Biophysical Chemistry for Life Scientists

Biotechnology Research Center, National Taiwan University

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Instructor: Sunney I. Chan

Vice President & Distinguished Research Fellow

Institute of Chemistry, Academia Sinica

Telephone: 2-2789-9402

E-mail: chans@chem.sinica.edu.tw

For 12/4 lecture
a) ✓ 40 cyrii
b) ✓ OH's done
c) give to Mr. Wu or Steve Yu

Lecture 10

Date: Monday December 4, 2000

Electrochemical Potential

For a neutral solute B,

$$\mu_B = \mu_B^{\circ}(T) + RT\ln a_B = \mu_B^{\circ}(T) + RT\ln\gamma_B{}^{m} + RT\ln m_B$$

For a charged species in solution where $\Phi = 0$, we can still write (e.g., B^+)

$$\mu_{B^+}(T) = \mu^\circ_{B^+}(T) + RT \ln a_{B^+} = \mu^\circ_{B^+}(T) + RT \ln \gamma_{B^+}{}^m + RT \ln m_{B^+}$$

Recall for an electrolyte that dissociates, we invoke the above result.

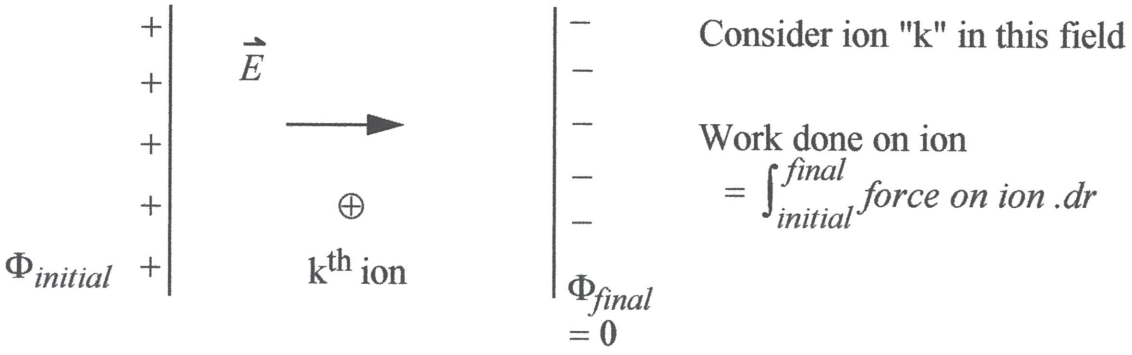
e.g. $NaCl \rightarrow Na^+(aq) + Cl^-(aq)$

$$\mu_{NaCl}(T) = \mu_{Na^+}(T) + \mu_{Cl^-}(T)$$

$$= \mu^\circ_{Na^+}(T) + \mu^\circ_{Cl^-}(T) + RT \ln a_{Na^+} a_{Cl^-}$$

$$= \mu^\circ_{Na^+}(T) + \mu^\circ_{Cl^-}(T) + RT \ln \left(\gamma \pm^m\right)^2 + RT \ln m_{Na^+} m_{Cl^-}$$

Now for an electrolyte under an electrostatic potential Φ, the expression for the chemical potential of an ion needs to be modified, because the electric field produced by the electrostatic potential can do work on the ion.



Force on ion $= \vec{F} = \vec{E} z_k |e|$

$\therefore$ work done on ion in placing ion in solution (reversible)

$$= \int_{initial}^{final} \vec{E}\, z_k |e| .\, d\vec{r} = z_k |e| \int_{initial}^{final} \vec{E} .\, d\vec{r}$$

$$= -z_k |e| \int_{initial}^{final} \vec{\nabla}\Phi .\, d\vec{r} = -z_k |e| \left(\Phi_{final} - \Phi_{initial} \right)$$

$$= z_k |e|\ \Phi_{initial}$$

So $\mu_k(T) = \mu_k^\circ(T) + RT \ln a_k +$ reversible work on ion in placing ion in solution x N_A

$$= \mu_k^\circ(T) + RT \ln a_k + z_k |e| N_A \Phi$$

$$= \mu_k^\circ(T) + RT \ln a_k + z_k F\ \Phi$$

$\boldsymbol{F}$ **= Faraday** = { charge associated with a mole of charged ion each of charge $|e|$

= 96,500 Coulomb or 96,500 joule/volt

= 23,052 cal/volt or 23.052 kcal/volt

(1 coulomb = 1 joule/volt = 0.23901 cal/volt).

Define $\bar{\mu}_k =$ electrochemical potential

$$= \mu_k + z_k F \Phi$$

$$= \mu_k^\circ(T) + RT \ln a_k + z_k F \Phi$$

Electrochemical Potential of Neutral Electrolyte

Suppose a neutral salt can ionize into υ_+ cations (C) and υ_- anions (A) is placed under electrostatic potential Φ. Then the electrochemical potential for neutral salt, μ_B,

$$\bar{\mu}_B = \upsilon_+ \bar{\mu}_C + \upsilon_- \bar{\mu}_A$$

$$= \upsilon_+ \mu_C^\circ + \upsilon_- \mu_A^\circ + RT \ln a_C{}^{\upsilon+} a_A{}^{\upsilon-} + (\upsilon_+ z_+ + \upsilon_- z_-) F \Phi$$

For a neutral salt,

$$(\upsilon_+ z_+ + \upsilon_- z_-) = 0$$

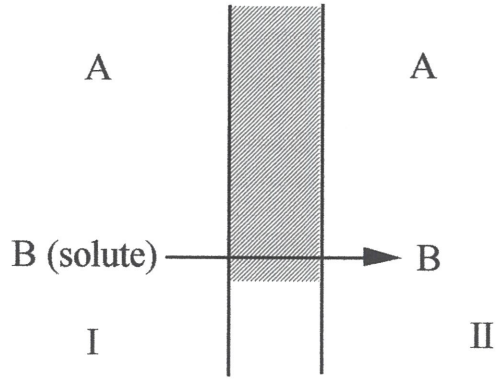
so last turn vanishes and dependence of $\bar{\mu}_B$ on Φ disappears

or $\bar{\mu}_B = \mu_B$**!**

A very important application of the above <u>innocent</u> result

Membrane Potential or Transmembrane Potential - Gibbs-Donnan equilibrium or Gibbs-Donnan Potential

• Suppose a membrane separate two components, and it is permeable to a solute that happens to be neutral. (We have already considered earlier the situation where the membrane is permeable to the solvent only ⇒ cf osmotic pressure). Here we consider a solute.



Denote the two compartments by I and II respectively.

Then

$$\mu_B{}^I(T) = \mu_B^\circ(T) + RT \ln a_B{}^I$$

and $$\mu_B{}^{II}(T) = \mu_B^\circ(T) + RT \ln a_B{}^{II}$$

and

$\Delta G =$ **Free energy change associated with transfer of a mole of B from compartment I to II**

$$= \mu_B{}^{II}(T) - \mu_B{}^I(T) = \mu_B^\circ(T) + RT \ln a_B{}^{II} - \left(\mu_B^\circ(T) + RT \ln a_B{}^I\right)$$

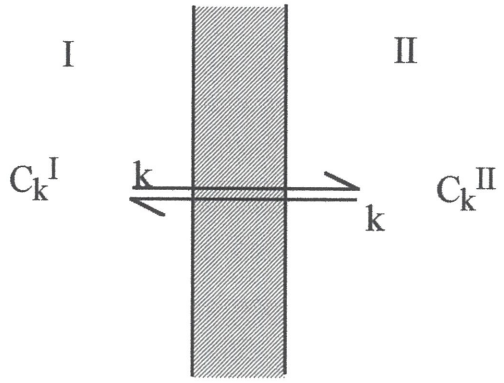
$$= RT\ln\left(\frac{a_B^{II}}{a_B^{I}}\right) = RT\ln\left(\frac{\gamma_B^{II}}{\gamma_B^{I}}\right) + RT\ln\left(\frac{m_B^{II}}{m_B^{I}}\right) \sim RT\ln\left(\frac{m_B^{II}}{m_B^{I}}\right)$$

Condition of equilibrium:

$$\Delta G = 0 \quad \text{or} \quad \left(m_B^{II} = m_B^{I}\right)_{eq}$$

• Now suppose B is strong electrolyte, and membrane is permeable to <u>either</u> the cation or the anion, but not both ⇒ semipermeable membrane.

Since we are dealing with charged species, we must



employed the electrochemical potential. Denote the membrane permeable species by k.

Then

$$\bar{\mu}_k^{I}(T) = \mu_k^{\circ}(T) + RT\ln a_k^{I} + z_k F \Phi_I$$

$$\bar{\mu}_k^{II}(T) = \mu_k^{\circ}(T) + RT\ln a_k^{II} + z_k F \Phi_{II}$$

and free energy associated with transfer of a mole of ion from compartment I to II

$$\Delta G = \bar{\mu}_k^{II}(T) - \bar{\mu}_k^{I}(T)$$

$$= RT \ln\left(\frac{a_k^{II}}{a_k^{I}}\right) + z_k F (\Phi_{II} - \Phi_I)$$

$$\cong RT \ln \frac{m_k^{II}}{m_k^{I}} + z_k F (\Phi_{II} - \Phi_I).$$

Suppose that $m_k^{I} > m_k^{II}$

and $\Phi_{II} - \Phi_I = 0$ **(no transmembrane potential)**

Then $\Delta G \cong RT \ln \frac{m_k^{II}}{m_k^{I}} < 0$

Therefore, there will be transfer by diffusion of ions k from compartment I to II.

Such a transfer of ions across the membrane (semipermeable) will lead to separation of charges across the membrane and set up a transmembrane potential, and this transmembrane potential will be of a sign as to counteract the ion flow drive by the

concentration gradient. Eventually an electro-chemical equilibrium will be established.

Equilibrium

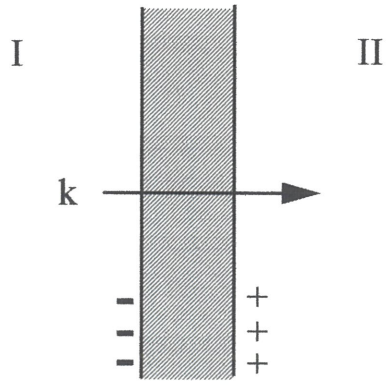
$$\Delta G = 0 \text{ or } RT\ln\left(\frac{m_k^{II}}{m_k^{I}}\right) + z_k F\left(\Phi_{II} - \Phi_I\right) = 0$$

or $$\boxed{(\Delta\Phi)_{eq} = (\Phi_{II} - \Phi_I)_{eq} = \left[RT\ln\left(\frac{m_k^{I}}{m_k^{II}}\right)_{eq}\right]\frac{1}{z_k F}}$$

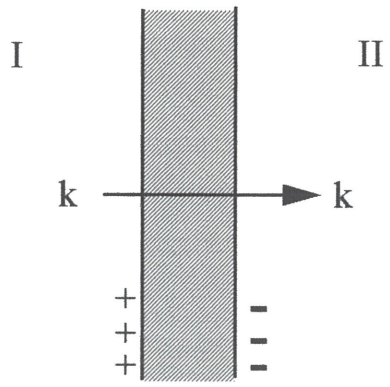
Gibbs-Donnan potential

Equilibrium called Gibbs-Donnan equilibrium

$z_k > 0$



$z_k < 0$



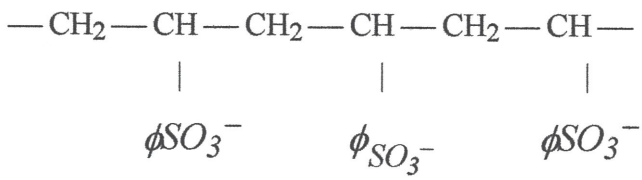
It turns out it takes the transfer of only a few ions to establish this electrochemical equilibrium so that

$$\left(\frac{m_k{}^{I}}{m_k{}^{II}}\right)_{eq} = \left(\frac{m_k{}^{II}}{m_k{}^{I}}\right)_{o}$$

Examples of semipermeable membranes that are permeable only to charged ions of a given sign

(A) Artificial Membranes

e.g. synthetic membranes made from ion exchange polymers such as sulfonated polystyrene



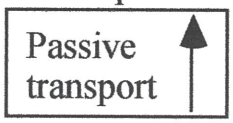
This membrane is a network of cross-linked polystyrenes containing a large number of sulfonic acid groups covalently bonded to the matrix. These strongly acidic groups readily dissociate and release their protons in exchange for other cations. Because of the high density of the negative SO_3^- groups on the matrix, anions are repelled and are excluded from

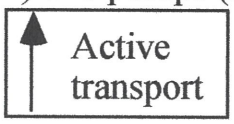
entering the membrane.

(B) Cell Membranes

The plasma membranes of cells, and organelle membranes as well, exhibit different permeability toward different ionic solutes, e.g. Na^+, K^+, Cl^-, HCO_3^-, **etc. Membrane permeability towards a given ionic solute is determined by many factors, e.g. properties of the charged species, properties of the membrane, including the**

presence of ion specific transporters (protein) and pumps (linked to ATP hydrolysis)





For example,

m_{K^+}	0.150 m	0.005 m
m_{Na^+}	0.015 m	0.150 m

The transfer of K^+ from inside to outside, and the transfer of Na^+ from outside to inside occurs by passive transport. However, to maintain the concentration of K^+ inside the cell and to keep the concentration of Na^+ inside the cell low, there is active

transport of the ions by ion pumps in the reverse direction. (We'll discuss these later.)

Now for a cell,

$$(\Phi_{II} - \Phi_I)_{ss} = (\Phi_{outside} - \Phi_{inside})_{ss} = 100mV$$

and the free energy change for the transfer of a charged species from the inside (I) to the outside (II) is

$$\Delta G = \bar{\mu}_k^{II}(T) - \bar{\mu}_k^{I}(T)$$

$$\cong RT \ln\left(\frac{m_k^{II}}{m_k^{I}}\right) + z_k F \underbrace{(\Phi_{II} - \Phi_I)}_{\Phi_{outside} - \Phi_{inside}}$$

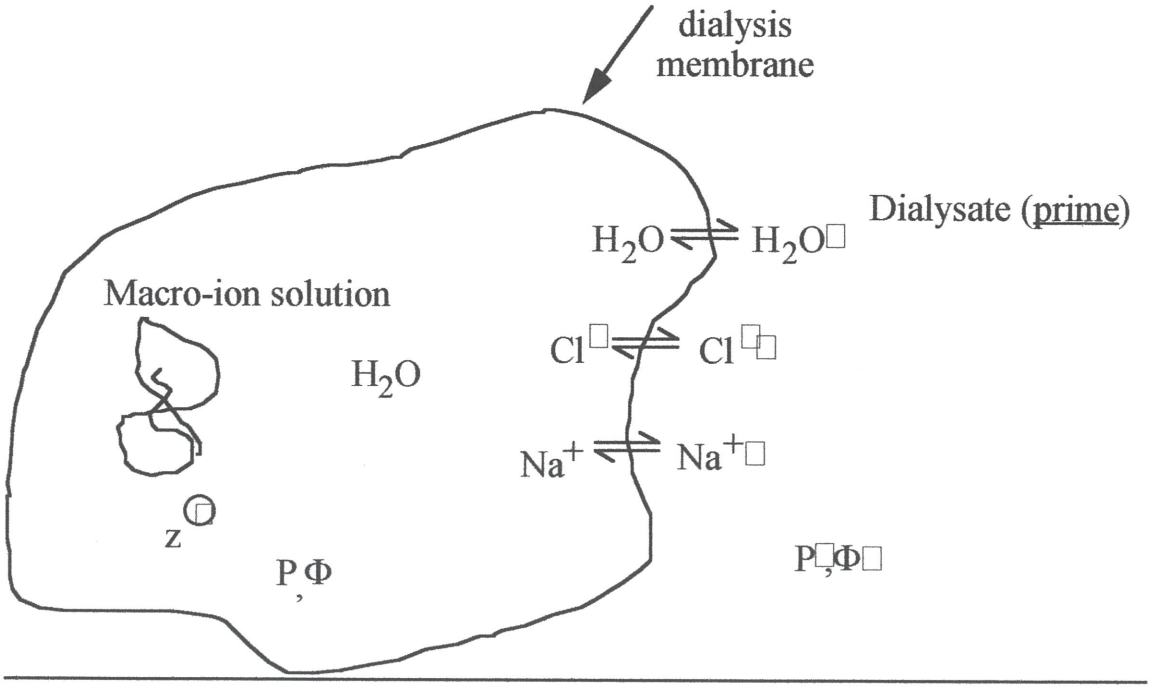
Ion	Concentration Free Energy $RT \ln\left(\frac{m_k^{II}}{m_k^{I}}\right)$	Electrical Free Energy $z_k F (\Phi_{II} - \Phi_I)$	Total Free Energy ΔG
Na^+	+1400 cal/mole	+2100 cal/mole	+3500 cal/mole
K^+	-2070 cal/mole	+2100 cal/mole	+30 cal/mole

Cell is essentially at electrochemical equilibrium with respect to K^+, but not so for Na^+.

Equilibrium Dialysis (Donnan effect; Donnan potential)

- **Thermodynamic analysis of membranes permeable to solvent and small ions, but impermeable to macro-ions such as proteins and DNA.**
- **Dialysis of polyelectrolytes leads to asymmetric concentration distribution of the small ions, call the Donnan effect, and a transmembrane potential, called the Donnan potential.**

- **Experiment**



Outcome

$$\left.\begin{array}{lll} C_{Na^+} & > & C'_{Na^+} \\ C_{Cl^-} & < & C'_{Cl^-} \\ P & > & P'(1\ atm) \\ \Phi & < & \Phi' \end{array}\right\} \text{at equilibrium } (\Pi = P - P')$$

- **Thermodynamic analysis**

At equil., $\bar{\mu}_i = \bar{\mu'}_i$ **for species free to permeate across dialysis membrane** $(H_2O,\ Na^+\ Cl^-)$

Now $\bar{\mu}_i = \mu_i^\circ + RT\ln a_i + \Pi \bar{V}_i + z_i F\ \Phi$

where $\bar{V}_i =$ **partial molal volume of species i**

and $\bar{\mu'}_i = \mu_i^\circ + RT\ln a_{i'} + z_i F\ \Phi'$

Hence $\mu_i^\circ + RT\ln a_i + \Pi \bar{V}_i + z_i F\ \Phi$

$$= \mu_i^\circ + RT\ln a_{i'} + z_i F\ \Phi'$$

Solving for $\Phi - \Phi$**:**

$$\boxed{\Phi' - \Phi = \frac{RT}{z_i F} \ln\left(\frac{a_i}{a_{i'}}\right) + \frac{\Pi \bar{V}_i}{z_i F}}$$

Donnan potential

second term $\left(\frac{\Pi \bar{V}_i}{z_i F}\right)$ **small compared to first term and ignore** $\gamma \pm$**'s**

Then $\Phi' - \Phi = \frac{RT}{z_i F} \ln\left(\frac{a_i}{a_{i'}}\right) = \frac{RT}{z_i F} \ln\left(\frac{C_i}{C_i'}\right)$

This result must hold for both Na^+ and Cl^-

i.e., $\Phi' - \Phi = \frac{RT}{F} ln\left(\frac{C_{Na^+}}{C'_{Na^+}}\right)$ for Na^+ $(z_i = +1)$

$\Phi' - \Phi = \frac{RT}{F} ln\left(\frac{C'_{Cl^-}}{C_{Cl^-}}\right)$ for Cl^- $(z_i = -1)$

Hence $\frac{C_{Na^+}}{C'_{Na^+}} = \frac{C'_{Cl^-}}{C_{Cl^-}}$ or $C_{Na^+}\, C_{Cl^-} = C'_{Na^+}\, C'_{Cl^-}$ as expected.

Now, for sake of electrical neutrality,

$C'_{Na^+} = C'_{Cl^-} = C'$ (except for a few ions)

and $z\, C_B + z_{Na^+}\, C_{Na^+} + z_{Cl^-}\, C_{Cl^-} = 0$

or $z\, C_B + C_{Na^+} - C_{Cl^-} = 0$

<u>So</u> $\underbrace{\left(C_{Na^+}\right)\left(zC_B + C_{Na^+}\right)}_{C_{Na^+} C_{Cl^-}} = \frac{\underbrace{C'^2}}{C'_{Na^+} C'_{Cl^-}}$

<u>Desire</u> $\frac{C_{Na^+}}{C'_{Na^+}}$ or $\frac{C_{Na^+}}{C'} \equiv Y$

$\left(\frac{C_{Na^+}}{C'}\right)\left(\frac{z\, C_B}{C'} + \frac{C_{Na^+}}{C'}\right) = 1$ at equilibrium

or $$Y^2 + \frac{z\,C_B}{C'}\,Y - 1 = 0$$

or $$Y = \frac{-z\,C_B}{2C'} + \sqrt{1 + \left(\frac{z\,C_B}{2C'}\right)^2}$$

Typically macro-ion charge concentration small compared to electrolyte concentration in dialysate, i.e., $\frac{z\,C_B}{C'} << 1$.

Therefore $$Y \cong 1 - \frac{z\,C_B}{2C'}$$

or $$\boxed{\Phi' - \Phi = \frac{RT}{z_{Na^+}F} ln\left(1 - \frac{z\,C_B}{2C'}\right)}$$

- **<u>Significance</u>**

For polyelectrolyte where z< 0, i.e., DNA

$$Y = \frac{C_{Na^+}}{C'_{Na^+}} = 1 + \frac{|z|C_B}{2C'} > 1 \quad \text{or} \quad C_{Na^+} > C'_{Na^+}$$

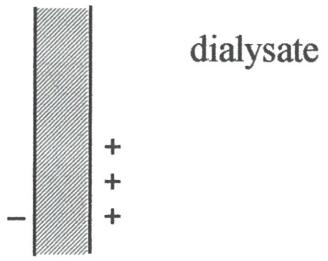
$$\frac{C_{Cl^-}}{C'_{Cl^-}} = Y^{-1} < 1 \quad \text{or} \quad C'_{Cl^-} > C_{Cl^-}$$

Hence

$C_{Na^+} > C'_{Na^+}$

$C_{Cl^-} < C'_{Cl^-}$

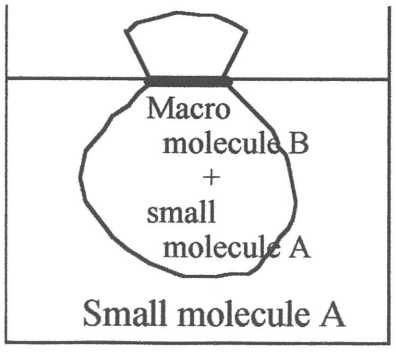
$\Phi < \Phi'$



macro-ion solution

dialysate

Equilibrium Dialysis (neutral solutes)



a) Dialysis membrane allows passage of H_2O and small molecule A

b) A is neutral!

c) Dialysis membrane is impermeable to macromolecule B

d) A binds to macromolecule B

e) $[A]_{outside}$ usually kept low. $[A]_{outside} = C_A^{outside}$.

f) N identical and independent binding sites for A on macromolecule

At equilibrium,

- $\mu_A^{outside} = \mu_A^{inside}$ (Note A = solute here, not solvent)

$$\mu_A^{outside} = \mu_A^{\circ}(T) + RT\ln a_A^{outside} = \mu_A^{\circ}(T) + RT\ln \gamma_A C_A^{outside}$$

$$\cong \mu_A^{\circ}(T) + RT\ln C_A^{outside}$$

for $[A]_{outside}$ sufficiently dilute.

- $\mu_A^{inside} = \mu_A^{\circ}(T) + RT\ln a_A^{inside}$

$$= \mu_A^{\circ}(T) + RT\ln C_A^{inside} + RT\ln \gamma_{A'}$$

- **Now $\gamma_{A'} \neq 1$ because of binding of A to macromolecule. But we may write**

$$\mu_A^{inside} = \mu_A^{\circ}(T) + RT\ln a_A^{inside}$$

$$= \mu_A^{\circ}(T) + RT\ln \gamma''_A C_A^{free}$$

$$\cong \mu_A^{\circ}(T) + RT\ln C_A^{free}$$

where C_A^{free} = concentration of <u>free</u> A inside the

dialysis at equilibrium $\equiv [A]_{inside}$

and $\gamma_{A''} \to 1$ for $[A]_{inside}$ **sufficiently dilute.**

So $C_A^{outside} = C_A^{free}$

or $[A]_{outside} = [A]_{inside}$ as expected.

Within dialysis bag

C_A^T = total concentration of A inside

$= C_A^{free} + C_A^{bound}$

Also

$$B + A \underset{}{\overset{K}{\rightleftharpoons}} BA; \quad [BA]_{eq} = K[B]_{eq}[A]_{inside}$$

$$BA + A \overset{K}{\rightleftharpoons} BA_2; \quad [BA_2]_{eq} = K[BA]_{eq}[A]_{inside}$$

$$\vdots$$

$$BA_N + A \overset{K}{\rightleftharpoons} BA_N; \quad [BA_N]_{eq} = K[BA_{N-1}]_{eq}[A]_{inside}$$

- Define $\upsilon \equiv$ number of small molecules bound per macromolecule

$$= C_A^{bound} / C_B = \frac{C_A^T - C_A^{free}}{C_B}.$$

Where $C_B \equiv$ stoichiometric concentration of B (macromolecule)

$$= [B]_{eq} + [BA]_{eq} + [BA_2]_{eq} + \cdots [BA_N]_{eq}$$

Then $\boxed{\theta \equiv \text{ fraction of sites bound } = \frac{\upsilon}{N}}$

Now the fraction of sites bound with A

$$\theta = \frac{[BA]_{eq}}{[B]_{eq} + [BA]_{eq}} = \frac{[BA_2]_{eq}}{[BA]_{eq} + [BA_2]_{eq}} = \cdot\cdot = \frac{[BA_N]_{eq}}{[BA_{N-1}]_{eq} + [BA_N]_{eq}}$$

and since $[BA]_{eq} = K[B]_{eq}[A]_{inside}$

$$\theta = \frac{K[B]_{eq}[A]_{inside}}{[B]_{eq} + K[B]_{eq}[A]_{inside}}$$

$$= \frac{K[A]_{inside}}{1 + K[A]_{inside}}$$

or $$\frac{\theta}{1-\theta} = K[A]_{inside}$$

or $$\frac{\nu}{N-\nu} = K[A]_{inside} = K\,C_A{}^{outside}$$

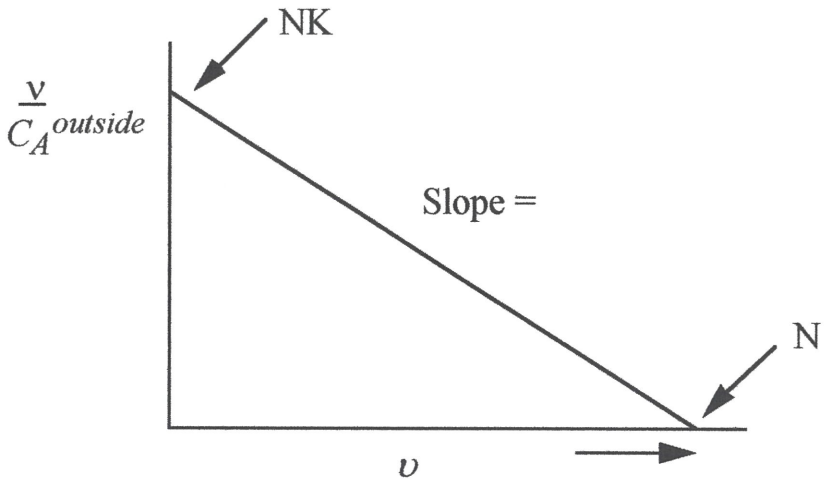
or $\boxed{\frac{\nu}{C_A{}^{outside}} = K \cdot (N - \nu)}$ "Scatchard equation"

- **Experimentally determine**

(a) $C_A^{outside}$

(b) $C_A^{T\ inside}$

$$\nu = \frac{C_A^T - C_A^{free}}{C_B} = \frac{C_A^T - C_A^{outside}}{C_B}$$



Above is called 涓 catchard plot"

x intercept $= N$

y intercept $= N \cdot K$

Slope $= -K$

If a "Scatchard plot" does not give a straight line, this indicates that the binding sites on the macromolecule for A are <u>not</u> independent or <u>not</u> identical. There can be positive or negative cooperativity. Will show how to handle this later!