

Lecture Notes

Introduction to Bioengineering

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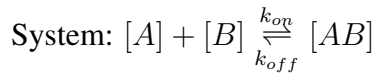
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1 Thermodynamics

1.1 The Basics



Dissociation constant: $K_d = \frac{[A][B]}{[AB]} = \frac{k_{off}}{k_{on}} = K_a^{-1}$

At equilibrium: association rate ($k_{on}[A][B]$) = dissociation rate ($k_{off}[AB]$)

Units:

parameter	Unit
$[A],[B],[AB]$	M
k_{on}	$M^{-1}s^{-1}$
k_{off}	s^{-1}
K_d	M
K_a	M^{-1}

Ranges for the equilibrium dissociation constant (K_d):

mM	non-specific
μM	protein - peptide (SH3)
nM	transcription factor - DNA
pM	antibody - antigen
fM	streptavidin - biotin

1.2 Fractional Binding

Fraction of B molecules bound by A is defined as:

$$Y_B = \frac{[AB]}{[B]_0}$$

where $[B]_0$ is the total amount of [B] in the system:

$$[B]_0 = [B] + [AB]$$

so that:

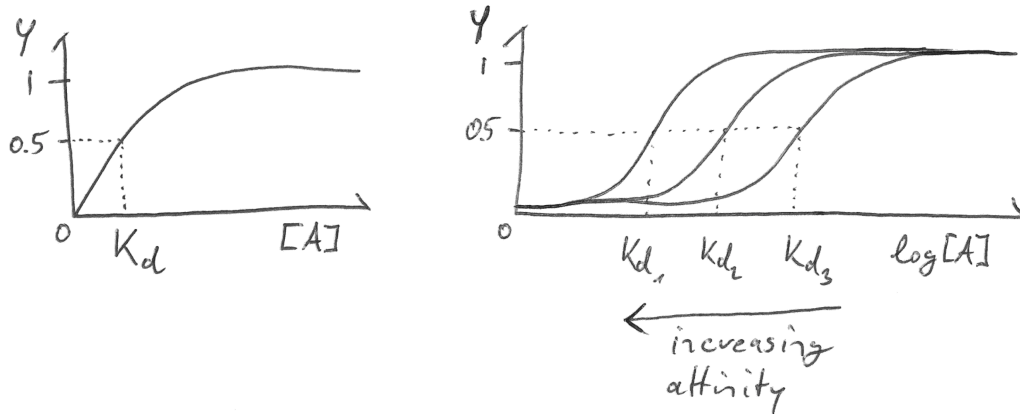
$$Y_B = \frac{[AB]}{[B] + [AB]}$$

$$K_d = \frac{[A][B]}{[AB]} \Rightarrow [B] = \frac{K_d[AB]}{[A]}$$

$$Y_B = \frac{\frac{[AB]}{K_d[AB] + [AB]}}{\frac{[AB]}{K_d[AB] + [AB]} + [AB]} = \frac{[AB][A]}{K_d[AB] + [A][AB]} = \frac{[A][AB]}{[AB](K_d + [A])} = \frac{[A]}{K_d + [A]}$$

$$Y_B = \frac{[A]}{K_d + [A]} \approx \frac{[A]_{total}}{K_d + [A]_{total}} \text{ if } [B]_{total} \ll K_d \text{ or } [A]_{total} \gg [B]_{total}$$

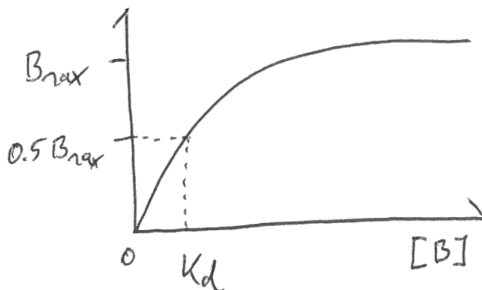
1.3 Fractional Saturation Binding Curves



→ B saturates at concentrations of A two orders of magnitude above K_d

→ Y_B changes drastically (is most sensitive) near concentrations of B equal to K_d

Note: $Y = \frac{[B]}{[B] + K_d} \approx \frac{[B]B_{max}}{[B] + K_d}$



1.4 Scatchard Plot

- linearization of the binding curve
- classic approach to determining K_d
- outdated and less accurate than current computer based methods

$$Y_B = \frac{[A]}{[A] + K_d} \Rightarrow Y_B[A] + Y_B K_d = [A]$$

$$K_d = \frac{[A] - Y_B[A]}{Y_B}$$

$$K_a = \frac{Y_B}{[A] - Y_B[A]}$$

$$K_a[A] - K_a Y_B[A] = Y_B$$

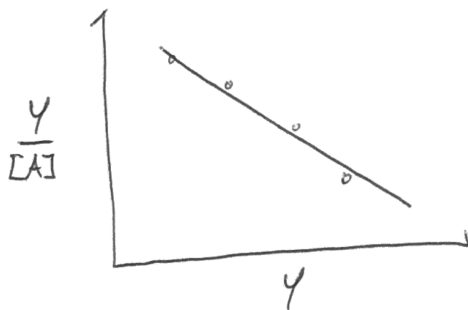
$$K_a - K_a Y_B = \frac{Y_B}{[A]} \quad (\text{this now looks similar to: } b + ax = y)$$

$$\frac{1}{K_d} - \frac{Y_B}{K_d} = \frac{Y_B}{[A]}$$

$$\text{slope} = -\frac{1}{K_d}$$

$$\text{x-intercept} = 1 \text{ or } B_{max}$$

$$\text{y-intercept} = -\frac{1}{K_d} \text{ or } \frac{B_{max}}{K_d}$$



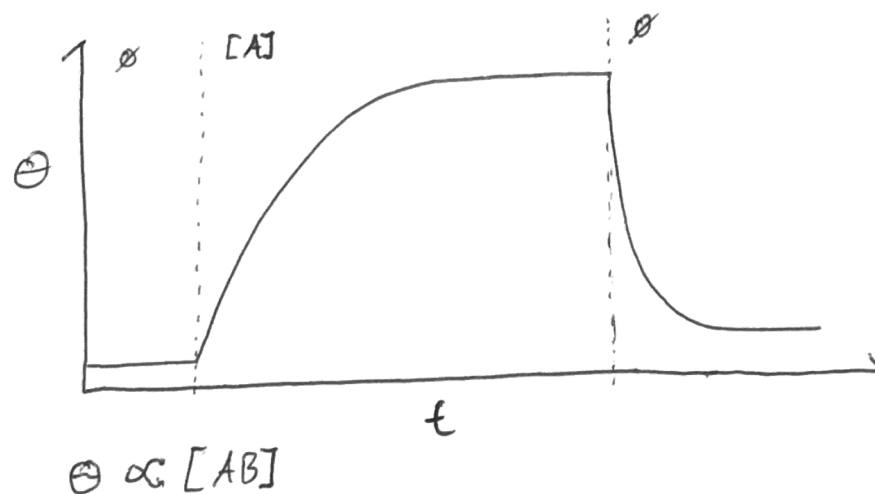
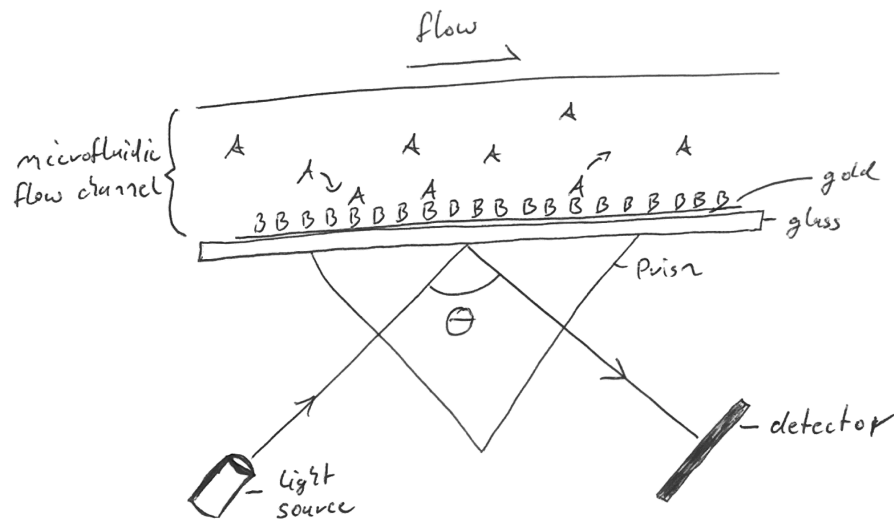
$$\text{slope} = -\frac{1}{K_d}$$

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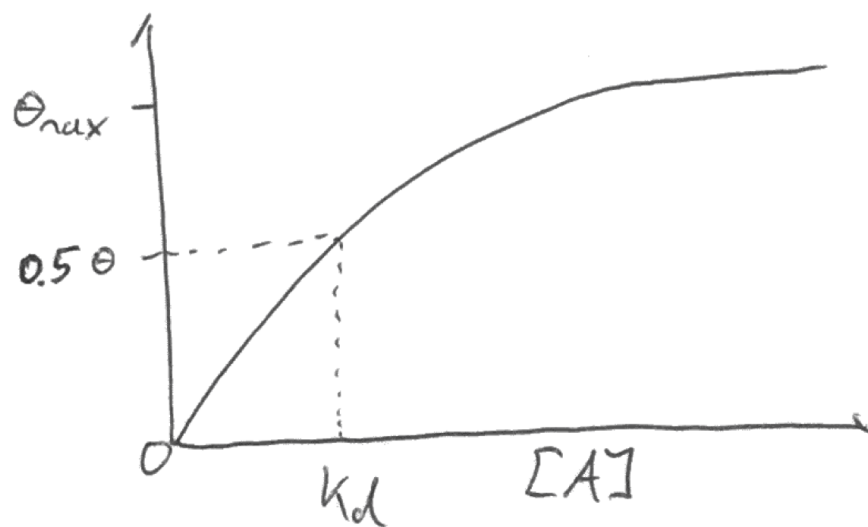
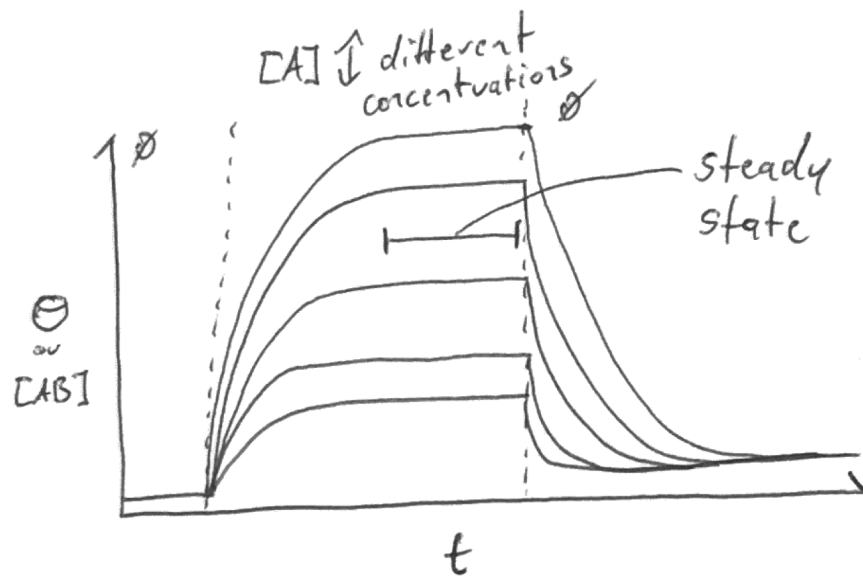
1.5 Measuring K_d , k_{on} , k_{off}

Surface Plasmon Resonance (SPR) based systems



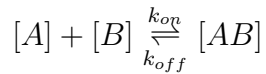
Order in which parameters should be determined:

- 1) measure off-rate \rightarrow obtain k_{off}
- 2) measure on-rate \rightarrow obtain k_{on}
- 3) determine $K_d = \frac{k_{off}}{k_{on}}$, determine K_d independently



1.6 Binding Kinetics

Chemical notation:

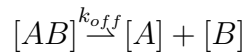


System of ordinary differential equations (ODEs):

$$\frac{d[AB]}{dt} = k_{on}[A][B] - k_{off}[AB]$$

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = k_{off}[AB] - k_{on}[A][B]$$

First let us consider dissociation only:



$$\frac{d[AB]}{dt} = -k_{off}[AB]$$

Write in standard form:

$$[AB]' + k_{off}[AB] = 0$$

Solving the above equation using the integration factor (IF) method:

Determine the integrating factor u:

$$u = e^{\int k_{off} dt}$$

$$u = e^{k_{off}t}$$

Multiply through with u:

$$e^{k_{off}t}[AB]' + k_{off}e^{k_{off}t}[AB] = 0$$

Apply the product rule $(uv)' = uv' + u'v$:

$$(e^{k_{off}t}[AB])' = 0$$

Integrate both sides:

$$e^{k_{off}t}[AB] = \int 0$$

$$e^{k_{off}t}[AB] = 0 + c$$

Solve for [AB]:

$$[AB] = ce^{-k_{off}t}$$

Determine c using initial values:

$$\text{at } t=0: [AB]_0 = c * 1$$

$$[AB] = [AB]_0 * e^{-k_{off}t}$$

Solving the same equation by separation of variables:

Separate variables:

$$\frac{d[AB]}{[AB]} = -k_{off}dt$$

Integrate both sides:

$$\int \frac{d[AB]}{[AB]} = \int -k_{off}dt$$

$$\ln|[AB]| + c = -k_{off}t + c$$

Solve for [AB]:

$$\ln[AB] = -k_{off}t + c$$

$$[AB] = e^{-k_{off}t+c}$$

$$[AB] = e^c e^{-k_{off}t}$$

$$[AB] = ce^{-k_{off}t}$$

1.7 First Order Linear Equations

A short reminder / explanation of how to solve first order linear equations using the integration factor method.

Standard first order linear equation:

$$a(x)y' + b(x)y = c(x)$$

If $c(x) = 0$ then the equation is a homogeneous first order linear equation.

Standard Linear Form:

$$y' + p(x)y = q(x)$$

Where the integrating factor $u(x)$ comes from:

$$uy' + p(x)uy = q(x)u$$

$$() = q(x)u$$

$$(uy)' = q(x)u$$

Product rule:

$$(uy)' = uy' + u'y$$

works if: $u' = p(x)u$

$$u' = p(x)u$$

$$\frac{u'}{u} = p(x)$$

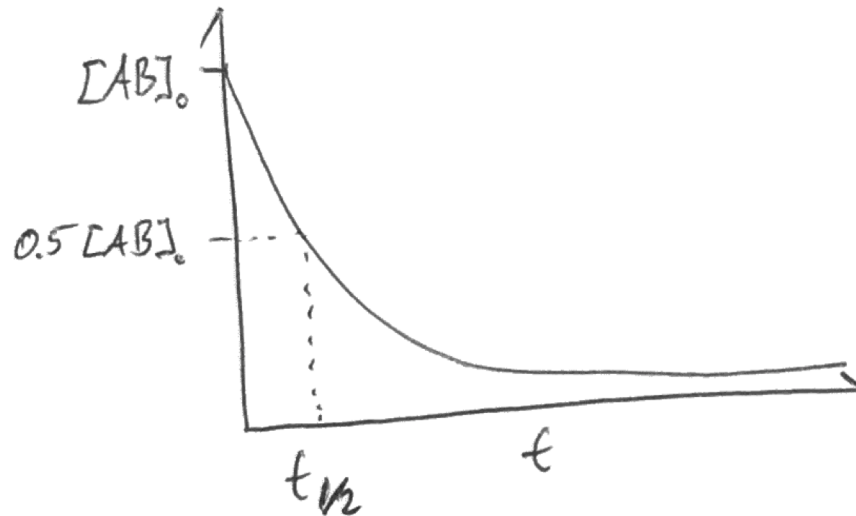
Integrate:

$$\int \frac{du}{u} = \int p(x)dx$$

$$\ln(u) = \int p(x)dx$$

$$u = e^{\int p(x)dx}$$

1.8 Half-life of Interactions



$$\frac{[AB]_0}{2} = [AB]_0 e^{-k_{off} t_{1/2}}$$

$$\frac{1}{2} = e^{-k_{off} t_{1/2}}$$

$$\ln\left(\frac{1}{2}\right) = -k_{off} t_{1/2}$$

$$\ln(1) - \ln(2) = -k_{off} t_{1/2}$$

$$0 - \ln(2) = -k_{off} t_{1/2}$$

$$t_{1/2} = \frac{\ln(2)}{k_{off}}$$

Some $t_{1/2}$ examples:

interaction	$t_{1/2}$	k_{off}
streptavidin-biotin	8 days	$1 \times 10^{-6} \text{ s}^{-1}$
antibody - antigen	20 hours	$1 \times 10^{-5} \text{ s}^{-1}$
Zif268 - DNA	11 minutes	$1 \times 10^{-3} \text{ s}^{-1}$
E12 - DNA	1 second	0.7 s^{-1}
Jun - Fos	0.1 second	7 s^{-1}

→ Interactions can have high affinity, i.e. low K_d values and high dissociation rates...

1.9 Binding Kinetics: on-rates

$$\frac{d[AB]}{dt} = k_{on}[A][B] - k_{off}[AB]$$

In order to solve the above equation we first need to make a couple of simplifying assumptions:

- 1) assume $[A]$ is constant
- 2) substitute $[B]_0$ into $[B] \rightarrow [B]_0$ is also constant

$$[B]_0 = [B] + [AB] \rightarrow [B] = [B]_0 - [AB]$$

$$\frac{d[AB]}{dt} = k_{on}[A]([B]_0 - [AB]) - k_{off}[AB]$$

$$\frac{d[AB]}{dt} = k_{on}[A][B]_0 - k_{on}[A][AB] - k_{off}[AB]$$

$$[AB]' = k_{on}[A][B]_0 - (k_{on}[A] + k_{off})[AB]$$

Write in standard linear form:

$$[AB]' + (k_{on}[A] + k_{off})[AB] = k_{on}[A][B]_0$$

Determine the integrating factor u:

$$u = e^{\int (k_{on}[A] + k_{off}) dt}$$

$$u = e^{(k_{on}[A] + k_{off})t}$$

Multiply through with u:

$$e^{(k_{on}[A] + k_{off})t} [AB]' + (k_{on}[A] + k_{off}) e^{(k_{on}[A] + k_{off})t} [AB] = k_{on}[A][B]_0 e^{(k_{on}[A] + k_{off})t}$$

Apply product rule:

$$(e^{(k_{on}[A] + k_{off})t} [AB])' = k_{on}[A][B]_0 e^{(k_{on}[A] + k_{off})t}$$

Integrate both sides:

$$e^{(k_{on}[A] + k_{off})t} [AB] + c_1 = k_{on}[A][B]_0 \int e^{(k_{on}[A] + k_{off})t} dt$$

$$e^{(k_{on}[A] + k_{off})t} [AB] + c_1 = \frac{k_{on}[A][B]_0}{k_{on}[A] + k_{off}} e^{(k_{on}[A] + k_{off})t} + c_2$$

$$[AB] = \frac{k_{on}[A][B]_0}{k_{on}[A] + k_{off}} + ce^{-(k_{on}[A] + k_{off})t}$$

Determine c using initial values:

at $t = 0$, $[AB]_0 = 0$

$$0 = \frac{k_{on}[A][B]_0}{k_{on}[A] + k_{off}} + c$$

$$c = -\frac{k_{on}[A][B]_0}{k_{on}[A] + k_{off}}$$

Solution:

$$[AB] = \frac{k_{on}[A][B]_0}{k_{on}[A] + k_{off}} (1 - e^{-(k_{on}[A] + k_{off})t})$$

and can be rewritten in a slightly more familiar form:

$$[AB] = \frac{[A][B]_0}{[A] + K_d} (1 - e^{-(k_{on}[A] + k_{off})t})$$

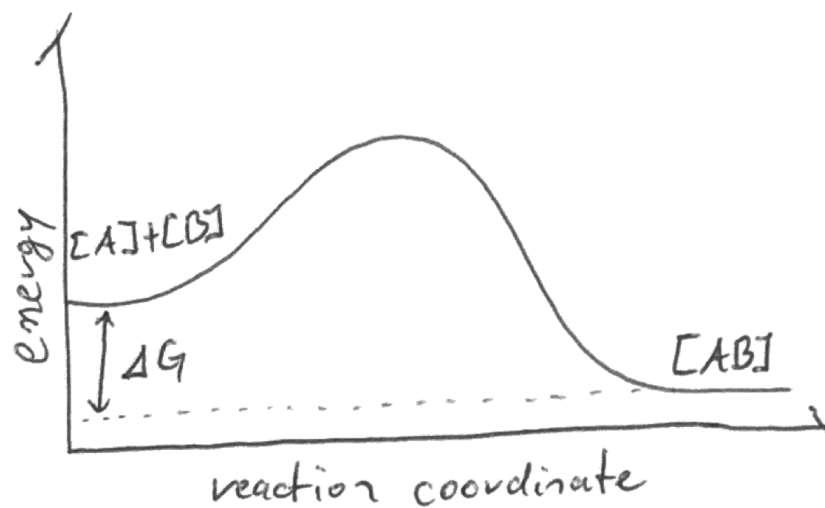
1.10 Gibbs Free Energy

Standard change in the Gibbs Free Energy is:

$$\Delta_r G^\ominus = -RT \ln K_{eq}$$

$$K_{eq} = e^{-\Delta_r G^\ominus / RT}$$

For brevity I will simply use ΔG instead of $\Delta_r G^\ominus$ when referring to the standard change in the Gibbs Free Energy.



ΔG depends on the steady-state ratio of products to reactants, i.e. $\frac{[AB]}{[A][B]}$. (Note that K_{eq} needs to be unitless...)

$$\frac{[AB]}{[A][B]} = K_a \text{ which is generally } 10^6 \rightarrow 10^{12} M^{-1}.$$

R is the gas constant: $8.31451 \text{ J K}^{-1} \text{ mol}^{-1}$

T is the temperature in Kelvin: 298 K (room temperature)

$$RT = k_b T N_A \approx 2.4790 \text{ k J mol}^{-1} \approx 0.592 \text{ k cal mol}^{-1}$$

Covalent bonds $\approx 100 \text{ k cal mol}^{-1}$

Hydrogen bonds $\approx 1 - 10 \text{ k cal mol}^{-1}$

Hydrophobic effect $\approx 1 - 5 \text{ k cal mol}^{-1}$

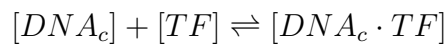
van der Waals forces $\approx < 1 \text{ k cal mol}^{-1}$

$\Delta G > 0 \Rightarrow$ endergonic reaction

$\Delta G < 0 \Rightarrow$ exergonic reaction (occur spontaneously)

K_{eq}	k cal mol^{-1}	k J mol^{-1}
10^{-12}	16.35	68.49
10^{-6}	8.18	34.25
10^{-3}	4.09	17.12
1	0	0
10^3	-4.09	-17.12
10^6	-8.18	-34.25
10^{12}	-16.35	-68.49

Example:



DNA_c is a consensus DNA molecule (CACGTG for example)

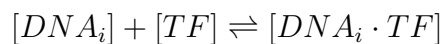
Let's assume the affinity of our transcription factor (TF) to DNA_c is:

$$K_{d,c} = 10^{-9} \text{ M}$$

and therefore the standard change in the Gibbs Free Energy for this reaction is:

$$\Delta G_c = -12.27 \text{ k cal mol}^{-1}$$

For another DNA target with a different sequence i (CACGAG for example) we have the following:



$$K_{d,i} = 10^{-8} \text{ M}$$

$$\Delta G_i = -10.91 \text{ k cal mol}^{-1}$$

By definition the consensus sequence is the target sequence to which the Transcription Factor binds with the highest affinity (by this definition there can be only one consensus sequence). All other target sequences are bound by the transcription factor with lower affinity.

From this we can calculate the difference in affinity ($\Delta\Delta G_i$) between the two DNA targets DNA_c and DNA_i :

$$\Delta\Delta G_i = \Delta G_i - \Delta G_c = -10.91 \text{ kcal mol}^{-1} + 12.27 \text{ kcal mol}^{-1} = 1.36 \text{ kcal mol}^{-1}$$

One order of magnitude difference in affinity equals an energy difference of $1.36 \text{ kcal mol}^{-1}$ or \approx one hydrogen bond.

More generally we define $\Delta\Delta G$:

$$\Delta\Delta G_i = \Delta G_i - \Delta G_c = -RT \ln K_i + RT \ln K_c = -RT(\ln K_i - \ln K_c)$$

$$\Delta\Delta G_i = -RT \ln \frac{K_i}{K_c}$$

1.11 Collision Theory

$$k(T) = Z\rho e^{-E_q/RT}$$

$$Z = N_a \sigma_{AB} \sqrt{\frac{8k_bT}{\pi\mu_{AB}}}$$

ρ = steric factor

Z = collision frequency

$$\sigma_{AB} = \text{reaction cross section} = \frac{1}{2}(d_A + d_B)$$

$$\mu_{AB} = \text{reduced mass} = \frac{m_A m_B}{m_A + m_B}$$

References:

[Wikipedia: Collision theory](#)

1.12 Arrhenius Equation

$$k(T) = A e^{-E_a/RT}$$

Reaction rate depends on A , the pre-exponential factor, which is related to $Z\rho$ of the collision theory.

It also depends on the activation energy E_a

In diffusion limited reactions, $k(T)$ only depends on A which is shown in the next section.

References:

[Wikipedia: Arrhenius equation](#)

1.13 Diffusion-limited Reactions

$$k(T) = 4\pi r D N_A$$

where:

$r = r_A + r_B$ reaction radius

and:

$D = D_A + D_B$ Diffusion coefficient

Example:

$$r = 10 \text{ nm} = 10^{-6} \text{ cm}$$

$$D = 10^{-6} \text{ cm}^2 \text{ s}^{-1} \text{ (diffusion coefficient for a small protein)}$$

$$k(T) = 4\pi 10^{-6} 10^{-6} 6 \times 10^{23} \text{ cm}^3 \text{ s}^{-1} \text{ mol}^{-1} = 7.5 \text{ cm}^3 \text{ s}^{-1} \text{ mol}^{-1} = 7.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$$

References:

[Wikipedia: Diffusion controlled reaction](#)