SUPPLEMENTARY INFORMATION

Table S1. Enzyme and substrate concentrations used in kinetics assays

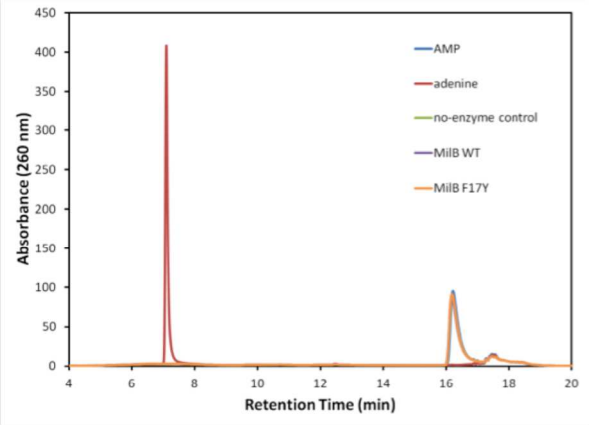
Protein (Substrate)	[Enzyme] (μM)	Substrate Range (mM)
BcmB WT (CMP)	20	0.02 to 1.5
BcmB WT (dCMP)	20	0.05 to 6
BcmB F6Y (CMP)	80	0.1 to 6
BcmB F6Y (dCMP)	10 or 20	0.03 to 1.2
MilB WT (CMP)	10 or 20	0.05 to 18
MilB WT (dCMP)	60	0.3 to 30
MilB F17Y (CMP)	175	0.3 to 30
MilB F17Y (dCMP)	1	0.1 to 8

Figure S1. No hydrolysis of AMP, IMP, or GMP is observed when incubated with MilB or MilB F17Y.

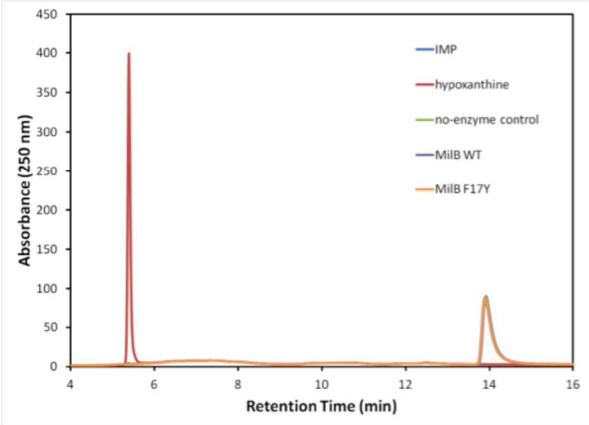
Figure S2. Structure of BcmB. (A) The BcmB protomer adopts the same α/β-twist fold as MilB. (B) The topology diagram of BcmB shows α-helices in purple and β-strands in orange. (C) The MilB dimer (blue) is shown superimposed with the BcmB dimer (green). The two molecules have a rmsd of 1.7 and display a highly similar overall fold. (D) A phosphate ion is bound in the active site and makes hydrogen bond interactions with a serine residue and an amide group.

Figure S3. Substrate specificity studies with BcmB. A) BcmB WT with CMP and dCMP. B) BcmB F6Y with CMP and dCMP

A



B



C

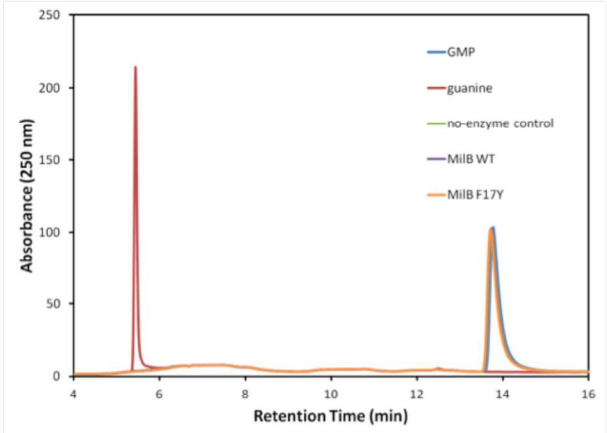


Figure S1

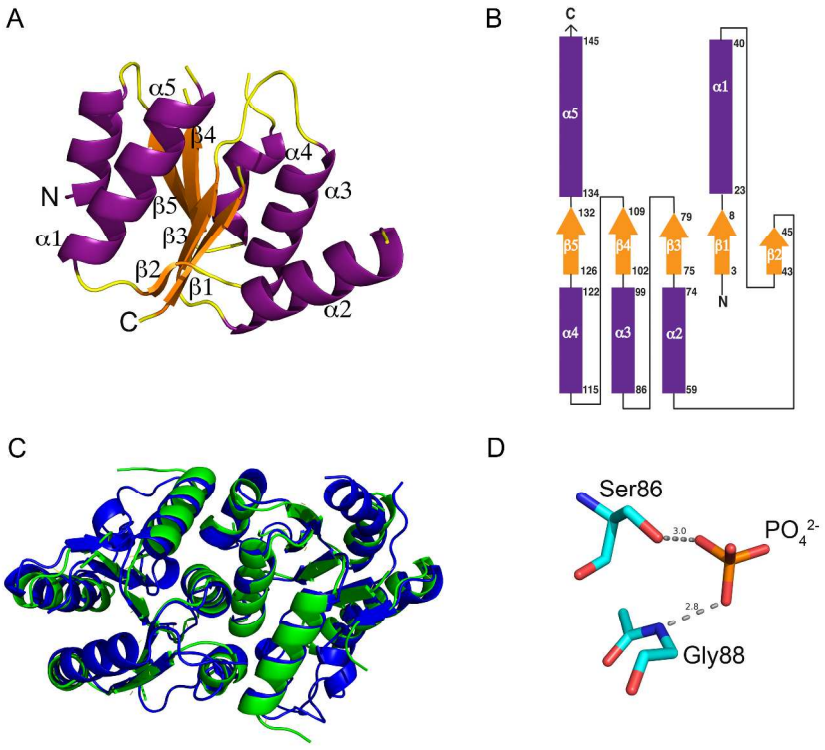

A
α5
β4
α4
N
α3
β5
α1
β3
β2
β1
C
α2
B
C
145
40
α5
α1
134
23
132
109
79
8
45
β5
β4
β3
β1
β2
126
102
75
3
43
N
122
99
74
α4
α3
α2
115
86
59
C
D
Ser86
3.0
PO4 2-
2.8
Gly88

Figure S2

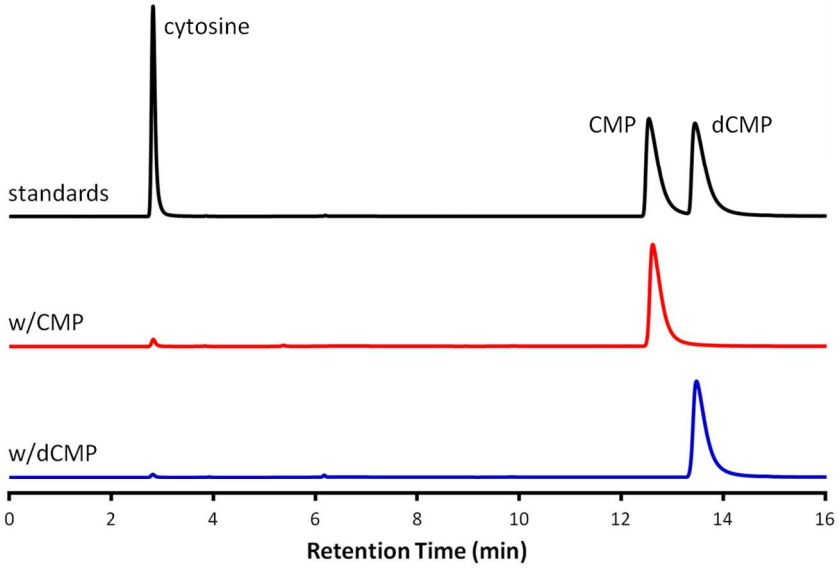


Figure S3A

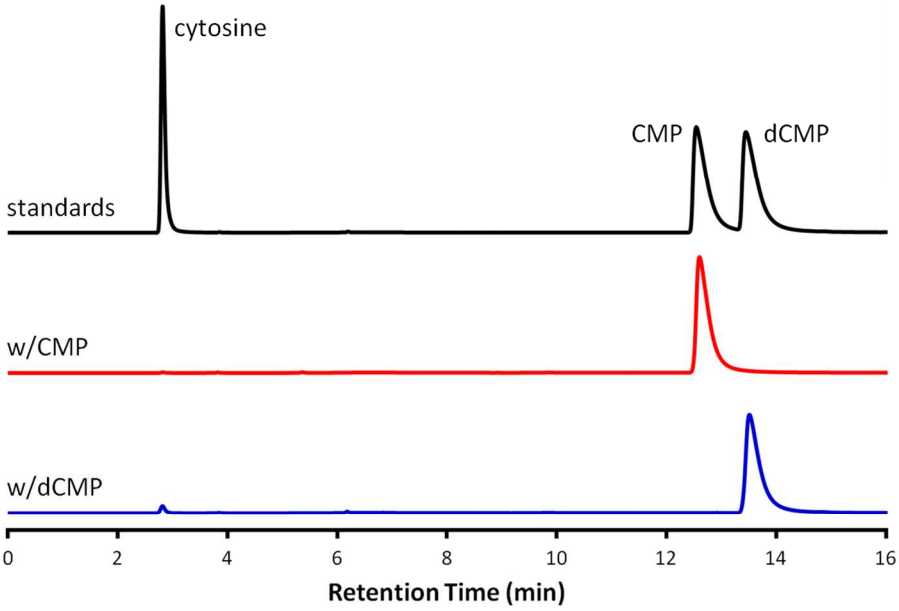


Figure S3B