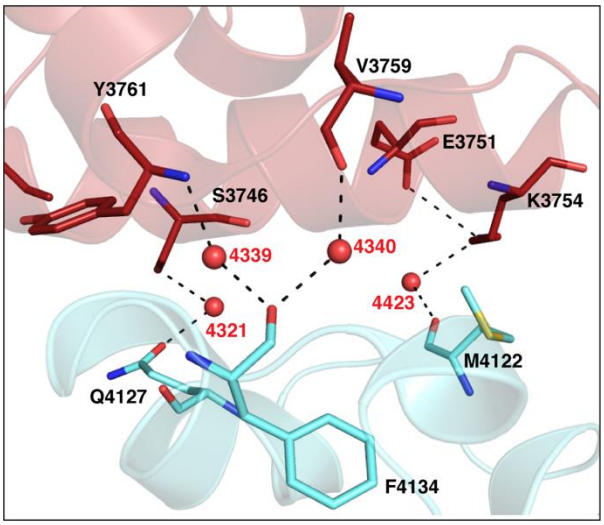

Figure S5 The 351-PCP-R PCP-reductase interface. Structured water molecules (red) mediating hydrogen bonds between residues of the PCP and reductase domain are shown.

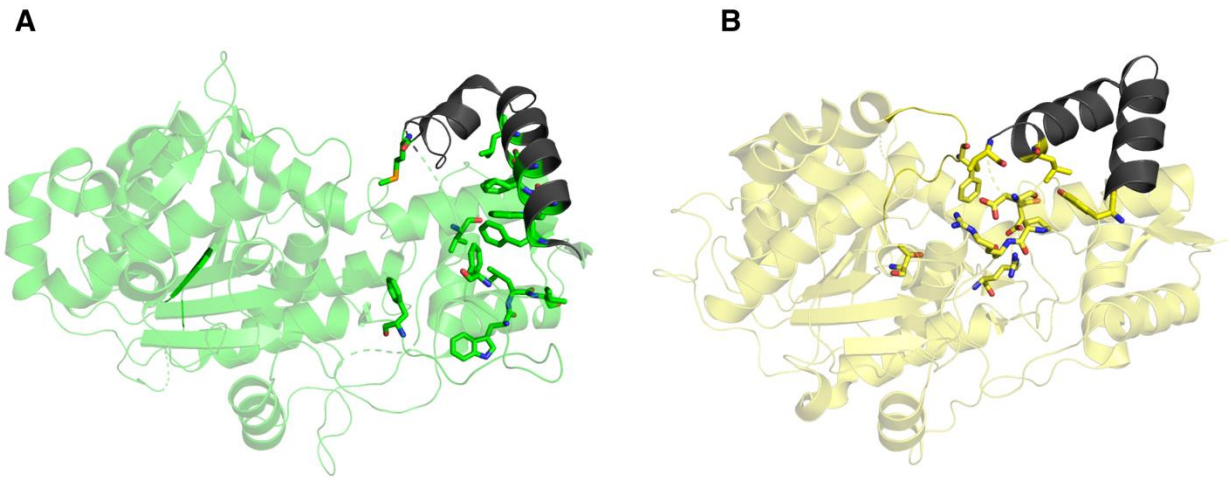

Figure S6 **A)** Residues lining the putative substrate binding hydrophobic groove in Mtb-R (27). The interface-HTH motif is shown in grey. **B)** Residues showing high probability of interaction with the substrate identified in MxaA-R (6). The interface-HTH motif is shown in grey.

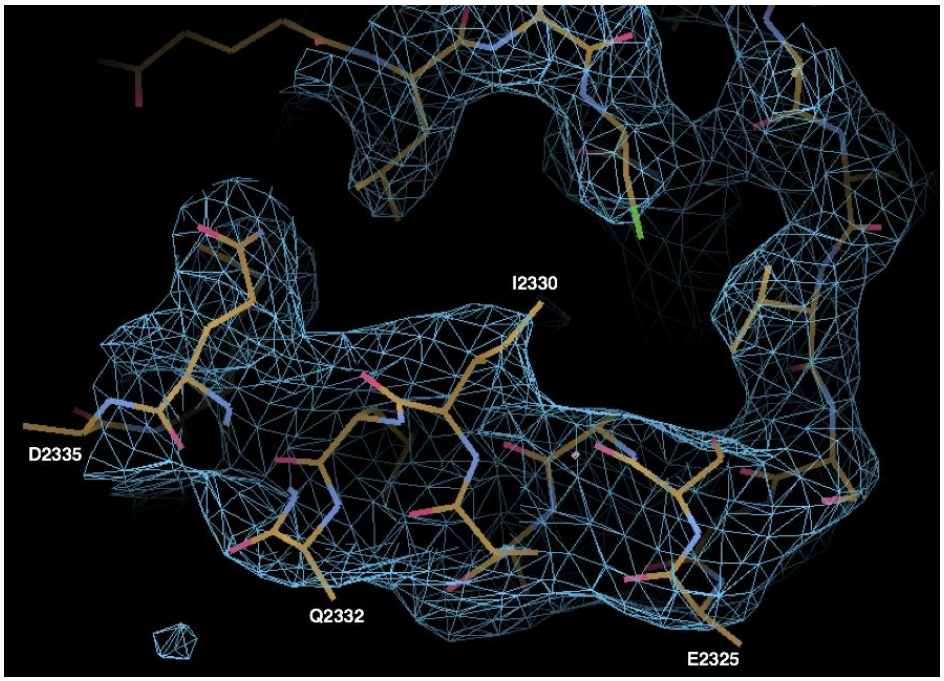

Figure S7 Figure showing weak electron density in the interface HTH region of AusA-R (8). The 2Fo-Fc map is contoured to 1.0 σ.

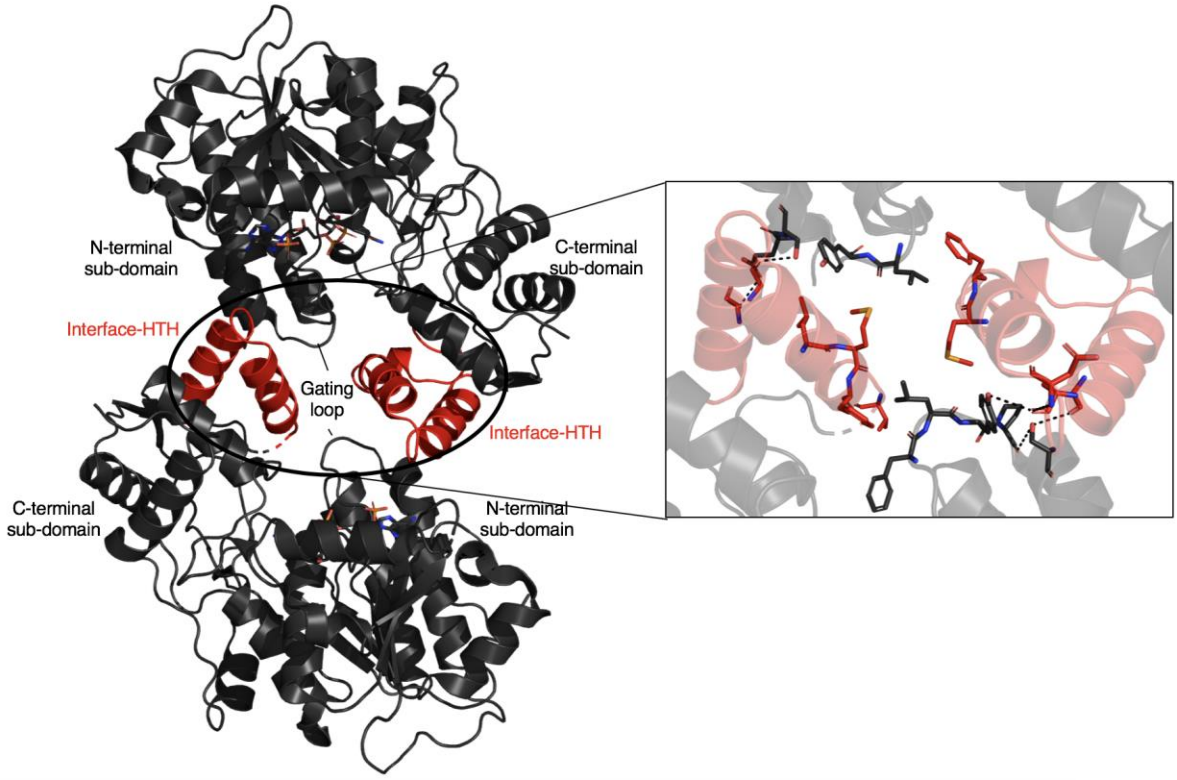

Figure S8 Ribbon diagram of Mxa-R reductase domain structure (6) showing both molecules of the asymmetric unit. Interface HTH is seen in close contact with the N-terminal sub-domain of the asymmetric partner molecule. The residues forming hydrogen bonding and hydrophobic interactions are shown in the inset figure. Hydrogen bonds are represented as black dotted lines.

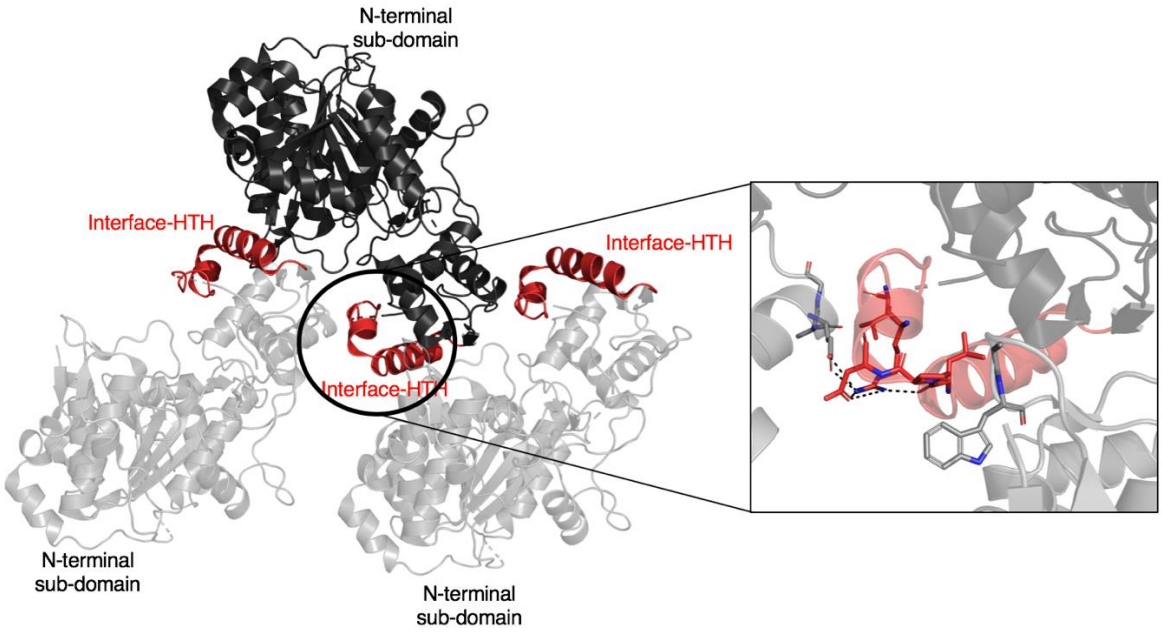

Figure S9 Ribbon diagram of Mtb-R reductase domain (27) (dark grey) structure shown with two symmetry-related molecules. Interface HTH is seen in close contact with the N-terminal sub-domain of one symmetry-related molecule and ordered C-terminal sub-domain region of the second symmetry-related molecule. The residues forming hydrogen bonding and hydrophobic interactions are shown in the inset figure. Hydrogen bonds are represented as black dotted line.

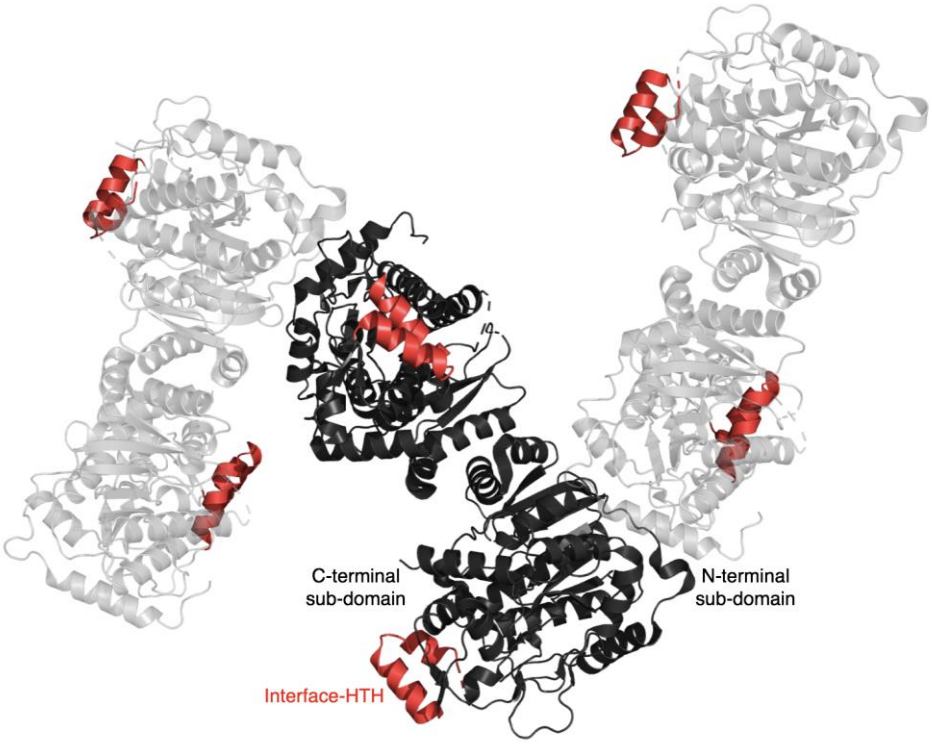

Figure S10 Ribbon representation of AusA-R (8) (dark grey) shown with two symmetry-related molecules (light grey). Interface HTH (red ribbon representation) shows no close contacts with symmetry-related molecules.

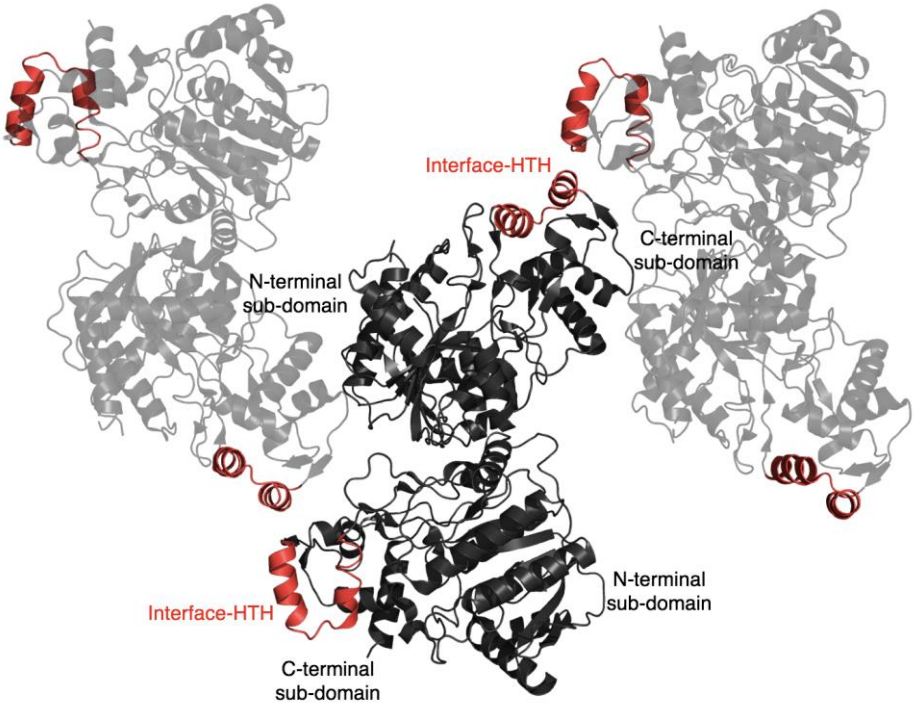

Figure S11 Ribbon representation of AusA-PCPR (8) (dark grey) shown with two symmetry-related molecules (light grey). Interface HTH (red ribbon representation) shows no close contacts with symmetry-related molecules.

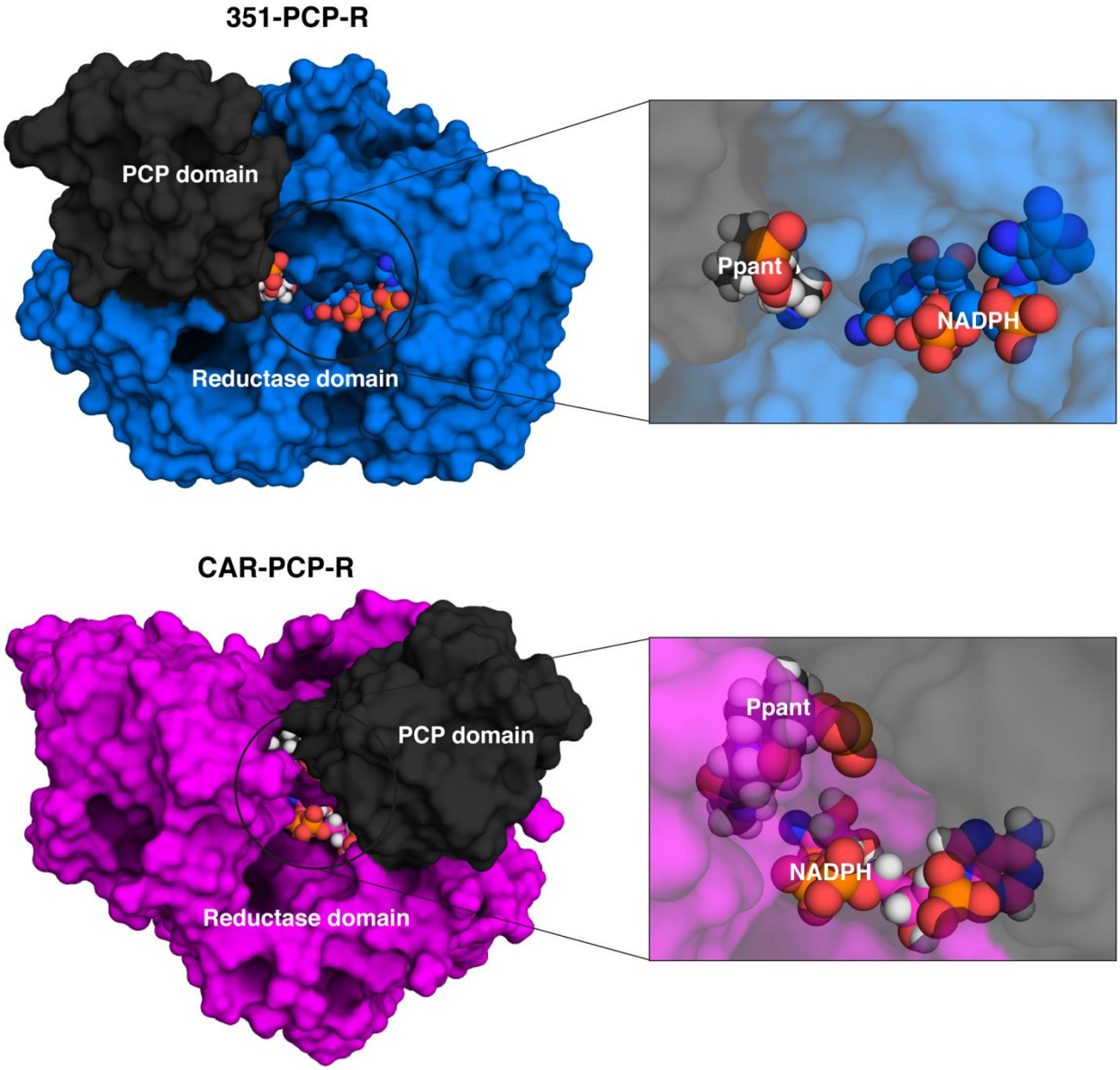

Figure S12 Surface diagram of Ppant-modified 351-PCP-R (blue, grey) and CAR-PCPR (20) (magenta, grey) showing the NADPH binding pocket. The position of the NADPH in the Ppant-modified 351-PCP-R structure is modelled based on the position of NADPH in the MxaA-R structure (6).

Table S2 Data collection and refinement statistics of the unmodified and Ppant-modified 351-PCP-R structures. Values in parentheses are for the highest-resolution shell.

Data collection statistics	**Unmodified 351-PCP-R**	**Ppant-modified 351-PCP-R**
Space group	*P* 3_2 2 1	*C* 1 2 1
Cell parameters		
Dimensions	139.92 139.92 71.75	98.95 71.83 82.73
Angles	90.00 90.00 120.00	90.00 90.26 90.00
Resolution range	45.80-2.65 (2.74-2.65)	47.50-1.95 (2.02-1.95)
$CC_{1/2}$	99.8% (42.2%)	99.3% (12.6%)
$\langle I/\sigma I \rangle$	12.00 (1.00)	7.70 (0.50)
R_{meas}	0.24 (3.08)	0.28 (4.28)
R_{merge}	0.23 (3.00)	0.26 (3.94)
Number of reflections	23792 (2359)	39217 (4098)
Multiplicity	29.0 (19.80)	7.50 (6.60)
Wilson *B* factor (Å^2)	75.77	32.22
Refinement statistics		
Resolution range	46.30-2.65 (2.74-2.65)	47.50-1.95 (2.02-1.95)
Number of reflections	23788 (2360)	38968 (3876)
Completeness	99.98% (100.00%)	91.94% (91.78%)
R_{work}	0.203 (0.323)	0.198 (0.369)
R_{free}	0.245 (0.321)	0.240 (0.385)
Ramachandran outliers	0.00%	0.00%
Ramachandran favoured	95.00%	97.21%
Rotamer outliers	0.32%	0.52%
RMS deviations from ideality		
Bonds (Å)	0.0079	0.0073
Angles (°)	0.89	0.88
Molprobity Clash score	4.85	2.74
Average *B* factor protein (Å^2)	73.7	36.9
Average *B* factor water (Å^2)	73.1	41.7
Average *B* factor ligand (Å^2)	-	38.6
Total number of atoms	2970	3986
Number of water molecules	5	248