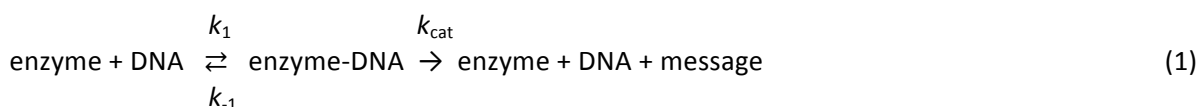


Origin Assignment 8 (Optional) – Due May 3, 2012, 5 PM

Kinetics of Message Synthesis by RNA Polymerase

The 'Central Dogma' of molecular biology teaches that DNA encodes the information for protein primary sequence, among other things, and that the process of *transcription* generates the messenger RNA, from which proteins are synthesized in the process of translation on ribosomes. (Goggle 'central dogma molecular biology', if you know nothing of this.)

The formation of RNA is a template-directed synthesis process; the polymerase enzyme uses the DNA as a template. The polymerase reads DNA in a *processive* manner – transcribing one base at a time as it moves along the backbone of the DNA. The kinetics of the process is undoubtedly complex, but the overall scheme can be reduced to:



The following data were obtained for the synthesis of an RNA message using saturating concentrations of nucleotide triphosphates (NTPs), at two different concentrations of RNA polymerase.

[DNA] _{tot} , μM	[Enz] _{tot} , μM	Velocity, μM·min ⁻¹
0.02	0.04	0.61 ± 0.12
0.04	0.04	1.03 ± 0.29
0.06	0.04	1.15 ± 0.36
0.08	0.04	1.25 ± 0.11
0.12	0.04	1.23 ± 0.07
0.16	0.04	1.34 ± 0.07

[DNA] _{tot} , μM	[Enz] _{tot} , μM	Velocity, μM·min ⁻¹
0.02	0.08	0.67 ± 0.27
0.04	0.08	1.31 ± 0.14
0.06	0.08	1.90 ± 0.03
0.08	0.08	2.25 ± 0.03
0.12	0.08	2.49 ± 0.10
0.16	0.08	2.83 ± 0.16

Procedure

1. Plot the Data, with the error bars.
2. Fit the data to the Michaelis-Menton Equation. You be able to find this equation, as well as derivations of it in biochemistry textbooks. Give the definitions of K_M and k_{cat} and discuss their meaning in general terms. You should generate three values of K_M and k_{cat} each. One for each concentration of $[\text{Enz}]_{\text{tot}}$ (two apiece altogether). Determine the third pair in a global fit of the data, in which K_M and k_{cat} are shared. Discuss (i) possible trends in the result, and (ii) whether the global fit was warranted or proved to be advantageous in this case.
3. Note that $[\text{Enz}]_{\text{tot}}$ is **not** always much less than $[\text{DNA}]_{\text{tot}}$ (substrate). Discuss why this might be a problem.
4. Derive an equation for the enzyme velocity (e.g. according to scheme 1), which does not assume $[\text{DNA}]_{\text{tot}} \gg [\text{Enz}]_{\text{tot}}$. Show all your work. A neat, handwritten derivation, step-by-step with variables full defined is fine. (if you don't want to use equation editor.) **Hint:** the solution involves a root to a quadratic equation in which $[\text{DNA}]_{\text{tot}}$ and $[\text{Enz}]_{\text{tot}}$ are fixed parameters, and

K_M and k_{cat} are adjustable parameters. The form of the solution, as well as the equations that you need to use in Origin, will resemble those used in Problem Set 5.

- 5.** *Refit the data sets to the equation derived in 5. Share the parameters K_M and k_{cat} between the two data sets. Compare the shared fit to the individual fits, and to the estimates of K_M and k_{cat} obtained in part 2. Discuss the significance of results, e.g. whether/why they are different in 2 & 5 (in one or two paragraphs).*