Supporting information for article:

*Bacillus licheniformis* trehalose-6-phosphate hydrolase structures suggest keys to substrate specificity

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Figure S1  The alignment of four BlTreAs in the crystallographic asymmetric unit. Chains A, B, C, and D are colored in green, cyan, yellow, and magenta, respectively.
Figure S2  Structural comparison between *Bi*TreA and its neighbor. Magnified view of the highly conserved catalytic residues in the active pocket of *Bi*TreA (green), *Bc*Ogl (magenta) and NX-5 (cyan). Residues from *Bi*TreA are labeled.
Figure S3  The overall structure of BlTreA in complex with pPNG. A, Schematic representation of the overall structure of BlTreA. The BlTreA subdomain is indicated in magenta, N-domain in green, and C-domain in red. pPNG (white) and the five conserved residues are shown as a stick model. B, The pPNG molecule in the active site. The 2Fo-Fc omit electron density maps are contoured at 1 σ level and are shown in blue.
**Figure S4** Structural comparison of the interacting residues between native *B*TreA and R201Q/pPNG. The native *B*TreA and R201Q/pPNG are colored in green and yellow, respectively. Residues from R201Q are labeled.
Figure S5  The models highlighting the binding cavity for BlTreA, BcOgl and NX-5. The enzymes are color coded with BlTreA in green, BcOgl in magenta, and NX-5 in cyan. The visualization of active site pockets are colored in gray.
Figure S6  The far-UV CD and fluorescence spectra of B/TreA and several mutants. Far-UV CD (A) and fluorescence (B) spectra were obtained with a protein concentration of approximately 0.2 mg/ml at 25°C.