STRUCTURAL AND KINETIC ANALYSIS OF *BACILLUS SUBTILIS N*-ACETYLGLUCOSAMINIDASE REVEALS A UNIQUE ASP-HIS DYAD MECHANISM

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Supplementary Table S1

Table S1 Michaelis-Menten Parameters for *Bs*NagZ and protein variants in the absence and presence of sodium azide*

Enzyme	Substrate	K_M [μM]	k_{cat} [s^{-1}]	k_{cat}/K_M [s^{-1}/mM]
wt	4-Mu β-GlcNAc	109.6 ± 4.3	6.42 ± 0.07	58.58
wt + azide	4-Mu β-GlcNAc	93.48 ± 5.53	1.20 ± 0.02	12.84
His234Gly	4-Mu β-GlcNAc	4.57 ± 0.39	$3.37 \times 10^{-3} \pm 4.8 \times 10^{-5}$	0.74
His234Gly + azide	4-Mu β-GlcNAc	7.84 ± 0.86	$3.03 \times 10^{-3} \pm 6.3 \times 10^{-5}$	0.39
Asp232Gly	4-Mu β-GlcNAc	56.24 ± 3.56	$1.40 \times 10^{-3} \pm 2.6 \times 10^{-5}$	0.025
Asp232Gly + azide	4-Mu β-GlcNAc	55.91 ± 4.07	$2.90 \times 10^{-3} \pm 6.0 \times 10^{-5}$	0.052

*condition were: 500 mM sodium azide at 0.1 M KH_2PO_4/0.1 M NaOH, pH 5.8 and 37°C

Supplementary Figure S1

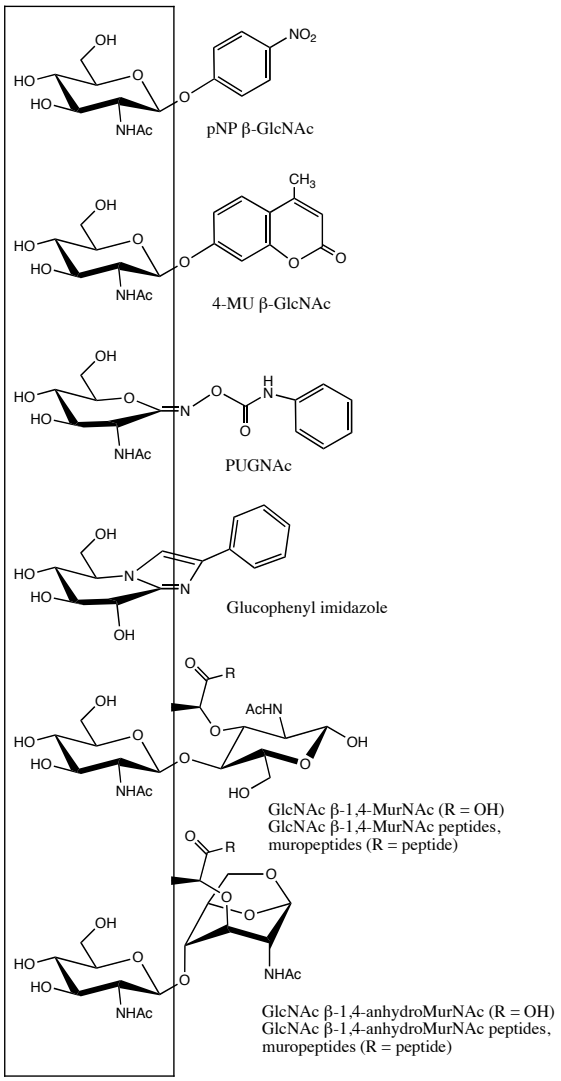

Fig. S1. Structure of selected substrates, natural and artifical, as well as transition state-like inhibitors of β-*N*-acetylglucos-aminidases of family 3.

Supplementary Figure S2

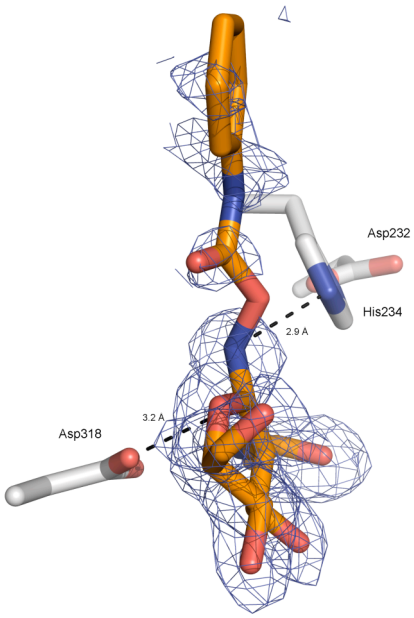

Fig. S2. A view of the $F_o - F_c$ omit map of the transition state mimicking inhibitor PUGNAc bound to *Bs*NagZ. The catalytic nucleophile Asp318 and the Asp232-His234 dyad of *Bs*NagZ lay in proximity to the inhibitor with distances to the C1 and N2 of the inhibitor as indicated. The map was generated on the basis of the Fc calculated from the model and was contoured at the 2.5 sigma level.

Supplementary Figure S3

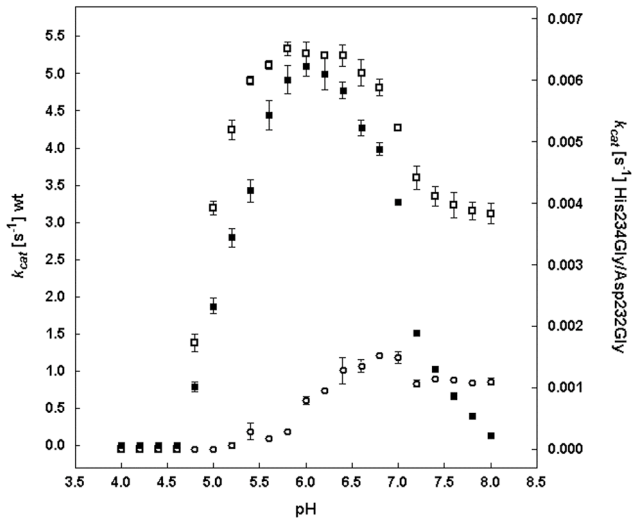

Fig. S3. pH activity profiles. The pH activity profiles of *Bs*NagZ (■) and the two mutants His234Gly (□) and Asp232Gly (○) using McIlvaine buffer ranging from pH 4.0-8.0). k_{cat} versus pH plot of wild-type NagZ corresponds to the left axis and the k_{cat} versus pH of the mutants to the right axis. Data shown were of three independent experiments with standard errors as indicated.

Supplementary Figure S4

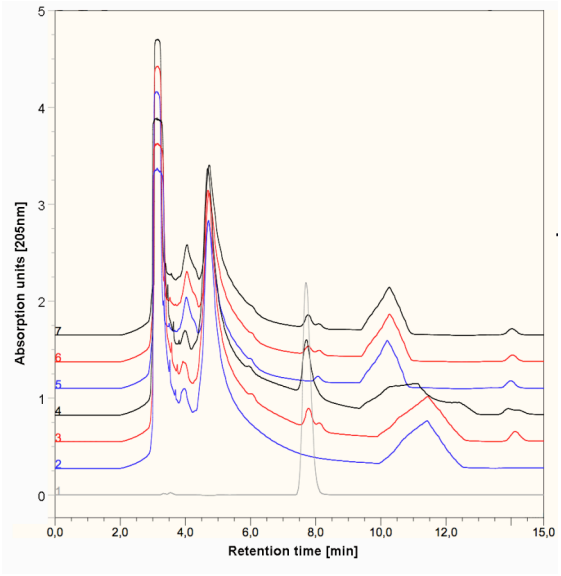

Fig. S4. Formation of β-GlcNAc azide by *Bs*NagZ variants. pNP GlcNAc (6 mM) was incubated with *Bs*NagZ-Asp232Gly (lines 2-4) and *Bs*NagZ-His234Gly (lines 5-7) in the presence of sodium azide (500 mM) for 2, 60 and 120 min at 25°C (blue, red and black lines, respectively). HPLC analysis revealed the formation of a product that elutes as the chemical synthesized β-GlcNAc azide standard (10 mM, line 1, grey line) at 8 min. This product was not observed in the absence of the enzyme. The other peaks indicate buffer compounds and sodium azide. GlcNAc elutes at 3.5 min and the substrate pNP GlcNAc elutes at 50 min.