

# Supplemental Material

## *myo*-inositol and D-ribose ligand discrimination in an ABC periplasmic binding protein

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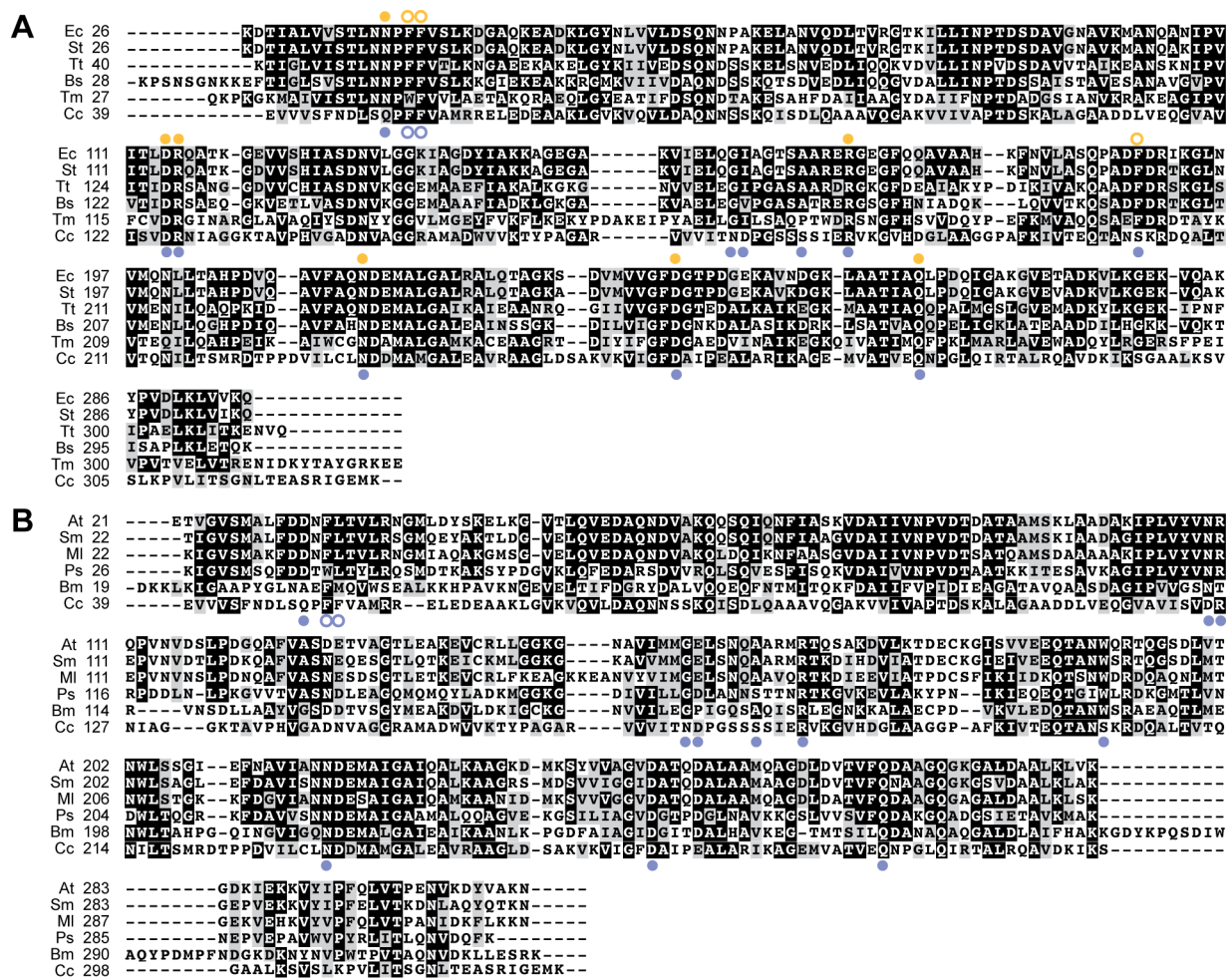
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Running title : Structure of *Caulobacter myo*-inositol binding protein.



**FIG S1** Amino acid sequence alignment of *C. crescentus* IbpA and RBP and IBP proteins. Solid circles highlight residues involved in polar interactions; open circles highlight residues that make hydrophobic interactions between IbpA and *myo*-inositol (in blue) and between RBPs and ribose (in orange). (A) Amino acid sequence alignment of the IbpA and RBP proteins. Sequences from from *Escherichia coli* (Ec), *Salmonella typhimurium* (St), *Thermoanaerobacter tengcongensis* (Tt), *Bacillus subtilis* (Bs), *Thermotoga maritima* (Tm) and *Caulobacter crescentus* (Cc) are included in this alignment. (B) Sequence alignment of IbpA and other IBP proteins. Sequences from *Agrobacterium tumefaciens* (At), *Sinorhizobium meliloti* (Sm), *Mesorhizobium loti* (MI), *Pseudomonas sp. GM48* (Ps), *Brucella melitensis* (Bm) and *Caulobacter crescentus* (Cc) are included in this alignment.

**Table S1 : primers.**

Primer	Sequence	Comment
IbpA1-UP	5'- <b>CATATG</b> GAGGTGGTGGTCAGCTTC-3'	Primers used to amplify <i>IbpA</i> without the sequence coding the peptide signal. Corresponding insert cloned into pET28c (NdeI-HindIII).
IbpA1-LO	5'- <b>AAGCTT</b> CTATTTTCATCTCGCCGAT-3'	
IbpA2-UP	5'- <b>AAGCTT</b> AGAACACGACCACGCCCTTG-3'	Primers used to amplify <i>IbpA</i> with 500 bp flanking regions. Corresponding insert cloned into pNPTS138 (HindIII-SphI).
IbpA2-LO	5'- <b>GCATGC</b> GCGCGTTCAGGGTCATGG-3'	
Q49>N49-UP	5'-GACCTTTCCAACCCCTTCTTC-3'	Primers used to substitute Q49 by Asn
Q49>N49-LO	5'-GAAGAAGGGGTTGGAAAGGTC-3'	
N168>G168-UP	5'-GTCATCACCGGCATCCCGGG-3'	Primers used to substitute N168 by Gly
N168>G168-LO	5'-CCCGGGATGCCGGTGATGAC-3'	
D169>I169 / S174>A174-UP	5'-ATCCCGGGCAGCTCCGCC-3'	Primers used to substitute D169 by Ile and S174 by Ala
D169>I169 / S174>A174-LO	5'-GGCGGAGCTGCCCGGGAT-3'	
S203>F203-UP	5'-CCGCCAACTTCAAGCGCGA-3'	Primers used to substitute S203 by Phe
S203>F203-LO	5'-TCGCGCTTGAAGTTGGCGG-3'	

**Table S2 : Strains.**

Strain number	Organism	Plasmid	Restriction sites	Genes	Comments	References
FC20	<i>CB15N</i>				CCWT, wild type strain	
FC405	<i>CB15N</i>			<i>iatP</i> (CC0861) – deleted for <i>iatP</i> (permease).	CC $\Delta$ <i>iatP</i> mutant strain	Boutte C. <i>et al.</i>
FC488	<i>CB15N</i>			<i>lbpA</i> (CC0859) – deleted for <i>lbpA</i> (periplasmic binding protein).	CC $\Delta$ <i>lbpA</i> mutant strain	Boutte C. <i>et al.</i>
FC489	<i>CB15N</i>			<i>iatA</i> (CC0860) – deleted for <i>iatA</i> (ATP binding cassette).	CC $\Delta$ <i>iatA</i> mutant strain	Boutte C. <i>et al.</i>
FC1898	<i>E. coli</i> Top10	pET28c	Ndel - HindIII	<i>lbpA</i> (CC0859)	Strain carrying the expression vector pET28c- <i>lbpA</i>	This study
FC1899	<i>Rosetta</i> (DE3)pLysS	pET28c	Ndel - HindIII	<i>lbpA</i> (CC0859)	Strain used to overexpress the His tagged version of the wild-type MIBP	This study
FC1900	<i>E. coli</i> Top10	pET28c	Ndel - HindIII	<i>lbpA</i> (CC0859) – mutations have been introduced in the ligand binding pocket (Q49>N49, D169>I169, S174>A174 and S203>F203).	Strain carrying the expression vector pET28c- <i>lbpACM1</i>	This study
FC1901	<i>Rosetta</i> (DE3)pLysS	pET28c	Ndel - HindIII	<i>lbpA</i> (CC0859) – mutations have been introduced in the ligand binding pocket (Q49>N49, D169>I169, S174>A174 and S203>F203).	Strain used to overexpress the His tagged version of the <i>lbpACM1</i>	This study
FC1902	<i>E. coli</i> Top10	pET28c	Ndel - HindIII	<i>lbpA</i> (CC0859) – mutations have been introduced in the ligand binding pocket (Q49>N49, N168>G168, D169>I169, S174>A174 and S203>F203).	Strain carrying the expression vector pET28c- <i>lbpACM2</i>	This study
FC1903	<i>Rosetta</i> (DE3)pLysS	pET28c	Ndel - HindIII	<i>lbpA</i> (CC0859) – mutations have been introduced in the ligand binding pocket (Q49>N49, N168>G168, D169>I169, S174>A174 and S203>F203).	Strain used to overexpress the His tagged version of the <i>lbpACM2</i>	This study
FC1904	<i>E. coli</i> Top10	pNPTS138	HindIII - SphI	<i>lbpA</i> (CC0859) – mutations have been introduced in the ligand binding pocket (Q49>N49, D169>I169, S174>A174 and S203>F203).	Strain used for allelic replacement of <i>lbpA</i> by <i>lbpACM1</i>	This study
FC1905	<i>CB15N</i>			<i>lbpA</i> (CC0859) – mutations have been introduced in the ligand binding pocket (Q49>N49, N168>G168, D169>I169 and S174>A174).	<i>lbpACM1</i> mutant strain	This study
FC1906	<i>E. coli</i> Top10	pNPTS138	HindIII - SphI	<i>lbpA</i> (CC0859) – mutations have been introduced in the ligand binding pocket (Q49>N49, N168>G168, D169>I169, S174>A174 and S203>F203).	Strain used for allelic replacement of <i>lbpA</i> by <i>lbpACM2</i>	This study
FC1907	<i>CB15N</i>			<i>lbpA</i> (CC0859) – mutations have been introduced in the ligand binding pocket (Q49>N49, N168>G168, D169>I169, S174>A174 and S203>F203).	<i>lbpACM2</i> mutant strain	This study