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Supplemental information

Molecular basis for the reversible

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Molecular basis for the reversible ADP-ribosylation of guanosine bases

Marion Schuller¹, Roberto Raggiaschi¹, Petra Mikolcevic², Johannes G. M. Rack¹, Antonio Ariza³, YuGeng Zhang¹, Raphael Ledermann⁴, Christoph Tang¹, Andreja Mikoc², Ivan Ahel^{1*}

¹Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

²Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

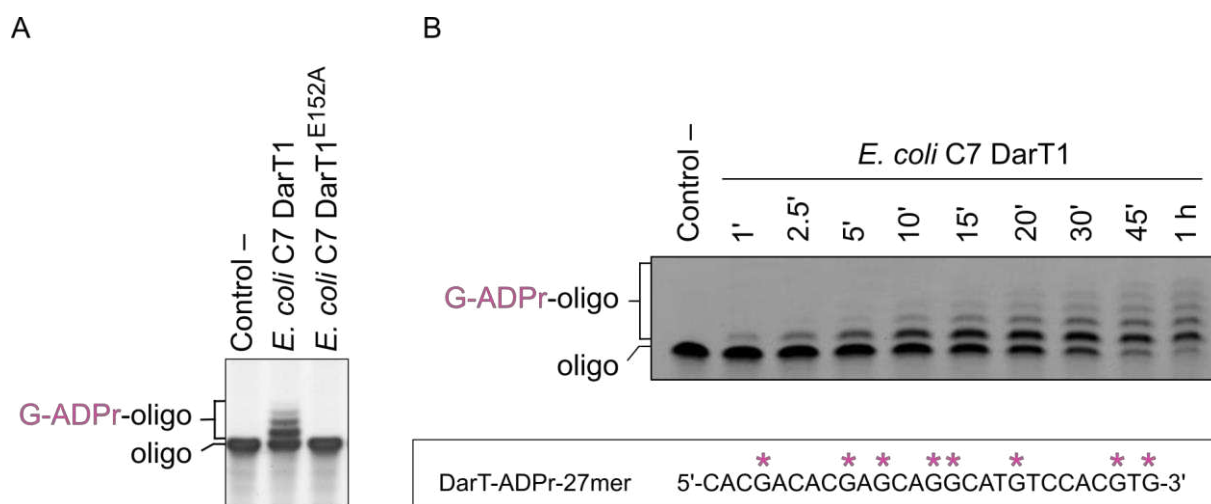
³School of Biosciences, University of Sheffield, Sheffield, United Kingdom

⁴Department of Biology, University of Oxford, Oxford, United Kingdom

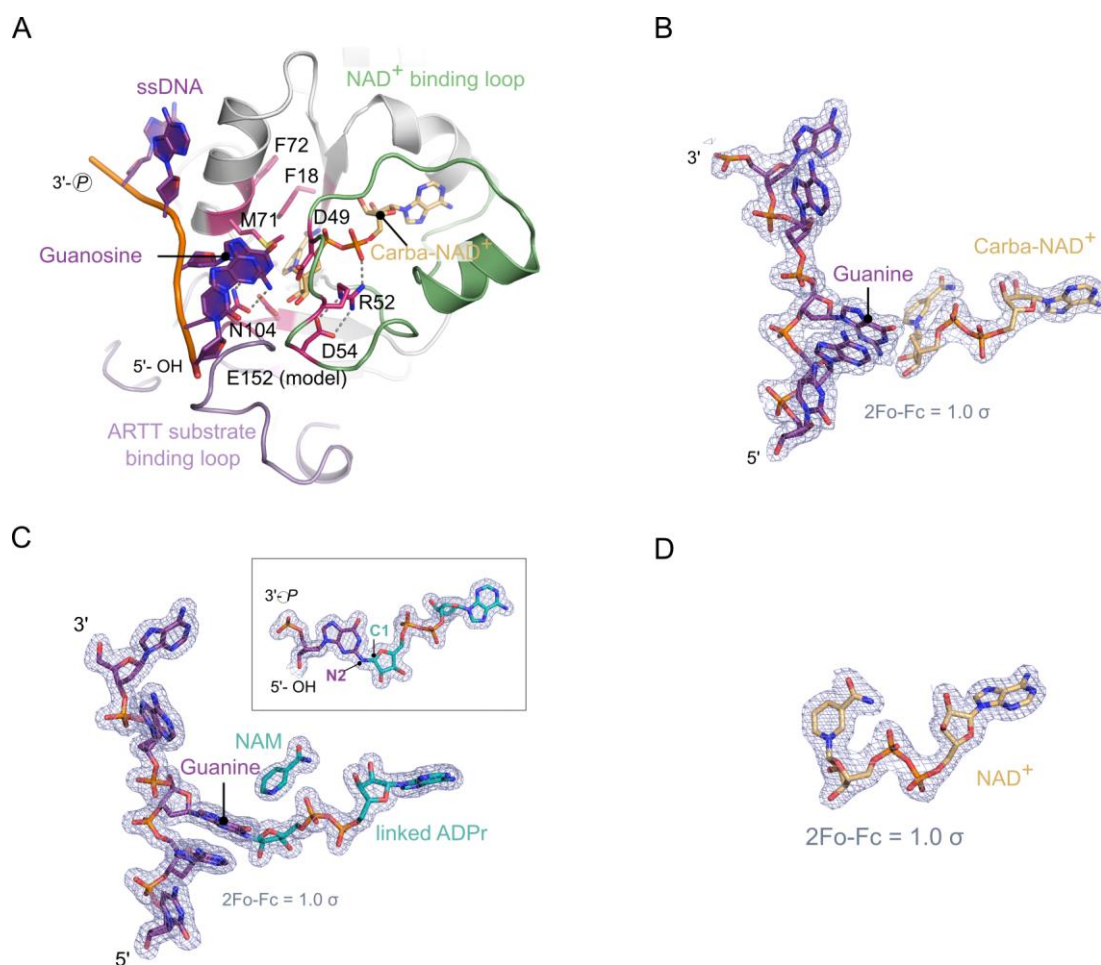
*Corresponding author and lead contact: ivan.ahel@path.ox.ac.uk

SUPPLEMENTAL INFORMATION

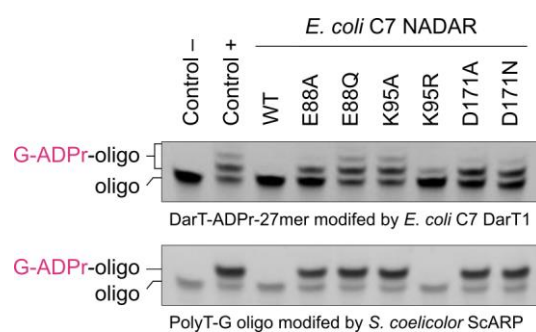
SUPPLEMENTARY FIGURES



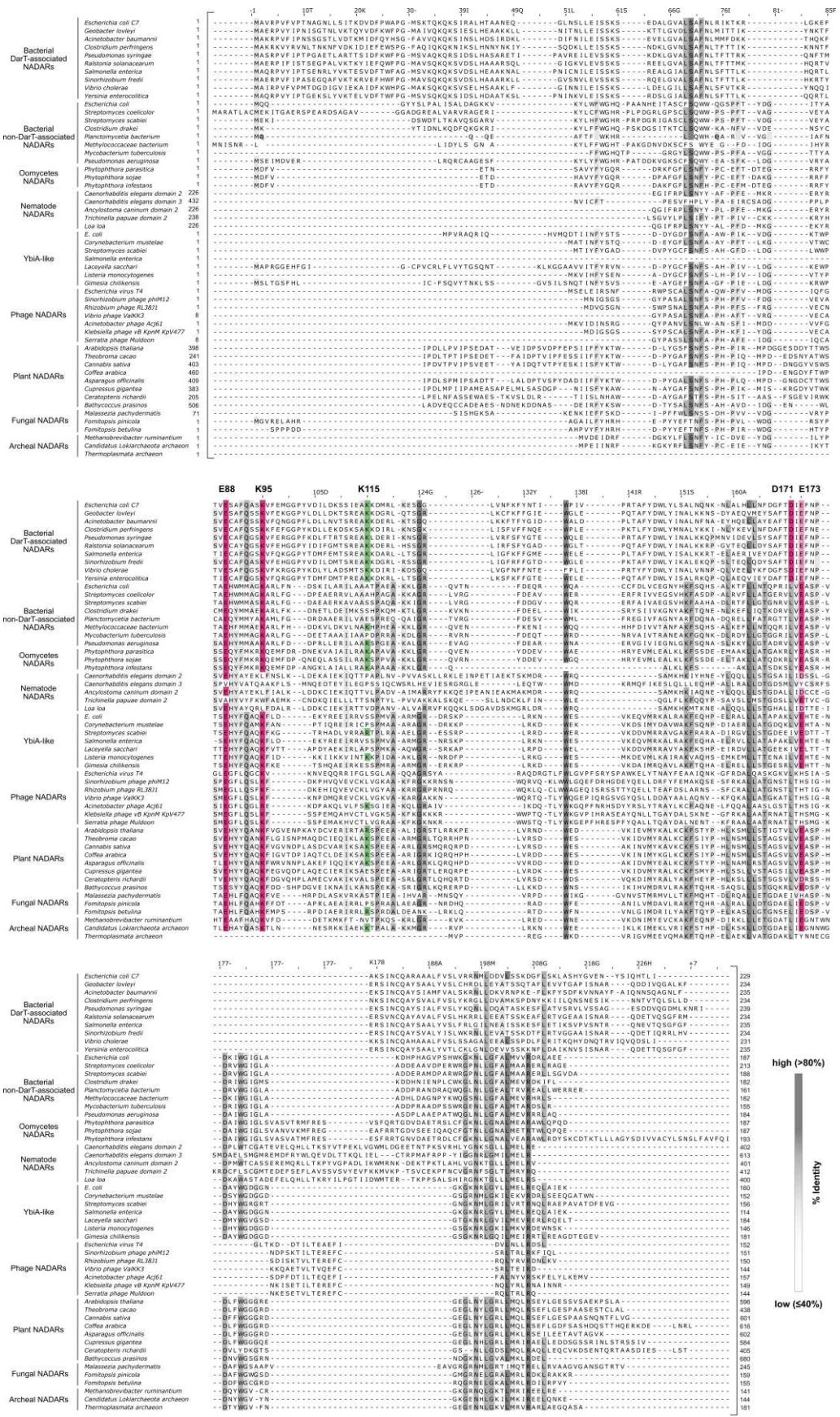
Supplementary Figure S1. ADP-ribosylation activity of *E. coli* C7 DarT1, related to Figure 2. (A) *In vitro* ADP-ribosylation activity of *E. coli* C7 DarT1 wild-type compared to mutant on the ssDNA substrate “DarT-ADPr-27mer”. Several distinct shifts of modified oligo compared to the unmodified oligo can be visualised, indicating the presence of multiple ADPr modifications on the substrate. Representative of three independent experiments. (B) *In vitro* ADP-ribosylation activity of *E. coli* C7 DarT1 on the substrate “DarT-ADPr-27mer” over a time course of 1h. Several ADP-ribose modifications are added onto the substrate over time leading to a laddering effect. Representative for three independent experiments.



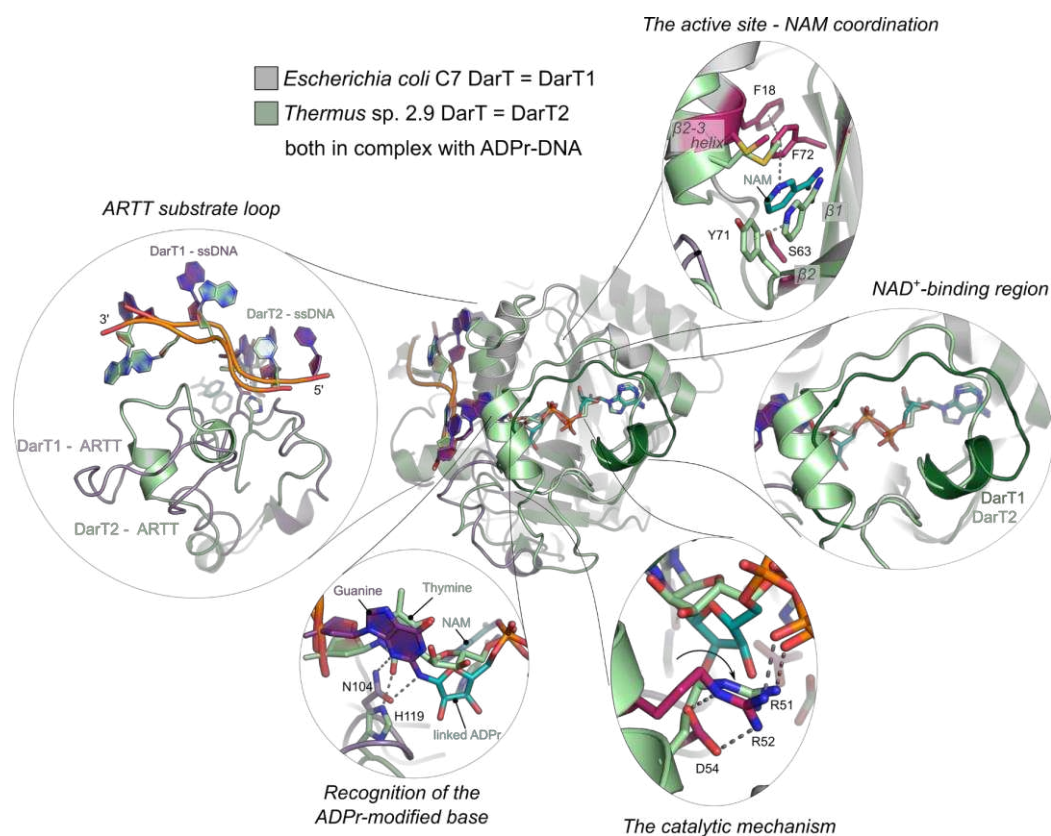
Supplementary Figure S2. DarT1 in substrate-bound pre- and post-reaction states, related to Figure 3. (A) Cartoon-stick model of the co-crystal structure of *E. coli* C7 DarT1^{E152A} with carba-NAD⁺ (brown sticks) and ssDNA (sequence AAGAC). The substrate-binding ARTT loop is highlighted in purple, and the NAD⁺-binding loop is in green. Active site residues are shown as pink sticks. (B) The 2Fo-Fc electron density map contoured at 1.0 σ around the ssDNA and the carba-NAD⁺ ligand as in the structure shown in (A) is displayed in grey. (C) The 2Fo-Fc electron density map contoured at 1.0 σ around the ADP-ribosylated DNA and the NAM ligand as in *E. coli* C7 DarT1^{E152A} co-crystallised with NAD⁺ and DNA is displayed in grey. The resolution of 1.63 Å allows revealing the DarT1-established connection of the distal-ribose C1 atom to the guanine N2 atom. The rectangular inset shows an enlarged view of this ADPr – DNA linkage. (D) The 2Fo-Fc electron density map contoured at 1.0 σ around the NAD⁺ ligand as in the DarT1-NAD⁺ co-crystal structure is shown in grey.



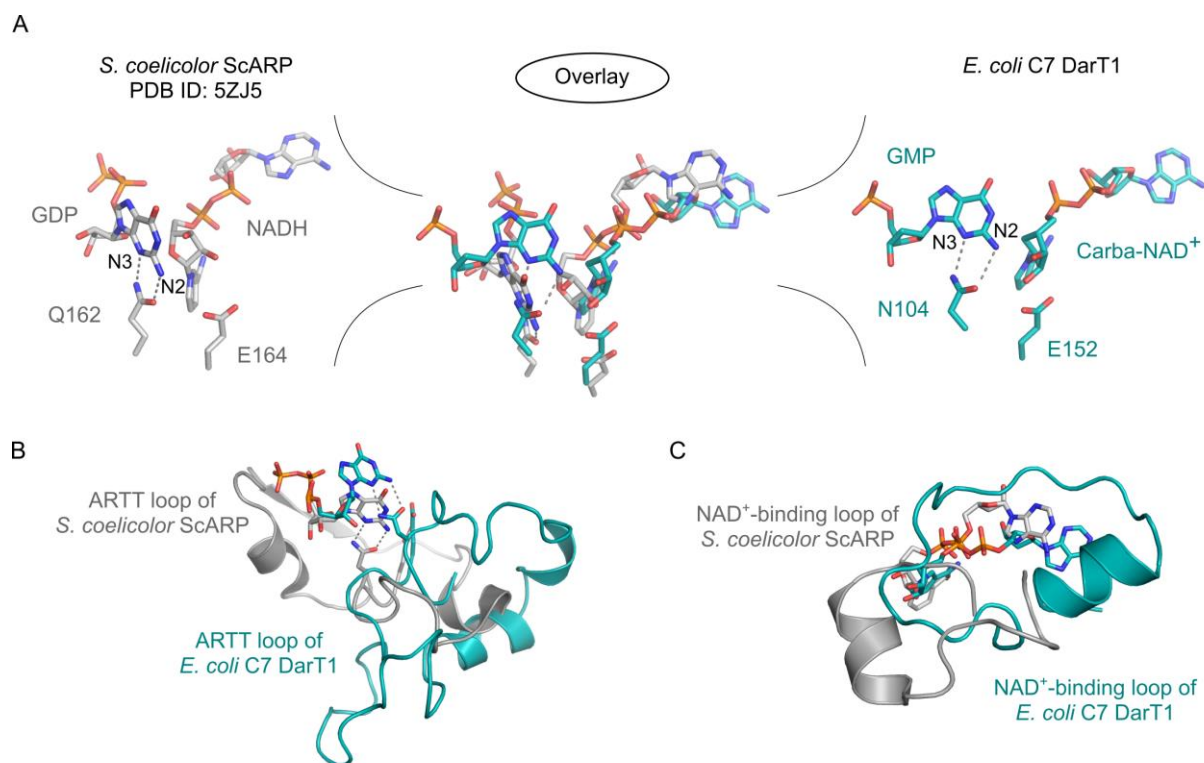
Supplementary Figure S3. Characterisation of catalytic residues of *E. coli* C7 NADAR, related to Figure 5. *In vitro* guanine-ADPr hydrolytic activity of *E. coli* C7 NADAR wild-type compared to catalytic mutants on the ssDNA substrates “DarT-ADPr-27mer” (modified by *E. coli* C7 DarT1) and “PolyT-G” (modified by *S. coelicolor* ScARP). Note, the de-ADP-ribosylation reaction of NADAR enzymes was only allowed to proceed for 15 min. Representative for three independent experiments.



Supplementary Figure S4. Multiple sequence alignment of selected members of the NADAR superfamily, related to Figures 1 and 5. Catalytically relevant residues in *E. coli* C7 NADAR are highlighted in pink, and residues specific for DarT1-associated NADARs are highlighted in green. The alignment was used to construct the phylogenetic tree shown in Figure 1E.



Supplementary Figure S5. Structural comparison of DarT1 and DarT2, related to Figure 3. In the middle of the figure, an overlay of *E. coli* C7 DarT, i.e. DarT1, with *Thermus* sp. 2.9 DarT, i.e. DarT2, both in complex with ADPr-DNA is shown. Differences in structural elements influencing the different catalytic functions are highlighted in the surrounding panels. The R51 side-chain flip observed in DarT2 compared to the pre-reaction state (not shown) is indicated by the black arrow. NAM coordination is relevant for considering NAD⁺ polarisation.



Supplementary Figure S6. Structural comparison of DarT1 and ScARP, related to Figure 6. (A) The co-crystal structure of *S. coelicolor* ScARP with GDP and NADH (PDB 5ZJ5) was overlaid with the *E. coli* C7 DarT1 structure in complex with GMP and carba-NAD⁺. The NAD⁺ derivatives and guanosine substrates take spatially similar positions although the transferases differ in their overall structural makeup, in particular regarding the ARTT substrate recognition loop (B) and the NAD⁺-binding loop (C). ScARP and DarT1 share the way of guanine positioning through N2 and N3 recognition and the orientation of the transferase-characteristic glutamate with respect to the distal-ribose of the NAD⁺-derived ligand.

SUPPLEMENTARY TABLES

Supplementary Table S1. Data collection and refinement statistics for crystal structures described in this study, related to Figures 3, 4 and S2.

	<i>E. coli</i> C7 DarT1: NAD ⁺	<i>E. coli</i> C7 DarT1: ADP- ribosylated DNA	<i>E. coli</i> C7 DarT1: Carba- NAD ⁺ and DNA	<i>G. lovleyi</i> NADAR apo
PDB accession code	8BAQ	8BAR	8BAS	8BAT
Data Collection				
Synchrotron/beam line	DLS/I03	DLS/I03	DLS/I03	DLS/I03
Wavelength (Å)	0.9763	0.9763	0.9763	0.9763
Space group	<i>P</i> 3 ₁ 2 1	<i>P</i> 4 ₃ 2 ₁ 2	<i>P</i> 4 ₃ 2 ₁ 2	<i>P</i> 2 ₁ 2 2 ₁
a (Å)	62.31	61.76	61.90	39.28
b (Å)	62.31	61.76	61.90	81.60
c (Å)	113.08	215.04	215.55	86.63
α (°)	90.00	90.00	90.00	90.00
β (°)	90.00	90.00	90.00	90.00
γ (°)	120.00	90.00	90.00	90.00
Content of AU	1	1	1	1
Resolution (Å) ^a	53.96 - 2.00 (2.05 - 2.00)	61.74 - 1.63 (1.66 - 1.63)	61.90 - 1.92 (1.97 - 1.92)	59.40 - 2.30 (2.38 - 2.30)
R _{sym} (%) ^{a,b}	12.9 (140.4)	7.2 (158.1)	15.9 (236.3)	19.1 (147.5)
I/σ(I)	5.8 (0.7)	14.0 (1.3)	8.3 (0.9)	6.2 (1.0)
Completeness (%) ^a	99.9 (99.9)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
Redundancy ^a	4.4 (4.4)	9.0 (9.0)	9.4 (9.8)	5.6 (5.3)
CC _{1/2} (%) ^a	99.7 (61.5)	99.9 (70.1)	99.8 (50.1)	99.4 (55.7)
Unique reflections ^a	17764 (1267)	53204 (2567)	33191 (2151)	12984 (1242)
Refinement				
R _{cryst} (%) ^c	19.6	15.5	17.0	18.1
R _{free} (%) ^d	24.8	17.8	21.0	23.2
RMSD bond length (Å)	0.0043	0.012	0.013	0.011
RMSD bond angle (°)	1.171	1.89	2.04	1.95
Amino acids ^e	208 [47.6]	1721 [28.5]	1716 [39.1]	1761 [46.9]
Water ^e	131 [47.21]	356 [44.7]	242 [47.9]	124 [46.1]
Ligands ^e	5 [47.3]	173 [30.90]	177 [49.3]	4 [72.8]
Ions ^e	-	-	-	1 [49.7]
Ramachandran plot				
Favoured (%)	95.6	97.6	97.1	98.6
Allowed (%)	3.9	1.9	2.4	1.4
Disallowed (%)	0.5	0.5	0.5	0.0

(a) Data for the highest resolution shell are given in parentheses.
(b) $R_{\text{sym}} = \sum |I| / \sum I$, where I is measured density for reflections with indices hkl .
(c) $R_{\text{cryst}} = \sum ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum |F_{\text{obs}}|$.
(d) R_{free} has the same formula as R_{cryst} , except that calculation was made with the structure factors from the test set.
(e) Number of atoms followed the average B factor in brackets.

Supplementary Table S1. Data collection and refinement statistics for crystal structures described in this study, related to Figures 3, 4 and S2. (Continuation)

	<i>P. nicotianae</i> <i>var. parasitica</i> NADAR: ADPr
PDB accession code	8BAU
Data Collection	
Synchrotron/beam line	DLS/I03
Wavelength (Å)	0.9763
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
a (Å)	44.83
b (Å)	66.59
c (Å)	72.89
α (°)	90.00
β (°)	90.00
γ (°)	90.00
Content of AU	1
Resolution (Å) ^a	49.17 - 1.60 (1.63 - 1.60)
R _{sym} (%) ^{a,b}	7.2 (212.8)
I/ σ (I)	13.0 (0.9)
Completeness (%) ^a	100.0 (99.9)
Redundancy ^a	9.6 (9.8)
CC _{1/2} (%) ^a	100.0 (59.6)
Unique reflections ^a	29538 (1421)
Refinement	
R _{cryst} (%) ^c	19.8
R _{free} (%) ^d	22.8
RMSD bond length (Å)	0.014
RMSD bond angle (°)	1.91
Amino acids	1539 [34.4]
Water	88 [36.6]
Ligands	56 [35.4]
Ions	-
Ramachandran plot	
Favoured (%)	98.4
Allowed (%)	1.6
Disallowed (%)	0.0

Supplementary Table S2. NCBI accession IDs of NADAR sequences used in this study, related to Figure 1.

Species	Accession number
Bacterial NADARs (DarT-associated)	
<i>Escherichia coli</i> C7	WP_032219797.1
<i>Geobacter lovleyi</i> [<i>Trichlorobacter lovleyi</i>]	WP_012470628.1
<i>Sinorhizobium fredii</i>	WP_014330845.1
<i>Vibrio cholerae</i>	WP_172778105.1
<i>Acinetobacter baumannii</i>	WP_001129309.1
<i>Clostridium perfringens</i>	MBS5923337.1
<i>Yersinia enterocolitica</i>	MBX9495195.1
<i>Pseudomonas syringae</i>	MCF9004830.1
<i>Ralstonia solanacearum</i>	WP_201016325.1
Bacterial NADARs (non-DarT-associated)	
<i>Escherichia coli</i>	WP_001183948.1
<i>Methylococcaceae bacterium</i>	NOQ36470.1
<i>Pseudomonas aeruginosa</i>	WP_116806626.1
<i>Planctomycetia bacterium</i>	MBL8863927.1
<i>Streptomyces caniscabiei</i>	WP_179201999.1
<i>Streptomyces coelicolor</i>	BDD75137.1
<i>Mycobacterium tuberculosis</i>	CNF61934.1
<i>Clostridium drakei</i>	WP_032077447.1
Bacterial YbiA-like	
<i>Listeria monocytogenes</i>	HAA3934926.1
<i>Gimesia chilikensis</i>	QDT22974.1
<i>Laceyella sacchari</i>	AUS10489.1
<i>Salmonella enterica</i>	WP_140040215.1
<i>Streptomyces scabiei</i>	WP_086756638.1
<i>Corynebacterium mustelae</i>	WP_047262349.1
<i>Escherichia coli</i>	HAW8155414.1
Oomycetes NADARs	
<i>Phytophthora sojae</i>	XP_009516941.1
<i>Phytophthora nicotianae</i> var. <i>parasitica</i>	XP_008911034.1
<i>Phytophthora infestans</i>	KAF4136563.1
Phage NADARs	
<i>Sinorhizobium</i> phage phiM12	YP_009143184.1
<i>Acinetobacter</i> phage Acj61	YP_004009822.1
<i>Rhizobium</i> phage RL38J1	QGZ13929.1
<i>Klebsiella</i> phage vB KpnM KpV477	YP_009288818.1
<i>Vibrio</i> phage ValKK3	YP_009201294.1
<i>Escherichia</i> virus T4	NP_049816.1
<i>Serratia</i> phage Muldoon	YP_009883850.1
Nematode NADARs	
<i>Caenorhabditis elegans</i>	NP_498348.1
<i>Ancylostoma caninum</i>	RCN47812.1
<i>Loa loa</i>	XP_003139559.1
<i>Trichinella papuae</i>	KRZ69666.1
Plant and fungal NADARs	
<i>Fomitopsis pinicola</i>	EPS93933.1
<i>Fomitopsis betulina</i>	KAI0715550.1
<i>Arabidopsis thaliana</i>	VYS59636.1
<i>Theobroma cacao</i>	EOY09810.1
<i>Cannabis sativa</i>	KAF4360218.1

Coffea arabica	XP_027089562.1
Asparagus officinalis	XP_020243908.1
Cupressus gigantea	ATG70670.1
Ceratopteris richardii	KAH7432347.1
Bathycoccus prasinos	XP_007514772.1
Malassezia pachydermatis	XP_017990970.1

Archaeal NADARs

Methanobrevibacter ruminantium	WP_012956765.1
Candidatus Lokiarchaeota archaeon	MBD3226909.1
Thermoplasma archaeon	MBE6519942.1

Supplementary Table S3. Oligonucleotides used in this study, related to STAR methods.

Oligo-ID	Sequence (5'→3')	Purpose
DarT_crist	AAGAC	Co-crystallisation with DarT1
DarT-ADPr-27mer	CACGACACGAGCAGGCATGTCCACGTG	ADP-ribosylation activity assay
DarT-ADPr-27mer-rc	CACGTGGACATGCCTGCTCGTGTCGTG	Reverse complement for ADP-ribosylation activity assay
PolyT-G	TTTTTTGTTTTTTTTTTTT	ADP-ribosylation activity assay
PolyT-GG	TTTTTTGGTTTTTTTTTTT	ADP-ribosylation activity assay
PolyT-GTG	TTTTTTGTGTTTTTTTTTT	ADP-ribosylation activity assay
PolyT-GTTG	TTTTTTGTTGTTTTTTTTT	ADP-ribosylation activity assay
PolyT-GTTTG	TTTTTTGTTTGTTTTTTTT	ADP-ribosylation activity assay
PolyT-GTTTTG	TTTTTTGTTTTGTTTTTTTT	ADP-ribosylation activity assay
DarT_Substrate_Motif1	CACTACACTATCATTCACTACCACTATC	ADP-ribosylation activity assay
DarT_Substrate_Motif2	CACTACACTATCATTACGACCACTATC	ADP-ribosylation activity assay
DarT_Substrate_Motif3	CACTACACTATCATTCGAGCCCACTATC	ADP-ribosylation activity assay
DarT_Substrate_Motif4	CACTACACTATCATTCAGGCCCACTATC	ADP-ribosylation activity assay
DarT_Substrate_Motif5	CACTACACTATCATTCATGTCCACTATC	ADP-ribosylation activity assay
DarT_Substrate_Motif6	CACTACACTATCATTCACGTCCACTATC	ADP-ribosylation activity assay
DarT_Substrate_Motif7	CACTACACTATCATTCGTGTCCACTATC	ADP-ribosylation activity assay
EcoliDarT_fwd	AGAACCTGTACTTCCAATCCATGACCATCCAAGAAATTATTC	Cloning of <i>E. coli</i> DarT1 into pBAD33 expression vector by Gibson Assembly
EcoliDarT_rev	CCGCCAAAACAGCCAAGCTTTCAACCCAGATAATAATGAC	
GeoDarT_fwd	AGAACCTGTACTTCCAATCCATGCGTACCGCAGTTGAAAATC	Cloning of <i>G. lovleyi</i> DarT1 into pBAD33 expression vector by Gibson Assembly
GeoDarT_rev	CCGCCAAAACAGCCAAGCTTTACAGCTGAAAGCTATTTGC	
pBAD33_fwd	AAGCTTGGCTGTTTTGGC	Vector amplification for Gibson Assembly
pBAD33_rev	GGATTGGAAGTACAGGTTC	
EcoliDarT-A152E-f001	CAGGCAGAGATTCTGGTGTTTGAGAAAATTCCGCCTAGCT	Mutagenesis of <i>E. coli</i> DarT1 to WT

EcoliDarT-A152E-r001	CAGAATCTCTGCCTGAACATCGGTGGTATATTCGCTCGGC	Mutagenesis of <i>E. coli</i> DarT1 to WT
GeoDarT-A152E-f001	CAGGCAGAGGTTCTGGTTTTTGGCACCATTTGAACCGGCAT	Mutagenesis of <i>E. coli</i> DarT1 to WT
GeoDarT-A152E-r001	CAGAACCTCTGCCTGCGGATGCGTCGGATAACTACGCGGA	Mutagenesis of <i>E. coli</i> DarT1 to WT
EcoliDarT-F18A-f001	TTTCATGCGACCCATAGCGATAATCTGACCAGCATTCTGG	Mutagenesis of <i>E. coli</i> DarT1 to WT
EcoliDarT-F18A-r001	ATGGGTGCGCATGAAACAGGCTGCGAATATTACGCTGCTGA	Mutagenesis of <i>E. coli</i> DarT1 to WT
EcoliDarT-D49A-f001	TGCAACGCGGAGGAACGCATTGATGGTCATCCTGATGCAA	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-D49A-r001	TTCTCCGCGTTGCAGTTATATTCATTGTTTTCGTTATCC	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-R52A-f001	GAGGAAGCGATTGATGGTCATCCTGATGCAATTTGTCTGA	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-R52A-r001	ATCAATCGCTTCCTCGTCGTTGCAGTTATATTCATTGTTT	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-S63A-f001	TGTCTGGCGGTTAGCTATCCGAATGCCAAAATGTTTTACA	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-S63A-r001	GCTAACCGCCAGACAAATTGCATCAGGATGACCATCAATG	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-M71A-f001	GCCAAAGCGTTTTACAAATACCGCTGTCTGAAACCTGGTG	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-M71A-r001	GTAAAACGCTTTGGCATTTCGGATAGCTAACGCTCAGACAA	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-F72A-f001	AAAATGGCGTACAAATACCGCTGTCTGAAACCTGGTGATT	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-F72A-r001	TTTGTACGCCATTTTGGCATTTCGGATAGCTAACGCTCAGA	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-N104A-f001	CCGACCGCGGCAGCCAGCAATAATGTGCGTTTTATCAATC	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-N104A-r001	GGCTGCCGCGGTCGGATAAAATGCACAATCTTTTGCCAC	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-D54A-f001	CGCATTGCGGGTCATCCTGATGCAATTTGTCTGAGCGTTAG	Mutagenesis of <i>E. coli</i> DarT1
EcoliDarT-D54A-r001	ATGACCCGCAATGCGTTCTCGTCGTTGCAGTTATATTCA	Mutagenesis of <i>E. coli</i> DarT1
PpaNADAR_GTWY_for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTGGAAGTTCTG	Cloning Phytophthora NADAR
PpaNADAR_GTWY_rev	TTCCAGGGTCCGATGGACTTTGTGGAGACGAATTCTGCCG GGGGACCACTTTGTACAAGAAAGCT GGGTATTAGTCCTGTGGCTGTAACCAAGCGCG	
NADAR-EcoliC7-E88A-f001	ACCGTTGCGAGCGCGTTTCAAGCGAGCAAAGTGTTGCGAAA	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-E88A-r001	CGCGCTCGCAACGGTGAACCTCCTTGCCCAGACGTTTGGTC	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K95A-f001	GCGAGCGCGGTGTTCGAAATGGGTGGCCCGTACGTTGACA	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K95A-r001	GAACACCGCGCTCGCTTGAAACGCGCTTTCAACGGTGAAC	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K115A-f001	GAGGCGGCGAAAGACATGCGTCTGAAGGAAAGCGGTGGCC	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K115A-r001	GTCTTTCGCCGCTCAATGCTGCTTTTATCCAGGATGTCA	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K116A-f001	GCGAAGGCGGACATGCGTCTGAAGGAAAGCGGTGGCCTGG	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K116A-r001	CATGTCCGCCTTCGCCTCAATGCTGCTTTTATCCAGGATG	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-R119A-f001	GACATGGCGCTGAAGGAAAGCGGTGGCCTGGTGAACTTCA	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-R119A-r001	CTTCAGCGCCATGTCTTTCTTCGCCTCAATGCTGCTTTTA	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K121A-f001	CGTCTGGCGGAAAGCGGTGGCCTGGTGAACTTCAAATTTT	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K121A-r001	GCTTTCGCCAGACGCATGTCTTTCTTCGCCTCAATGCTG	Mutagenesis of <i>E. coli</i> NADAR

NADAR-EcoliC7-D171A-f001	TTTACCGCGATCGAGTTTAACCCGGCGAAAAGCATTAACT	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-D171A-r001	CTCGATCGCGGTAAAGCCGTCGAAGTTCAGCAGGTGCAGC	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-E173A-f001	GATATCGCGTTTAACCCGGCGAAAAGCATTAACTGCCAAG	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-E173A-r001	GTAAACGCGATATCGGTAAAGCCGTCGAAGTTCAGCAGG	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K178A-f001	CCGGCGGCGAGCATTAACTGCCAAGCGCGTGCGGCGGCGC	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K178A-r001	AATGCTCGCCGCCGGTTAAACTCGATATCGGTAAAGCCG	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-D171N-f001	TTTACCAATATCGAGTTTAACCCGGCGAAAAGCATTAACT	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-D171N-r001	CTCGATATTGGTAAAGCCGTCGAAGTTCAGCAGGTGCAGC	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-E88Q-f001	ACCGTTCAGAGCGCGTTTCAAGCGAGCAAAGTGTTGAAA	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-E88Q-r001	CGCGCTCTGAACGGTGAACCTTGGCCAGACGTTTGGTC	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K95R-f001	GCGAGCCGCGTGTTCGAAATGGGTGGCCCGTACGTTGACA	Mutagenesis of <i>E. coli</i> NADAR
NADAR-EcoliC7-K95R-r001	GAACACGCGGCTCGCTTGAAACGCGCTTTCAACGGTGAAC	Mutagenesis of <i>E. coli</i> NADAR

Supplementary Table S4. Strains and plasmids used in this study, related to STAR methods.

Strain or plasmid-ID	Description	Source
DH5α	<i>huA2 a(argF-lacZ)U169 phoA glnV44 a80a(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i>	NEB
DH5α-macro	DH5α with integrated <i>T. aquaticus</i> DarG macrodomain at P21 site	Schuller <i>et al.</i> , 2021 [S1]
BL21	<i>fhuA2 [lon] ompT gal [dcm] ΔhsdS</i>	NEB
BL21(DE3)	<i>fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHI ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5</i>	NEB
Rosetta™ BL21 (DE3)	<i>F-ompT hsdSB(rB- mB-) gal dcm (DE3) pRARE (cam^R)</i>	Novagen
Plasmids		
pBAD33	Medium copy plasmid with an arabinose-inducible promoter; cam ^R	Guzman <i>et al.</i> , 1995 [S2]
pET28a	Medium copy plasmid containing the IPTG-inducible promoter; kan ^R	Novagen
pNIC28-Bsa4	Medium copy plasmid containing the IPTG-inducible promoter; kan ^R	Addgene [S3]
pBAD33_Taq_darT	pBAD33 carrying <i>T. aquaticus</i> darT full-length; cam ^R	Jankevicius <i>et al.</i> , 2017 [S4]
pET28_SC_SCO5461	pET28a carrying <i>S. coelicolor</i> scarp (SCO5461) full-length; kan ^R	Lalić, J. <i>et al.</i> , 2016 [S5]
pET28_Taq_darG_macro	pET28a carrying <i>T. aquaticus</i> darG macrodomain (aa 1-155); kan ^R	Jankevicius <i>et al.</i> , 2017 [S4]
pBAD33_Ecoli_darT1	pBAD33 carrying <i>E. coli</i> C7 darT1 full-length; cam ^R	This study
pBAD33_Ecoli_darT1 ^{F18A}	pBAD33 carrying <i>E. coli</i> C7 darT1 ^{F18A} full-length; cam ^R	This study
pBAD33_Ecoli_darT1 ^{D49A}	pBAD33 carrying <i>E. coli</i> C7 darT1 ^{D49A} full-length; cam ^R	This study
pBAD33_Ecoli_darT1 ^{R52A}	pBAD33 carrying <i>E. coli</i> C7 darT1 ^{R52A} full-length; cam ^R	This study
pBAD33_Ecoli_darT1 ^{D54A}	pBAD33 carrying <i>E. coli</i> C7 darT1 ^{D54A} full-length; cam ^R	This study
pBAD33_Ecoli_darT1 ^{S63A}	pBAD33 carrying <i>E. coli</i> C7 darT1 ^{S63A} full-length; cam ^R	This study
pBAD33_Ecoli_darT1 ^{M71A}	pBAD33 carrying <i>E. coli</i> C7 darT1 ^{M71A} full-length; cam ^R	This study
pBAD33_Ecoli_darT1 ^{F72A}	pBAD33 carrying <i>E. coli</i> C7 darT1 ^{F72A} full-length; cam ^R	This study
pBAD33_Ecoli_darT1 ^{N104A}	pBAD33 carrying <i>E. coli</i> C7 darT1 ^{N104A} full-length; cam ^R	This study
pBAD33_Ecoli_darT1 ^{E152A}	pBAD33 carrying <i>E. coli</i> C7 darT1 ^{E152A} full-length; cam ^R	This study
pNIC28_Ecoli_darT1 ^{E152A}	pNIC28-Bsa4 carrying <i>E. coli</i> C7 darT1 ^{E152A} full-length; kan ^R	This study
pBAD33_Glov_darT1	pBAD33 carrying <i>G. lovleyi</i> darT1 full-length; cam ^R	This study
pBAD33_Glov_darT1 ^{E152A}	pBAD33 carrying <i>G. lovleyi</i> darT1 ^{E152A} full-length; cam ^R	This study
pDEST17_Pnp_nadar	pDEST17 carrying <i>P. nicotianae</i> var. <i>parasitica</i> nadar full-length; kan ^R	This study
pET28_Glov_nadar	pET28a carrying <i>G. lovleyi</i> nadar full-length; kan ^R	This study
pET28_SinoR_nadar	pET28a carrying <i>S. fredii</i> nadar full-length; kan ^R	This study
pET28_Ecoli_nadar	pET28a carrying <i>E. coli</i> C7 nadar full-length; kan ^R	This study
pET28_Ecoli_nadar ^{E88A}	pET28a carrying <i>E. coli</i> C7 nadar ^{E88A} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{E88Q}	pET28a carrying <i>E. coli</i> C7 nadar ^{E88Q} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{K95A}	pET28a carrying <i>E. coli</i> C7 nadar ^{K95A} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{K95R}	pET28a carrying <i>E. coli</i> C7 nadar ^{K95R} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{K115A}	pET28a carrying <i>E. coli</i> C7 nadar ^{K115A} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{K116A}	pET28a carrying <i>E. coli</i> C7 nadar ^{K116A} full-length; kan ^R	This study

pET28_Ecoli_nadar ^{R119A}	pET28a carrying <i>E. coli</i> C7 nadar ^{R119A} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{K121A}	pET28a carrying <i>E. coli</i> C7 nadar ^{K121A} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{D171A}	pET28a carrying <i>E. coli</i> C7 nadar ^{D171A} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{D171N}	pET28a carrying <i>E. coli</i> C7 nadar ^{D171N} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{E173A}	pET28a carrying <i>E. coli</i> C7 nadar ^{E173A} full-length; kan ^R	This study
pET28_Ecoli_nadar ^{K178A}	pET28a carrying <i>E. coli</i> C7 nadar ^{K178A} full-length; kan ^R	This study

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