Ligand binding pocket bridges DNA-binding and dimerization domains of the urate-responsive MarR homolog MftR from *Burkholderia thailandensis*

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Supplemental Tables

Table S1. Primers used

A		
MftR	MftR_Fw	CAGGAGGG***CATATG***GATCGC
	MftR_Rev	GCTTGAC***GAATTC***GGCGAGTC
W11F	W11F_Fw	GCGGTCGAGCAGT**TC**CGCAGCGAGCGCCCGGATCTCGATC
	W11F_Rev	GCGCTCGCTGCG**GA**ACTGCTCGACCGCATGAGCTGCGCGATC
D56S	BTh_D56S_Fw	GCCGGGCGAGTTC**TC**CGTGCTCGCGACGCTGC
	BTh_D56S_Rev	GCGAGCACG**GA**GAACTCGCCCGGCTGCAGGCCGTAGC
R63S	BTh_R63S_Fw	GCTGCGC**A**GCAGCGGCGCGCCGTATGCGCTGAC
	BTh_R63S_Rev	GCGCCGCTGC**T**GCGCAGCGTCGCGAGCACGTCGAACTC
R89N	BTh_R89N_Fw	GCATGACGAAC**AAC**ATCGATCGGCTCGAGAAGGCGGGGTGGGTC
	BTh_R89N_Rev	GAGCCGATCGAT**GTT**GTTCGTCATGCTGCCCGACGAAATCATCGC
B		
mftR	Fw	GATCGATCGGCTCGAGAA
	Rev	CTGCCCTTGCAACAGCTT
mftP	Fw	GCCCATGCTTAATTCTCCTG
	Rev	ATCAGCAGGATCGGCAAG
gapdH	Fw	CTTCACGTCGAAGGAAAAGG
	Rev	TGATAGACGTCCGTCAGCAC
C		
mftrO	*mftrO*_Top	CGTCCAAGTTATCTTGACGTAGAGACATGTCGAATC
	*mftrO*_Bot	GATTCGACATGTCTCTACGTCAAGATAACTTGGACG
mftpO	*mftpO*_Top	TGTCGAATCTATCTTGATGTCGAGACAATTATACGC
	*mftpO*_Bot	GCGTATAATTGTCTCGACATCAAGATAGATTCGACA
mftO	Fw	GAGCT***ACGCGT***TCCATTAACCC
	Rev	GATGCG***ACGCGT***CCGGAC

A. Gene cloning and mutagenesis. B. qRT-PCR. C. Primers for amplification of intergenic region *mftO* and oligonucleotides containing each cognate site. Restriction sites indicated in bold italics and substitutions required for site-directed mutagenesis in boldface.

Table S2. Melting temperature of MftR and variants determined by CD spectroscopy

MftR variants	Melting temperature (°C)
MftR WT	50.4 ± 1.0 (D1)
	60.0 ± 0.1 (D2)
W11F	27.0 ± 0.7
D56S	52.1 ± 0.2
R63S	45.4 ± 0.6
R89N	56.1 ± 0.4

Table S3. Apparent dissociation constants of HucR and PecS variants

HucR variants[a]	K_d (nM)	PecS variants[b]	K_d (nM)
HucR WT	0.6 ± 0.2	WT PecS	0.4 ± 0.0
W20F	1.7 ± 0.3	W18F	**
D73S	0.3 ± 0.0	D62S	3.0 ± 0.2
R80S	0.4 ± 0.0	R69S	0.8 ± 0.1
R106N	*	R95N	***

*DNA binding was significantly compromised; non-specific complex was formed at μM concentration of HucR-R106N.
**Mutation resulted in protein aggregation.
***DNA binding to PecS promoter was completely abolished.
[a]From Perera et al. (2009*) J. Mol. Biol. 390*, 1019-1029. Apparent Kd for HucR binding to a single site.
[b]From Perera & Grove (2010) *J. Mol. Biol. 402*, 539-551. Apparent Kd for PecS binding to DNA with three cognate sites.

Supplemental Figures

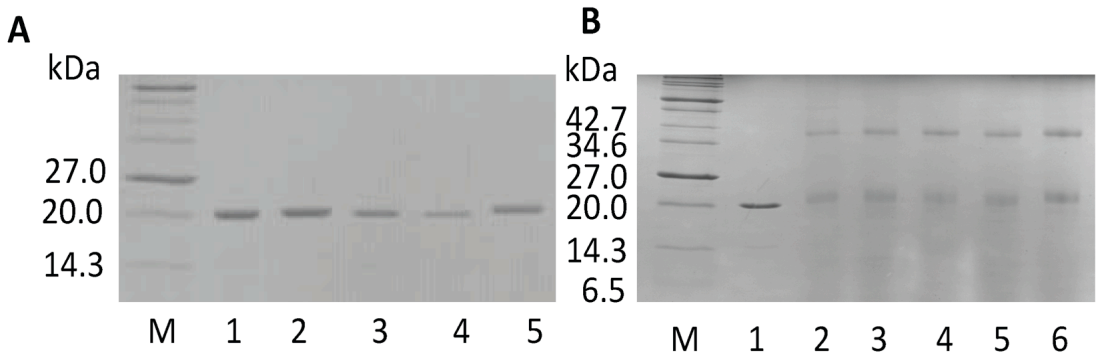

Figure S1. SDS-PAGE gel (18%) showing purified WT MftR and single residue mutants and their crosslinked products. (A) Left lane is protein marker (M) and lanes 1-5 are purified WT MftR, W11F, D56S, R63S and R89N, respectively. (B) Left lane (M) is protein marker. Lane 1 is WT MftR and lanes 2-6 contain 3.0 μg protein crosslinked with glutaraldehyde: WT MftR, W11F, D56S, R63S and R89N, respectively. Proteins were crosslinked with 0.5% (v/v) glutaraldehyde.

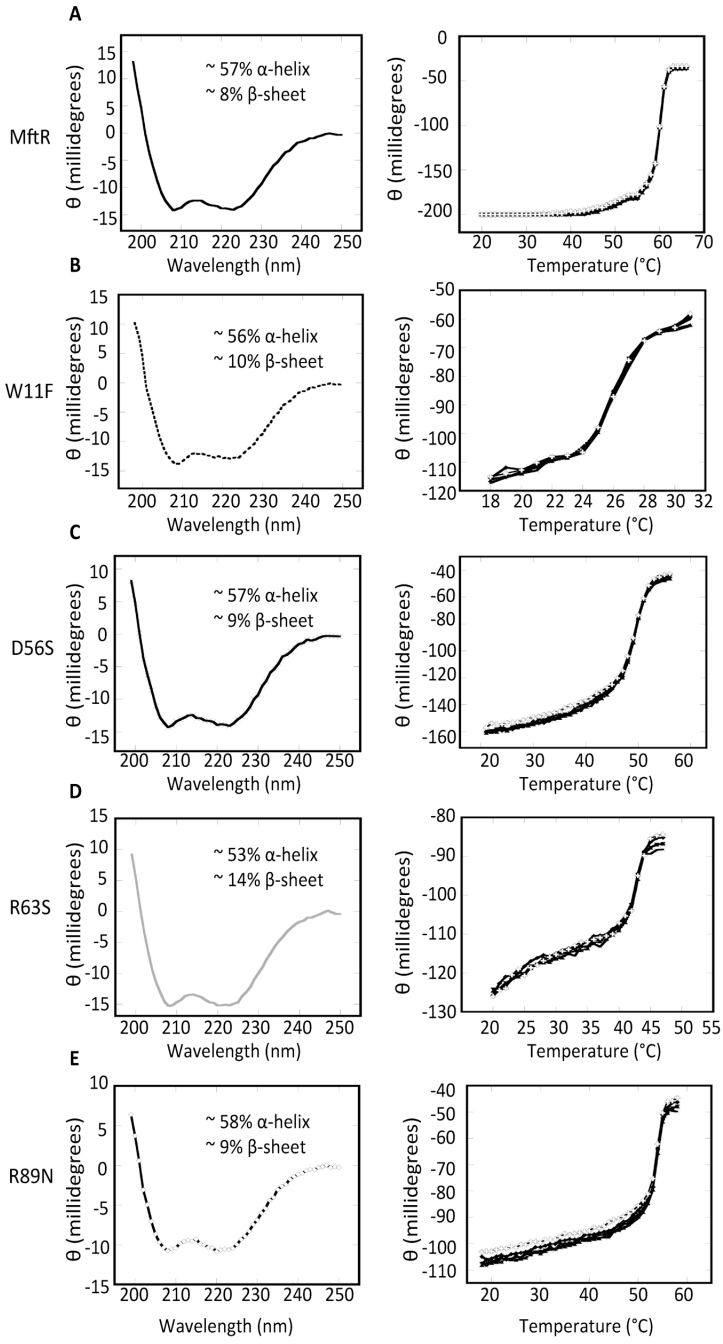

Figure S2. CD spectral analysis of MftR and variants. Left and right panels show the far UV CD spectrum and melting profile of MftR and variants, respectively. Machine units (millidegrees) were used to express ellipticity measurements. Ellipticity measurements were collected over the temperature range of 20-65 °C at five wavelengths spanning the negative ellipticity maximum characteristic of α-helices: 220 nm (×); 221 nm (▲); 222 nm (▼); 223 nm (◆); and 224 nm (✞). (A) Far-UV CD spectrum of MftR (solid black line) and thermal unfolding transition in right panel. (B) W11F (black dotted line). (C) D56S (×; solid black line). (D) R63S (solid gray line). (E) R89N (◇; solid black line).

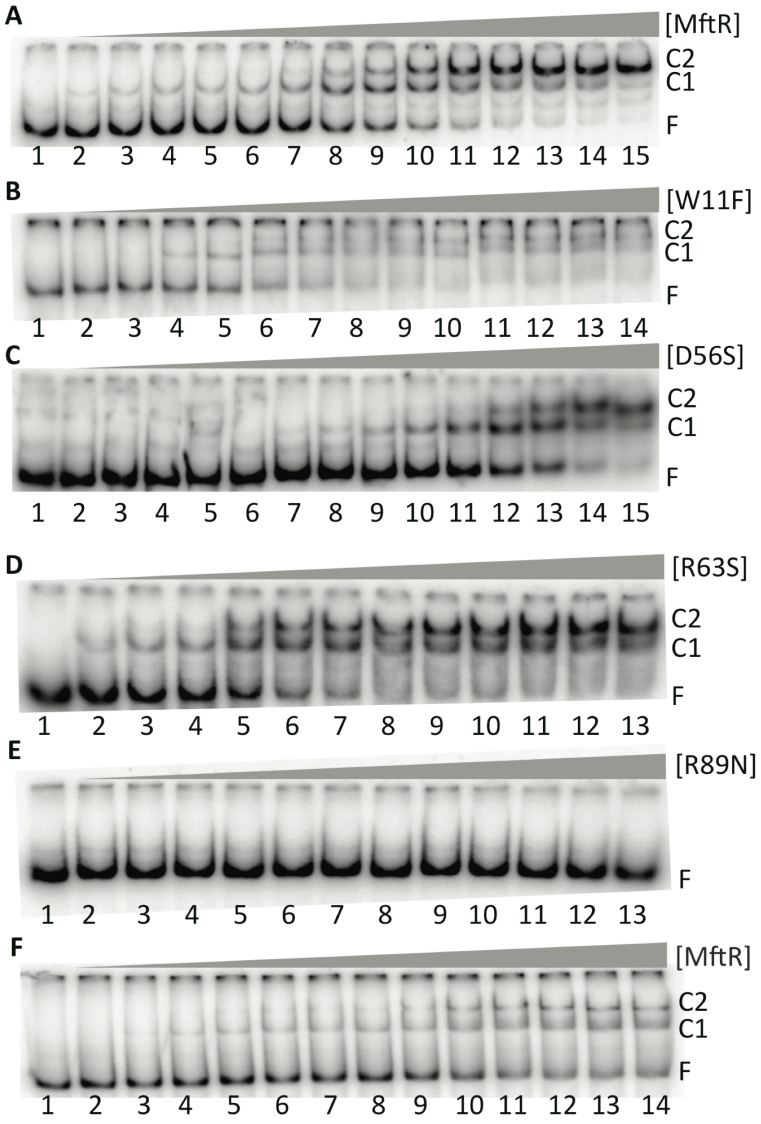

Figure S3. MftR-*mftO* complexes with WT and MftR variants W11F, D56S, R63S, R89N. (A) EMSA showing *mftO* (3.0 nM) titrated with increasing concentration of MftR (0.1 – 50 nM; lanes 2-15). F, C1 and C2 represent free DNA, complex 1 and complex 2, respectively. Reactions in panels A, C, D and E were incubated at room temperature. Reactions in the first lane of each panel contain DNA only. (B) *mftO* titration with increasing concentration of W11F (0.5 - 3000 nM; lanes 2-14; reactions were incubated at 4°C). (C) Increasing concentration of D56S with mftO (1 - 2000 nM; lanes 2-15). (D) R63S titrated with *mftO* (2 - 2000 nM; lanes 2-13). (E) *mftO* with increasing concentration of R89N (2 – 2000 nM; lanes 2-13). (F) *mftO* titration with increasing concentration of MftR (0.1 - 30 nM; lanes 2-14); reactions were incubated at 37°C and gel electrophoresis was performed at 37°C.

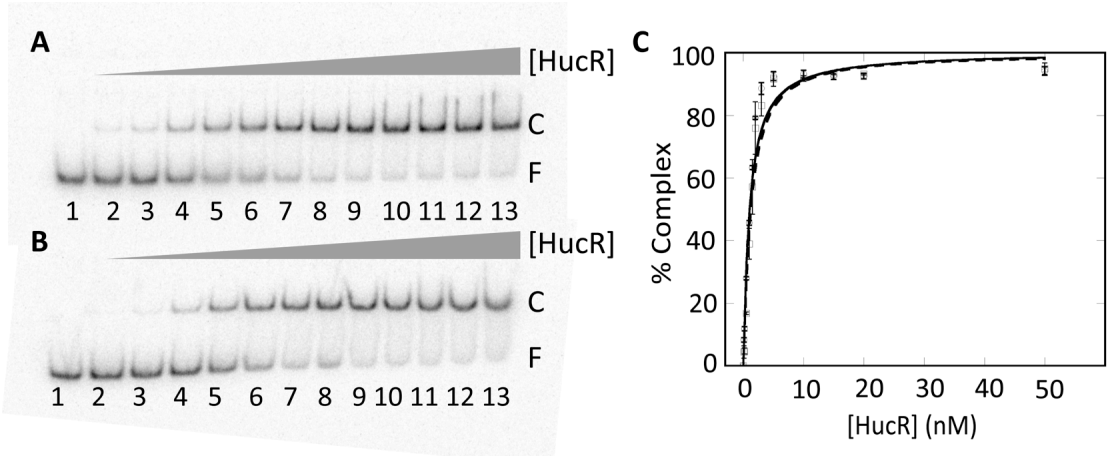

Figure S4. HucR binds *HucO* comparably at room temperature and 37 °C. (A) HucR-*HucO* complex (where *HucO* refers to 77 bp DNA containing HucR-binding site in *HucR* promoter) obtained by titration with increasing concentration of HucR (0.1 - 50 nM; lanes 2-13); reactions were incubated at room temperature and gel electrophoresis was performed at room temperature (22 °C). F and C represent free DNA and complex respectively. (B) HucR-*HucO* complex obtained by titration with increasing concentration of HucR (0.1 - 50 nM; lanes 2-13); reactions were incubated at 37 °C and gel electrophoresis was performed at 37 °C. (C) Fractional complex formation plotted as a function of HucR concentration. Binding isotherm with *HucO* at room temperature (□; solid line) and at 37 °C (○; dashed line). Error bars represent the standard deviation of three independent repeats.

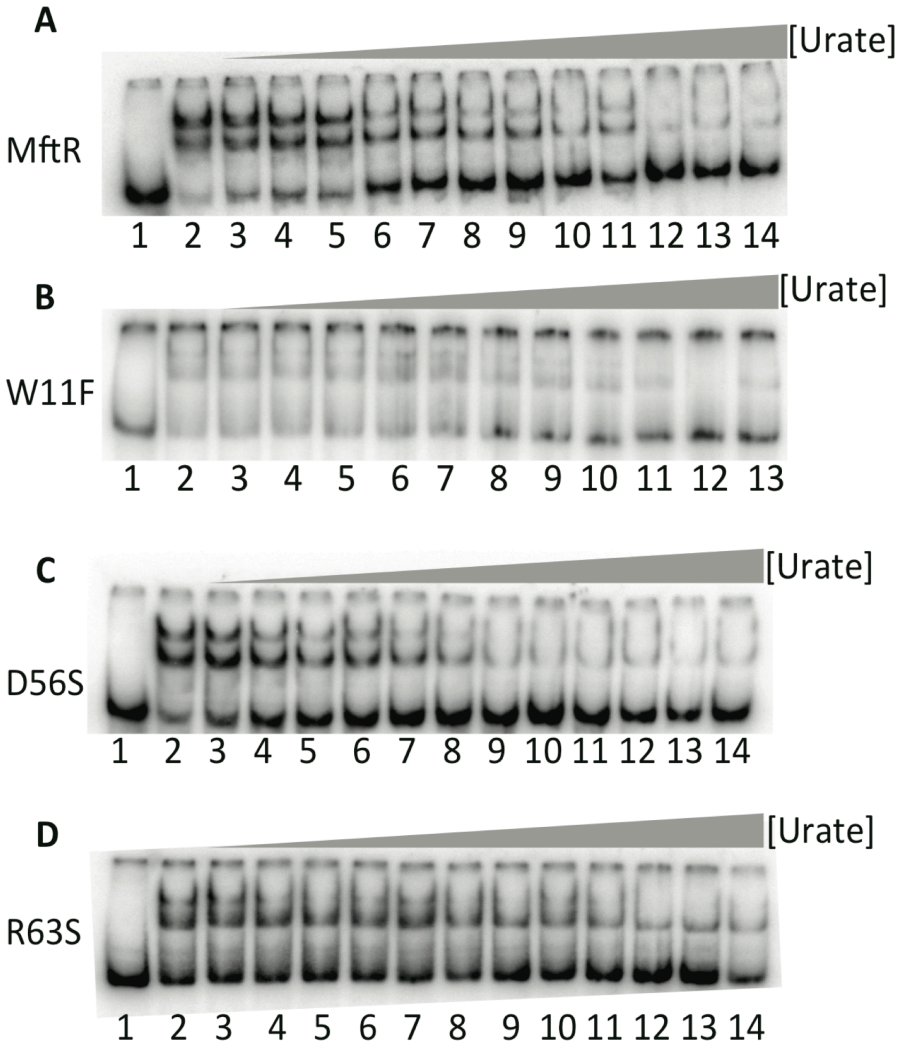

Figure S5. Sensitivity of WT and MftR variants to urate. (A) MftR-*mftO* complex titrated with increasing concentration of urate (1 - 25 mM; lanes 3-14). Reactions in lane 1 of each panel contain free DNA only (F), while lanes 2 of each panel is MftR-*mftO* without urate. (B) W11F-*mftO* complex titrated with increasing concentration of urate (1 - 30 mM; lanes 3-13). (C) D56S-*mftO* complex titrated with increasing concentration of urate (1 - 25 mM; lanes 3-14). (D) R63S-*mftO* complex titrated with increasing concentration of urate (1 - 25 mM; lanes 3-14).