Origin Assignment 8 (Optional) – Due May 10, 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:

$$\text{enzyme + DNA} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{enzyme-DNA} \xrightarrow{k_{cat}} \text{enzyme + DNA + message} \qquad (1)$$

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-Menten 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 $[Enz]_{tot}$ (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 $[Enz]_{tot}$ is **not** always much less than $[DNA]_{tot}$ (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 $[DNA]_{tot} >> [Enz]_{tot}$. 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 $[DNA]_{tot}$ and $[Enz]_{tot}$ 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).*

Answers

1. *Plot the Data, with the error bars.*

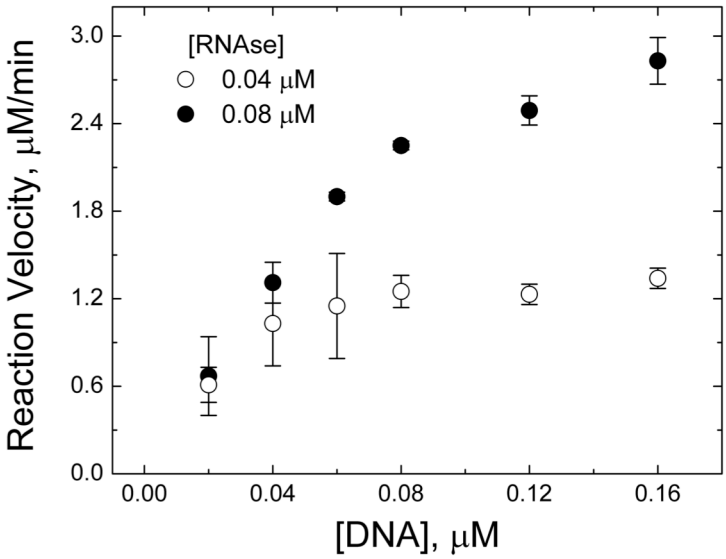

Figure A1. Plot of the enzyme-catalyzed reaction rate as a function of the total template [DNA], at two different concentrations of the enzyme (0.04 and 0.08 μM).

2. *Fit the data to the Michaelis-Menten 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 $[Enz]_{tot}$ (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.*

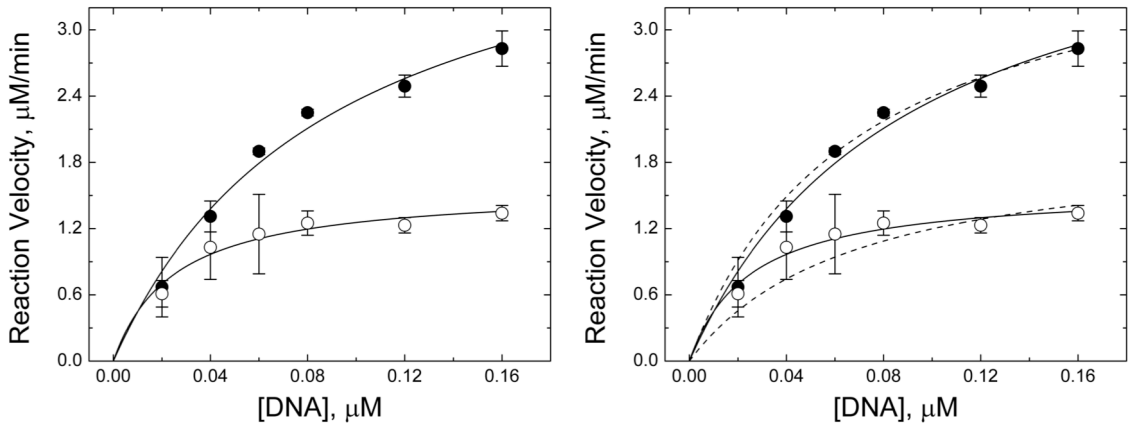

Figure A2. Fits of reaction velocity according to the Michaelis-Menten model (Eq. A5), where each data set is fit individually (solid lines, left and right panels) or globally (dashed lines, right panel). In the right panel, the form of the equation used to fit the data was $v = k_{cat}[Enz]_{tot}[DNA]/(K_M + [DNA])$, so that the different total enzyme concentrations could be accommodated in the global fit.

Table A1. Michaelis-Menten ($v = V_{max}[DNA]/(K_M + [DNA])$ **Left Panel**

$[Enz]_{tot}$, μM	V_{max}, μM/min	K_M, μM	Red. χ^2	Adj. R^2
0.04	1.567 ± 0.099	0.0249 ± 0.0058	0.0054	0.922
0.08	4.478 ± 0.466	0.0900 ± 0.0191	0.0159	0.975

Table A2. Michaelis-Menten ($v = k_{cat}[Enz]_{tot}[DNA]/(K_M + [DNA])$ **Right Panel**, Individual Fits, Solid Lines

$[Enz]_{tot}$, μM	k_{cat}, min^{-1}	K_M, μM	Red. χ^2	Adj. R^2
0.04	39.18 ± 2.47	0.0249 ± 0.0058	0.0054	0.922
0.08	55.97 ± 5.84	0.0900 ± 0.0191	0.0159	0.975

The values of V_{max} in individual fits in Table A1 can be verified equal to $k_{cat}[Enz]_{tot}$ in Table A2 by carrying out the multiplication ($V_{max} = k_{cat}[Enz]_{tot}$).

Table A3. Michaelis-Menten ($v = k_{cat}[Enz]_{tot}[DNA]/(K_M + [DNA])$ **Right Panel**, Global Fit, Dashed Lines

$[Enz]_{tot}$, μM	k_{cat}, min^{-1}	K_M, μM	Red. χ^2	Adj. R^2
0.04 0.08	50.37 ± 5.33	0.0682 ± 0.0165	0.0286	0.943

3. *Note that $[Enz]_{tot}$ is **not** always much less than $[DNA]_{tot}$ (substrate). Discuss why this might be a problem.*

The Michaelis-Menten equation assumes that $[DNA]_{tot} >> [Enz]_{tot}$, so that $[ED] \approx [Enz]_{tot}$ and $[DNA]_{free} \approx [DNA]_{tot}$. This is no longer true when $[DNA]_{tot} \sim [Enz]_{tot}$.

4. *Derive an equation for the enzyme velocity (e.g. according to scheme 1), which does not assume $[DNA]_{tot}$ >> $[Enz]_{tot}$. 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 $[DNA]_{tot}$ and $[Enz]_{tot}$ 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.*

The rate of the enzyme-catalyzed reaction is given by

$$v = \frac{d[\mathrm{M}]}{dt} = k_{cat}[\mathrm{ED}] \tag{A1}$$

This reaction generates messenger RNA, M, by the action of RNA polymerase, Enz or E, using DNA, D, as a template. The conventional derivation of the Michaelis-Menten equation (or more precisely the Henri-Michaelis-Menten equation) assumes that the total substrate concentration ($[D]_{tot}$) is much larger than the total enzyme concentration ($[E]_{tot}$). In this limit, the approximation $[D]_{free} = [D]_{tot}$ is valid because the concentration of enzyme-substrate complex ([ED]) can never become large enough have a significant impact $[D]_{tot}$. In practice, this corresponds to situations where $[E]_{tot} \sim 0.1[D]_{tot}$, or less. The Michaelis-Menten approximation also assumes that the concentration of the enzyme-substrate complex, [ED], achieves a steady state during the initial rate measurement, *i.e.* $d[\mathrm{ED}]/dt = 0$.

$$\frac{d[\mathrm{ED}]}{dt} = k_1[\mathrm{E}][\mathrm{D}] - k_{-1}[\mathrm{ED}] - k_{cat}[\mathrm{ED}] = 0 \tag{A2}$$

The mass conservation equation for the total enzyme concentration

$$[\mathrm{E}]_{tot} = [\mathrm{E}]_{free} + [\mathrm{ED}] \quad \rightarrow \quad [\mathrm{E}]_{free} = [\mathrm{E}]_{tot} - [\mathrm{ED}] \tag{A3}$$

is employed to solve for the concentration of the enzyme substrate complex, which leads to the result

$$[\mathrm{ED}] = \frac{[\mathrm{E}]_{tot}[\mathrm{D}]}{(K_M + [\mathrm{D}])} \tag{A4}$$

where $K_M = (k_{-1} + k_{cat})/k_1$. Combining Eqs. A1 and A4, gives the Michaelis-Menten equation.

$$v = \frac{k_{cat}[\mathrm{E}]_{tot}[\mathrm{D}]}{(K_M + [\mathrm{D}])} \tag{A5}$$

As long as $[E]_{tot} << [D]_{tot}$, it can be assumed that $[D]_{tot} = [D]_{free}$. If this condition is not satisfied, then the mass conservation equation for the substrate, D, must also be used in the equation for the steady state concentration of ED.

$$[\mathrm{D}]_{tot} = [\mathrm{D}]_{free} + [\mathrm{ED}] \quad \rightarrow \quad [\mathrm{D}]_{free} = [\mathrm{D}]_{tot} - [\mathrm{ED}] \tag{A6}$$

$$k_1([E]_{tot} - [ED])([D]_{tot} - [ED]) - k_{-1}[ED] - k_{cat}[ED] = 0 \quad \text{(A7)}$$

$$k_1([E]_{tot}[D]_{tot} - ([E]_{tot} + [D]_{tot})[ED] + [ED]^2) - k_{-1}[ED] - k_{cat}[ED] = 0 \quad \text{(A8)}$$

$$[ED]^2 - ([E]_{tot} + [D]_{tot} + K_M)[ED] + [E]_{tot}[D]_{tot} = 0 \quad \text{(A9)}$$

The appropriate root to the quadratic equation (A9), inserted in equation (A1), gives the equation for enzyme velocity without assuming that $[E]_{tot} = [E]_{free}$

$$v = \frac{1}{2}k_{cat}\left[([E]_{tot} + [D]_{tot} + K_M) - \sqrt{([E]_{tot} + [D]_{tot} + K_M)^2 + 4[E]_{tot}[D]_{tot}}\right] \quad \text{(A10)}$$

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).*

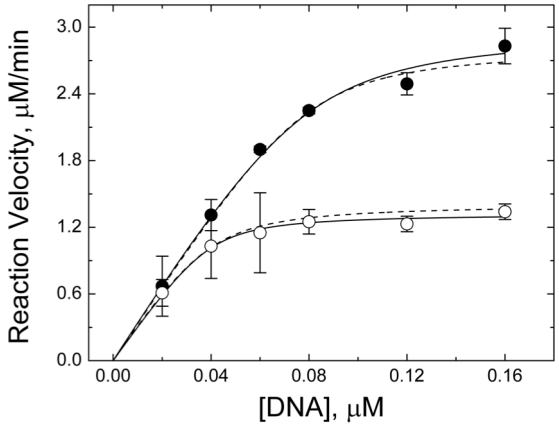

Figure A3. RNA polymerase data fit to modified Michaelis-Menten model, Eq. A10, individually (solid lines) and in a global fit (dashed lines).

To illustrate the problem in using the Michaelis-Menten equation when $[D]_{tot}$ is *not* larger $[E]_{tot}$, the data were fit to both models, Eq. A5 (as shown above in Figure A2) and Eq. A10 (as shown in Figure A3). The tables and plots are the results of fits to (i) Eq. A5 *versus* Eq. A10, and (ii) sharing k_{cat} and K_M between both sets of data *versus* allowing them to vary independently among the 0.04 and 0.08 μM data sets.

Table A4. Modified Michaelis-Menten (Eq. A10) Individual Fits, Solid Lines

$[Enz]_{tot}$, μM	k_{cat}, min^{-1}	K_M, μM	Red. χ^2	Adj. R^2
0.04	33.11 ± 0.96	0.00288 ± 0.00113	0.0017	0.976
0.08	37.23 ± 2.28	0.00667 ± 0.00373	0.0068	0.989

Table A5. Modified Michaelis-Menten (Eq. A10) Global Fit, Dashed Lines

$[Enz]_{tot}$, μM	k_{cat}, min^{-1}	K_M, μM	Red. χ^2	Adj. R^2
0.04 0.08	35.30 ± 0.06	0.0043 ± 0.0001	0.0016	0.997

The results demonstrate there is a significant difference in the treatment of the data without the assumptions. The total DNA concentration was not larger than the total Enzyme concentration in these experiments, so [ED] ≠ $[Enz]_{tot}$ and $[DNA]_{free} \neq [DNA]_{tot}$, even approximately. Plots of the residuals would undoubtedly show that the data are much better explained by Eq. A10 than Eq. A5. The global vs. individual fit of the data to Eq. A10 do not add significantly to this, it is the improvement of Eq. A10 relative to Eq. A5 that has the greatest impact.

K_M is poorly estimated with Eq. A5, because the approximation has a great impact on the effective concentrations of the Enzyme-DNA complex, which influences substantially the estimate of the strength of the enzyme-DNA interaction. K_M is found to be **10-fold** smaller in the absence of the assumption. The impact on V_{max} (k_{cat}) is smaller, but still significant, because Eq. A5, a simple hyperbolic equation, provides an inadequate description of the system at intermediate substrate concentrations [DNA] ~ $[Enz]_{tot}$. Overall, the ability to conduct the nonlinear least squares analysis computationally leads to a better result.