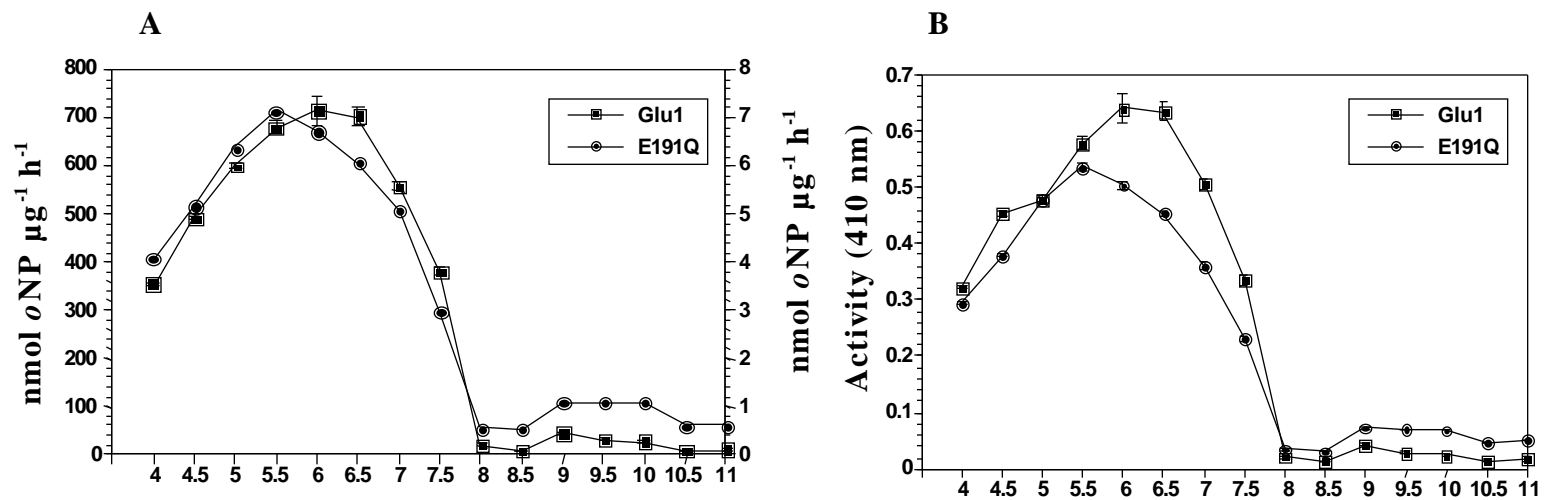


Table I. Kinetic parameters of para/orto-nitrophenyl- $\beta$ -D-glycopyranosides obtained with rGlu1 and rGlu1 E191Q enzymes.

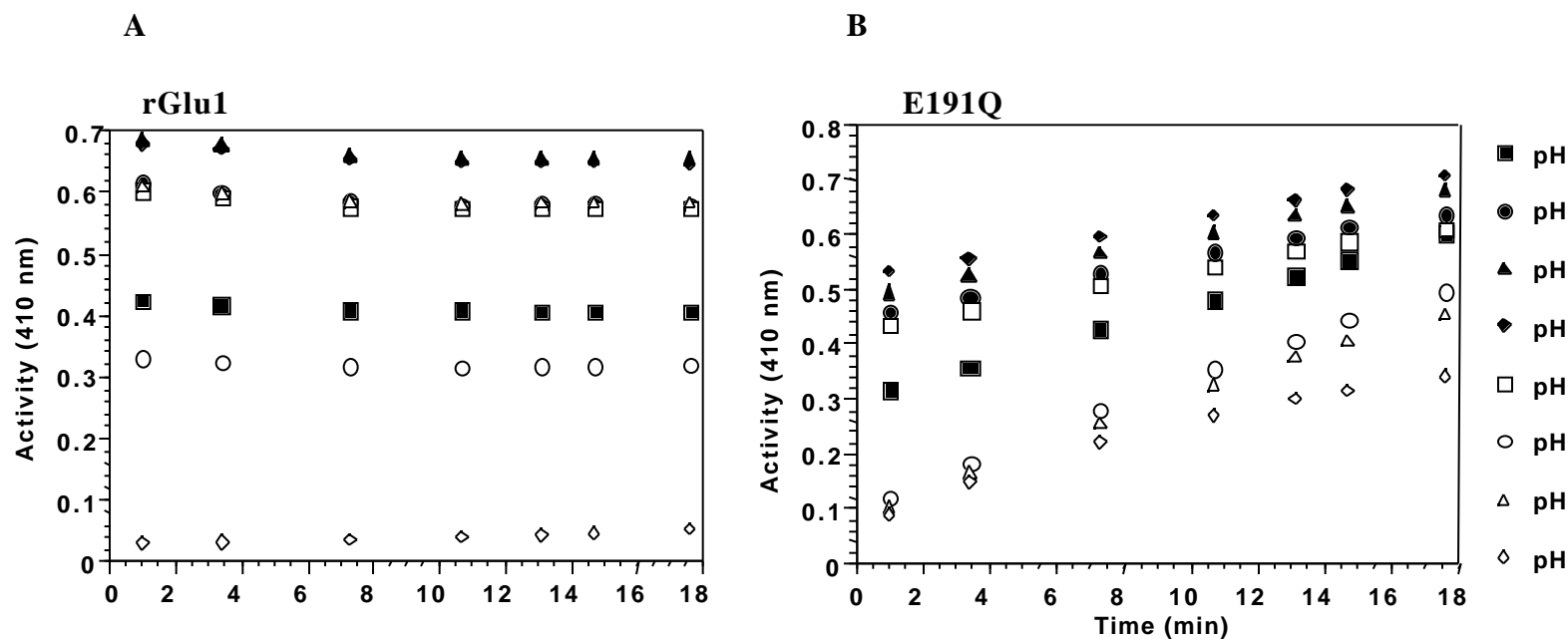
Para/orto-nitrophenyl $\beta$ -D-glycopyranoside	rGlu1			E191Q			$\frac{(k_{cat}/K_m)_{rGlu1}}{(k_{cat}/K_m)_{E191Q}}$
	$K_m$ (mM)	$k_{cat}$ (min <sup>-1</sup> )	$k_{cat}/K_m$ (min <sup>-1</sup> .mM <sup>-1</sup> )	$K_m$ (mM)	$k_{cat}$ (min <sup>-1</sup> )	$k_{cat}/K_m$ (min <sup>-1</sup> .mM <sup>-1</sup> )	
<i>p</i> NPGlc	0.47	1452	3089	0.44	7.2	16.36	188.8
<i>o</i> NPGlc	1.27	1185	930	1.33	10.8	8.12	114.5
<i>p</i> NPGal	5.66	163.2	28.8	5.84	1.0	0.18	160.0
<i>o</i> NPGal	9.90	108.6	10.9	8.33	0.6	0.08	136.2
<i>p</i> NPFc	0.57	2.5x10 <sup>4</sup>	4.3x10 <sup>4</sup>	0.56	102	81.17	237.4
<i>o</i> NPFc	1.90	1538	809	1.93	87.7	45.44	17.8

Table II. Inhibition of Glu1 and its E191Q mutant by *p*-nitro-phenyl-thio-Glc at pH 5.8

Substrates	Type of inhibition	Glu1 $k_i$ (mM)	E191Q $k_i$ (mM)	$k_i$ ratio (Glu1/E191Q)
<i>p</i> NPGlc	Competitive	0.157	0.008	19.60
<i>o</i> NPGlc	Competitive	0.194	0.050	3.88
<i>p</i> NPFuc	Competitive	0.141	0.077	1.83
<i>o</i> NPFuc	Competitive	0.295	0.089	3.31



**Fig. 6.** **A**, pH dependence of specific activity for native Glu1 versus E191Q. The Y axis on the left and right presents specific activity data for native Glu1 and E191Q, respectively; **B**, pH profile of native Glu1 and E191 Q mutant after activity is normalized at pH 5.2 in Na-acetate buffer.



**Fig. 7.** Time-dependent activity enhancement of Glu1(A) and E191Q mutant (B) with *o*NPG at different pHs conditions. The reaction mixtures were first incubated for 10 min and then 0.4 M Na-carbonate was added. Time-dependent changes in *o*NP release were monitored at 410 nm. Note that no change in *o*NP absorbance is detected after addition of Na-carbonate to the reaction mix containing Glu1 (A). However *o*NP absorbance increases in the reaction mix containing the E191Q mutant (B) after addition of Na-carbonate.