# Supplementary Data

**HPLC-based kinetics assay facilitates analysis of systems with multiple reaction products and thermal enzyme denaturation**

Klingaman, Chase A.[1†]; Wagner, Matthew, J.[1†]; Brown, Justin R.[1‡]; Klecker, John B.[1‡]; Pauley, Ethan H.[1]; Noldner, Colin J.[1]; Mays, Jared R.[1*]

[1] Augustana University, Department of Chemistry, 2001 S. Summit Ave., Sioux Falls, SD 57197, Phone: 605-274-4815, Fax: 605-274-4492, Email: jmays@augie.edu

* Corresponding author

† These authors contributed equally to this work

‡ These authors contributed equally to this work

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$$[\mathrm{Gluc}]_t = \mathrm{K_m} W(x)$$

**Equation SD-1**

$$W(x) \cong (1+\varepsilon)\left(\ln\left\{\frac{6\cdot x}{5\cdot\ln\left[(12/5)\cdot\left(x/\ln(1+(12/5)\cdot x)\right)\right]}\right\}\right) - \varepsilon\left(\ln\left\{\frac{2\cdot x}{\ln(1+2\cdot x)}\right\}\right)$$

**Equation SD-2**

$$x = \left(\frac{[\mathrm{Gluc}]_0}{K_\mathrm{m}}\right)\exp\left(\frac{[\mathrm{Gluc}]_0 - V_\mathrm{max}\cdot t}{K_\mathrm{m}}\right)$$

**Equation SD-3**

Y=(KM)*((1+0.4586887)*(ln((6*((Y0/KM)*(exp((Y0-(VMAX*X))/KM))))/(5*ln((12/5)*(((Y0/KM)*(exp((Y0-(VMAX*X))/KM)))/ln(1+(12/5)*((Y0/KM)*(exp((Y0-(VMAX*X))/KM)))))))-(0.4586887*ln((2*((Y0/KM)*(exp((Y0-(VMAX*X))/KM))))/(ln(1+(2*((Y0/KM)*(exp((Y0-(VMAX*X))/KM))))))))))

**Equation SD-4.** Textual definition of Equation SD-1 (with included Eq. SD-2 and Eq. SD-3) for use in calculating reaction progress curves for $[\mathrm{Gluc}]_t$ in GraphPad Prism 6.0 and other nonlinear regression software. This equation is based on Equation 7 with the improved Lambert *W* function (Equations 10-11) in Goličnik, M. *Anal. Biochem.* **2010**, *406*, 94–96. Initial variable values for nonlinear regression were $[\mathrm{Gluc}]_0$ = Y0 = 500, $K_\mathrm{m}$ = KM = 1.0, and $V_\mathrm{max}$ = VMAX = 8.0.

$$[\mathrm{ITC}]_t = [\mathrm{nitrile}]_t = [\mathrm{Gluc}]_0 - [\mathrm{Gluc}]_t$$

**Equation SD-5**

Y=Y0-((KM)*((1+0.4586887)*(ln((6*((Y0/KM)*(exp((Y0-(VMAX*X))/KM))))/(5*ln((12/5)*(((Y0/KM)*(exp((Y0-(VMAX*X))/KM)))/ln(1+(12/5)*((Y0/KM)*(exp((Y0-(VMAX*X))/KM)))))))-(0.4586887*ln((2*((Y0/KM)*(exp((Y0-(VMAX*X))/KM))))/(ln(1+(2*((Y0/KM)*(exp((Y0-(VMAX*X))/KM))))))))))

**Equation SD-6.** Textual definition of Eq. SD-5 (with included Eq. SD-1, Eq. SD-2, and Eq. SD-3) for use in calculating reaction progress curves for $[\mathrm{ITC}]_t$ or $[\mathrm{nitrile}]_t$ in GraphPad Prism 6.0 and other nonlinear regression software. This equation is based on Equation 7 from Goličnik, M. *Anal. Biochem.* **2010**, *406*, 94–96. Initial variable values for nonlinear regression were $[\mathrm{Gluc}]_0$ = Y0 = 500, $K_\mathrm{m}$ = KM = 1.0, and $V_\mathrm{max}$ = VMAX = 8.0.

$$V_0 = -\frac{d[\mathrm{Gluc}]}{dt} = \frac{V_\mathrm{max}\cdot[\mathrm{Gluc}]_0}{K_\mathrm{m} + [\mathrm{Gluc}]_0}$$

**Equation SD-7.** The Michaelis–Menten equation.

Y=(KM)*((1+0.4586887)*(ln((6*((Y0/KM)*(exp((Y0-((VMAX*(exp(-KD*X)))*X))/KM))))/(5*ln((12/5)*(((Y0/KM)*(exp((Y0-((VMAX*(exp(-KD*X)))*X))/KM)))/ln(1+(12/5)*((Y0/KM)*(exp((Y0-((VMAX*(exp(-KD*X)))*X))/KM)))))))-(0.4586887*ln((2*((Y0/KM)*(exp((Y0-((V0*(exp(-KD*X)))*X))/KM))))/(ln(1+(2*((Y0/KM)*(exp((Y0-((VMAX*(exp(-KD*X)))*X))/KM))))))))))

**Equation SD-8.** Textual definition of Eq. SD-1 (with included Eq. SD-2, Eq. SD-3, and Eq. 1) for use in calculating reaction progress curves of $[\mathrm{Gluc}]_t$ in GraphPad Prism 6.0 and other nonlinear regression software. This equation is based on Equation 7 with the improved Lambert *W* function (Equations 10-11) in Goličnik, M. *Anal. Biochem.* **2010**, *406*, 94–96 and includes a term to account for the first-order denaturation of enzyme (Eq. 1). Initial variable values for nonlinear regression were $[\mathrm{Gluc}]_0$ = Y0 = 250, $K_\mathrm{m}$ = KM = 500, $V_\mathrm{max}$ = VMAX = 8.0, and $k_\mathrm{d}$ = KD = 1 x $10^{-7}$. The following constraints were placed on nonlinear regression variables: Y0 = 0–400, KM = 0–1200, VMAX = 0–50, and KD = 0–0.1.