Supplementary information

PrgB Promotes Aggregation, Biofilm Formation, and Conjugation through DNA Binding and Compaction

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Tables

Table S1. Thermodynamic parameters of ITC experiments

Protein	DNA	K_d [µM]	ΔS [kJ/mol/K]	ΔH [kJ/mol]	ΔG [kJ/mol]	n
$PrgB_{188-1233}$ Monomer	19	364.0 ± 73.8	0.24 ± 0.02	52.71 ± 12.24	-18.82 ± 4.73	1
$PrgB_{188-1233}$ Dimer	19	163.6 ± 42.4	0.13 ± 0.03	17.14 ± 3.75	-21.6 ± 5.53	1
$PrgB_{246-558}$	19	46.2 ± 14.5	0.14 ± 0.05	17.6 ± 2.93	-24.12 ± 7.75	1
Glycerol phosphate		**K_d [µM]**				
$PrgB_{188-1233}$		309.2 ± 55.3	0.12 ± 0.06	15.27 ± 1.62	-20.49 ± 3.58	8
$PrgB_{246-558}$		276.1 ± 71.2	0.10 ± 0.05	10.04 ± 1.83	-19.76 ± 5.22	8

Table S2. dsDNA sequences (only 1 strand is shown).

DNA_{10}

CGGCCCGGGG

DNA_{19}

GGGGGCGGGGCGGGCGGCG

oriT

TCGCAACATGCTAGCATGTTGCTCCGCTTGCAAAAAGAAA

DNA_{100}

TCGCAACATGCTAGCATGTTGCTCCGCTTGCAAAAAGAAAGCCTACCCTTGGGTATA
ACCAATTGTCAAACTAAGGAGACTACTTATTATGTAAAAGAAA

DNA_{250}

TCGCAACATGCTAGCATGTTGCTCCGCTTGCAAAAAGAAAGCCTACCCTTGGGTATA
ACCAATTGTCAAACTAAGGAGACTACTTATTATGTAAAAGAAAGAAGAAATAATTAT
GGAGAATAAACAAAAACTAAAACGTCCTATTCAAAGAATAGTTAGACTATCAGAAG
AAGAAAATAACTTAATCAAACGAAAAATTGAGGAAAGTTTTTTTCCAAACTTTCAAA
ATTTTGCCTTGCACCTTTTGATT

DNA-1

Biotin-
TGAACGAAAAATACGAGCAATTAAACCAGTATTTAAATCAAGTGGCTTCGTTGAAGC

AAAGCATTCGAAACGCCAACAACATTGAGCTGGTCAATAGCTCTTTAAACTATTTAA
AAAGCTTTACCAGCAACAACTACAACAGCACCACCCAATCGCCCATCTTTAACGCCG
TGCAAGCCGTTATCACTTCGGTATTGGGTTTTTGGAGTCTTTATGCGGG

DNA-2

CGTTCTTGTTTTAGTTTGGCTTTATTCCCATTAAATACTGATGATAAAATTCAAAAGA
TGATTTTATCGTTACAAAAATCAACGCTTCAAAGATAAATAGGTTAAAATACTCCAA
AATCTTTTTTTTTTTTTGGAAATCCAATAAATTTATAGTAAAATTAGGTTCATTGTAA
ATATATTATCACTTCATGATATTCTTACAACAAAAACATTACTTTAAGGAACACTTTT
ATGAAAAAG-Biotin

Figures

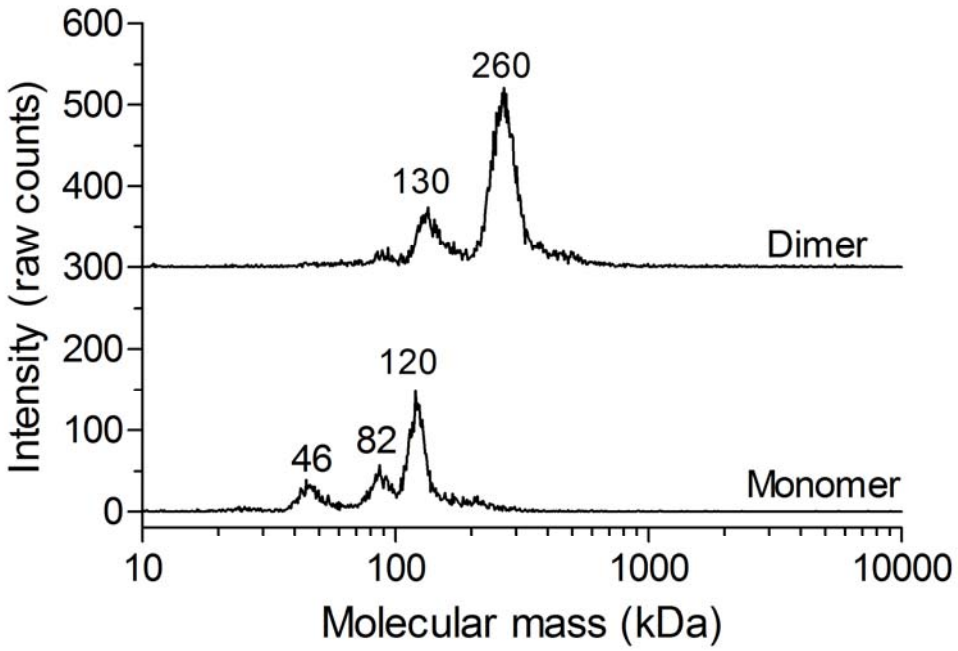

Figure S1 GEMMA analysis of PrgB. The two samples are taken from size exclusion chromatography fractions corresponding to dimers and monomers, respectively, and the GEMMA analysis was performed with a protein concentration of 0.05 mg/mL. The determined molecular masses (in kDa) are written above the peaks. The baseline is shifted vertically to fit two experiments in the same graph.

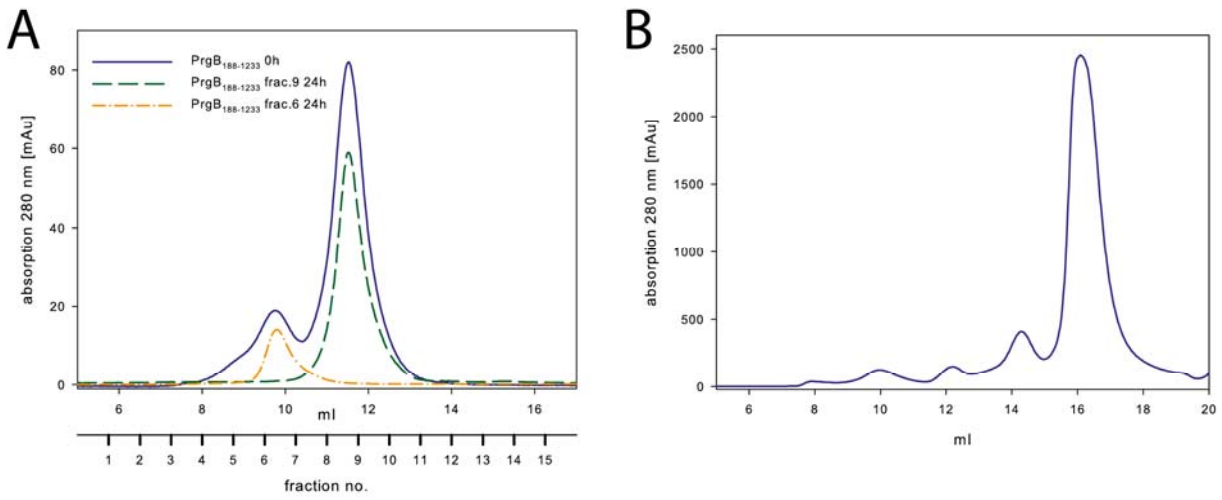

Figure S2. Size exclusion chromatography of PrgB on an Superdex S200 (10/300) Increase column. The absorption at 280 nm is plotted against the elution volume. **A:** $PrgB_{188-1233}$ at timepoint 0 (blue solid line), the two peaks at 9.8 and 11.7 ml represent dimeric and monomeric PrgB, respectively. Dashed yellow and green line represent fraction 6 and 9, respectively, both rerun after 24 h. **B:** Chromatogram of $PrgB_{246-558}$, the elution volume of 16.2 ml corresponds to the expected molecular weight of a monomer.

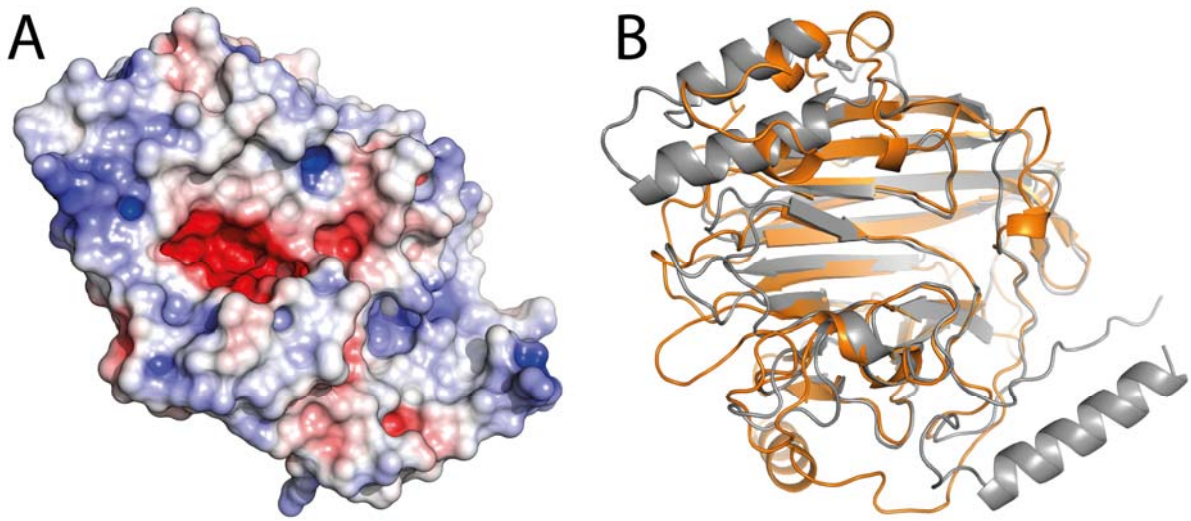

Figure S3. A: Surface charge distribution of SspB (PDB code 2WD6) calculated by APBS, scaled from -5 (red) to 5 k_bT (blue). Compared to PrgB, the negatively charged pocket in SspB is smaller and more constricted (compare with Fig. 2b). **B:** Superposition of $PrgB_{246\text{-}558}$ (orange) and SspB (grey).

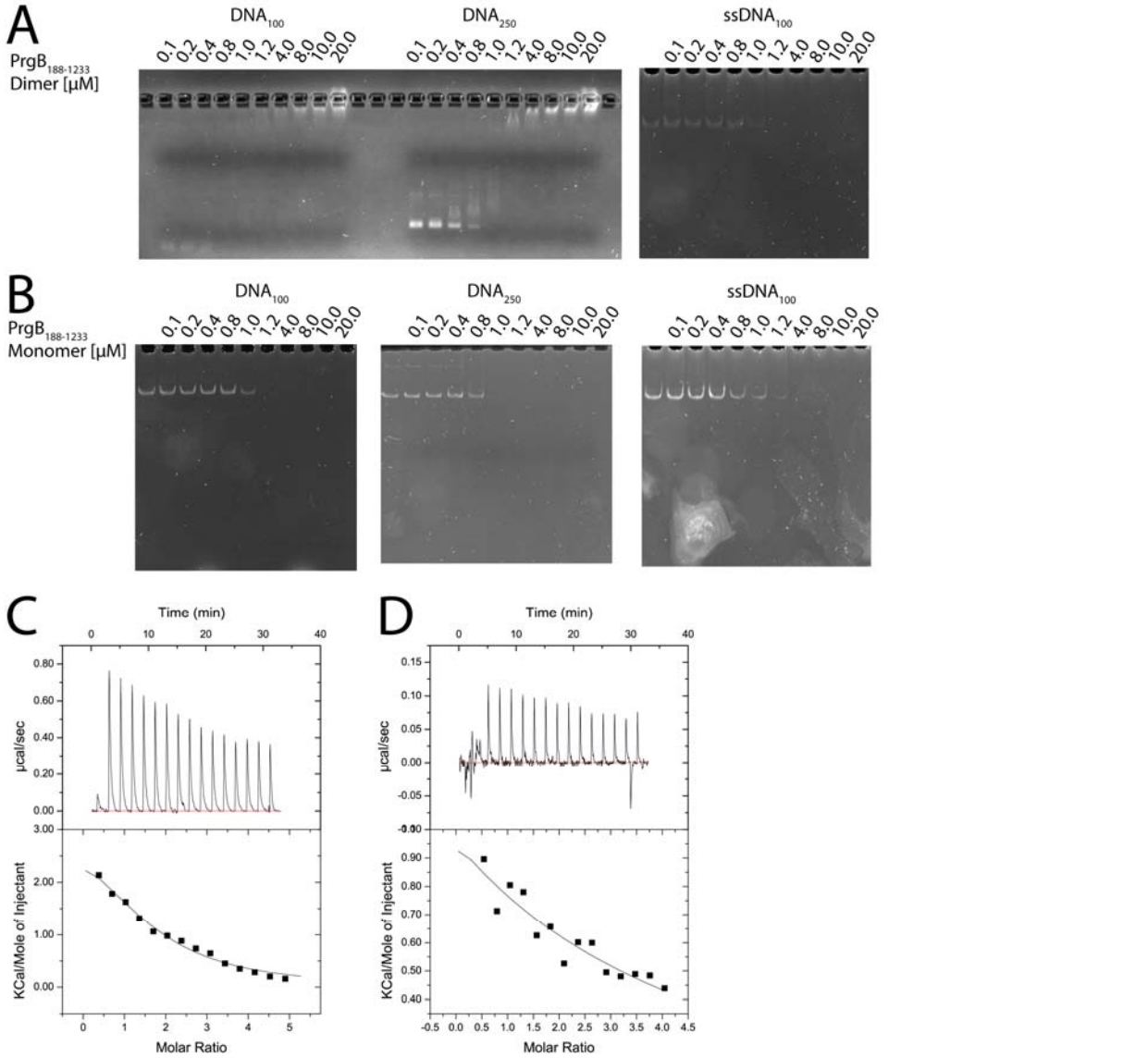

Figure S4. EMSA gels and ITC traces of DNA binding to PrgB. **A:** EMSA of $PrgB_{188\text{-}1233}$ dimer with DNA_{100} and DNA_{250}. **B:** EMSA of $PrgB_{188\text{-}1233}$ monomer with DNA_{100} and DNA_{250} **C:** ITC trace and curve fit of $PrgB_{246\text{-}558}$ binding to DNA_{19} **D:** ITC trace and curve fit of $PrgB_{188\text{-}1233}$ monomer to DNA_{19}

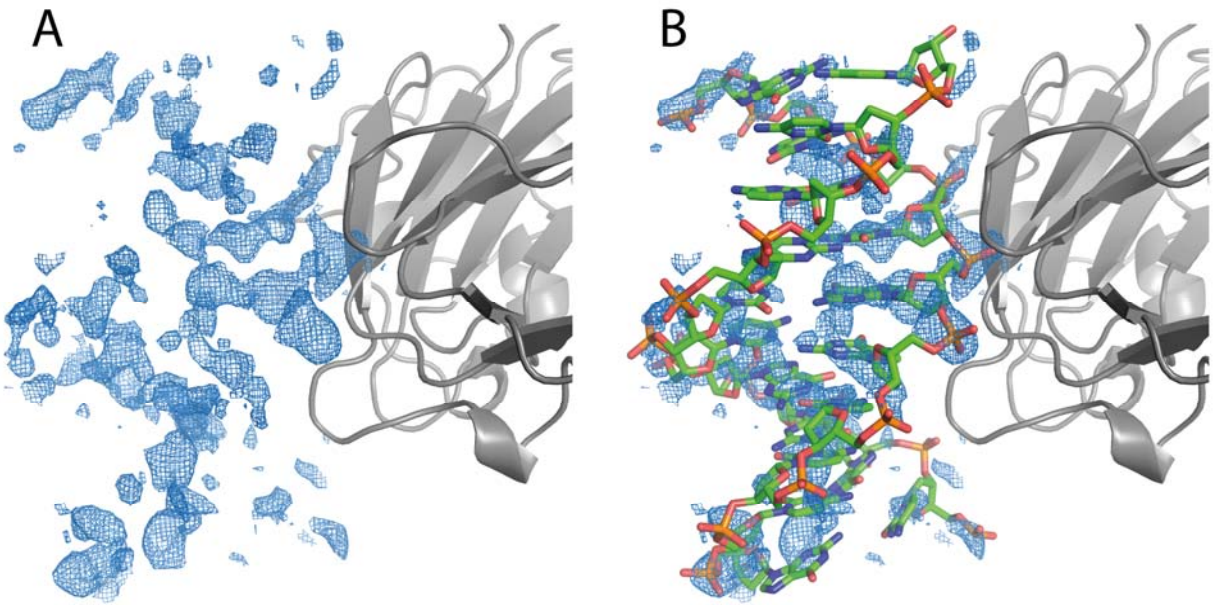

Figure S5 2mFo-DFc simulated annealing composite omit-map of the region of $PrgB_{246-558}$ co-crystallized with a 10 bp dsDNA, where extra electron density was observed during refinement. **A:** Simulated annealing composite omit-map, at σ-level 1.0. **B:** Model of the dsDNA molecule built in the electron density.

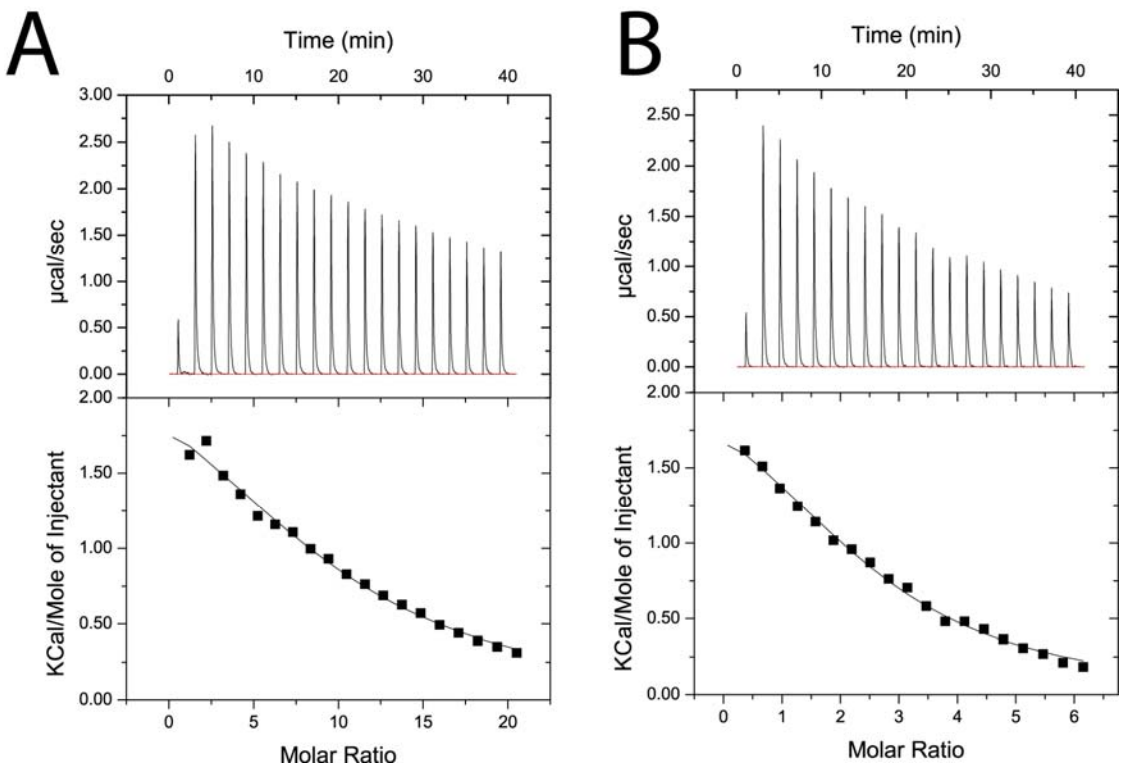

Figure S6. ITC traces and curve fits of glycerol phosphate binding to **A:** $PrgB_{188-1233}$ and **B:** $PrgB_{246-558}$

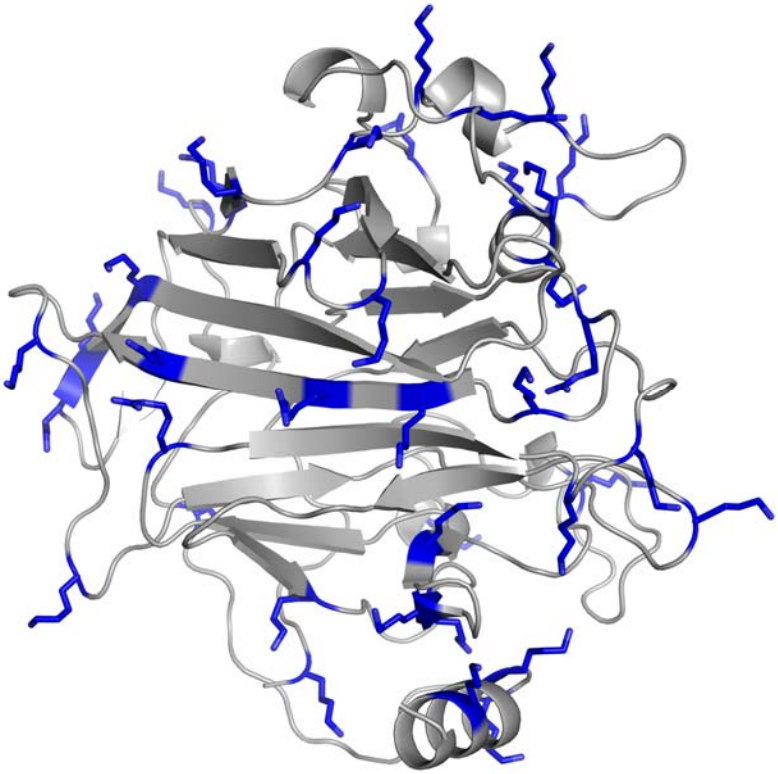

Figure S7. Surface exposed arginine and lysine residues of $PrgB_{246-558}$. The protein is shown in grey and all surface exposed arginines and lysines are shown as blue sticks. The orientation of the protein is the same as the back view in Fig. 2.