Title: A tale of tails: Deciphering the contribution of terminal tails to the biochemical properties of two Dps proteins from *Streptomyces coelicolor*.

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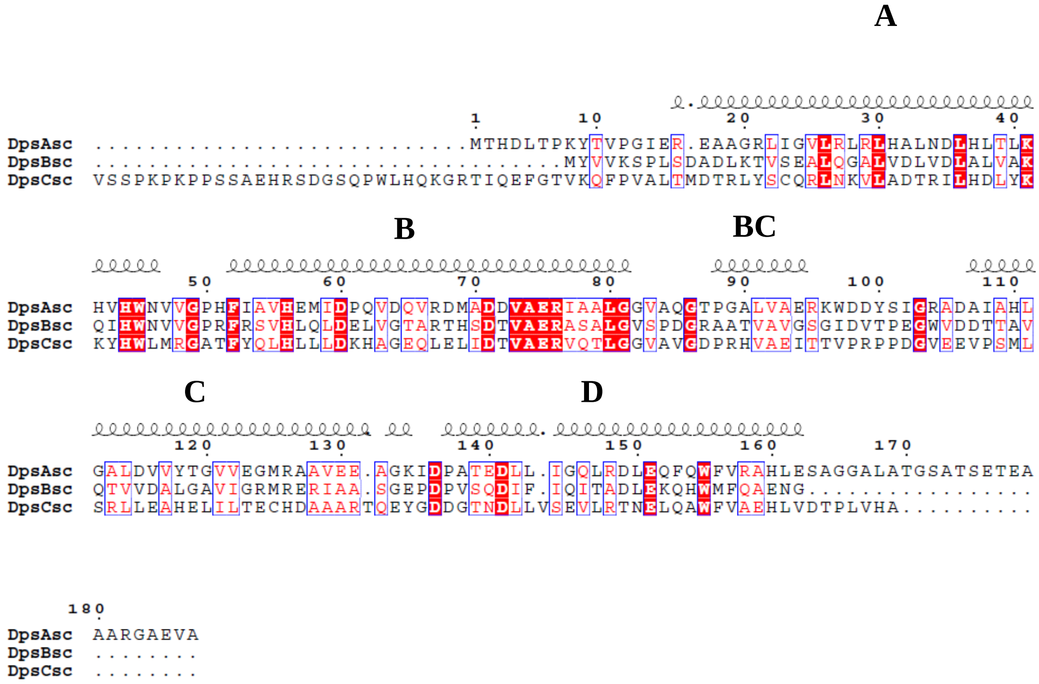
Supplementary Fig. 1: Aligned amino acid sequences of DpsA, DpsB and DpsC (A). Predicted α-helices are indicated. **Synthetic Dps mutant coding sequences used in this study (B).** NdeI and BglII sites introduced at 5' and 3' ends are underlined.

A



B

dpsA-DNT

cat ATG CGT GAA GCG GCT GGT CGT CTG ATT GGG GTT CTC CGC TTA CGC CTC CAT GCC CTG AAT GAC CTT CAT CTG ACG CTG AAA CAC GTG CAC TGG AAC GTC GTT GGC CCG CAC TTT ATC GCG GTG CAT GAG ATG ATC GAT CCG CAA GTC GAT CAG GTC CGC GAC ATG GCG GAT GAT GTT GCC GAA CGC ATT GCT GCG TTG GGA GGT GTA GCG CAA GGT ACA CCA GGC GCG CTG GTA GCA GAA CGC AAG TGG GAT GAC TAC TCG ATT GGT CGC GCA GAT GCC ATT GCG CAT CTG GGC GCA CTT GAC GTG GTG TAT ACC GGC GTA GTG GAA GGA ATG CGT GCA GCC GTT GAA GAA GCC GGG AAA ATC GAC CCT GCT ACC GAG GAT CTG CTG ATT GGC CAG TTA CGG GAT TTG GAG CAG TTC CAG TGG TTT GTG CGT GCC CAT CTG GAG AGC GCA GGC GGT GCA CTG GCC ACT GGC AGT GCG ACG TCC GAA ACC GAA GCG GCT GCC CGT GGT GCG GAA GTC GCa gatct

dpsA-DTM

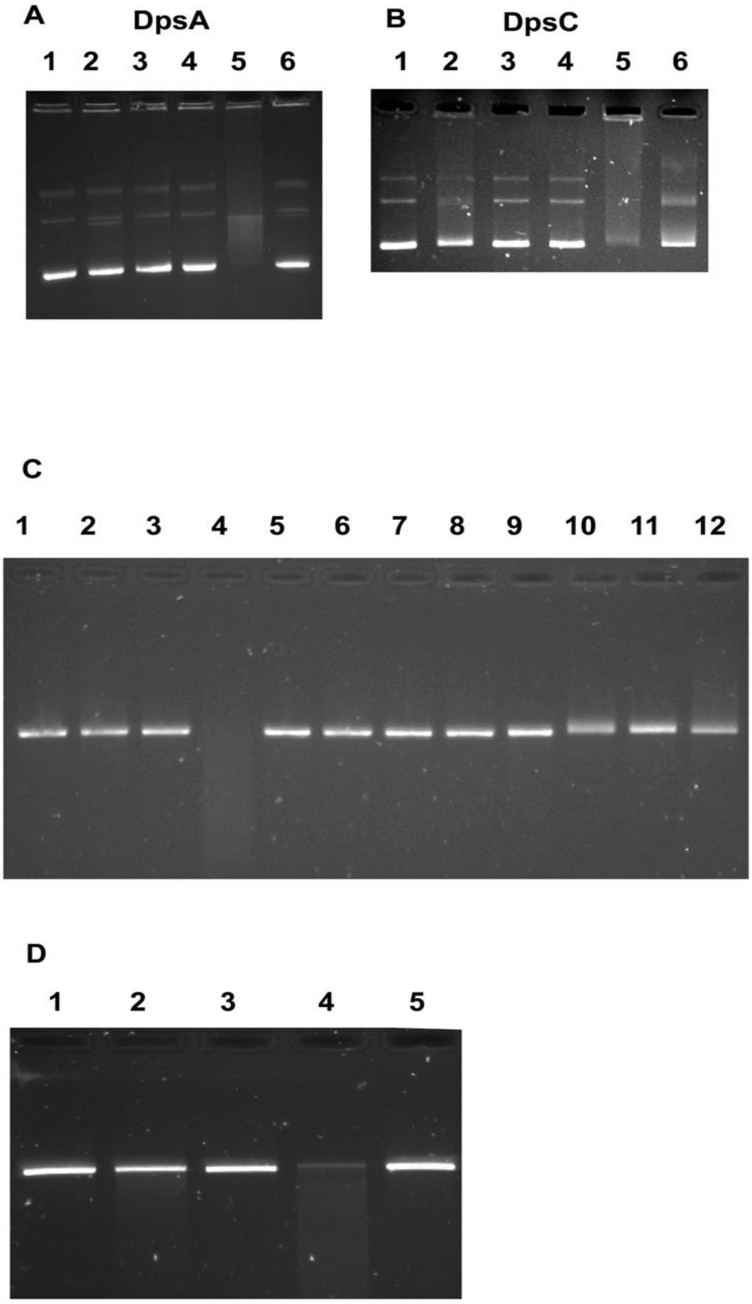
cat ATG CGC GAA GCT GCT GGT CGC CTG ATT GGC GTG TTA CGC CTG CGT CTG CAT GCG TTG AAC GAC CTC CAT CTG ACC CTG AAA CAC GTT CAC TGG AAT GTC GTT GGG CCG CAC TTT ATC GCC GTT CAT GAG ATG ATC GAT CCT CAG GTG GAC CAA GTG CGC GAT ATG GCT GAC GAT GTC GCG GAA CGC ATT GCA GCC CTC GGA GGA GTT GCA CAA GGG ACT CCA GGT GCG CTT GTA GCG GAG CGT AAA TGG GAC GAT TAC AGC ATT GGC CGT GCA GAT GCC ATT GCC CAT TTA GGT GCG CTG GAT GTG GTG TAT ACG GGT GTC GTG GAA GGC ATG CGT GCC GCG GTA GAA GAA GCC GGC AAG ATC GAT CCG GCA ACC GAG GAC CTG TTG ATT GGC CAG CTT CGG GAT CTG GAA CAG TTC CAG TGG TTT GTA CGC GCG CAT CTG GAAgat ct

dpsC-DNT

cat ATG ATG GAT ACT CGC CTG TAT AGC TGT CAA CGC CTG AAC AAA GTC CTC GCA GAT ACG CGG ATT CTG CAT GAC CTC TAC AAG AAA TAC CAC TGG CTG ATG CGT GGA GCG ACC TTC TAT CAG CTG CAC CTG TTA CTG GAC AAA CAC GCT GGT GAA CAG TTG GAG CTG ATT GAC ACG GTT GCG GAA CGC GTA CAG ACG TTA GGT GGC GTA GCG GTT GGT GAT CCG CGT CAT GTG GCG GAA ATC ACA ACC GTT CCG CGT CCT CCG GAT GGG GTG GAA GAG GTG CCC AGT ATG CTG TCC CGT TTG TTG GAA GCC CAC GAA CTG ATC CTT ACC GAG TGC CAT GAT GCC GCT GCA CGC ACT CAG GAG TAT GGC GAT GAT GGC ACC AAC GAC TTA CTG GTT TCG GAA GTG CTT CGC ACC AAT GAA CTG CAA GCC TGG TTT GTC GCG GAA CAT CTC GTG GAT ACA CCA CTG GTC CAT GCAgatct

Supplementary Fig. 2: DpsA and DpsC protect DNA from H_2O_2-mediated oxidative damage. Reactions were set using 40 ng of pUC18 (supercoiled in A and B, linearised with HindIII in C and D), 2 μg of protein (DpsA or DpsC), 50 μM $FeSO_4$ and initiated by adding 5 mM H_2O_2 as indicated followed by 5 minutes incubation at room temperature. Samples in panels A and B are: Lane 1- DNA, lane 2- DNA with $FeSO_4$, lane 3-DNA with H_2O_2, lane 4-DNA with Dps protein, lane 5- DNA with $FeSO_4$ and H_2O_2 and lane 6-DNA with Dps protein, $FeSO_4$ and H_2O_2. Protein used in each panel is indicated.

Samples in panel C are: Lane 1-plasmid DNA, lane 2- DNA and $FeSO_4$, lane 2- DNA with H_2O_2, lane 4- DNA with $FeSO_4$ and H_2O_2, lane 5- DNA with DpsA, lane 6- DNA with DpsA-DCT, lane 7- DNA with DpsA-DNT, lane 8- DNA with DpsA-DTM, lane 9- DNA with $FeSO_4$, H_2O_2 and DpsA, lane 10- DNA with $FeSO_4$, H_2O_2 and DpsA-DCT, lane 11- DNA with $FeSO_4$, H_2O_2 and DpsA-DNT, lane 12- DNA with $FeSO_4$, H_2O_2 and DpsA-DTM. Panel D shows DpsA protection against oxidative damage of DNA mediated by $CuSO_4$. Samples in lanes are as panels A and B, but using 50 μM $CuSO_4$.



Supplementary Table 1: Specific activity of DpsA and mutant variants. Specific activities were calculated as change in absorbance per second/mg of protein used in the reaction, using the linear portion of the curves. The data used was collected from three independent replicates using 276 µM substrate concentration and 0.2 mg/ml of protein (~0.74 µM).

Protein	**SA (ΔAbs$_{310}$ /s/mg)**	**St dev**
DpsA	0.00012	1.50E-05
DpsA-DCT	0.00058	2.2E-05
DpsA-DTM	0.00008	7.86E-06
DpsA-DNT	0.00005	3.93E-06

Supplementary Table 2: Data collection, phasing and refinement statistics

	DpsA	DpsA-DCT	DpsC
Data Collection			
X-ray source	i04	i04	i02
Wavelength (Å)	0.9795	0.9795	0.9795
Space group	F 41 3 2	P 42 3 2	P 21 21 21
Cell dimensions			
a, b, c (Å)	209.66 209.66 209.66	105.33 105.33 105.33	92.22 153.12 170.28
α, β, γ (°)	90.00 90.00 90.00	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	1.78	1.86	1.78
R_{merge} (%)[a,b]	0.090 (0.390)	0.084 (0.821)	0.077 (0.974)
I/σ(I)[a]	19.4 (6.5)	19.8 (4.2)	15.6 (2.4)
Completeness (%)[a]	99.9 (99.8)	99.9 (99.9)	100 (100)
Multiplicity[a]	11.4 (11.3)	14.0 (14.7)	6.7 (6.6)
Refinement			
Resolution (Å)	1.78	1.86	1.78
No. measurements	437943	241851	1537883
No. unique	38268	17268	228634
R_{work} / R_{free}	0.153 / 0.171	0.159 / 0.180	0.159 / 0.194
No. atoms			
Protein	1333	1257	16574
Ligand/ion	18	0	16
Water	243	135	1541
Average B-factor	18	28	29
R.m.s. deviations			
Bond lengths (Å)	0.03	0.0210	0.018
Bond angles (°)	2.8	1.7	1.7
PDB codes	4CY9	4CYA	4CYB

[a] Number in parentheses is for the last shell, [b] R = ∑(Abs(I - <I>) / ∑(I))

Supplementary Table 3: A summary of interface characteristics found in DpsA, DpsC and DpsA-DCT dodecamers.

	Interface type	**interface area, Å^2**	**$\Delta^{i}G$ kcal/mol**	**$\Delta^{i}G$ P-value**	**N_{HB}**	**N_{SB}**	**CSS**
DpsA	**Dimer interface**	1310.3	-7.9	0.552	14	8	0.660
	Dps-like trimer	512.0	-7.1	0.260	2	2	0.697
	Ferritin-like trimer	742.0	-3.5	0.552	8	7	0.295
DpsC	**Dimer interface**	1870.5	-12.8	0.699	27	27	0.525
	Dps-like trimer	820.4	-14.2	0.135	5	0	0.706
	Ferritin-like trimer	735.0	-4.9	0.637	8	9	0.593
DpsA-DCT	**Dimer interface**	1340.3	-7.7	0.554	12	2	1.000
	Dps-like trimer	447.2	-5.7	0.291	2	3	0.320
	Ferritin-like trimer	665.9	-2.0	0.628	9	6	0.311

Supplementary Table 4: DpsA N-terminal tail residue inter-subunit interfaces. Shown are the N-tail residues and the residues they interact with from a single symmetry related subunit per interface. C-terminal tail residues are shown in italics. Hb indicates predicted hydrogen bonds.

N-tail Residue	**Dimer Interface**	**Ferritin-like trimer**	**Total Interfacing Residues**
Asp-4	-	-	**0**
Leu-5	-	-	**0**
Thr-6	-	-	**0**
Pro-7	Arg-104	-	**1**
Lys-8	-	*Gly-165 (Hb), Gly-166, Ala-167*	**3**
Tyr-9	-	Arg-158,*Glu-162, Gly-166, Leu-168*	**4**
Thr-10	-	Arg-158 (Hb)	**1**
Val-11	-	Glu-151	**1**
Pro-12	-	Thr-119, Val-122, Glu-123, Arg-126 (Hb), Gln-154,	**5**
Gln-13	-	Arg-126	**1**
Ile-14	-	Arg-126	**1**
Glu-15	-	-	**0**
		Total Interfacing residues with N-tail	**17**

Supplementary Table 5: DpsA C-terminal tail residue inter-subunit interfaces. Shown are the C-tail residues and the residues they interact with from a single symmetry related subunit per interface. N-terminal tail residues are shown in italics. Hb and Sb indicate predicted hydrogen bonds and salt bridges.

C-Tail Residue	**N-terminal trimer**	**Dps-like trimer**	**Total Interfacing Residues**
Glu-162	*Tyr-9*	Val-48 Arg-104 (Hb, Sb x 2)	**4**
Ser-163	-	Val-48, Arg-104	**2**
Ala-164	-	Arg-104, Ala-105	**2**
Gly-165	*Lys-8 (Hb)*	Arg-104	**2**
Gly-166	*Lys-8*, *Tyr-9*	Arg-104	**2**
Ala-167	*Lys-8*	-	**1**
Leu-168	*Tyr-9*	-	**1**
Ala-169	-	-	**0**
Ala-170	-	-	**0**
		Total Interfacing Residues with C-tail	**14**

Supplementary Table 6: DpsA-DCT N-terminal tail residue inter-subunit interfaces. Shown are the N-tail residues and the residues they interact with from a single symmetry related subunit per interface. Hb indicates predicted hydrogen bonds.

N-tail Residue	**Dimer Interface**	**Ferritin-like trimer**	**Total Interfacing Residues**
Asp-4	-	-	**0**
Leu-5	-	-	**0**
Thr-6	-	-	**0**
Pro-7	Arg-104	-	**1**
Lys-8	-	-	**0**
Tyr-9	-	Arg-158, Glu-162 (Hb)	**2**
Thr-10	-	Arg-158 (Hb)	**1**
Val-11	-	Glu-151	**1**
Pro-12	-	Thr-119, Val-122, Glu-123, Arg-126 (Hb), Gln-154,	**5**
Gln-13	-	Arg-126	**1**
Ile-14	-	Arg-126	**1**
Glu-15	-	-	**0**
		Total Interfacing residues	**12**

Supplementary Table 7: DpsC N-terminal tail residue inter-subunit interfaces. C-terminal tail residues are shown in italics. Hb and Sb indicates predicted hydrogen bonds and salt bridges.

N-tail Residue	**Dimer Interface**	**Ferritin-like trimer**	**Total Interfacing Residues**
Arg-28	Glu-135 (2Hb) (Sb)	-	**1**
Thr-29	Glu-134, Val-133, Gly-132	-	**3**
Ile-30	Glu-135, Arg-77, Val-133 (Hb)	*Thr-195, Pro-196*	**5**
Gln-31	Gly-132, Arg-77, Val-133 (2Hb)	-	**3**
Glu-32	-	-	**0**
Phe-33	-	-	**0**
Gly-34	-	-	**0**
Thr-35	-	-	**0**
Val-36	-	-	**0**
Lys-37	Arg-77	Glu-190 (Hb), (2Sb)	**2**
Gln-38	-	-	**0**
Phe-39	-	Trp-186, Ala-189, Glu-190	**3**
Pro-40	-	Leu-144, Glu-148, Ala-185	**3**
Val-41	-	Glu-148, Leu-151, Glu-182	**3**
Vla-42	-	Glu-148 (Hb), Leu-151, Thr-152, His-155	**4**
Leu-43	-	His-155, Glu-182	**2**
Thr-44	-	-	**0**
		Total Interfacing Residues with N-tail	**29**

Supplementary Table 8: DpsC C-terminal tail residue inter-subunit interfaces. N-terminal tail residues are shown in italics. Hb indicates predicted hydrogen bonds.

C-Tail Residue	**N-terminal trimer**	**Dps-like trimer**	**Total Interfacing Residues**
Val-193	*Ile-30, Lys-37*	Gly-78, Arg-77	**4**
Asp-194	-	-	**0**
Thr-195	*Ile-30, Lys-37*	Gly-78, Arg-77, Val-136	**5**
Pro-196	*Ile-30, Lys-37*	Glu-135, Val-136, Pro-137	**5**
Leu-197	-	Pro-137	**1**
Val-198	-	Pro-137, Leu-192, Asp-194	**3**
His-199	-	Ser-138, Pro-137, Ser-141 (Hb) Leu-192	**4**
		Total Interfacing Residues with C-tail	**22**

Supplementary Table 9: PISA statistics for Dps encoded by of mesophilic or extremophilic (*) organisms.

Organism (PDB ID)	**Interface type**	**Interface area, Å^2**	**Δ^iG kcal/mol**	**Δ^iG P-value**	**N_{HB}**	**N_{SB}**	**CSS**
S. coelicolor DpsA	**Dimer interface**	1310.3	-7.9	0.552	14	8	0.66
	Dps-like trimer	512.0	-7.1	0.260	2	2	0.697
	Ferritin-like trimer	742.0	-3.5	0.552	8	7	0.295
S. coelicolor DpsC	**Dimer interface**	1870.5	-12.8	0.699	27	27	0.525
	Dps-like trimer	820.4	-14.2	0.135	5	0	0.706
	Ferritin-like trimer	735.0	-4.9	0.637	8	9	0.593
M. smegmatis Dps1 (1VEI)	**Dimer interface**	1303.9	-7.0	0.673	14	8	0.153
	Dps-like trimer	482.9	-6.6	0.268	4	2	0.276
	Ferritin-like trimer	793.5	-8.5	0.181	6	7	0.341
E. coli Dps (1DPS)	**Dimer interface**	1531.1	-11.8	0.262	16	7	0.598
	Dps-like trimer	399.2	-6.2	0.179	3	0	0.238
	Ferritin-like trimer	682.0	-0.2	0.652	12	9	0.210
M. smegmatis Dps2 (2Z90)	**Dimer interface**	1266.4	-4.7	0.496	17	13	0.368
	Dps-like trimer	495.6	-1.0	0.561	8	5	0.320
	Ferritin-like trimer	970.2	2.2	0.751	17	15	0.197
L. innocua Dps (1QGH)	**Dimer interface**	1141.5	-17.1	0.116	14	10	0.510
	Dps-like trimer	492.5	-5.4	0.380	5	3	0.202
	Ferritin-like trimer	429.4	-3.7	0.472	4	5	0.157
K. radiotolerans Dps * (4A25)	**Dimer interface**	1257.9	-7.3	0.498	22	17	0.372
	C-terminal trimer	573.5	-2.9	0.542	9	5	0.390
	N-terminal trimer	1079.4	-6.6	0.447	11	6	0.569

T. elongatus Dps * (2C41)	**Dimer interface**	1167.1	-14.5	0.327	12	10	1.000
	C-terminal trimer	798.7	-12.0	0.191	9	5	1.000
	N-terminal trimer	471.8	-3.0	0.670	4	4	0.198
D. radiodurans Dps * (2C2F)	**Dimer interface**	1627.5	-14.8	0.158	20	8	0.221
	C-terminal trimer	533.5	-0.8	0.675	10	9	0.117
	N-terminal trimer	784.1	-5.0	0.249	14	3	0.206
S. solfataricus Dps * (2CLB)	**Dimer interface**	1293.0	-9.2	0.317	23	19	0.326
	C-terminal trimer	507.1	-8.8	0.086	4	2	0.309
	N-terminal trimer	904.2	-7.2	0.249	9	2	0.333
H. salinarum Dps * (1MOJ)	**Dimer interface**	1748.4	-11.8	0.219	32	14	0.465
	C-terminal trimer	878.2	-8.7	0.115	12	5	0.543
	N-terminal trimer	826.9	-3.3	0.442	16	14	0.484