Supplementary material

The recognition domain of the methyl-specific endonuclease McrBC flips out 5-methylcytosine

Rasa Sukackaite, Saulius Grazulis, Gintautas Tamulaitis, Virginijus Siksnys*

Department of Protein-DNA Interactions, Institute of Biotechnology,
Vilnius University, Graiciuno 8, 02241 Vilnius, Lithuania

* Corresponding author

Supplementary figures S1-S4
Supplementary table 1

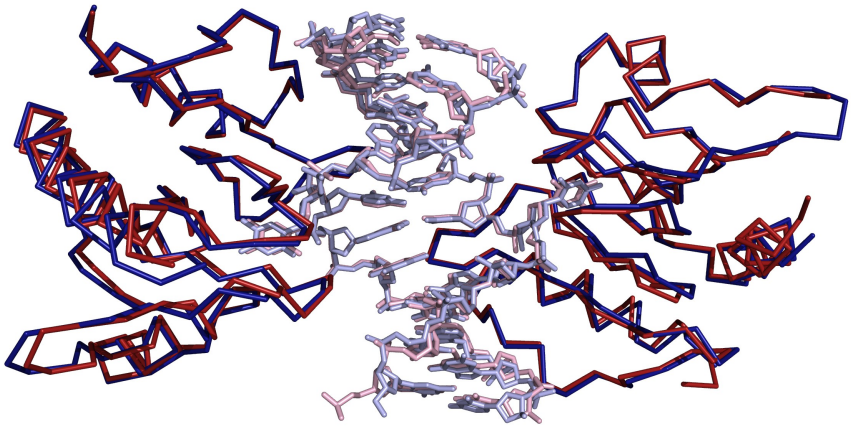

Figure S1. Overlay of the McrB-N/(m/m) and McrB-N/(m/-) structures. The McrB-N complex with di-methylated (m/m) DNA (Table 1) is shown in red, while the McrB-N complex with hemi-methylated (m/-) DNA is shown in blue. The hemi-methylated DNA can be also bound in the opposite orientation.

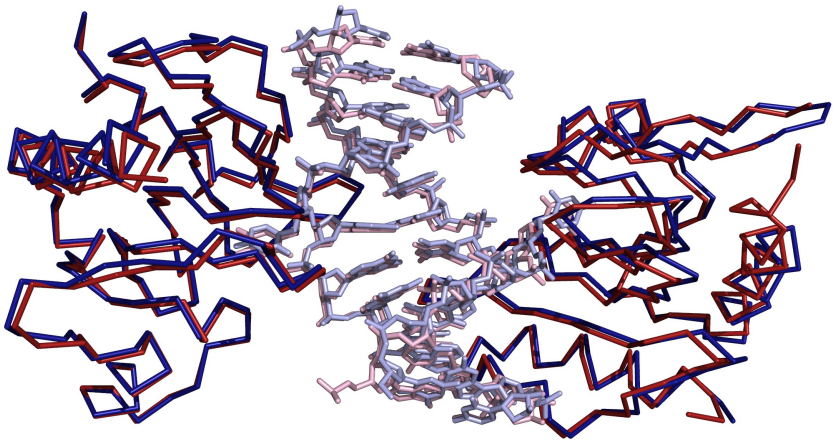

Figure S2. Overlay of the McrB-N/(m/m) and McrB-N/(-/-) structures. The McrB-N complex with di-methylated (m/m) DNA (Fig. 1B) is shown in red, while the McrB-N complex with non-methylated (-/-) DNA is shown in blue.

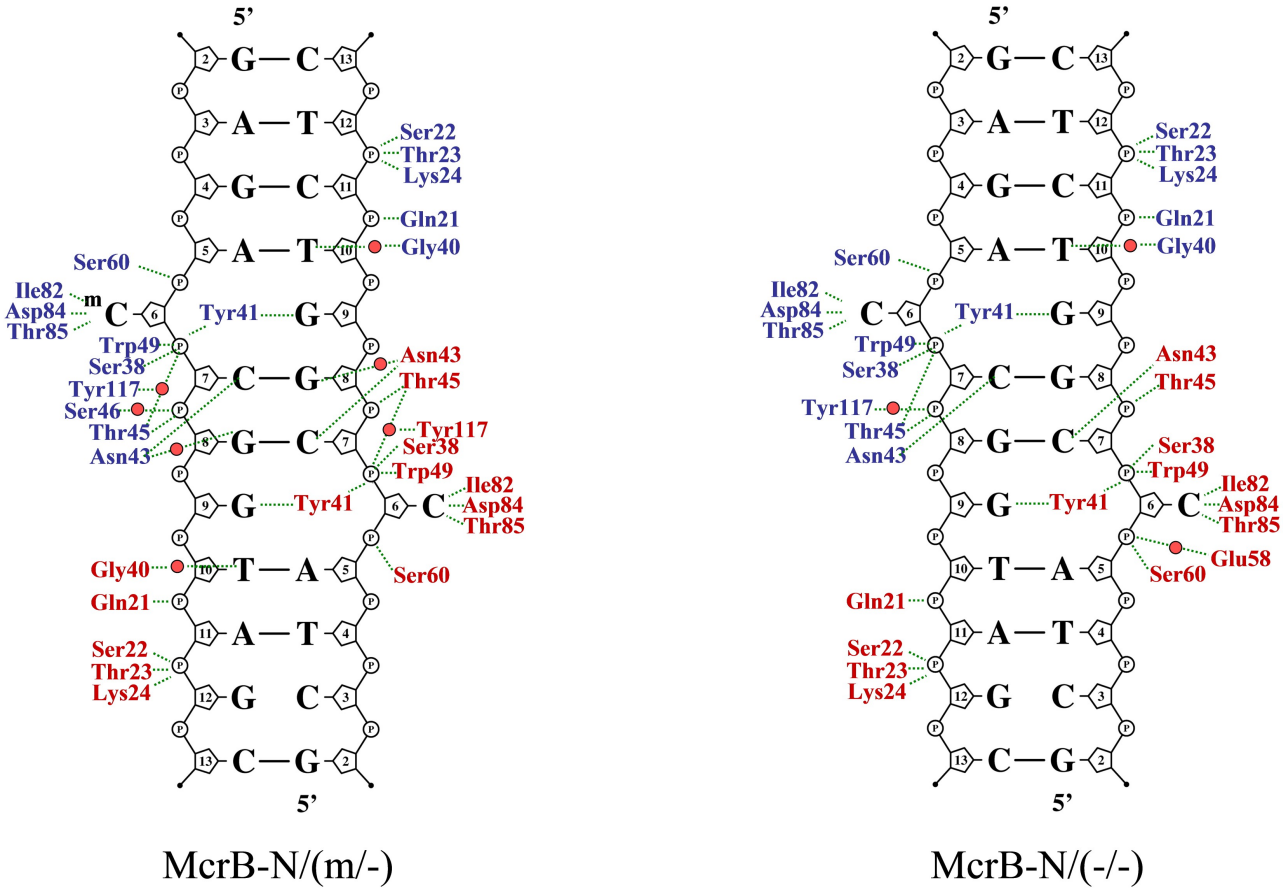

Figure S3. Schemes showing hydrogen bonds in the McrB-N/hemi-methylated DNA (m/-) (Fig. 1B) and McrB-N/non-methylated DNA (-/-) complexes. Residues from different McrB-N monomers are shown in blue and red, water molecules are shown as red spheres.

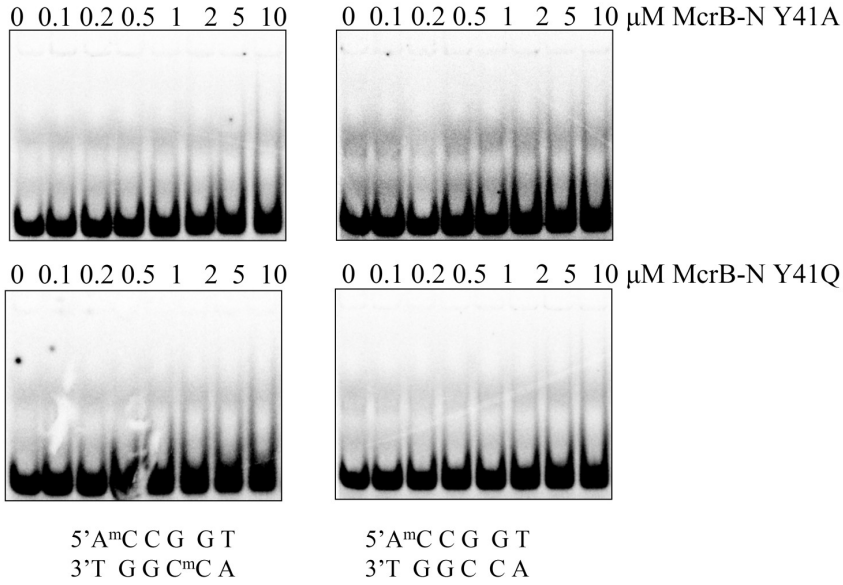

Figure S4. Gel mobility shift analysis for McrB-N Tyr41 mutants binding to DNA. McrB-N Y41A and Y41Q mutants at concentrations indicated above the relevant lanes were mixed with the di-methylated (m/m) or hemi methylated (-/m) DNA (Figure 1B) at the final concentration of 100 nM. The samples were electrophoresed through 8% PAA gels under native conditions.

Table S1. Datasets

Dataset	**I**	**II**	**III**	**IV**	**Pt-peak**	**Pt-infl**
Oligoduplex (Fig. 1B)	(m/m)	(m/-)	(m/-)	(-/-)	(m/-)	
Crystallization conditions	0.1 M Bis-Tris (pH=5.5), 0.2 M NH_4Cl, 22% PEG3350	0.2 M LiCl, 18% PEG4000	0.2 M LiCl, 20% PEG4000	0.1 M Bis-Tris (pH=5.5), 0.2 M NH_4Cl, 30% PEG4000	0.2 M LiCl, 22% PEG4000	
X-ray source	EMBL X11	EMBL X11	EMBL X12	RU-H3R	EMBL X12	
Wavelength (Å)	0.81480	0.81500	1.72196	1.5418	1.07009	1.07065
Spacegroup	$P2_12_12_1$					
Cell constants (Å)						
a	35.40	36.09	36.09	36.05	36.32	
b	67.46	67.19	68.58	69.09	69.43	
c	140.77	142.41	143.64	143.89	144.36	
Resolution (Å)	35.3–2.1	29–2.3	68.5–2.6	62.3–2.7	62.6–2.5	62.6–2.5
Completeness (%)	94.4 (51)[a]	99.8 (99.9)	95.6 (94.8)	100 (100)	100 (100)	100 (100)
Multiplicity	4.2 (2.1)	5.6 (5.3)	43.1 (42.6)	5.6 (5.7)	13.8 (14.2)	13.8 (14.1)
I/σ_I	3.1 (2.3)	6.1 (1.9)	7.7 (2.1)	5.3 (1.7)	7.5 (2.9)	6.5 (2.1)
R_{merge}[b]	12.9 (25.1)	7.0 (25.4)	7.9 (36.0)	13.2 (44.4)	8.5 (32.3)	8.6 (36.4)

[a]Values in parentheses refer to data in the highest resolution shell.

[b]$R_{merge}=\sum|I_{hi}-\langle I_h\rangle|/\sum I_{hi}$, where I_{hi} is an intensity value of the i-th measurement of reflection h and $\langle I_h\rangle$ is the average measured intensity of reflection h.