Appendix A: Derivation of quadratic equations leading to an expression for rate constant for hydrolysis of glycosidic bond.

In this section, as in submitted manuscript [21], it may appear that the main text is repeated: But in order to avoid confusion and to enable verification there is no need jumping into useful derived final equations. Thus stepwise approach is far better as against "ambush-style presentation". However, the issue addressed here is entirely different despite the fact that the form of the equations appears to be the same until subsequent simple steps may show on the contrary. Therefore, separate presentation in this section is worthwhile. Equation (A.1) below is similar to Eq. (4) in the text and here M_S is strongly assumed to be the molar mass of the polysaccharide or rather the fragment of the polysaccharide transformed.

$$[P]N_A = \frac{[S_0]}{M_S}\left(1 - \frac{1}{\exp(k\,t)}\right)\left(\frac{M_S}{162} - 1\right)N_A \tag{A.1}$$

Equation (A.1) is premised on the fact that for every mole of maltose yielded, one mole of the glycosidic bond is hydrolyzed.

$$[P] = \frac{\dfrac{[S_0](\exp(kt)-1)}{\exp(kt)}\left(\dfrac{M_S}{162} - 1\right)}{M_S} \tag{A.2}$$

Rearrangement of Eq. (A.2) gives:

$$\frac{[S_0]}{162} - \frac{[S_0]}{M_S} = \frac{\exp(k\,t)[P]}{(\exp(k\,t)-1)} \tag{A.3}$$

$$\frac{[S_0]}{M_S} = \frac{[S_0]}{162} - \frac{\exp(k\,t)[P]}{(\exp(k\,t)-1)} \tag{A.4}$$

Let Eq. (A.5) below holds temporarily for the purpose of brevity.

$$\zeta = [S_0]\frac{1}{162} - \frac{\exp(k\,t)[P]}{(\exp(k\,t)-1)} \tag{A.5}$$

$$M_S = \frac{[S_0]}{\zeta} \tag{A.6}$$

$$k_{2[s]} = \frac{2\Delta[S_0]\,k_2}{2vtM_3\phi} \tag{A.7}$$

As expressed elsewhere (submitted manuscript [21]), if $\left[S_0\right]/M_3$ is the molar concentration of maltose yielded, division by $\phi k_{2[S]}$ is postulated to give perhaps crudely but reasonably the molar concentration of the substrate which formed complex and got transformed to product. The integer 2 in the denominator and nominator in Eq. (A.7) is introduced to account for the fact that the degree of polymerization of maltose is two. In Eq. (A.7), M_3 is = 324 g/mol (*i.e.* 2 ×162g/mol). This implies that 162 ϕ = relative molecular mass of the substrate, but in this case, it may conjecturally be the relative molar mass of the fragment of the polysaccharide hydrolyzed because within the short duration of assay, it is not likely that the entire chain plus branch is digested. Consequently, Eq. (A.7) can be re-written as:

$$k_{2[s]} = \frac{\Delta\left[S_0\right] k_2}{2vtM_S}$$
(A.8)

where M_S is the molar mass described earlier. The term transformation is simply the breaking and making of bonds before the departure of the product. In Eq. (A.7), $\dfrac{v}{k_2} : \dfrac{\Delta\left[S_0\right]}{tM_3 k_{2[S]}\phi} = 1$ is simply an expression of the ratio of the molar concentration of the enzyme involved in complex formation to the molar concentration of the substrate that was transformed to product or more precisely, the number of moles per liter of hydrolyzed glycosidic bonds (for every one molecule of maltose produced, one molecule of water is utilized and one glycosidic bond is hydrolyzed), given that the value of M_3 is known.

$$v\,t = \frac{k_2\Delta\left[S_0\right]}{2M_S K_{2[S]}}$$
(A.9a)

$$k_{2[s]} = \frac{\phi\left[S_0\right] k_2}{2vtM_S}$$
(A.9b)

Where M_S is the molar mass of the part of the polysaccharide transformed.

In Eq. (9b), $k_{2[S]} \; \alpha \; 1/M_S$. Thus,

$$k_{2[s]} = \frac{\Theta}{M_S}$$
(A.10)

In Eq. (A.9a) $k_2 \neq k_{2[S]}$. This calls for further elucidation taking into cognizance the mass conservation law [33-36]. If one mole of the substrate is hydrolyzed by appropriate hydrolase, the number of times hydrolytic actions occur is ϕ-1which, represents the number of glycosidic bonds per molecule of the polysaccharide or the number of moles of water molecules needed. If $\Delta[S_0]$ is hydrolyzed, then $N_A(\Delta[S_0]/M_S)(\phi$-1) is the number of glycosidic bonds if M_S is the molar mass of the part of the polysaccharide hydrolyzed. The purpose of Eq.

(A.9a) is the determination of a slope ($S_{L(1)}$) which could be used to find an alternative expression for M_S. The slope from the plot of $v\,t$ (or $[P]$) versus $\Delta[S_0]$ ($[S_0]\exp((k\,t)-1)/\exp(k\,t)$) can be expressed as:

$$S_{L(1)} = \frac{k_2}{2M_S k_{2[S]}} \tag{A.11}$$

Equation (A.11) leads to Eq. (A.12).

$$k_{2[S]} = \frac{k_2}{2M_S S_{L(1)}} \tag{A.12}$$

From Eq. (A.11)

$$M_S = \frac{k_2}{2S_{L(1)}k_{2[S]}} \tag{A.13}$$

By replacing $k_{2[S]}$ in Eq. (19) in the text with $\boldsymbol{k_{2[S]}}$ and substitution of Eq. (A.13) into it gives:

$$\Delta[S_0] = \left(\Theta[S_0]\frac{k_2}{162 S_{L(1)}\boldsymbol{k_{2[S]}}} - \frac{\Theta[P]\exp(k\,t)k_2}{\left(\exp(k\,t)-1\right)S_{L(1)}\boldsymbol{k_{2[S]}}} \right) v\frac{t}{[S_0]k_2} \tag{A.14}$$

It should be noted that $k_{2[S]}$ (in the main text) $\neq \boldsymbol{k_{2[S]}}$. Meanwhile, $[P] = v\,t$ and Eq. (A.13) is substituted into Eq. (A.10) to give:

$$\boldsymbol{k_{2[S]}} = \frac{2\Theta}{k_2}\,S_{L(1)}k_{2[S]} \tag{A.15}$$

$$\Theta = \frac{k_2}{2S_{L(1)}} \tag{A.16}$$

Rearrangement of Eq. (A.14) and substitution of Eq. (A.16) into it gives the following after simplification,

$$\Delta[S_0] = \Theta\left(\frac{[S_0]k_2}{162 S_{L(1)}\boldsymbol{k_{2[S]}}} - \frac{[P]\exp(k\,t)k_2}{\left(\exp(k\,t)-1\right)S_{L(1)}\boldsymbol{k_{2[S]}}} \right) v\frac{t}{[S_0]k_2}$$

$$= \frac{k_2}{2\boldsymbol{k_{2[S]}}S_{L(1)}^2}\left(\frac{[S_0]}{162} - \frac{[P]\exp(k\,t)}{\left(\exp(k\,t)-1\right)} \right) v\frac{t}{[S_0]} \tag{A.17}$$

Appendix B: Determination of proportionality constant.

Looking carefully at the derivations in the theoretical sub-section, one may feel that there is no substantial difference between what is in that section and appendix section: But there is a difference with strong implication analogous to what is expected when there is a single point genetic mutation. The derivation and application of the equation in this section unlike theoretical section enable the determination of $k_{2[S]}$ ($k_{2[S]} \neq k_2$).

A plot of $\Delta[S_0]$ versus $\left(\dfrac{[S_0]}{162} - \dfrac{[P]\exp(kt)}{(\exp(kt)-1)} \right) v \dfrac{t}{[S_0]}$ in Eq. (A.17) gives a 2nd slope, $S_{L(2)}$ expressed as:

$$S_{L(2)} = \frac{k_2}{2k_{2[s]} S_{L(1)}^2} \tag{B.1}$$

Thus,

$$k_{2[s]} = \frac{k_2}{2S_{L(1)}^2 S_{L(2)}} \tag{B.2}$$