

Basic Principles of Kinetics and Thermodynamics

Arthur L. Haas, Ph.D.

Department of Biochemistry and Molecular Biology

Review: Garrett and Grisham, "Enzyme Kinetics" (Chapt. 13), in *Biochemistry*, Third Edition, Saunders, Fort Worth, 2005.

This lecture will be devoted to an overview of the principles of kinetics and thermodynamics as they apply to biochemical and cell biological systems. The main focus of our considerations will center on how these fundamental physical properties can be applied to practical laboratory situations including experimental design, interpretation of results, and solving technical problems in experimental protocols.

I. First order reactions

- A. The most fundamental of chemical reactions are **monomolecular** or **first order reactions** involving the conversion of a single reactant species (A) to product (P). First order reactions can be represented by a simple **kinetic scheme**. The formation of product with time (and the loss of reactant, since at any time $A_{\text{initial}} = P_{\text{formed}} + A_{\text{remaining}}$) follows **exponential kinetics**.

The **instantaneous rate** or **velocity** of the reaction (amount of P formed per unit time) at any time t can be graphically represented as a tangent to the progress curve at that time t . Intuitively we recognize that the more A present, the greater the rate of formation of P, which leads to a simple mathematical expression termed the **first order rate equation**.

The **first order rate constant** (k) is inversely related to energy that A must have in order to react and be converted to P. [The standard convention to avoid confusion is *always* to represent rate constants as lower case k and equilibrium constants by upper case K .] The overall process can be represented by a **reaction coordinate diagram** defining the **transition state** and the **energy of activation** for the reaction.

Consideration of the first order rate equation over time results in the **integrated first order rate equation** which defines the amount of reactant remaining at time t (A_t) with respect to time and the first order rate constant. In the equation the amount of A at zero time (that is the amount one started with) is defined as A_0 . Since $A_{\text{initial}} = P_{\text{formed}} + A_{\text{remaining}}$ one can substitute and rearrange this equation to express formation of product at any time t (P_t).

The **half life** of the reaction ($t_{1/2}$) is defined as the time required for half of the reactant to be converted to product, leading to a useful mathematical relationship between k and $t_{1/2}$.

First order processes are common in nature such as the decay of radioactive isotopes and the spontaneous denaturation/inactivation of proteins.

Application

Background: You are studying the phosphorylation of a regulatory protein within a cultured cell line. To follow phosphorylation of the protein, the cells are incubated with radioactive inorganic phosphate ($^{32}\text{P}_i$) which is taken up by the cells, rapidly incorporated into ATP from which it is used by a specific protein kinase to transfer ^{32}P to your protein. After several hours you prepare an extract from the cells and isolate your protein by immunoprecipitation (the jargon is to “IP” the protein) using an antibody specific for the protein. Radioactivity in the immunoprecipitate is too small to measure directly since most proteins are present at exceedingly small levels within cells so you resolve the sample by **Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE)** to separate by relative molecular weight. The gel is dried and placed against x-ray film to produce an autoradiogram.

Problem: You originally did this experiment on July 23 but find while writing the paper that you need to compare this result to a later experiment conducted under different conditions. This requires you to run a new SDS-PAGE containing an aliquot of your earlier sample and a new sample from the more recent experiment conducted with fresh $^{32}\text{P}_i$. You recall that the half life for ^{32}P is 14 days. How would you directly compare the two samples by autoradiography?

-
- B. Frequently one is interested in comparing the first order rate constants or half lives for reactions. One example is comparing the rates of degradation for different proteins in cells. Such comparisons can be made from single time points by using the integrated first order rate equation and solving for k if one knows the amount of A at two different times.

However, more accurate estimates of k (and therefore $t_{1/2}$) can be made by using data over the entire time course of the reaction. This involves graphically analyzing the data by a **first order** or **semi-log plot**. The integrated first order rate equation can be rearranged to a linear form that provides a precise determination of k from the slope of the plot. Semi-log paper was developed to make allow one to plot the data directly rather than to calculate log values.

C. Problems and interpretations of first order data can assume three forms.

1. The semi-log plot is linear except at long times where it appears to plateau.

2. The semi-log plot shows two distinct segments.

3. The reaction proceeds to a non-zero end point.

II. Second order reactions

- A. **Second order or bimolecular reactions** involve the conversion of two reactants (A and B) to form one or more products. The rules for writing the kinetic scheme and rate equation for second order reactions are the same as for unimolecular reactions. However, since the rate of product formation depends on the concentrations of two reactants, such reactions can show complex forms rather than exponential behavior.
- B. The **integrated second order rate equation** is equally complex and is rarely used. Instead, second order reactions are analyzed by making the **pseudo first order assumption**.

III. Equilibrium reactions

- A. The kinetics of equilibrium reactions can be measured even though they are complicated by the fact that the reverse reaction is also occurring. Consider the simple monomolecular interconversion of two species (A and B). The time course for the reaction will not proceed to completion but to an equilibrium position at which point the forward and reverse rates are equal and there is no **net** formation of either A or B.

The rate equation for equilibrium reactions reflect the contributions from the two opposing reactions.

If one chooses an observation time for which the reverse reaction is negligible, it is possible to analyze the forward reaction. This approach is called **approach to equilibrium kinetics**.

- B. Binding processes are common in biological systems and are a type of reversible reaction. Binding processes can be described by a simple kinetic scheme that is applicable to a wide range of systems. The kinetic equation describing such mechanisms defines the mathematical form of a **rectangular hyperbola**.

A mathematical property of rectangular hyperbolic equations is that they can be transformed into a linear form by rearranging the equation to a **double reciprocal**.

Application

Background: You have purified the protein kinase involve in Application 1. In the presence of ATP and MgCl_2 , the kinase readily phosphorylates your regulatory protein. You notice that during the course of the reaction (analyzed by SDS-PAGE and autoradiography), the protein kinase is also becoming phosphorylated.

Problem: Your dissertation committee requires you to determine whether this autophosphorylation is intramolecular or intermolecular. Devise a kinetic experiment to distinguish among all possible models for the autophosphorylation.

IV. Statistics and graphical analysis

A. Definitions used in experimental measurements

Accuracy is the validity of your measurement and the degree to which the measurement approximates the theoretical **true value** for a parameter. Accuracy is determined by how carefully you perform the measurement and the experimental error inherent in the measurement technique, since no measurements possesses absolute accuracy.

Precision is the number of significant figures in the measurement. There is much confusion regarding precision and significant figures. Even if the person making a measurement is as accurate as humanly possible, limitations imposed by the measuring instrument, variations in parameter being measured, and variations in the conditions under which the measurement is being made dictate that the result should not imply greater precision than circumstances warrant. Therefore one needs to be concerned about **implied precision**. For example, a value of 24.5 has an implied precision that the true value is greater than 24.45 but less than 24.55 (following the convention that numbers less than 5 round down and those greater than 5 round up). Stated simply: **The last digit recorded should be the one containing the variability due to experimental error.**

Reproducibility is the normal distribution of replicate measurements; *i.e.*, the degree to which one can consistently obtain the same number.

B. Measurements cluster in a **Gaussian distribution** about some mean value. The **Standard Deviation** (SD) defines the magnitude of this clustering. In replicate sets of measurements, independent Standard Deviations also cluster in a Gaussian distribution for which the magnitude is defined by the **Standard Error of the Mean** (SE).

C. **Linear Least Squares Fit method.**

D. **Non-linear Least Squares Fit method.**

E. **Graphical analysis.**

V. Review of thermodynamics

A. ***First Law of Thermodynamics***- The energy of a system is conserved.

System- The process under consideration.

Surroundings- Everything else.

Application

Background: You have measured the K_d for binding of two structurally related drugs to a receptor. The two drugs differ only by the substitution of a -H for a -COOH group at one position. The drug with the carboxyl group binds with greater affinity. From the ΔG° values for each drug, you calculate a $\Delta\Delta G^\circ = 5$ kcal/mole and state in a manuscript that the carboxyl group contributes 5 kcal/mole to the drug binding.

Problem: A referee rejects your manuscript on the basis that this statement is incorrect. Why?

B. **Second Law of Thermodynamics**- All processes proceed with a net increase in entropy.

C. **Third Law of Thermodynamics**- All processes proceed to the lowest free energy.

D. The **Gibbs Free Energy Equation** relates the K_{eq} to ΔG°

E. Implications of **microscopic reversibility**.