Supplementary information for:

A phage encoded GapR nucleoid-associated protein binds a bacterial GapR to deactivate it via an inhibitory hetero-oligomer mechanism

Maria A. Schumacher[1], Raul Salinas[1], Sunny Yadav[1], Katie Leung[2], Porter K. Ellis[1], Hengshan Zhang[1] and Monica S. Guo[2]

[1]Department of Biochemistry, 307 Research Dr., Box 3711, Duke University Medical Center, Durham, NC 27710, USA.

[2]Department of Microbiology, 750 Republican St., F830, University of Washington School of Medicine, Seattle, WA 98109, US

Correspondence:

E-mail: maria.schumacher@duke.edu

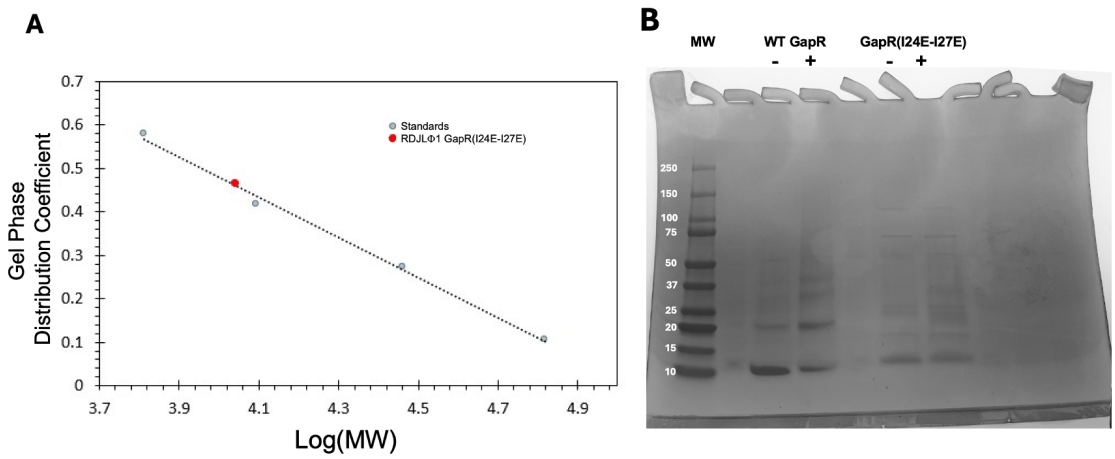

Figure S1. Roseophage dimer mutant supports structural model. Glutaraldehyde **A** SEC analyses of the Roseophage Gapr(I24E-I27E) mutant showing it eluted as a monomer. **B** crosslinking of Roseophage GapR WT and GapR(I24E-I27E) mutant. Samples were crosslinked with 0.05% glutaraldehyde for 0 and 10 min and then run on a 4-20% Mini-PROTEAN TGX Stain-free precast gel (BIO-RAD) (Cat # 456-8093). The proteins crosslinked are labeled above the gel and – and + indicate non-crosslinked and crosslinked for 15 min. The samples were then added to SDS protein running buffer and boiled for 2 min prior to loading. Note, the WT shows clear crosslinking to a dimer (with some dimer even present in the non-crosslinked lane) with some higher trimer and tetramer, while there is no visible crosslinked species for the mutant.

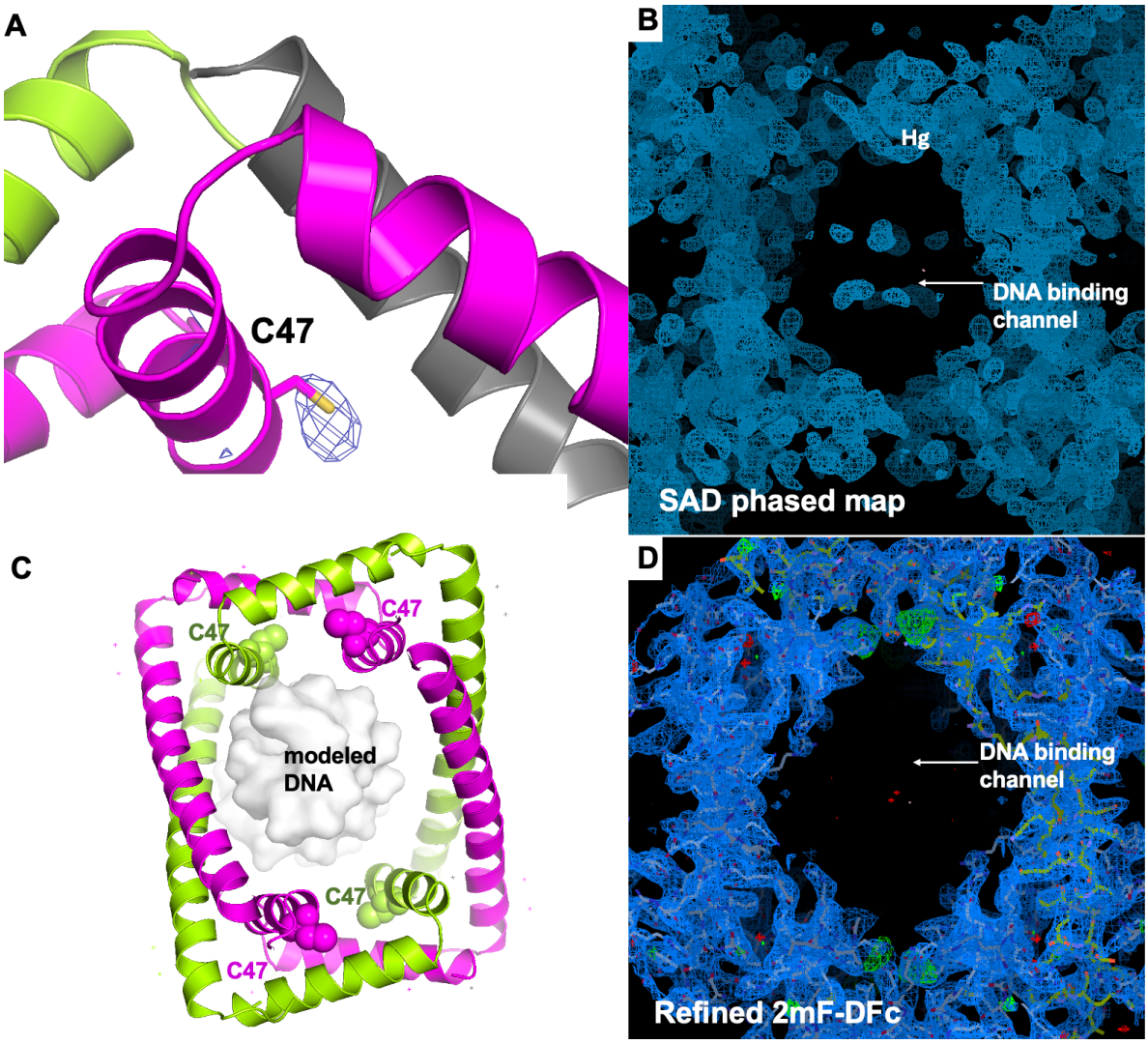

Figure S2. *R. denitrificans* GapR-DNA structure contains disordered DNA. **A** Close up of the anomalous difference map contoured at 15 σ and shown as blue mesh. B SAD phased map of data collected for a $HgCl_2$ soak contoured at 1 σ. the map shows clear density for the protein but not for the DNA. **C** Model of the *R. denitrificans* GapR-DNA complex showing the location of the cysteines and where the DNA would bind. **D** 2mFo-DFc map (blue mesh contoured at 1 σ) and mFo-DFc map (green mesh contoured at 4 σ) of the Hg soaked data without adding the Hg. Again, no DNA density is evident in the map.

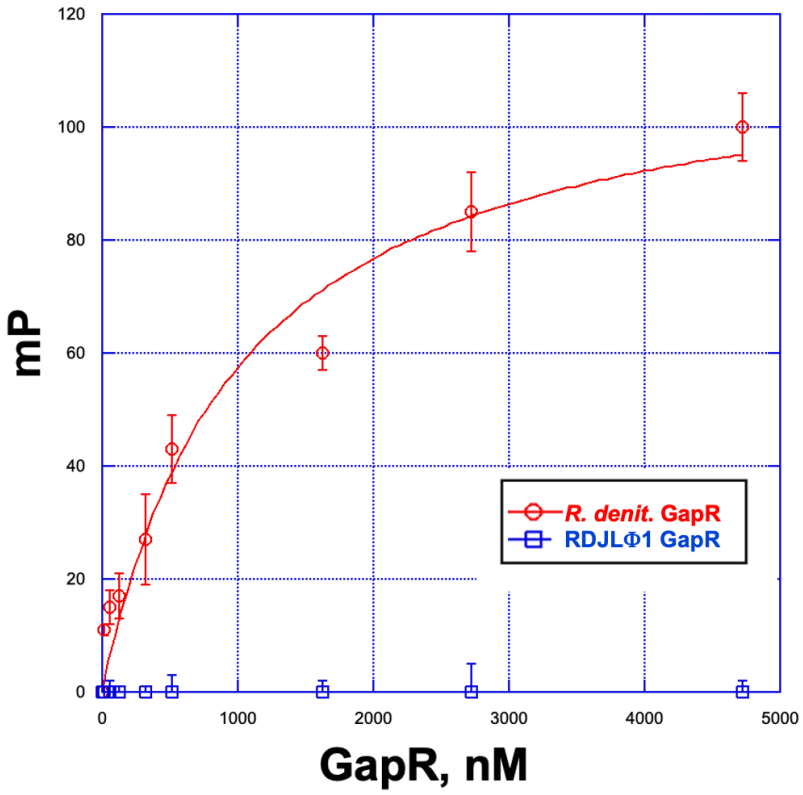

Figure S3. FP binding isotherms of *R. dentrificans* GapR and RDJLΦ1 GapR binding to the fluoresceintly tagged DNA duplex (top strand: 5´-CGCGCCGGCCGGCCGGCGCG-3´). *R. dentrificans* GapR bound with a K_d of 151± 34 nM while RDJLΦ1 GapR showed no detectable binding. The *x* and *y* axes are concentration of each GapR protein in nM and millipolarization units, respectively. Three technical repeats were performed for each (shown is a representative binding curve) and the standard errors from the three affinities were determined.

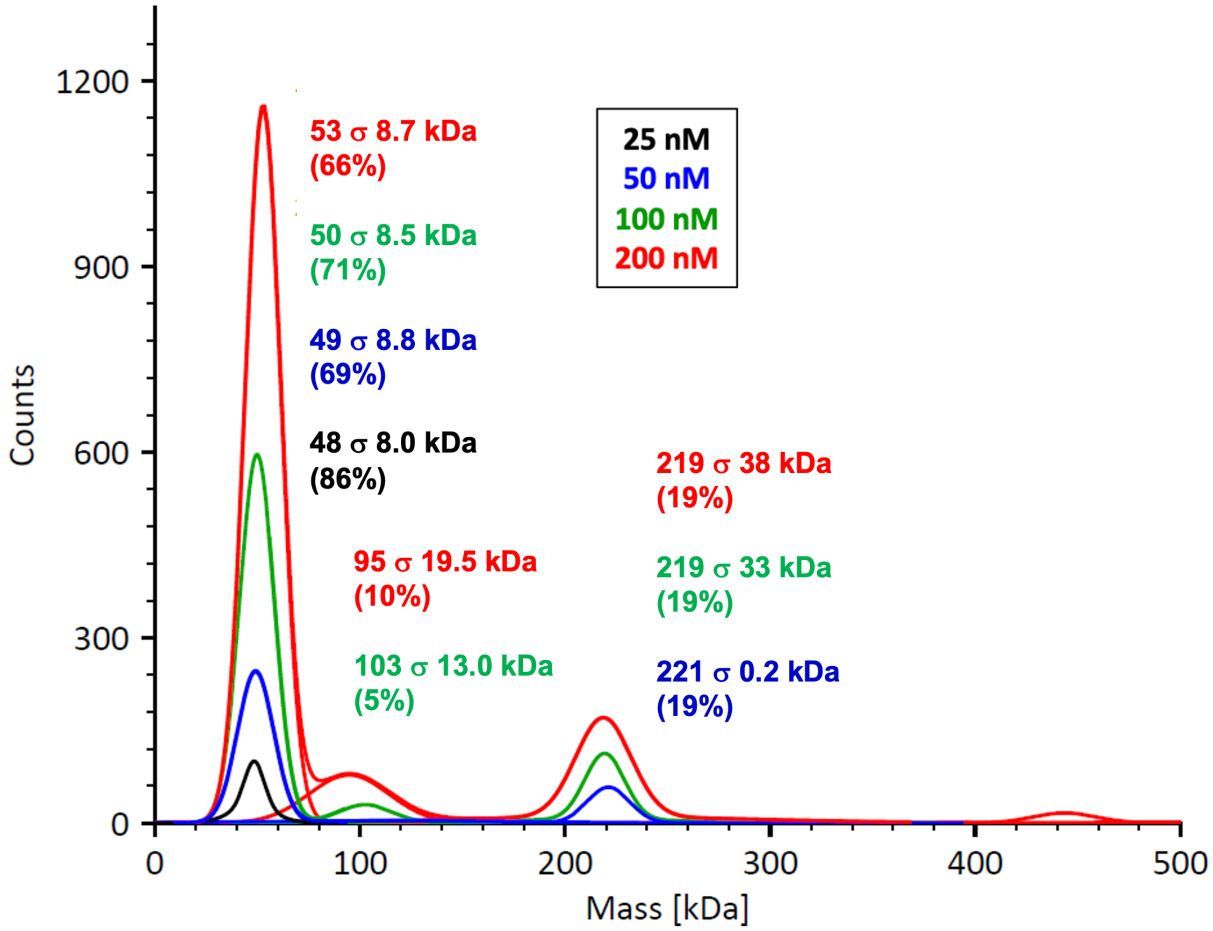

Figure S4. MP data analyses of MNP-*R. dentrificans* GapR at concentration of 25 nM (black), 50 nM (blue), 100 nM (green) and 200 nM (red) after 4 h incubation at rt.

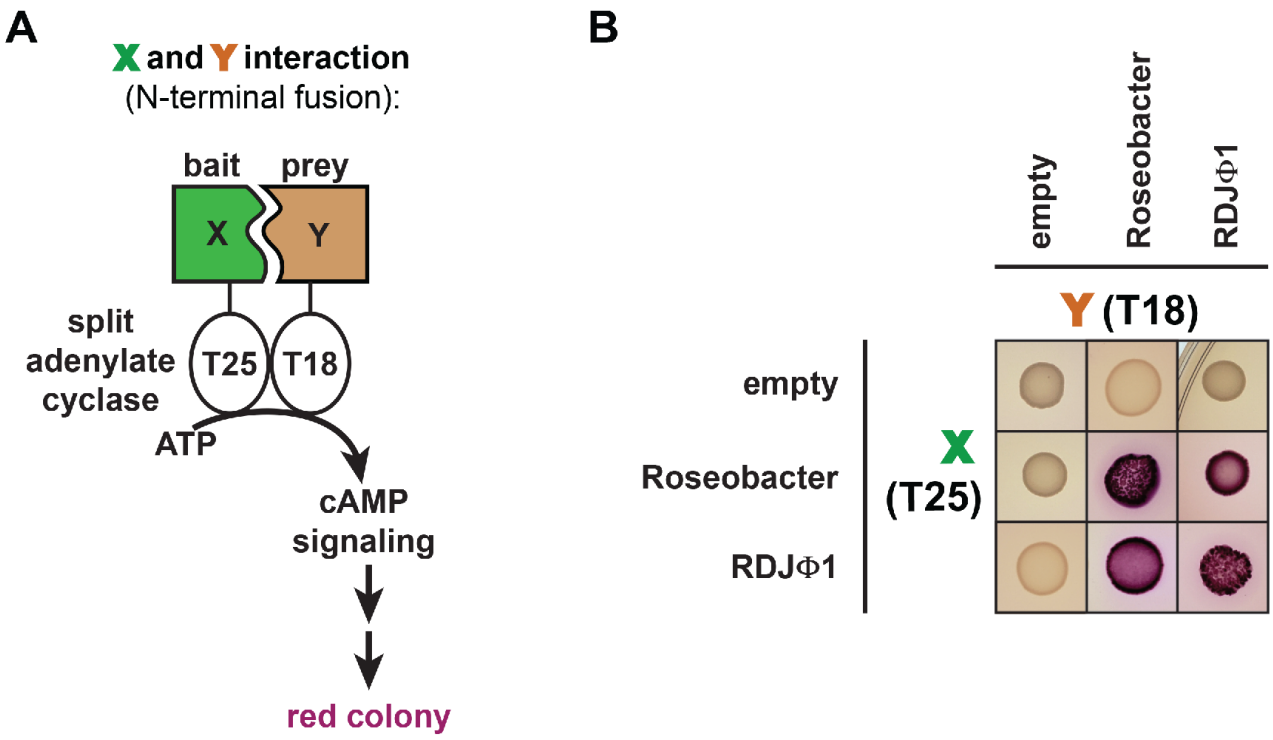

Figure S5. B2H analyses of RDJLΦ1 and *Roseobacter* GapR. **A** Schematic of the bacterial two-hybrid (B2H) assay. Briefly, adenylate cyclase is split into T25 and T18 subunits and N-terminally fused to bait (X) and prey (Y) proteins. On MacConkey agar, no interaction results in beige colonies while interactions reconstitute the adenylate cyclase resulting in a red colony. **B** B2H results for *Roseobacter* or RDJLΦ1 GapR interactions.

Table S1. Crystallographic data collection statistics: Data collection for *Roseobacter* and RDJLΦ1 GapR structures

	RDJLΦ1 GapR/crystal form 1	RDJLΦ1 GapR/crystal form 2	*Roseoacter* GapR apo	*Roseoacter* GapR with DNA
Data collection				
Pdb code	10OK	10ON	10OS	10PH
Space group	$I2_12_12_1$	$P4_12_12$	$P4_12_12$	I222
Wavelength (λ)	0.9797	1.0200	1.0200	3.5418
Cell dimensions				
a,b,c (Å)	41.8, 56.6, 90.1	48.8,48.8, 182.4	57.2, 57.2,169.5	37.3,63.0,84.3
α, β, γ (°)	90.0,90.0,90.0	90.0,90.0,90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	37.95-2.20(2.35-2.20)*	47.12-3.00(3.11-3.00)	39.34-2.94(3.08-2.98)	18.6-2.94(3.10-2.94)
R_{sym}	0.065 (0.680)	0.090 (0.230)	0.045 (1.016)	0.058 (.321)
R_{pim}	0.017 (0.256)	0.031 (0.055)	0.021 (0.597)	0.014 (0.076)
I/σI	23.7 (2.1)	20.2 (2.2)	22.7 (1.9)	50.2 (2.7)
Completeness (%)	97.4 (94.2)	98.1 (97.8)	99.8 (99.2)	99.8 (100.0)
Redundancy	12.1 (9.8)	9.0 (9.0)	9.3 (5.6)	20. 1(19.0)
CC(1/2)	1.000 (0.821)	0.996 (0.995)	0.998 (0.886)	1.000 (0.996)
Refinement				
Resolution (Å)	37.95-2.20	47.12-3.00	39.34-2.98	18.6-2.94
No. reflections	9705 (384)	4753 (445)	6238 (1347)	2307 (384)
R_{work}/R_{free}	23.1/25.6	22.7/26.6	22.7/26.7	23.8/26.4
Rms deviations				
Bond lengths (Å)	0.008	0.001	0.007	0.010
Bond angles (°)	1.37	0.440	0.91	1.134
Ramachandran				
Favored (&)	100.0	98.0	98.7	94.6
Disallowed (%)	0.0	0.0	0.0	0.0

*Highest resolution shells

Table S2. Strains, plasmids, and primers used for B2H

Strains

Identifier	Genotype	Source	Resistance
MSG1208	Dh5α	Lab collection	
MSG1052	Dh5α pKT25	Lab collection	kan
MSG1053	Dh5α pNKT25	Lab collection	kan
MSG1054	Dh5α pUTC18	Lab collection	amp
MSG1055	Dh5α pUT18	Lab collection	amp
MSG1056	BTH101 (B2H assay strain)	Euromedex	strep
KL3005	Dh5α pUT18-*R. denitrificans* GapR	This work	carb
KL3006	Dh5α pUT18-RDJLΦ1 GapR	This work	carb
KL3008	Dh5α pNKT25-*R. denitrificans* GapR	This work	kan
KL3009	Dh5α pNKT25-RDJLΦ1 GapR	This work	kan
KL3018	Dh5α pUT18C-*R. denitrificans* GapR	This work	carb
KL3020	Dh5α pKT25-*R. denitrificans* GapR	This work	kan
KL3021	Dh5α pKT25-RDJLΦ1 GapR	This work	kan
KL3025	Dh5α pUT18C-RDJLΦ1 GapR	This work	carb
KL3040	Dh5α pKT25-*C. crescentus* GapR	This work	kan
KL3055	Dh5α pKT25-Cr30 GapR	This work	kan
KL3077	Dh5α pKT25-JessA GapR	This work	kan
KL3083	Dh5α pKT25-RW GapR	This work	kan
KL3010	BTH101 pNKT25-*R. denitrificans* GapR + pUT18-*R. denitrificans* GapR (positive control)	This work	carb, kan
KL3022	BTH101 pNKT25 pUT18-*R. denitrificans* GapR (negative control)	This work	carb, kan

Plasmids

Identifier	Name	Source
EUP-18N	pUT18	Euromedex
EUP-18C	pUT18C	Euromedex
EUP-25N	pNKT25	Euromedex
EUP-25C	pKT25	Euromedex

Primers

Name	Sequence 5' to 3'	Source	Usage
oAM1620	tatgcttccggctcgtatg	Sigma-Aldrich	sequencing
oAM1625	atgtactggaaacggtgc	Sigma-Aldrich	sequencing
oAM1623	ggtgaccagcggcgattc	Sigma-Aldrich	sequencing
oAM1624	gtgctgcaaggcgattaag	Sigma-Aldrich	sequencing
oAM1626	gggctggcttaactatgc	Sigma-Aldrich	sequencing
oAM1622	aacaagtcgatgcgttcg	Sigma-Aldrich	sequencing
oAM1621	gcgtttgcgtaaccagc	Sigma-Aldrich	sequencing
MSG631	actctagagatgttcggccacgagaccctg	IDT	cloning
MSG632	ctcggtacccg catgcccagggcctccttgtac	IDT	cloning
MSG633	actctagag atgaacgtcaaccgcgactatgaccagc	IDT	cloning
MSG634	ctcggtacccg gtcgggcttctgcgcgatgccg	IDT	cloning
KL32	actctagagatgactgaagtagcatcagtagatg	IDT	cloning

KL33	ctcggtaccaatttcaccaagagcggtgaga	IDT	cloning
KL34	gtcgactctagagatgagcggtatcggccacaactc	IDT	cloning
KL35	cttaggtacccgagacttggtcgtcttcgtcg	IDT	cloning
KL36	gtcgactctagagatgatcggacacaacgc	IDT	cloning
KL37	cttaggtacccgtcagacgaggtcttcgcaacc	IDT	cloning
KL38	gtcgactctagaggccgacgacgccattccccacac	IDT	cloning
KL39	gagctcggtacccggatctcgccgatcgccgacagatagag	IDT	cloning