Optical Microscopy

1. Ray tracing

Components

- Detector
- Light source
- Specimen
- Optical lenses

→ **Earliest microscope**

↳ Van Leeuwenhoek

- $n \sin(\theta_1) = n' \sin(\theta_2)$ Snell

- $a + b = \theta_1$ | using $\sin(x) \approx x \approx \tan(x)$

$$\sin(a) + \sin(b) = \sin(\theta_1) = \frac{h}{S_o} + \frac{h}{R}$$

$a \approx \sin(a)$ $a \approx \tan(a)$

- $b - c = \theta_2$ | using $\sin(x) \approx x \approx \tan(x)$

$$\sin(b) - \sin(c) = \sin(\theta_2) = \frac{h}{R} - \frac{h}{S_i}$$

$\sin(b) \approx b$ $b \approx \tan(b)$
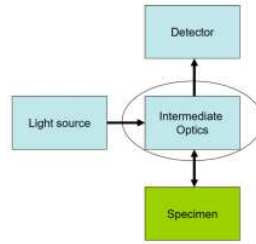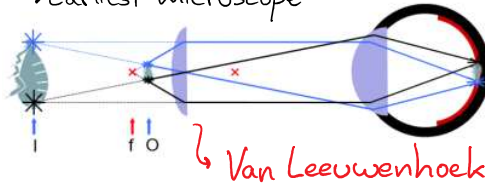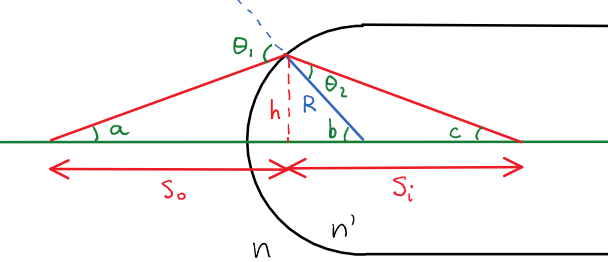
→ $\sin(\theta_1) = \frac{h}{S_o} + \frac{h}{R}$

$\frac{n'}{n} \sin(\theta_2) = \frac{h}{S_o} + \frac{h}{R}$

$\sin(\theta_2) = \frac{n}{n'}\left(\frac{h}{S_o} + \frac{h}{R}\right)$

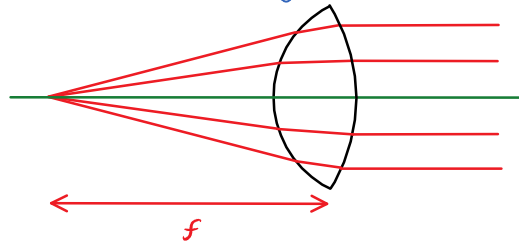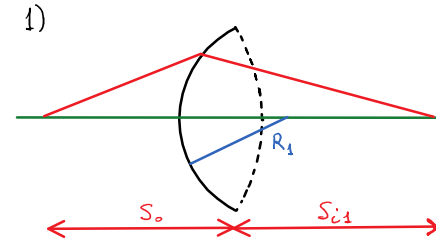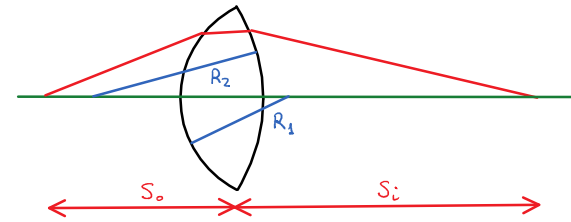→ $\frac{n}{n'}\left(\frac{h}{S_o} + \frac{h}{R}\right) = \frac{h}{R} - \frac{h}{S_i}$

$\frac{n}{S_o} + \frac{n}{R} = \frac{n'}{R} - \frac{n'}{S_i}$

$\Rightarrow \frac{n}{S_o} + \frac{n'}{S_i} = \frac{n'-n}{R}$

$$\boxed{\frac{n}{S_o} + \frac{n'}{S_i} = \frac{n'-n}{R}}$$

$S_o > nR/(n'-n)$ $S_o = nR/(n'-n)$ $S_o < nR/(n'-n)$
$S_i > 0$ $S_i \to \infty$ $S_i < 0$

→ For two spherical interfaces: Use the image from the first refraction as the object for the second

1)

$$\boxed{\frac{n}{S_o} + \frac{n'}{S_{i1}} = \frac{n'-n}{R_1}}$$

2)
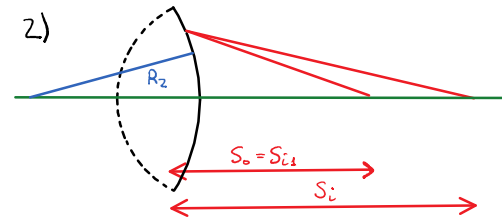
$$\boxed{\frac{n'}{S_{i1}} + \frac{n}{S_i} = \frac{n-n'}{R_2}}$$

⚠ → $R_2 < 0$ for convex lens
→ $n'-n$ has opposite sign

$$\boxed{\frac{1}{S_o} + \frac{1}{S_i} = \frac{1}{f} = \frac{(n'-n)}{n}\left(\frac{1}{R_1} - \frac{1}{R_2}\right)}$$

- f = focal length

f

⇒ focal length → R_o at $R_i \to \infty$

$$\boxed{\frac{1}{f} = \frac{(n'-n)}{n}\left(\frac{1}{R_1} - \frac{1}{R_2}\right)}$$

↳ f is S_o limit for convergent/divergent lens

→ Types of spherical lenses

Plano Convex
$R_1 > 0$
$R_2 \to \infty$
$f > 0$

Plano Concave
$R_1 \to \infty$
$R_2 > 0$
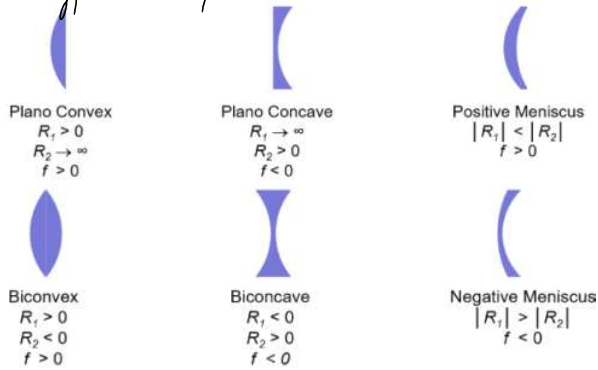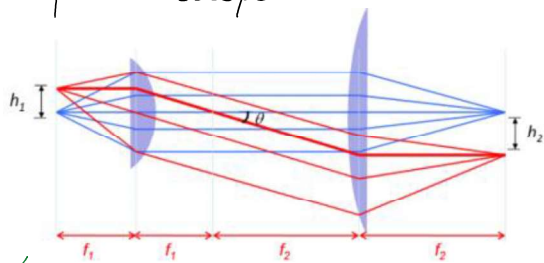$f < 0$

Positive Meniscus
$|R_1| < |R_2|$
$f > 0$

Biconvex
$R_1 > 0$
$R_2 < 0$
$f > 0$

Biconcave
$R_1 < 0$
$R_2 > 0$
$f < 0$

Negative Meniscus
$|R_1| > |R_2|$
$f < 0$

→ Simple microscope

↳ Sharp focus where the specimen is placed at the focal length of the first lens f_1 ←

↝ In this case is the magnification:
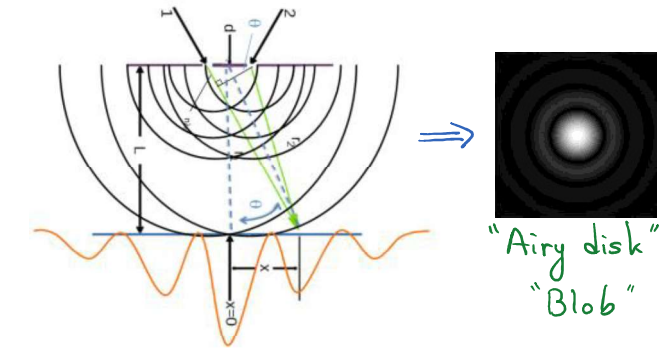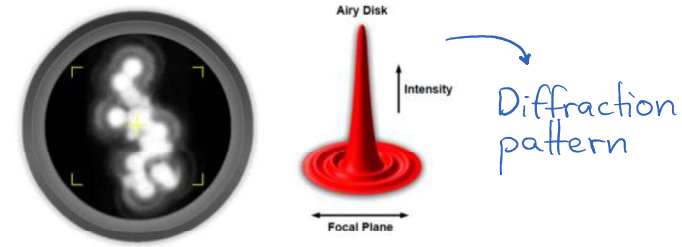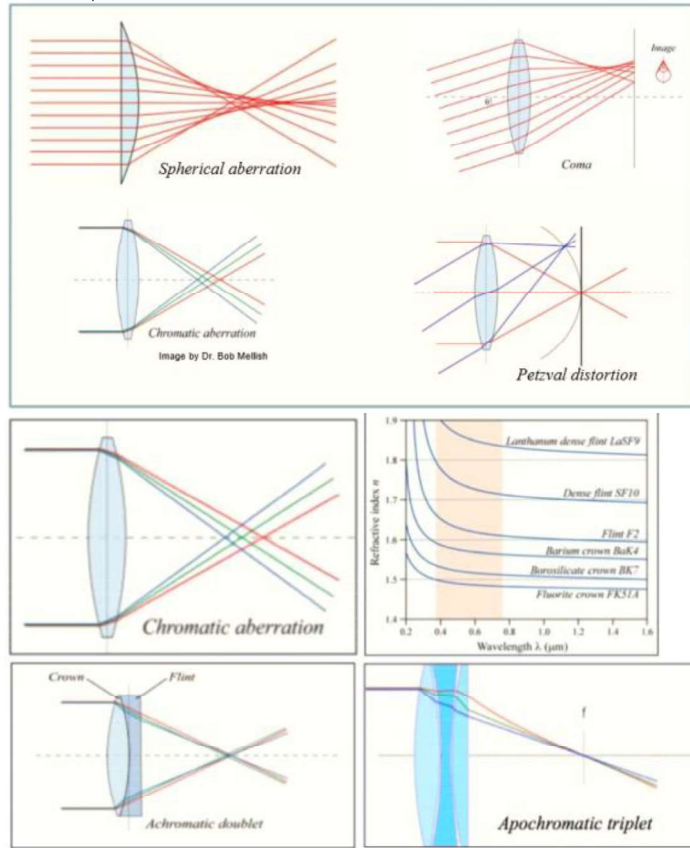
$$\sin(\theta) = \frac{h_1}{f_1} = \frac{h_2}{f_2} \implies \boxed{M = \frac{h_2}{h_1} = \frac{f_2}{f_1}}$$

↝ WD = working distance ↴

The distance from the front lens element of the objective to the closest surface of the coverslip when the specimen is in sharp focus.

→ Aberrations: Imperfections on the lenses (deformation) and the different refractive indexes for different colors (ie. wavelengths) can distort the final image

→ Optical aberrations

Spherical aberration

Coma

Chromatic aberration
Image by Dr. Bob Mellish

Petzval distortion

Chromatic aberration

Lanthanum dense flint LaSF9
Dense flint SF10
Flint F2
Barium crown BaK4
Borosilicate crown BK7
Fluorite crown FK51A

Crown   Flint

Achromatic doublet

Apochromatic triplet

2. Limits of lenses & resolution

→ Linear superposition of EM-waves + Huygens-Fresnel principle ⟹ Diffraction

d_2

d_1

Airy Disk

Intensity

Focal Plane

Diffraction pattern

⟹

"Airy disk"
"Blob"

$$I(\theta) = I_0 \left(\frac{2J_1(ka\sin(\theta))}{ka\sin(\theta)} \right)^2 \rightsquigarrow \sin(\theta) \approx \frac{1.22\lambda}{d}$$

first zero of $I(\theta)$ ←

Numerical aperture
↓
┌ constant across surface limits (Snell)

$$NA_{obj} = n\sin(\theta) = n\sin\left(\arctan\left(\frac{D}{2f}\right)\right) \approx \frac{nD}{2f}$$

$$\boxed{R \approx \frac{1.22\lambda_0}{2NA_{obj}}} \qquad \boxed{NA_{obj} \approx \frac{nD}{2f}}$$
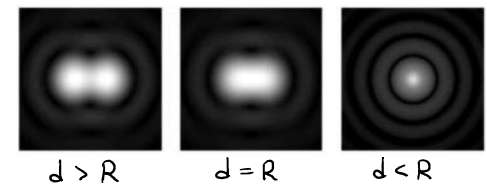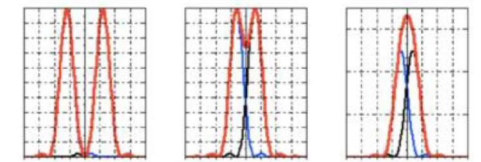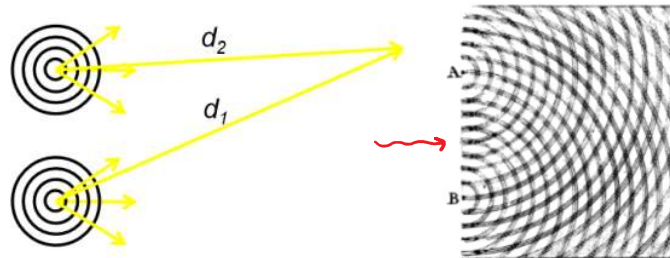
$d > R$ $d = R$ $d < R$

→ R is the separation at which the maximum of the airy disk made by point 2 lies on the first zero of the disk made by point 1

↳ = Rayleigh Criterion

→ To increase resolution of the microscope

- make illumination have small λ
- Increase lens' aperture D ⎤
- Decrease focal length f ⎦ Increase NA_{obj}

Signal & Noise

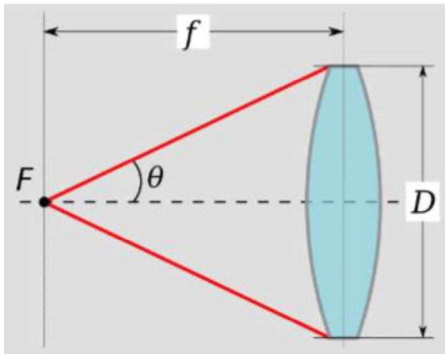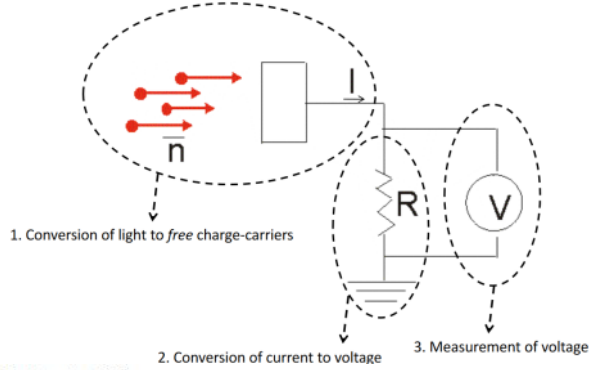→ Maximum information you can extract from any experiment is dictated by Signal and noise

1. Conversion of light to *free* charge-carriers

2. Conversion of current to voltage   3. Measurement of voltage

(1) Shot noise (N_s) –
inherent noise in counting a finite number of photons/electrons per unit time

(2) Dark current noise (N_d) –
thermally induced "firing" of the detector

(3) Johnson noise (N_J) –
thermally induced current fluctuation in the load resistor

(4) Technical noise and 1/f noise
due to various detector imperfections – can be avoided by good design

1. Photon Shot Noise

Most light sources — Random Light

Exotic quantum light sources — Squeezed Light
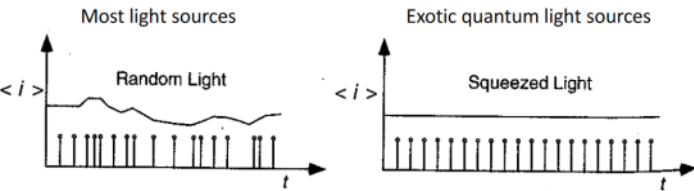
• Random events obey Poisson distribution

• If the average number of photons in the time interval t is $\bar{n}$, then the probability of observing n photons is

$$P(n|\bar{n}) = e^{-\bar{n}}\frac{\bar{n}^n}{n!}$$

• $\sigma_n = \sqrt{\bar{n}}$

• Converting it to noise in current

$\bar{n}$ = # of incident photons q = electron charge $(1.6\times10^{-19}\,C)$
Δt = acquisition time η = "quantum efficiency"

→ Signal current = $\langle I_{signal}\rangle = \dfrac{\eta q\bar{n}}{\Delta t}$

→ Noise current $\langle I_{noise}\rangle = \dfrac{\eta q\sqrt{\bar{n}}}{\Delta t}$

$$\langle I_{noise}^2\rangle = \left(\frac{\eta q}{\Delta t}\right)^2 \bar{n} = \frac{\eta q}{\Delta t}\langle I_{signal}\rangle$$

↳ $\langle I_{noise}\rangle \propto \sqrt{\langle I_{signal}\rangle}$
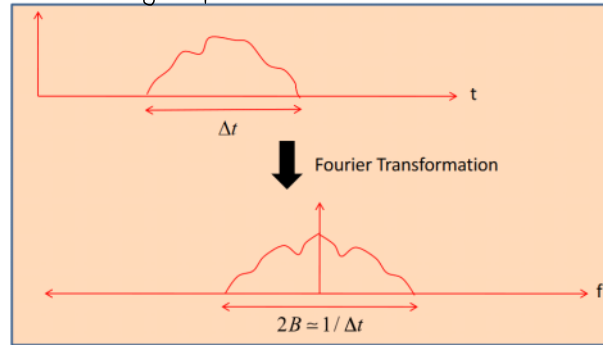
• **Signal-to-Noise Ratio (SNR)**

$$SNR := \frac{signal\ power}{noise\ power} = \frac{S}{N}$$

$$S = \langle I_{signal}^2\rangle R \qquad\qquad N = \langle I_{noise}^2\rangle R$$

• **Noise Equivalent Power (NEP)**

NEP := Signal power at SNR = 1

Δt

Fourier Transformation

$2B = 1/\Delta t$

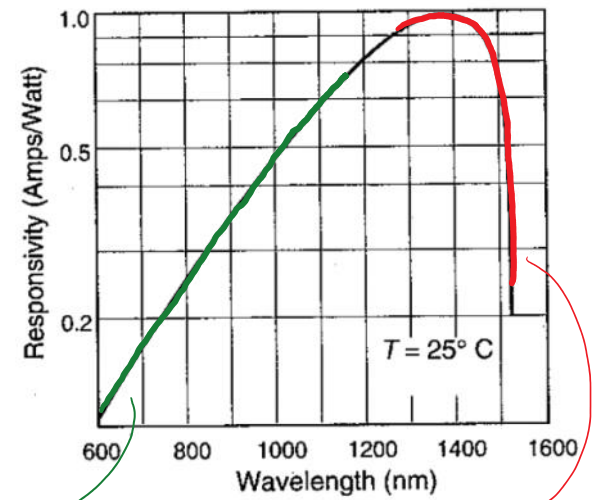• $\langle I_{noise}^2\rangle = \dfrac{\eta q}{\Delta t}\langle I_{signal}\rangle = 2\eta q B\langle I_{signal}\rangle$

$$\Rightarrow N_s = \langle I_{noise}^2\rangle R = 2R\eta q B\langle I_{signal}\rangle$$

• Relating current to optical power

$\langle I\rangle \approx q\dfrac{\eta\bar{n}}{\Delta t}, \qquad P \approx \dfrac{\bar{n}hc}{\lambda\Delta t} \rightsquigarrow \dfrac{hc}{\lambda} = 1$ photon's energy

• Responsivity = $\dfrac{\langle I\rangle}{P} = \dfrac{\eta q\lambda}{hc}$

$T = 25°\,C$

$\dfrac{\langle I\rangle}{P} \approx \dfrac{\eta q}{hc}\lambda$

η depends on λ, since no photons are emitted with energies below the energy gap E_g

$$SNR_{current} = \left(\frac{N_s}{N_c}\right)_{current} = \sqrt{\frac{\langle I_{signal}\rangle}{2\eta q B}}$$

$$SNR_{power} = \left(\frac{N_s}{N_c}\right)_{power} = \bar{n} \rightarrow \text{number of incident photons}$$

2. Dark Current Noise

• Generated electrons fluctuate obeying Poisson distribution

• Same equations as shot noise:

$\langle I_{noise}^2\rangle = 2\eta q B\langle I_{signal}\rangle, \quad N_s = \langle I_{noise}^2\rangle R = 2R\eta q B\langle I_{signal}\rangle$

$$N_d = 2R\eta q B\langle I_d\rangle$$

3. Johnson Noise

• Temperature dependent fluctuations in the load resistor R.

Thermal energy: $\dfrac{kT}{2}$

Thermal power: $\tilde{N}_T = kTB$

$$I_J \propto \sqrt{\frac{kTB}{2}}$$

$$\tilde{N}_J \propto I_J^2 R \propto \frac{kTB}{2}R$$

→ Sum of all noises

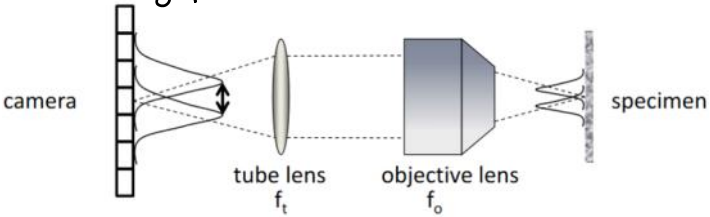$$I_{noise} = I_{shot} + I_{dark} + I_{Johnson}$$

$$\langle I_{noise}^2 \rangle = \langle (I_s + I_D + I_j)^2 \rangle = \langle I_s^2 \rangle + \langle I_D^2 \rangle + \langle I_j^2 \rangle$$

uncorrelated cross noise average to zero

$$\boxed{\langle I_{noise}^2 \rangle = \langle I_s^2 \rangle + \langle I_D^2 \rangle + \langle I_J^2 \rangle}$$

$$\boxed{N_{noise} = N_s + N_D + N_J} \quad \text{(N = noise power)}$$

→ Choosing pixel size

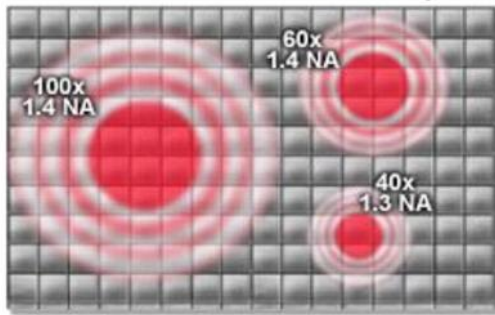camera — tube lens f_t — objective lens f_o — specimen

Airy-disk size: $R \approx \dfrac{1.22\,\lambda_0}{2\,NA}$ (before magnification)

Final airy-disk size: $R \approx \dfrac{1.22\,\lambda_0}{2\,NA} M \quad \left(M = \dfrac{f_t}{f_o} \right)$

$\Rightarrow$ We should be able to see "notice" differences
d of d = R

$$\boxed{\text{maximum pixel size} = \frac{1}{2} \cdot \frac{1.22\,\lambda_0}{2\,NA} M}$$

Camera Sensor CCD Pixel Array

100x 1.4 NA

60x 1.4 NA

40x 1.3 NA

6 Micrometers

$$\boxed{SNR = \frac{\langle Signal \rangle}{\sigma_{signal}} = \frac{Total\ Signal}{\sqrt{Total\ Noise}}}$$

standard deviation of signal = error

- No noise $\Rightarrow \sigma_{signal} = 0 \Rightarrow SNR \to \infty$
not possible, since shot noise $\sigma_{shot} = \sqrt{signal} \neq 0$

- Signal = Counts, # photons, # electrons etc
with quantum efficiency $\Rightarrow Signal_{tot} = QE \cdot signal$

- Noise = $\sigma_{noise}^2 = \sigma_{shot}^2 + \sigma_{dark}^2 + \sigma_{Johnson}^2$

also counts, # photons etc $\sigma_{shot} = \sqrt{Signal} \Rightarrow \sigma_{shot}^2 = $ total signal

With quantum efficiency $\Rightarrow \sigma_{shot} = \sqrt{QE \cdot Signal}$

$$\boxed{\sigma_{noise}^2 = QE \cdot Signal + \sigma_{dark}^2 + \sigma_{Johnson}^2}$$

$$\Rightarrow \boxed{SNR = \frac{QE \cdot Signal}{\sqrt{QE \cdot Signal + \sigma_{dark}^2 + \sigma_{Johnson}^2}}}$$

Molecular Adsorption and Electron Transfer

adsorption ❓ | molecule | ❓ electron transfer
1st hour | | 2nd hour
electrode
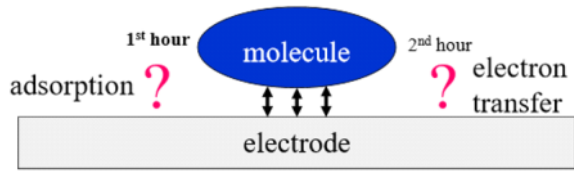
→ **Schrödinger Equation**

• Stationary

$$\hat{H}|\Psi\rangle = E|\Psi\rangle \qquad H = T + V$$

(hamiltonian; kinetic energy; potential)

$$H = -\frac{\hbar^2}{2m}\nabla^2 + V, \quad \nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}$$

• Probability $\Psi^*(\vec{r_o})\Psi(\vec{r_o})\,dx\,dy\,dz$ (Born interpretation) (Wave → probability)

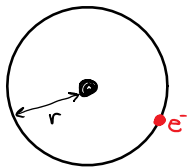$$\rightarrow \int \Psi^*\Psi\,dx\,dy\,dz \overset{!}{=} 1$$

• Time dependent $$\hat{H}|\Psi\rangle = i\hbar\frac{\partial|\Psi\rangle}{\partial t}$$

→ **Hydrogen atom**

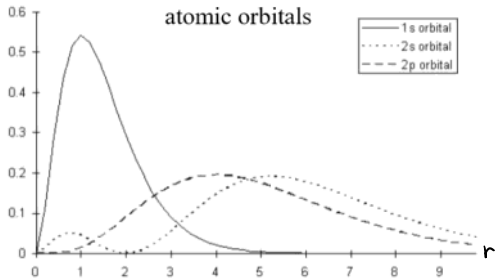$$H = T_n + T_e + V_{n-e} \rightarrow \text{Potential between nucleous/electron}$$

kinetic energies (nucleous/electron)

$$= -\frac{\hbar^2}{2m_n}\nabla_n^2 - \frac{\hbar^2}{2m_e}\nabla_e^2 - \frac{Ze^2}{4\pi\varepsilon_0 r}$$

• reduced mass $\mu = \frac{m_n m_e}{m_n + m_e}$

$$\psi(r,\theta,\phi) = R(r)\gamma(\theta,\phi)$$

atomic orbitals

$$R(\rho) = N_{n,l}\,\rho^l\,L_{n+1}^{2l+1}(\rho)\,e^{-\frac{\rho}{2n}}$$

$$\rightarrow R(\rho) \propto e^{-\frac{\rho}{2n}}$$

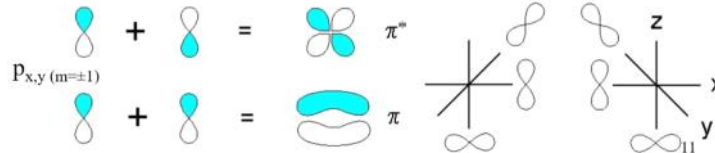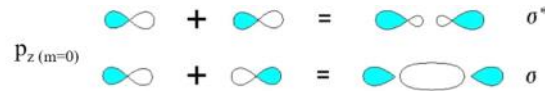$$E_n = -\frac{Z^2\mu e^4}{32\pi^2\varepsilon_0^2 h^2}\frac{1}{n^2} = -13.6\frac{1}{n^2}\,eV$$

$$1\,eV = 1.602\times10^{-19}J = 23.06\,kcal\cdot mol^{-1}$$

→ **Born-Oppenheimer Approximation**

• For more than one hydrogen atom there is no analytical solution ⇒ approximation

• Nucleous is much heavier than electrons
  ⇒ Approximation = fixed/stationary nuclei

→ **LCAO MO** (linear combination of atomic orbitals)

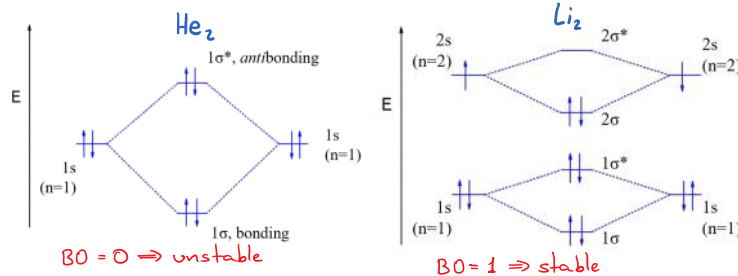• Sum of all interactions: $\Phi_i = \sum_j C_{ij}\Psi_j$
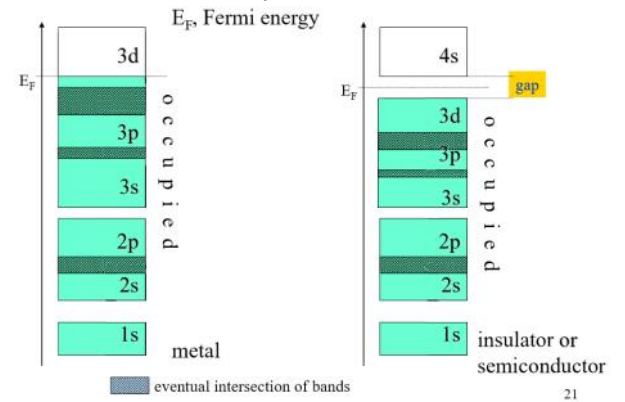

• bonding and antibonding states

• Bond order

$$\text{Bond order} = \frac{\begin{pmatrix}\text{number of electrons}\\\text{in bonding MOs}\end{pmatrix} - \begin{pmatrix}\text{number of electrons}\\\text{in anti-bonding MOs}\end{pmatrix}}{2}$$

$\neq 0 \Rightarrow$ stable, $= 0 \Rightarrow$ unstable

He₂ — BO = 0 ⇒ unstable

Li₂ — BO = 1 ⇒ stable

• Occupation of Crystal Bands

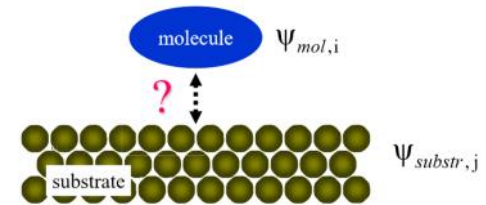metal | insulator or semiconductor

eventual intersection of bands

21

• Fermi-Dirac Distribution
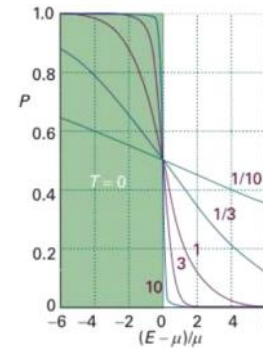
$$f(E) = \frac{1}{e^{(E-E_F)/k_B T} + 1}$$

k_B = Boltman constant
$$= 1.38\times10^{-23}\frac{J}{K} \approx 8.6\times10^{-5}\frac{eV}{K}$$

Molecular Adsorption

molecule $\Psi_{mol,i}$

substrate $\Psi_{substr,j}$

→ **Physiosorption** (Van-der-Waals interaction)

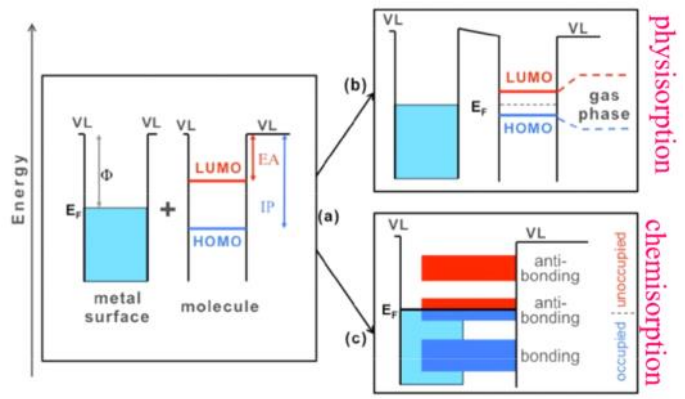$$U_{Keesom} = -\frac{\mu_1^2\mu_2^2}{6(4\pi\varepsilon_0)^2 k_B T d^4}$$

$$U_{Debye} = -\frac{\mu^2\alpha}{(4\pi\varepsilon_0)^2 d^6}$$

$$U_{London} = -\frac{3}{2}\frac{\alpha_1\alpha_2}{(4\pi\varepsilon_0)^2 d^6}\frac{h\nu_1\nu_2}{(\nu_1+\nu_2)}$$

μ, dipole
α, polarizability
hν, ionization energy

→ **Chemisorption**

- Electron transfer between surface and molecule
- Depending on $S_{ij} = \int \varphi_{mol,i} \, \Psi_{substr,j} \, dx\,dy\,dz$ overlapping integral
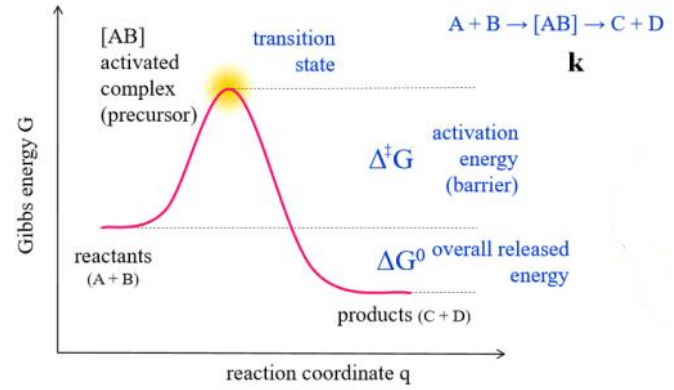

physisorption
chemisorption

→ **Gibbs Free Energy G**

T, temperature
U, internal energy
P, pressure
V, volume
S, entropy

$$G = U + pV - TS$$

↳ thermodynamic potential

- Every system (T, p constant!) seeks to achieve a minimum of free energy ⇒ Chemical reactions
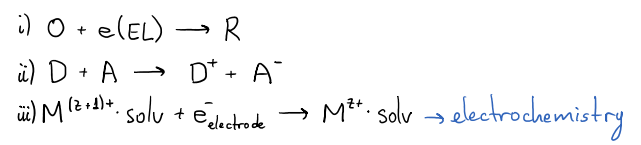

$A + B \rightarrow [AB] \rightarrow C + D$ **k**

$$k \propto e^{-\frac{\Delta^{\ddagger}G}{RT}}$$

$R = \dfrac{k_B}{N_A} = 8.3 \; J\cdot K^{-1}\cdot mol^{-1}$
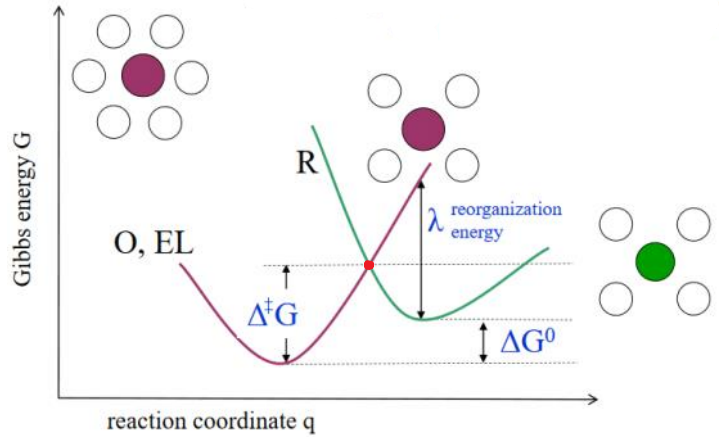
↳ ↑Gibbs energy ⇒ ↓k

→ **Redox**: Describes all chemical reactions in which atoms have their oxidation number changed

- Ways of writing a redox reaction

  i) $O + e(EL) \rightarrow R$

  ii) $D + A \rightarrow D^+ + A^-$

  iii) $M^{(z+1)+} \cdot solv + e^-_{electrode} \rightarrow M^{z+} \cdot solv \rightarrow$ electrochemistry

1. Marcus Theory

$$k_{et} = K_{P,0} \, \nu_n \, K_{el} \, e^{-\frac{\Delta^{\ddagger}G}{RT}}$$

$K_{P,0}$ = precursor equilibrium constant (ratio of the reactant concentration in the precursor state with respect to its bulk concentration)
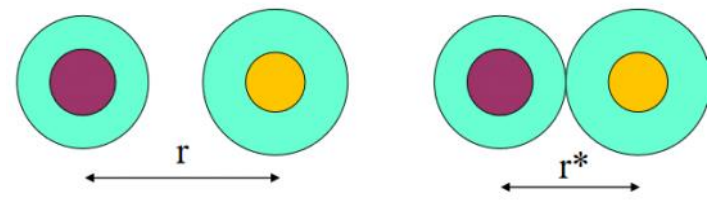
ν_n = nuclear frequency factor (frequency attempts on the energy barrier)

K_{el} = electronic transmission coefficient (probability of electron tunneling)

- from the perturbation theory (Landau-Zener)

$K_{el} \propto \langle H_{EL\text{-}0}\rangle^2, \quad H_{EL\text{-}0} \equiv \int_V \varphi^*_{EL} \, H_{EL\text{-}0} \, \varphi_0$

$\langle H_{EL\text{-}0}\rangle^2 = \langle H^*_{EL\text{-}0}\rangle^2 e^{-\beta(r-r^*)} \longrightarrow \boxed{k_{el} \propto e^{-\beta(r-r^*)}}$
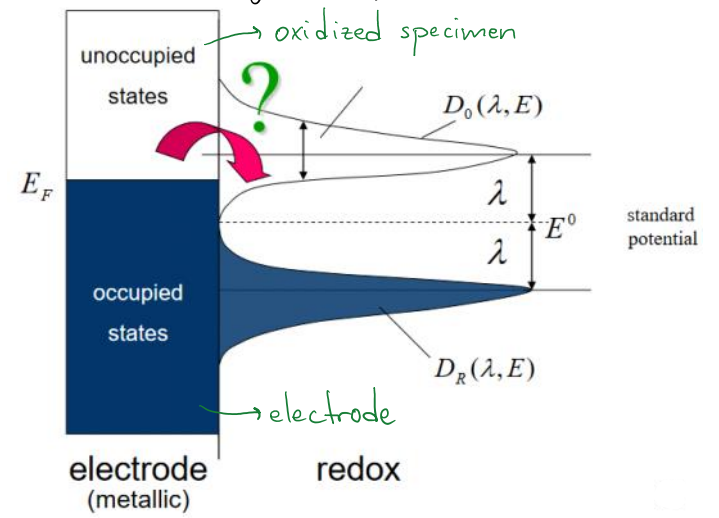

- for an effective tunneling (and consequently redox reaction) both reactants need to be very close one from the another

$$\Delta^{\ddagger}G = \frac{(\Delta G^0 + \lambda)^2}{4\lambda}$$

2. Gerischer Theory

- tries to quantify k_{et} using the energy levels

→ oxidized specimen
→ electrode

- Considering $N_{occ} = f(E)\rho(E)$

$D_O(\lambda, E) = N_A C_o(0,t) W_o(\lambda, E)$

E_O, energy obtained adding an electron from the infinity to the *unoccupied* state of the oxidized form

E_R, energy spent removing an electron from the *occupied* state of the reduced form to the infinity

$D_R(\lambda, E) = N_A C_R(0,t) W_R(\lambda, E)$
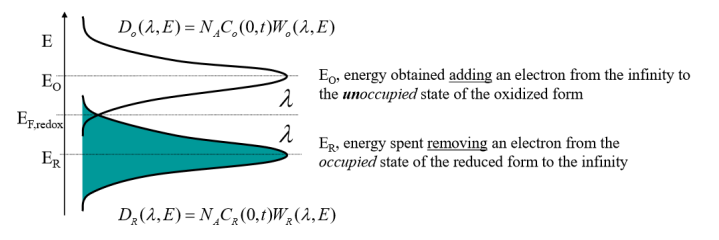
$$k_{et} = \frac{1}{\Delta t} \int_{-\infty}^{\infty} \mathcal{E}_{red}(E) \, W_0(\lambda, E) \, f(E) \, \rho(E) \, dE$$
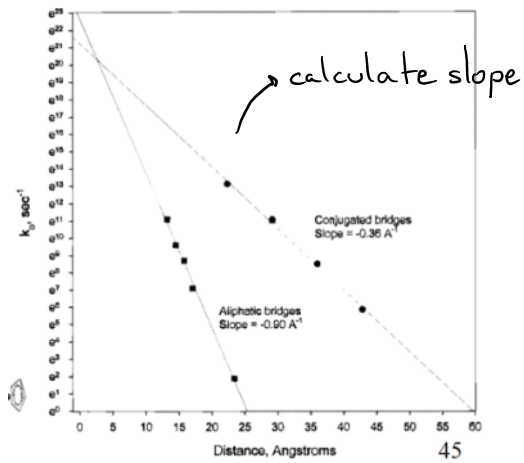
↳ ! (proportionality function)

• at the end we get the same values as Marcus

→ Electron transfer through a molecular wire

$$k_{el}(x) = k_{el}^{\circ} e^{-\beta x}$$

$$\beta \approx \frac{4\pi \sqrt{2m\emptyset}}{h}$$

$$\approx 1.02 \, \text{Å}^{-1} \text{eV}^{-1/2} \sqrt{\emptyset}$$

• ∅ = barrier in vacuum

→ Determining β

→ calculate slope

Conjugated bridges
Slope = -0.36 Å⁻¹

Aliphatic bridges
Slope = -0.90 Å⁻¹

45

Potentiometric Biosensors

→ **Chemical equilibrium:** State in which the chemical activities or concentrations of the reactants and products have no net change over time.

- Forward chemical process proceeds at the same rate as the reverse reaction → *dynamic equilibrium*

→ Chemical Potential

- A form of energy that can be absorbed or released during a chemical reaction or phase transition due to a change of particle number.
- It is defined as the rate of change of the free Gibbs energy with respect to the change in the number of atoms/molecules of the species
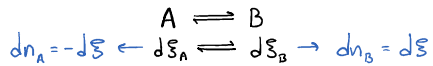
$$\mu_j \equiv \left[\frac{\partial G}{\partial n_j}\right]_{p,T,n'}$$

- Considering $d\xi = \frac{n_j - n_j^o}{v_j}$ = extent of reaction

measures the progress of the reaction ↵

n_j^o = initial mole of substance j
n_j = mole of substance j when extent is reached
v_j = stoichiometric coefficient

$d\xi$ is constant for reactants/products $(d\xi_A = d\xi_B)$
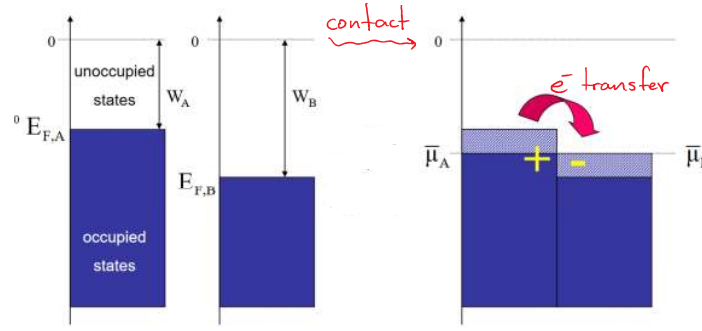(in absolute value)

$$A \rightleftharpoons B$$

$dn_A = -d\xi \leftarrow d\xi_A \rightleftharpoons d\xi_B \rightarrow dn_B = d\xi$

$dG|_{p,T} = \mu_A dn_A + \mu_B dn_B = (\mu_B - \mu_A)d\xi$

equilibrium ⇒ minimum of Gibbs energy ⇒ $dG|_{p,T} \overset{!}{=} 0$

$$\boxed{\mu_A = \mu_B} \quad \rightarrow \text{condition for chemical equilibrium}$$

- The chemical potential can be interpreted (or is) the fermi energy at 0°K. When brought into contact both materials need to have the same fermi energy → $E_{F,A} = E_{F,B}$ $(\mu_A = \mu_B)$

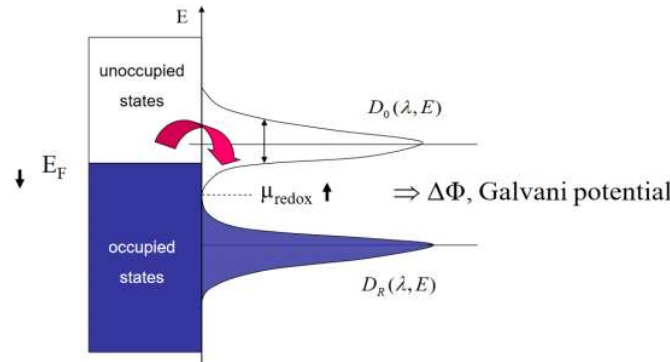---

contact
e⁻ transfer

$$\boxed{\overline{\mu} \equiv \mu_o + ZF\Delta\varphi}$$

$\overline{\mu}$ = chemical potential at equilibrium
Z = number of charges for each ion (electron = -1)
F = Faraday's constant

→ Electrode-Solution Interface

- μ_{redox} = redox fermi energy = energy E^* where the densities of states match ⟶ $D_{red}(\lambda, E^*) = D_{metal}(\lambda, E^*)$

$\Rightarrow \Delta\Phi$, Galvani potential

- Galvani Potential: the potential generated as consequence of the electrons jumping into the available levels at lower energy

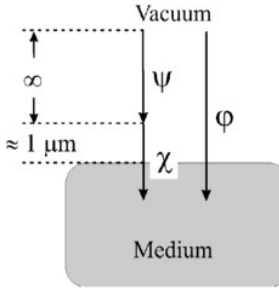$$\overline{\mu} \equiv \mu_o + ZF\Delta\varphi \longrightarrow \boxed{\Delta\varphi = -\frac{\Delta_r G}{ZF}}$$

- The different potentials

i) **Volta/external potential Ψ:** Potential difference when bringing a charge from infinity $(\Psi(\infty) = 0)$

---

to a region close to the surface ($\sim 1\mu m$)

ii) **Surface potential χ:** Potential at the internal phase passing through the interface → Determined by dipoles aligned at the surface and by surface charges

iii) **Galvani potential φ:** Sum of all potentials

$$\boxed{\varphi = \Psi + \chi}$$

→ We only work with the Galvany potential (we can ignore these other potentials ☺)

→ Nernst Equation

$\mu_j = \mu_j^o + RT \ln(a_j)$ ↝

$$\Delta\varphi = \Delta\varphi_c^o + \frac{RT}{nF}\left[\sum_{ox}\ln(a_{ox,j}) - \sum_{red}\ln(a_{red,j})\right]$$

$\begin{bmatrix} v_{prod} > 0 \\ v_{react} < 0 \end{bmatrix} = \Delta\varphi_c^o - \frac{RT}{nF}\ln(Q), \quad Q = \left(\prod_j a_j^{v_j}\right)_{equilibrium}$

measures the relative amounts of reactants and products → can be compared to the rate of the reaction k, but k is only for equilibrium. Q is for any point in time → But in this equation Q is $Q_{equilibrium}$, which is k.

$$\boxed{\Delta\varphi_j = \Delta\varphi_c^o - \frac{RT}{ZF}\ln(Q)}$$

n = # of electrons transferred in one reaction

→ $\Delta\varphi$ and concentration

- Since the activity a_j is proportional to the concentration of ions $(a_j \propto r_{ion}\cdot[ion])$

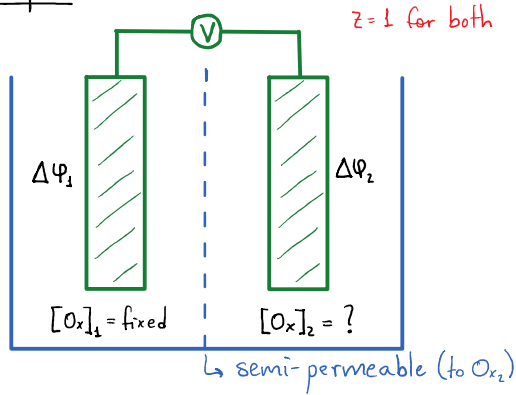$$\Rightarrow \Delta\varphi_j = C_1 + \frac{RT}{ZF}\ln(a_{ion}) = C' + \frac{RT}{ZF}\ln([ion])$$

~59 mV when using log (without z)

$$\boxed{\Delta\varphi_j = C' + \frac{0.059}{Z}\log([ion])}$$

↳ $\frac{RT}{F} = 0.05916$ V

- **example:**

$z = 1$ for both

$[O_x]_1 = $ fixed $[O_x]_2 = ?$

↳ semi-permeable (to O_{x_2})

$\Delta\varphi_1 = \Delta\varphi° - \dfrac{RT}{F}\ln[O_x]_1$

$\Delta\varphi_2 = \Delta\varphi° - \dfrac{RT}{F}\ln[O_x]_2$ } $V = \Delta\varphi_1 - \Delta\varphi_2$

$\Rightarrow V = \dfrac{RT}{F}\ln\left(\dfrac{[O_{ox}]_2}{[O_x]_1}\right)$

→ **Nernst at semi permeable membranes**

$j_{total} = j_{diffusion} + j_{drift} \xrightarrow{equilibrium} 0$

$= -|z|D\dfrac{d[C]}{dt} - \mu|z|F[C]\dfrac{dV}{dx}$

Diffusion constant ↰   ↳ mobility, not potential

- Integrating from outside to inside

$\displaystyle\int_{V_{out}}^{V_{in}} dV = -\dfrac{k_BT}{Q}\int_{C_{out}}^{C_{in}}\dfrac{d[C]}{[C]} \Rightarrow \boxed{\Delta\varphi = V_{out} - V_{in} = \dfrac{k_BT}{Q}\ln\left(\dfrac{[C]_{out}}{[C]_{in}}\right)}$

- **Ion-selective Electrodes (ISE)**
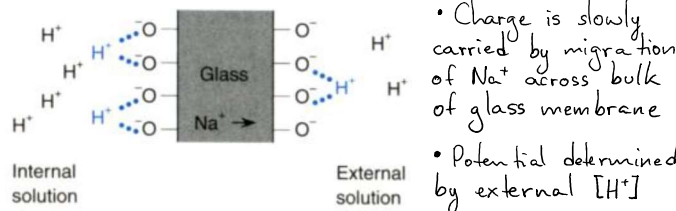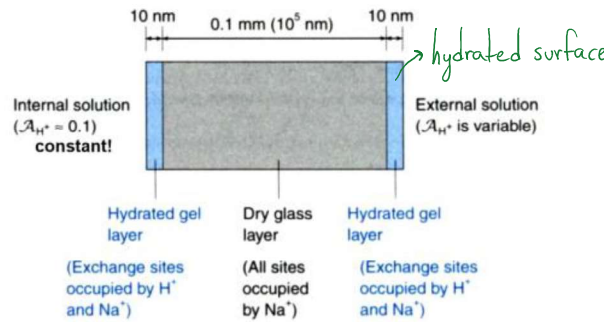

constant []

$\boxed{\Delta\varphi_m = \dfrac{RT}{zF}\ln\left(\dfrac{[A]_i}{[A]_o}\right)}$

→ **pH Electrode**

- When in aqueous solution, Corning 015 (a type of glass) forms a hydrated surface, resulting in the formation of negatively charged sites ($-SiO^-$). Na^+ serve as counter ions. Because H^+ binds more strongly to $-SiO^-$ than does Na^+, they displace the sodium ions

$$H^+ + SiO^-Na^+ \rightleftharpoons SiO^-H^+ + Na^+$$
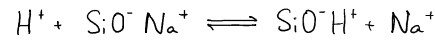
- The transport of charge across the membrane is carried by the Na^+ ions

- Charge is slowly carried by migration of Na^+ across bulk of glass membrane
- Potential determined by external $[H^+]$

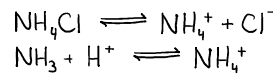- Potential of a glass electrode obeys the equation

$\Delta\varphi = $ constant $+ 0.059\log([H^+])$ ($pH := -\log[H^+]$)

$\Rightarrow \boxed{pH = \dfrac{constant - \Delta\varphi}{0.059}}$ → constant
$\Rightarrow$ needs calibration

→ **Gas Sensors**

1. NH_3

$NH_4Cl \rightleftharpoons NH_4^+ + Cl^-$
$NH_3 + H^+ \rightleftharpoons NH_4^+$
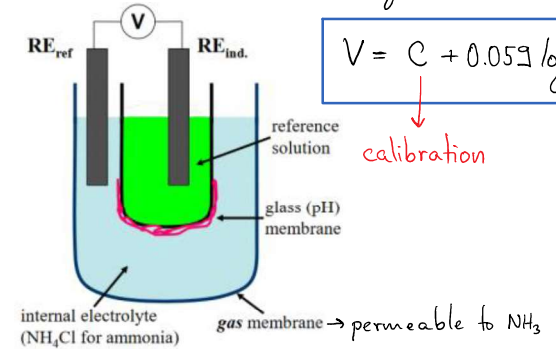
$E_{cell} = E_{ind} - E_{ref}$
↓
Indicator is H^+ sensitive

} Use a pH sensor to calculate change in $[H^+]$, consequently $[NH_3]$

Nernst equation $\longrightarrow K_{el} = \dfrac{a_{NH_3}\,a_{H^+}}{a_{NH_4^+}}$

$\log(a_{H^+}) = \log(K_{el}) + \log(a_{NH_4^+}) - \log(a_{NH_3})$

$\Rightarrow E_{cell} = $ constant $+ 0.059\log(a_{H^+})$
    $= $ constant' $+ 0.059\log(a_{NH_3})$

gas membrane → permeable to NH_3

$\boxed{V = C + 0.059\log([NH_3])}$
↓
calibration

2. CO_2

$CO_2 + H_2O \rightleftharpoons H_2CO_3$
$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$ } $K = \dfrac{[H^+][HCO_3^-]}{[H_2CO_3]}$
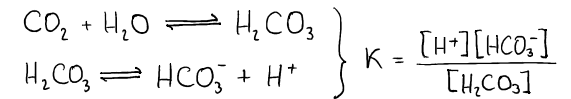
$pH = pK + \ln\left(\dfrac{[HCO_3^-]}{[H_2CO_3]}\right) \rightsquigarrow$ at equilibrium $[H_2CO_3] \propto [CO_2]$

$\boxed{pH = pK' + \ln\left(\dfrac{[HCO_3^-]}{[CO_2]}\right)}$

- For different gases

| Gas	Inner Solution	Sensor
CO_2	$NaHCO_3$	pH glass
SO_2	$NaHSO_3$	pH glass
HF	H^+	F-LaF_3
H_2S	pH 5 buffer	S^{2-}-Ag_2S
HCN	$KAg(CN)_2$	Ag^+-Ag
NH_3	NH_4Cl	pH glass

→ **Enzymes**: Proteins that catalyze (i.e. accelerate) chemical reactions by lowering the activation energy (E_a or $\Delta^{\ddagger}G$)

• They are not consumed by the reactions nor do they alter the equilibrium of these reactions

Substrate entering active site of enzyme — Enzyme/substrate complex — Enzyme/products complex — Products leaving active site of enzyme
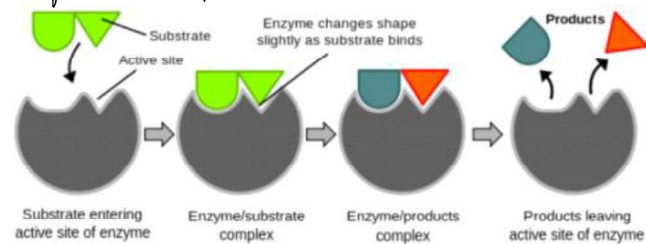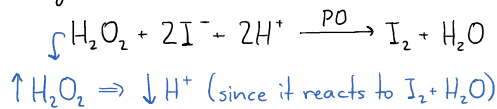
1. Urease

• We can get the urea concentration by using urease and an ammonia-selective gas electrode

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_3^{2-}$$
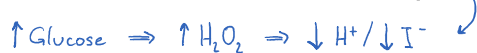
$\uparrow$ Urea $\Rightarrow$ $\uparrow NH_4^+$ ↵
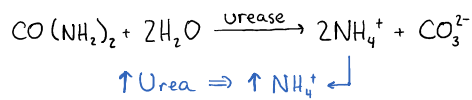
2. Peroxidase (PO)

• Get H_2O_2 concentration by measuring pH (or using an iodide electrode)

$$H_2O_2 + 2I^- + 2H^+ \xrightarrow{PO} I_2 + H_2O$$

$\uparrow H_2O_2 \Rightarrow \downarrow H^+$ (since it reacts to $I_2 + H_2O$)

3. Glucose Oxydase (GOx)

• Use same sensor as before

$$\text{glucose} + O_2 \xrightarrow{GOx} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + 2I^- + 2H^+ \xrightarrow{PO} I_2 + H_2O$$

$\uparrow$ Glucose $\Rightarrow$ $\uparrow H_2O_2$ $\Rightarrow$ $\downarrow H^+ / \downarrow I^-$
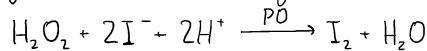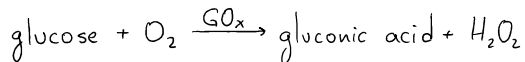
→ **Helpful tips**

• For reactions of the form

$$A \cdot ox + B \cdot X + z\,e^- \rightleftharpoons C \cdot red + D \cdot Y$$
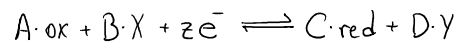
we have

$$\boxed{E = E° + \frac{RT \cdot 2.303}{F \cdot z} \log\left(\frac{a_{ox}^A \cdot a_x^B}{a_{red}^C \cdot a_y^D}\right)}$$

• Values for the activity a

i) Ions $\Rightarrow$ $a = \frac{r_{ion}[ion]}{1M}$ (normally is $r_{ion} = 1$)

ii) Gases $\Rightarrow$ $a =$ partial pressure $p_{gas} = \frac{P(gas)}{P_{atm}}$

iii) Solids/fluids $\Rightarrow$ $a \approx 1$ (H_2O, metals etc)

• <u>Example</u>: ZnCu cell

$Zn \rightarrow Zn^{2+} + 2e^-$ $Cu \rightarrow Cu^{2+} + 2e^-$
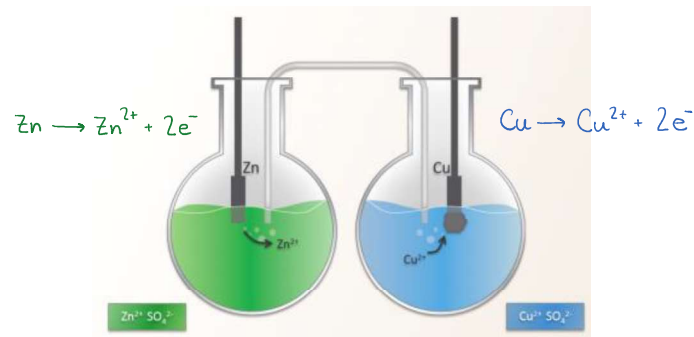
<u>for Zn</u>:

$$E_{Zn} = E_{Zn}° + \frac{0.059}{2}\log\left(\frac{[Zn^{2+}]}{[Zn]}\right)$$
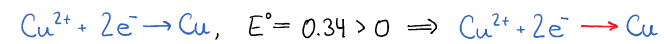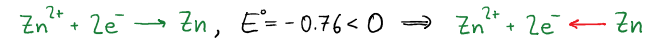$$= -0.76 + \frac{0.059}{2}\log([Zn^{2+}])$$

<u>for Cu</u>:

$$E_{Cu} = E_{Cu}° + \frac{0.059}{2}\log\left(\frac{[Cu^{2+}]}{[Cu]}\right)$$
$$= 0.34 + \frac{0.059}{2}\log([Cu^{2+}])$$

• Initial values for $E°$ are the redox numbers of the reaction $\Rightarrow$ table

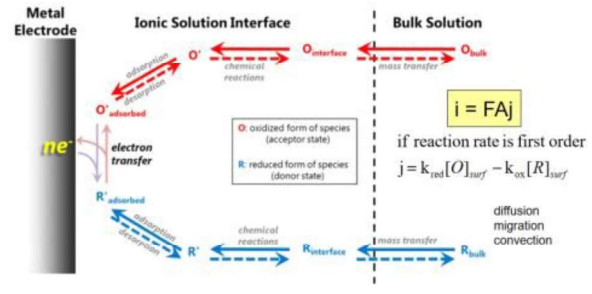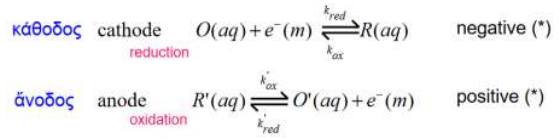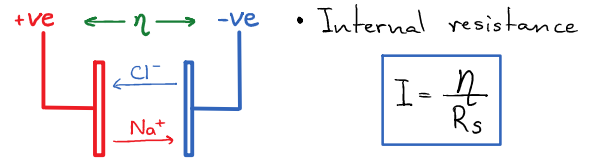|		
$I_2(s) + 2e^-$	$\longrightarrow 2I^-(aq)$	0.54
$O_2(g) + 2H_2O(l) + 4e^-$	$\longrightarrow 4OH^-(aq)$	0.40
$Cu^{2+}(aq) + 2e^-$	$\longrightarrow Cu(s)$	0.34
$Sn^{4+}(aq) + 2e^-$	$\longrightarrow Sn^{2+}(aq)$	0.15
$2H^+(aq) + 2e^-$	$\longrightarrow H_2(g)$	0
$Pb^{2+}(aq) + 2e^-$	$\longrightarrow Pb(s)$	−0.13
$Ni^{2+}(aq) + 2e^-$	$\longrightarrow Ni(s)$	−0.26
$Cd^{2+}(aq) + 2e^-$	$\longrightarrow Cd(s)$	−0.40
$Fe^{2+}(aq) + 2e^-$	$\longrightarrow Fe(s)$	−0.45
$Zn^{2+}(aq) + 2e^-$	$\longrightarrow Zn(s)$	−0.76
$2H_2O(l) + 2e^-$	$\longrightarrow H_2(g) + 2OH^-(aq)$	−0.83

• The sign of the redox number tells us in which direction the reaction normally happens (according the direction given in the table)

$Zn^{2+} + 2e^- \rightarrow Zn$, $E° = -0.76 < 0 \implies Zn^{2+} + 2e^- \leftarrow Zn$

$Cu^{2+} + 2e^- \rightarrow Cu$, $E° = 0.34 > 0 \implies Cu^{2+} + 2e^- \rightarrow Cu$

Amperometric Sensors

analyte — Δi — transducer — (V) signal — signal processor

biological detection element

The external power source causes this electrode to have a deficiency of electrons.

The external power source causes this electrode to have an excess of electrons, so it is negatively charged.

ANODE Reductant is oxidised
CATHODE Oxidant is reduced

overpotential
$$\eta \equiv \varphi_S - \varphi_M$$
$$\equiv \Delta\varphi\big|_{appl} - \Delta\varphi\big|_{equil}$$

Key:
Current consists of moving electrons.
Current consists of moving ions in electrolyte.

↳ $\eta = 0 \Rightarrow$ net current equals zero
$\eta \neq 0 \Rightarrow$ net current different from zero

+ve ← η → -ve   • Internal resistance

Cl^-
Na^+

$$I = \frac{\eta}{R_s}$$

| κάθοδος | cathode (reduction) | $O(aq) + e^-(m) \underset{k_{ox}}{\overset{k_{red}}{\rightleftharpoons}} R(aq)$ | negative (*) |
| άνοδος | anode (oxidation) | $R'(aq) \underset{k_{red}}{\overset{k_{ox}}{\rightleftharpoons}} O'(aq) + e^-(m)$ | positive (*) |

$i = FAj$

if reaction rate is first order
$j = k_{red}[O]_{surf} - k_{ox}[R]_{surf}$

O: oxidized form of species (acceptor state)
R: reduced form of species (donor state)

$$I = FAj = FA\left(k_{red}[O]_{surf} - k_{ox}[R]_{surf}\right)$$

→ η in the Gerischer's view

• The **applied** overpotential η shifts the μ_{redox} up or down (positive/negative applied voltage) to a state with bigger $D(\lambda, E)$

• Consequently more levels are empty $\Rightarrow$ more e^- jumping $\Rightarrow$ higher current
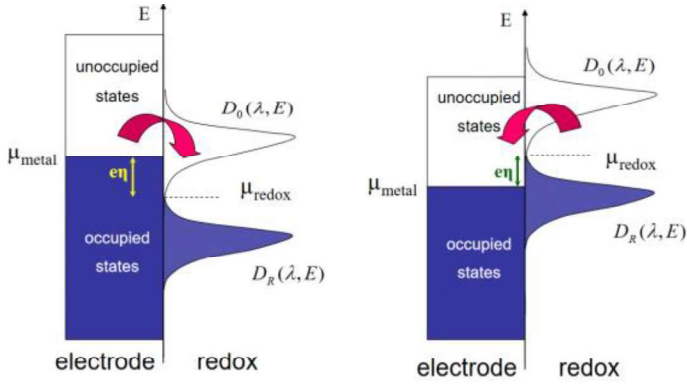
• In general $Current_{tot} = Current_{ohmic} + Current_{DOS}$

But $Current_{DOS}$ is only noticeable after a threshold overpotential η^{tt}

ohmic (linear)
ohmic + DOS
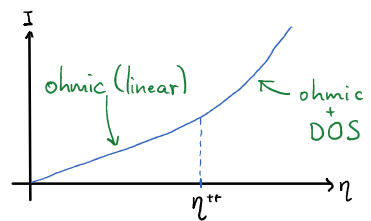
→ Faraday's Laws

1. The quantities of substances involved in the chemical change are proportional to the quantity of electricity which passes through the electrolyte

2. The masses of different substances set free or dissolved by a given amount of electricity are proportional to their chemical equivalents

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$$m = \frac{q \times EW}{F} = \frac{I \cdot t \cdot EW}{F}$$

$EW = \frac{molecular\ weight}{valency}$

• $EZ = \frac{M}{z}$

$$\Rightarrow m = \frac{qM}{Fz} = \frac{I \cdot \Delta t \cdot M}{Fz}$$

• **Example:** Amount of Ni deposited in 45min with a current of 1.5A ($Ni^{2+} + 2e^- \longrightarrow Ni$, $M = 58.7 g \cdot mol^{-1}$)

$$\Rightarrow m = \frac{qM}{F \cdot z} = \frac{1.5 \cdot 45 \cdot 60}{96485\ C \cdot mol^{-1}} \cdot \frac{58.7 g \cdot mol^{-1}}{2} = \underline{1.23 g}$$

• If we want the quantity in moles
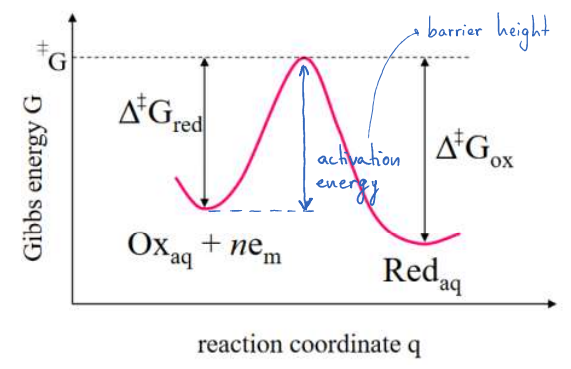
$$\Rightarrow n = \frac{m}{M} = \frac{qM}{Fz} \cdot \frac{1}{M} = \frac{q}{Fz} = \frac{I \cdot \Delta t}{Fz}$$

$$[mol] \rightarrow n = \frac{Q}{Fz} = \frac{I \cdot \Delta t}{F \cdot z}$$
$$[g] \rightarrow m = \frac{QM}{Fz} = \frac{I \cdot \Delta t}{Fz}$$

→ Butler-Volmer Equation

• Calculates the electron flow in the presence of of an electrode potential
• We want to find the barrier height (intersection of the Gibbs energies for reactants/products) when a potential is applied
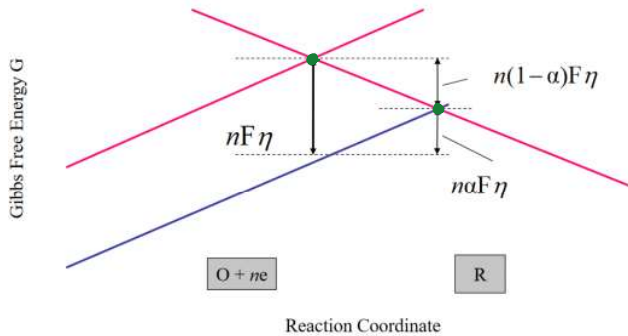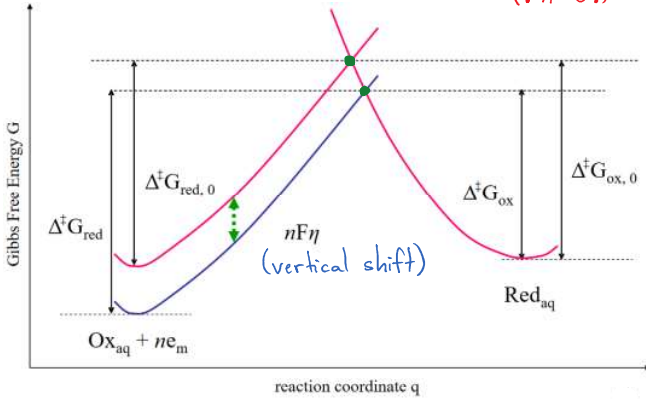

barrier height
$\Delta^{\ddagger}G_{red}$
$\Delta^{\ddagger}G_{ox}$
activation energy
$Ox_{aq} + ne_m$
Red_{aq}
Gibbs energy G
reaction coordinate q

→ Considering

- $O^{z+}(aq) + ne^-(m) \rightleftharpoons R^{(z-n)+}(aq)$
- φ_s = potential of the ions in the solution
- φ_m = potential of the electrons in the electrode
- z = valency of the oxidized species, n = number of transferred electrons $\Rightarrow$ $z-n$ = valency of the oxidized species
- $\eta = \varphi_s - \varphi_m = \Delta\varphi|_{appl} - \Delta\varphi|_{equiv}$
- New Gibbs energy → $G = G_{no\ potential} + G_{Coulombian\ potential}$

$$G_{react} = G'_{react} + zF\varphi_s - nF\varphi_m$$
$$= G'_{react} + (z-n)F\varphi_s - nF(\varphi_m - \varphi_s)$$
$$G_{prod} = G'_{prod} + (z-n)F\varphi_s$$

$\hookrightarrow nF\eta$ (vertical shift)
(! $n<0$!)

- We want to study the intersection, since this defines the height of the barrier

$\hookrightarrow$ Introduce transfer coefficient α (unknown)

$\Rightarrow nF\eta = n(1-\alpha)F\eta + \alpha F\eta$

$\hookrightarrow$ We can now find the barrier height seen from the reactant minimum and seen from the product minimum

$$\Delta^{\ddagger}G_{red} = \Delta^{\ddagger}_{red,0} + n\alpha F\eta$$
$$\Delta^{\ddagger}G_{ox} = \Delta^{\ddagger}_{ox,0} - n(1-\alpha)F\eta$$

$$k_{red} = A_{red}\,e^{-\frac{\Delta^{\ddagger}G_{red}}{RT}} = A_{red}\,e^{-\frac{\Delta^{\ddagger}G_{red,0}}{RT}}\,e^{-\frac{n\alpha F\eta}{RT}}$$
$$k_{ox} = A_{ox}\,e^{-\frac{\Delta^{\ddagger}G_{ox}}{RT}} = A_{ox}\,e^{-\frac{\Delta^{\ddagger}G_{ox,0}}{RT}}\,e^{\frac{n(1-\alpha)F\eta}{RT}}$$

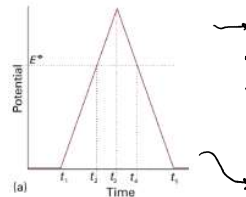considering equilibrium $C(0,t) = C^*$, $\eta = 0 \Rightarrow k_{red} = k_{ox} = k_0$

$$\boxed{k_{red} = k_0\,e^{-\frac{n\alpha F\eta}{RT}} \quad \text{and} \quad k_{ox} = k_0\,e^{\frac{n(1-\alpha)F\eta}{RT}}}$$

using $I = F\cdot A\cdot j = F\cdot A\cdot (k_{red}[O]_{surf} - k_{ox}[R_{surf}])$

$$\boxed{\Rightarrow I = FAk_0\left(C_{ox}(0,t)\,e^{-\frac{\alpha n F\eta}{RT}} - C_{red}(0,t)\,e^{\frac{(1-\alpha)nF\eta}{RT}}\right)}$$
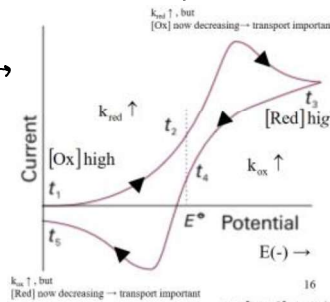
→ Cyclic Voltammetry (CV)

- Type of potentiodynamic electrochemical measurement
- The idea is to apply a positive cyclic sawtooth voltage and record the current
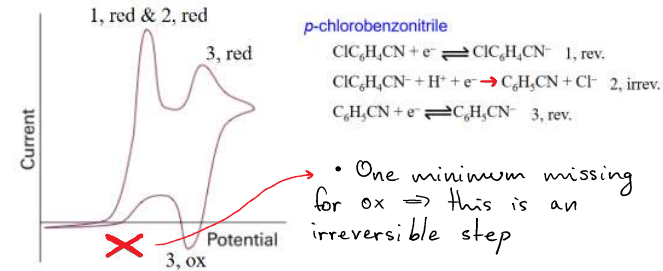

$\hookrightarrow$ assumption / initial condition (t):
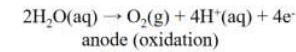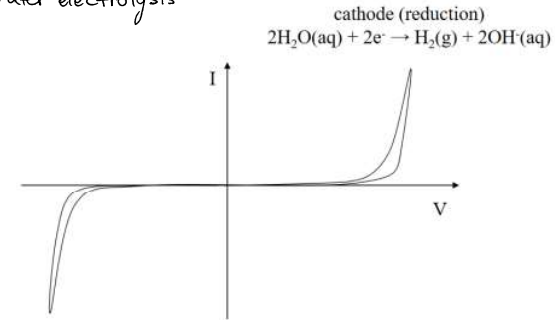- $\eta = 0$
- Redox species only in its oxidized form

- CV can help to find irreversible steps

$ClC_6H_4CN + e^- \rightleftharpoons ClC_6H_4CN^-$ 1, rev.
$ClC_6H_4CN^- + H^+ + e^- \rightarrow C_6H_5CN + Cl^-$ 2, irrev.
$C_6H_5CN + e^- \rightleftharpoons C_6H_5CN^-$ 3, rev.

- One minimum missing for ox $\Rightarrow$ this is an irreversible step

- Water electrolysis

cathode (reduction)
$2H_2O(aq) + 2e^- \rightarrow H_2(g) + 2OH^-(aq)$

$2H_2O(aq) \rightarrow O_2(g) + 4H^+(aq) + 4e^-$
anode (oxidation)
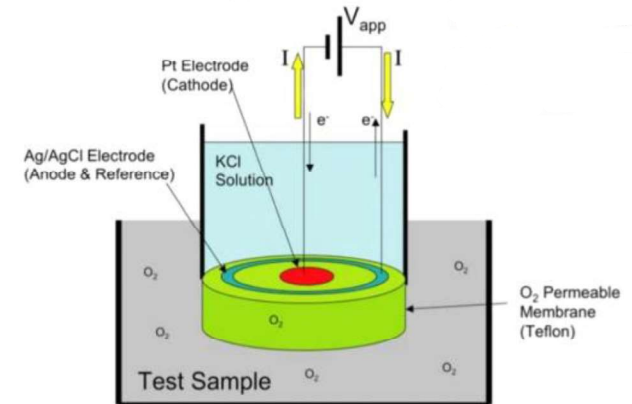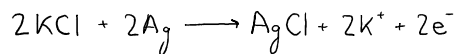
→ Clark Electrode

$V_{app} = -0.6V$

- Platinum and silver electrodes submerged in a concentrated KCl solution
- O_2 permeable membrane (teflon)
- Voltage source

- By applying a voltage between the Pt (cathode, electron receiver) and Ag (anode, electron donor) electrodes, one oxidizes the silver

$$2KCl + 2Ag \longrightarrow AgCl + 2K^+ + 2e^-$$

- Platinum is a strong catalyst for the covalent dissociation or reassociation of water. If there are electrons present, O_2 will reduce

$$\frac{1}{2}O_2 + 2e^- + H_2O \longrightarrow 2OH^-$$
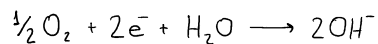
- formation of K^+ (at anode) and consumption of H^+ at cathode $\Rightarrow$ charge balance

- This current flowing through the electrodes will only happen if there is enough O_2 to be reduced

$$\uparrow [O_2] \Rightarrow \uparrow Current$$

- The voltage applied helps to move the electrons. At too high voltages we get the electrolysis of KCl so that the current generated will be governed by moving K^+ and Cl^- and not $[O_2]$. The voltage that is normally applied is the mean of the Nernst potentials of both electrodes.

- Cathode is normally made out of nobel metals like platinum, so it does not participate in the reactions
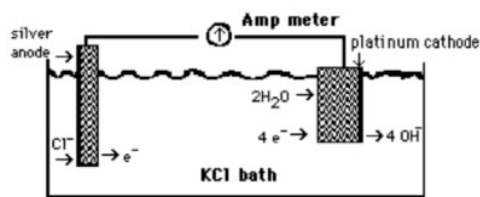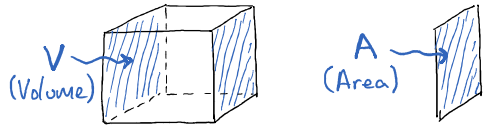

for more details

Membranes and Transport

1. Concentration & Diffusion

V (Volume)   A (Area)

concentration:
$$c(x,t) = \lim_{V \to 0} \frac{\text{amount of substance}}{V} \to \text{Molar}$$

$$[c(x,t)] = \text{mole} \cdot L^{-1} = M$$

$$1\,M = 10^{-3}\,\text{mole} \cdot cm^{-3}$$

flux:
$$\phi(x,t) = \lim_{\substack{A \to 0 \\ \Delta t \to 0}} \frac{\text{amount of substance flowing through A in } \Delta t}{\Delta t \cdot A}$$

$$[\phi(x,t)] = \text{mol} \cdot s^{-1} cm^{-3}$$

→ Fick's First Law

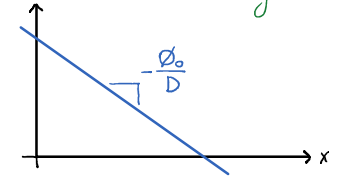$$\boxed{\phi(x,t) = -D\,\frac{\partial c(x,t)}{\partial x}} \quad D = \text{Diffusion constant}$$

$c(x,t)$ $\phi(x,t) > 0$

$c(x,t)$ $\phi(x,t) < 0$
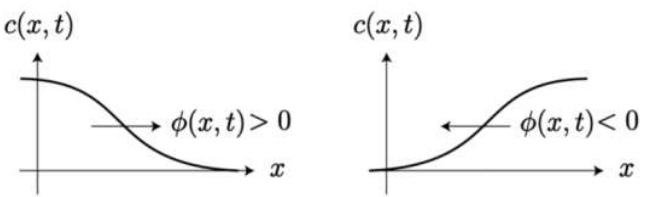
In general ↑Molecule size ⇒ ↓D

→ Continuity equation: for $\Delta x, \Delta t \to 0$

$$\boxed{-\frac{\partial \phi(x,t)}{\partial x} = \frac{\partial c(x,t)}{\partial t}}$$

• Steady state: outcome for large times t ("$t \to \infty$") ⟶ time independent (flux and concentration not changing)

$$\frac{\partial \phi(x,t)}{\partial t} = \frac{\partial c(x,t)}{\partial t} \to 0 \Rightarrow \frac{\partial \phi(x,t)}{\partial x} = 0$$

$$\Rightarrow \phi(x,t) \text{ is constant (in x)}$$

$$\Rightarrow -D\,\frac{\partial c(x,t)}{\partial x} = \text{constant} = \phi_0$$

$$\Rightarrow c(x,t) = \text{linear function of } x = c(x, t_0)$$

$c(x,t) \to t \to \infty$ for steady state

$-\frac{\phi_0}{D}$

Steady state ≠ Equilibrium

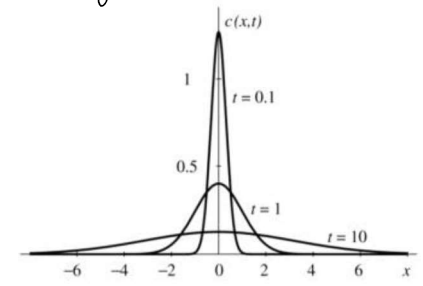• Time-dependent solution (no steady-state)

$$\left.\begin{array}{l} -\dfrac{\partial \phi(x,t)}{\partial x} = \dfrac{\partial c(x,t)}{\partial t} \\[2mm] \phi(x,t) = -D\,\dfrac{\partial c(x,t)}{\partial x} \end{array}\right\} \quad \boxed{\frac{\partial c(x,t)}{\partial t} = D\,\frac{\partial^2 c(x,t)}{\partial x^2}}$$

• Example: given $c(x,t=0) = n_0 \delta(x)$

delta-like initial distribution

$c(x, t=0)$ ⟶ n_0

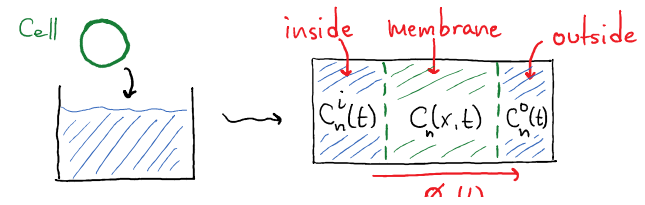solution: $c(x,t) = \dfrac{n_0}{\sqrt{4\pi D t}} e^{-\frac{x^2}{4Dt}}$

→ gaussian mean = 0   → $\sigma = \sqrt{2Dt}$

$$\boxed{t_{1/2} = \frac{x_{1/2}^2}{D}}$$

$D \approx 10^{-5} cm^2 \cdot s^{-1}$ for small solutes (eg. Na^+)

$t_{1/2}$ = time it takes for half (50%) of the solute diffuse to $|x| > x_{1/2}$

2. Diffusion accross membrane

Cell

inside   membrane   outside

$C_n^i(t)$ $C_n(x,t)$ $C_n^o(t)$

$\phi_n(t)$

→ Equilibrium

substance
solute n
Substance

$$\boxed{k_{A:B} := \frac{C_n^A}{C_n^B}}$$

partition coefficient

$t=0$ $\quad t=0^+$

$t = \tau_{ss}$ $\quad t=0^{++}$
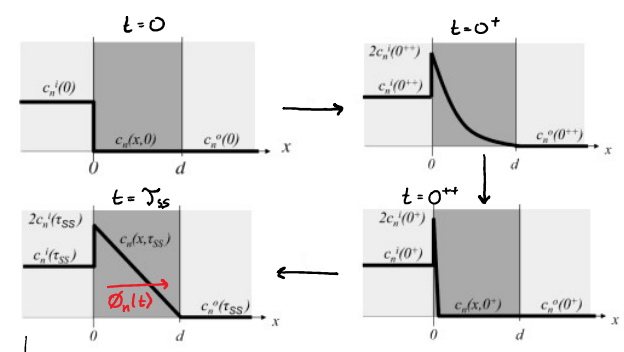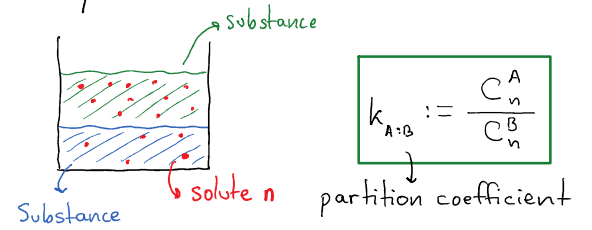
⟶ $t = \tau_{ss}$ ⟶ steady state $\boxed{\tau_{ss} = \frac{d^2}{\pi^2 D}}$

$$\boxed{P_n = \frac{D_n k_n}{d}} \quad \boxed{\phi_n(t) = P_n\left(C_n^i(t) - C_n^o(t)\right)}$$

⟶ permeability of membrane to solute n

3. Osmosis

→ Movement of solvent/water across membranes

→ Membrane may be only permeable to one single substance (only water or only solvent, for example)

4. Ion Transport

$$-\frac{\partial \phi(x,t)}{\partial x} = \frac{\partial c(x,t)}{\partial t} \longrightarrow \phi = \frac{\#\,Ions}{A \cdot \Delta t} = \frac{I}{A} = J$$

$$\Rightarrow \boxed{\frac{\partial J(x,t)}{\partial t} = -z_n F \frac{\partial c(x,t)}{\partial t}}$$

Continuity
$$\frac{\partial J_n(x,t)}{\partial x} = -z_n F \frac{\partial c_n(x,t)}{\partial t}$$

Remember:
$$-\frac{\partial \phi(x,t)}{\partial x} = \frac{\partial c(x,t)}{\partial t}$$

A difference in- versus out- flux of charge (i.e. gradient in current density)
➜ Increase/decrease in local charge

Poisson's Equation
$$\frac{\partial^2 \psi(x,t)}{\partial x^2} = -\frac{1}{\epsilon}\sum_n z_n F c_n(x,t)$$

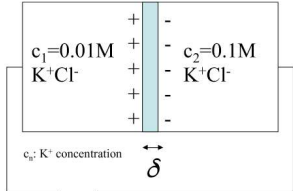Electric field potential ← Local charge density

Nernst-Planck Equation

Molar mechanical mobility
$$J_n(x,t) = -z_n F D_n \frac{\partial c_n(x,t)}{\partial x} - u_n z_n^2 F^2 c_n(x,t)\frac{\partial \psi(x,t)}{\partial x}$$

Current ← Diffusion of charge + Mobility of charge in E-field

Membrane permeable only to K^+

$c_1=0.01M$ $c_2=0.1M$
K^+Cl^- K^+Cl^-

c_n: K^+ concentration δ

$$V_n \approx \frac{60}{z_n}\log_{10}\left(\frac{c_s^o}{c_s^i}\right)\ mV$$

↓

Nernst Potential for a specific ion

$V_m = \Delta\psi_{12} = \psi_1 - \psi_2$

$$\Delta\psi_{12} = \psi_1 - \psi_2 = \frac{D_n}{u_n z_n F}\ln\left(\frac{c_2}{c_1}\right) = \frac{RT}{z_n F}\ln\left(\frac{c_2}{c_1}\right)$$ **Nernst Potential**

$$\begin{array}{cc} c_{K^+}^i & c_{K^+}^o \\ c_{Na^+}^i & c_{Na^+}^o \\ c_{Cl^-}^i & c_{Cl^-}^o \\ \vdots & \vdots \\ \vdots & \vdots \\ \hline + V_m - \end{array}$$

$V_m = V_K = \frac{RT}{F}\ln\left(\frac{c_{K^+}^o}{c_{K^+}^i}\right)$ No K^+ diffusion

$V_m = V_{Na} = \frac{RT}{F}\ln\left(\frac{c_{Na^+}^o}{c_{Na^+}^i}\right)$ No Na^+ diffusion

$V_m = V_{Cl} = \frac{RT}{(-1)F}\ln\left(\frac{c_{Cl^-}^o}{c_{Cl^-}^i}\right)$ No Cl^- diffusion

Donnan Equilibrium

$\left(\frac{c_{K^+}^o}{c_{K^+}^i}\right) = \left(\frac{c_{Na^+}^o}{c_{Na^+}^i}\right) = \left(\frac{c_{Cl^-}^o}{c_{Cl^-}^i}\right)^{-1} = \left(\frac{c_{Mg^{2+}}^o}{c_{Mg^{2+}}^i}\right)^{1/2} = ... = \left(\frac{c_X^o}{c_X^i}\right)^{\frac{1}{z_X}} = e^{\left(\frac{zFV_m}{RT}\right)}$

→ Ion concentrations

Component	Intracellular Concentration (mM)	Extracellular Concentration (mM)	Nernst Potential (mV)
Cations			
Na^+	5-15	145	58 ~ 86
K^+	140	5	-85
Mg^{2+}	0.5	1-2	17.8 ~ 35.5
Ca^{2+}	10^{-4}	1-2	236
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)	-14
Anions			
Cl^-	5-15	5-15	-

• Resting potential is at about −70 mV and is mostly dependend on potassium concentrations (c_K^i and c_K^o)
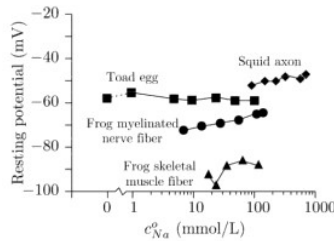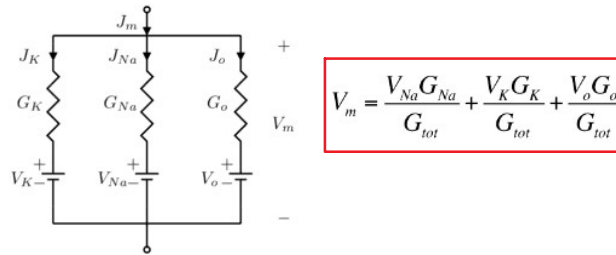

Largely independent on C_{Na}
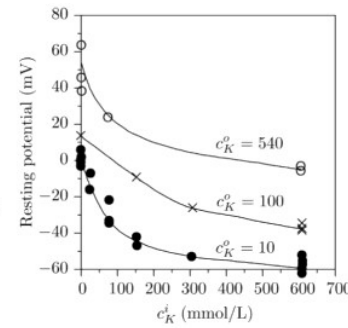
Dependent on both $C_k{}^i$ and $C_k{}^o$
(Squid giant axon)

→ Multiple-ion Model

$$V_m = \frac{V_{Na}G_{Na}}{G_{tot}} + \frac{V_K G_K}{G_{tot}} + \frac{V_o G_o}{G_{tot}}$$

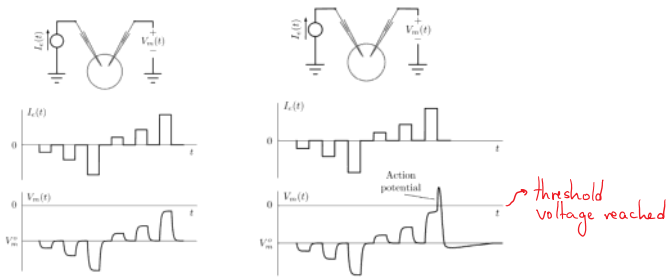| Ion	G_n (S/cm^2)	G_n/G_m	c_n^o/c_n^i	V_n (mV)
K^+	3.7×10^{-4}	0.55	0.05	−72
Na^+	1×10^{-5}	0.016	9.8	+55
leakage	3.0×10^{-4}	0.44	—	−49

5. Active Transport

→ Active-driven pump for each ion to maintain quasi-equilibrium ($J^a + J^p \approx 0$)
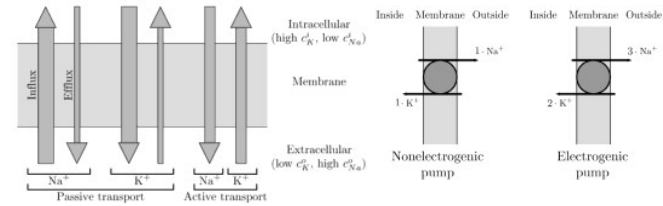
AP & HH

→ Non-excitable vs excitable cells (i.e. neurons)

Graded Response vs **Action Potential**
(Electrically non-excitable cells) (Electrically excitable cells)


→ threshold voltage reached

→ **Properties of action potentials**

1. They are universal → same concept for all animals

2. All-or-none Threshold
 • Very sharp threshold ↪ too high current will also not generate an action potential   → all-or-none
 • After reaching threshold, APs are pretty much the same

threshold
Top 6 traces : "1.00"
0.96, 0.85, 0.71, 0.57, 0.43, 0.21
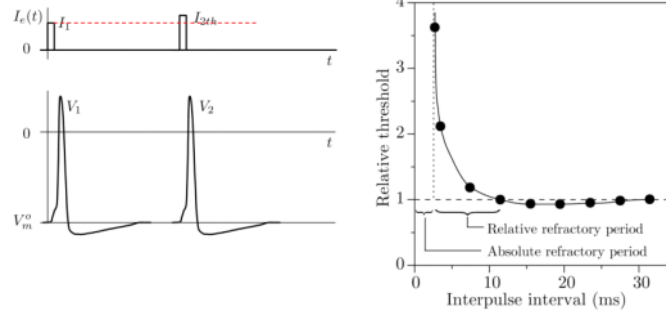-1, -0.71, -0.57, -0.43, -0.21
amount of current injected
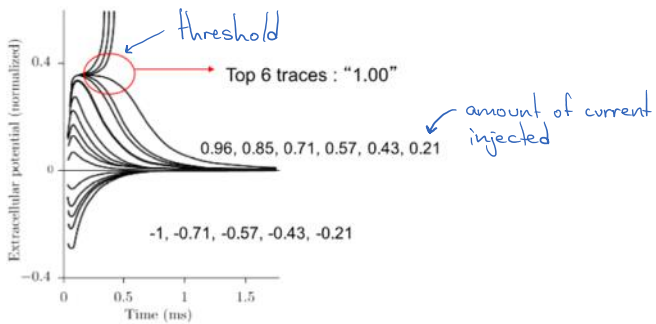
3. Accomodation
 • Cells ignore slow increasing currents → they are "smart"
 • "Threshold" is not a simple definition (constant voltage, for example)

Current

Accomodation
No AP
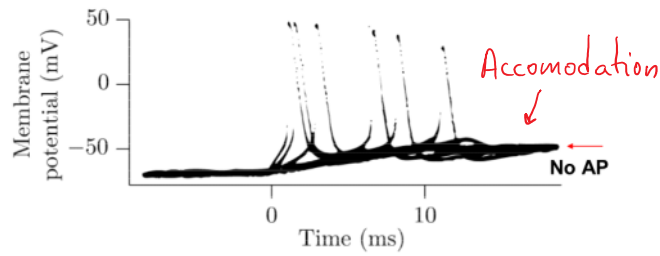
4. Refractoriness
 • After firing an AP it is more difficult to fire subsequent APs
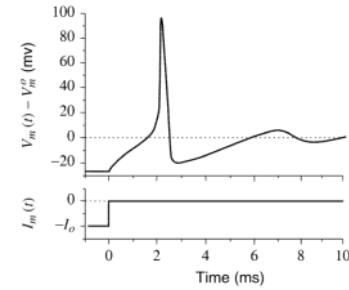

Relative refractory period
Absolute refractory period

5. Decrement-free conduction
 • Graded potential : Conducts decrementally (decaying)
 • Action potential : Conducts dissipation-free (amplification?)

Decremental vs **Decrement-free conduction**

non-excitable cell   excitable cell
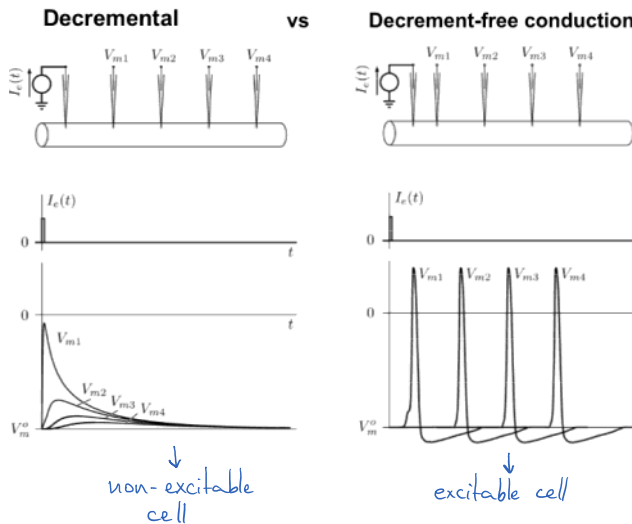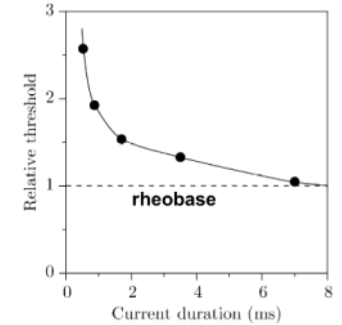
6. Anode Break

 • By hyperpolarizing (more negative) and then repolarizing back to resting potential, you could elicit action potentials as well

7. Strength-duration relation

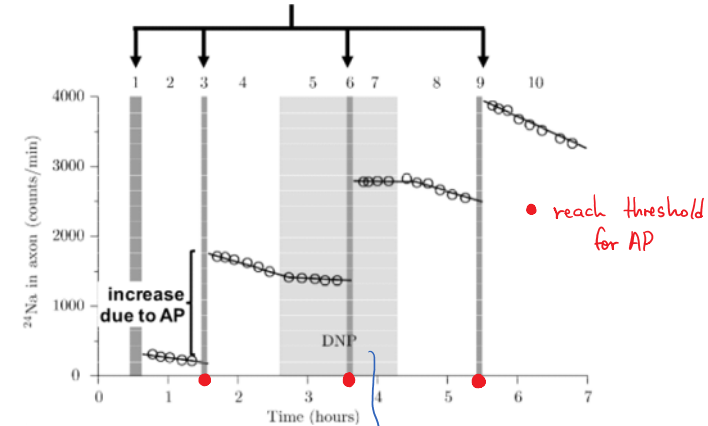For longer duration current pulse, the lower the current threshold for stimulation of AP.

Rheobase : threshold in response to a current of arbitrarily long duration

rheobase

8. Active pumps are not responsible for APs
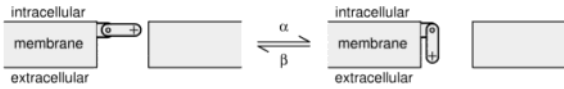 • DNP blocks active pump functionality

Action potential is inducible repeatedly even after DNP treatment

increase due to AP
DNP
• reach threshold for AP
AP even though we blocked active pumps

 • One action potential causes only a little change in Na concentration

→ Channel Modeling

intracellular
membrane
extracellular
$\rightleftharpoons$ (α, β)
intracellular
membrane
extracellular

Assume $\mathcal{N}$ channels per unit area, of which $n(t)$ are open.

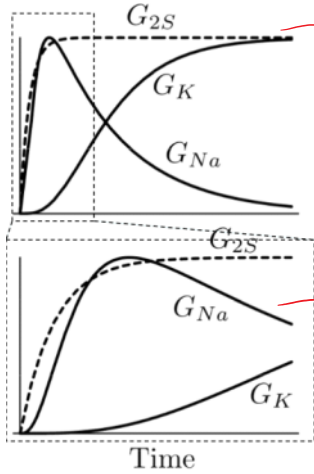$$\frac{dn(t)}{dt} = \alpha(\mathcal{N} - n(t)) - \beta n(t)$$

$$n(t) = n_\infty + (n(0) - n_\infty)e^{-t/\tau_x} \; ; \quad n_\infty = \frac{\alpha}{\alpha+\beta}\mathcal{N}, \quad \tau_x = \frac{1}{\alpha+\beta}$$

Assume $\mathcal{N}$ is large.

$$x(t) = \text{probability gate is open} \approx \frac{n(t)}{\mathcal{N}}$$

$$x(t) = x_\infty + (x(0) - x_\infty)e^{-t/\tau_x} \; ; \quad x_\infty = \frac{\alpha}{\alpha+\beta}, \quad \tau_x = \frac{1}{\alpha+\beta}$$

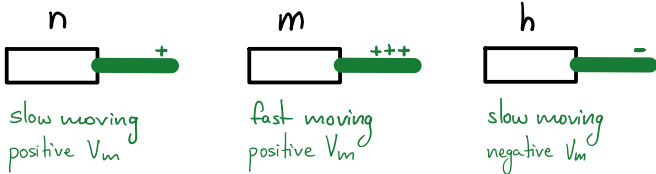• The 2-state ion-channel model could not perfectly describe $G_K(V_m,t)$ and $G_{Na}(V_m,t)$

G_{2S}, G_K, G_{Na} — Does not explain this actual slow increase (G_{2s} much faster as reality)

G_{2S}, G_{Na}, G_K — Does not explain this Na-channel inactivation (no inactivation for G_{2s})

Time

• Solution: Multi-stage ion-channels

n — slow moving positive V_m
m — fast moving positive V_m
h — slow moving negative V_m

$$\frac{dn}{dt} = \alpha_n(V_m)(1-n) - \beta_n(V_m)n$$
$$\frac{dm}{dt} = \alpha_m(V_m)(1-m) - \beta_m(V_m)m$$
$$\frac{dh}{dt} = \alpha_h(V_m)(1-h) - \beta_h(V_m)h$$
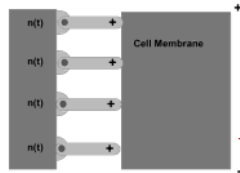
• Original parameters used by Huxley

$$\alpha_n(V_m) = \frac{0.01(10-V_m)}{\exp\left(\frac{10-V_m}{10}\right)-1} \quad \alpha_m(V_m) = \frac{0.1(25-V_m)}{\exp\left(\frac{25-V_m}{10}\right)-1} \quad \alpha_h(V_m) = 0.07\exp\left(\frac{-V_m}{20}\right)$$

$$\beta_n(V_m) = 0.125\exp\left(\frac{-V_m}{80}\right) \quad \beta_m(V_m) = 4\exp\left(\frac{-V_m}{18}\right) \quad \beta_h(V_m) = \frac{1}{\exp\left(\frac{30-V_m}{10}\right)+1}$$

• Nowadays it is generalized to $\dfrac{A_p(V_m - B_p)}{\exp\left(\frac{V_m - B_p}{C_p}\right) - D_p}$
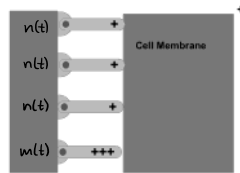
same for α, β ←

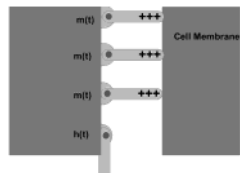↳ values determined experimentally

• Slow opening & closing
• $x(t) = [n(t)]^4$

$$\boxed{G_K(V_m,t) = \overline{G_K} \cdot [n(V_m,t)]^4}$$

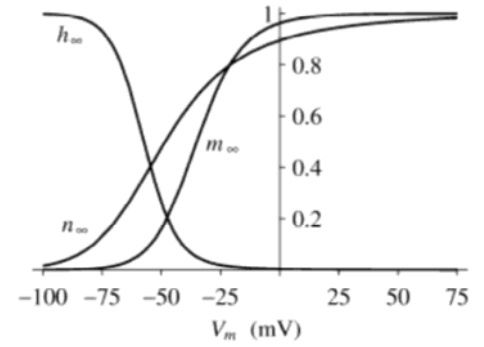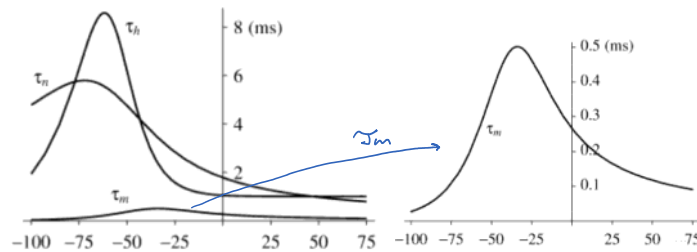• Slow opening & fast closing
• $x(t) = [n(t)]^3 \cdot m(t)$

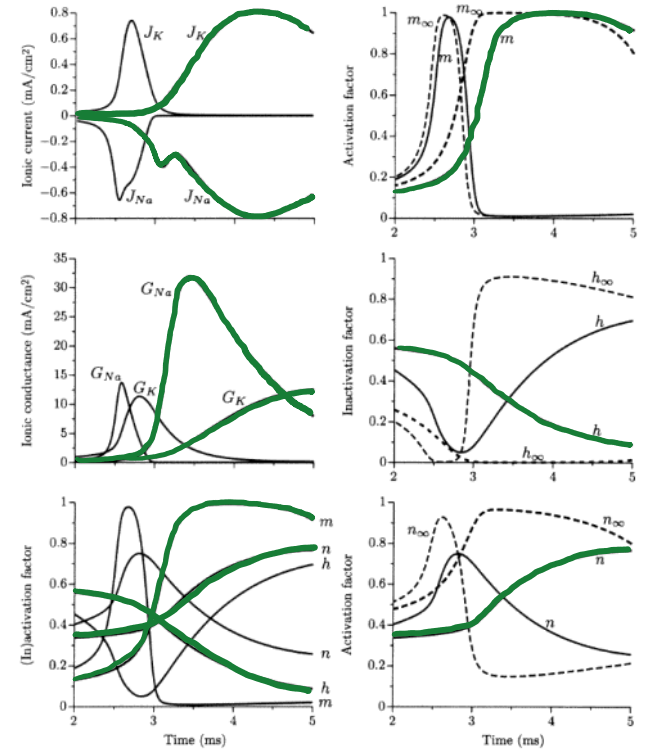• Fast opening & closing
  + slow inactivation
• $x(t) = [m(t)]^3 \cdot h(t)$

$$\boxed{G_{Na}(V_m,t) = \overline{G_{Na}} \cdot [m(V_m,t)]^3 \cdot h(V_m,t)}$$

τ_n, τ_m, τ_h, h_∞, m_∞, n_∞, change with V_m, hence with time during AP
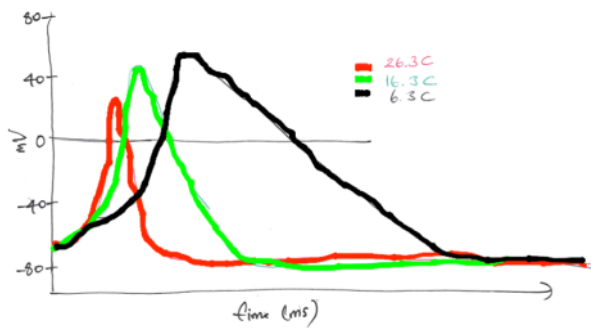

• Temperature dependence

Green ⇒ 6.3°C   Black ⇒ 26.3°C

→ Temperature increase affects the speed of an AP

→ Temperature can affect both the Nernst Potential and the different rate constants (h, n, m)

↳ Change caused by Nernst Potential is negligible!

C. Outward potassium current (I_K) – after TTX

D. Inward sodium current (I_{Na}) – after TEA

- **Concentration dependence**
  ↪ Affects the Nernst Potential $\emptyset_{ion} = \dfrac{RT}{zF} \ln\left(\dfrac{[ion]^o}{[ion]^i}\right)$

  * Na concentrations affect depolarization maximum, since at depolarization the membrane voltage V_m reaches V_{Na}

  * K concentrations affect hyperpolarization minimum, since at hyperpolarization the membrane voltage V_m reaches V_k
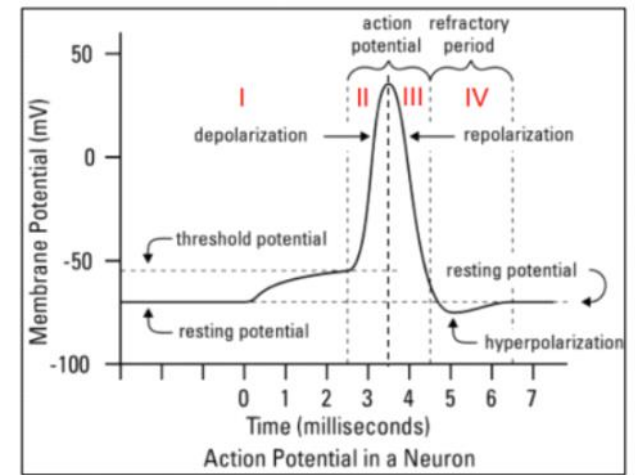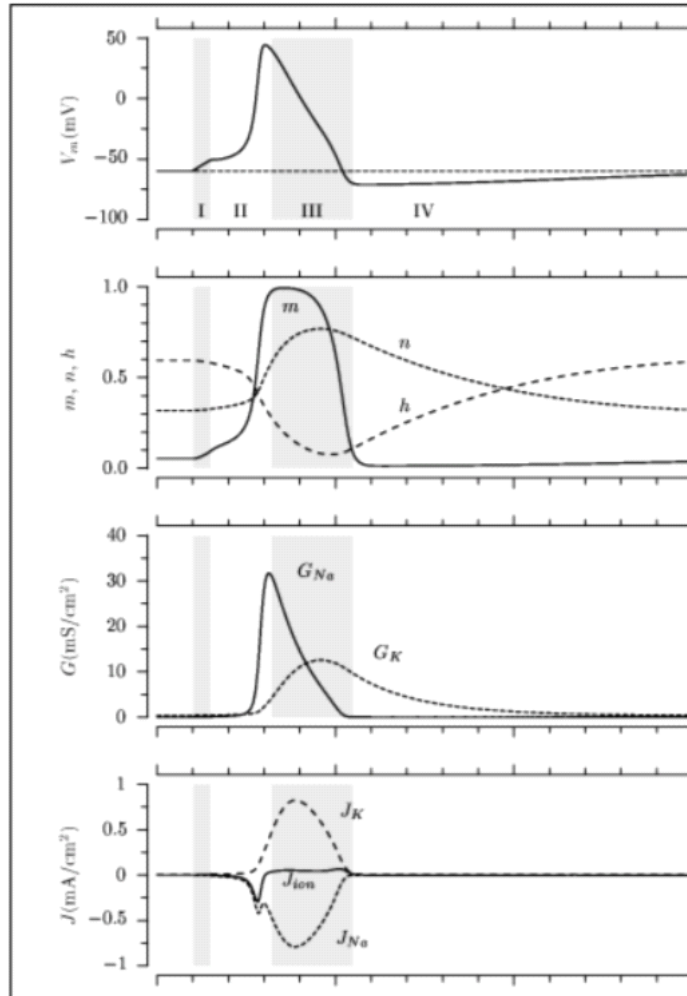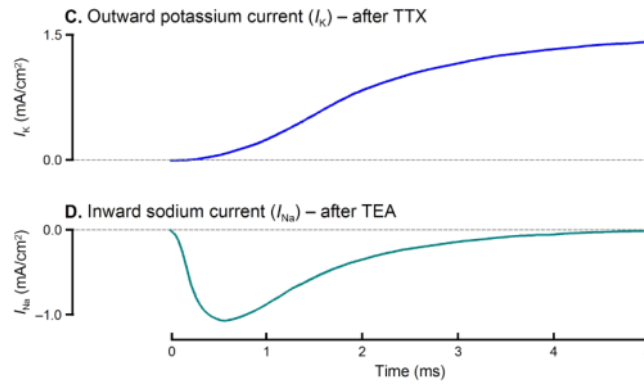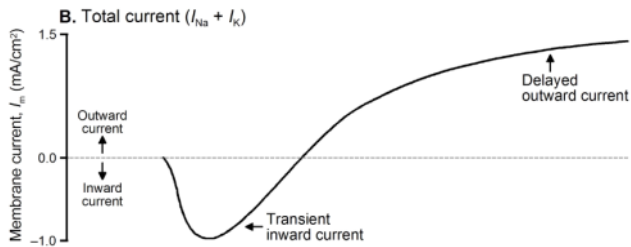
$$J_m \propto G_{Na}(V_m(t)-V_{Na}) + G_k(V_m(t)-V_k)$$

⇒ ↑intracellular $[Na]^i$ ⇒ ↓V_{Na} ⇒ ↑$V_{m,peak}$

⇒ ↑extracellular $[k]^o$ ⇒ ↑V_k ⇒ ↓$V_{m,hyper}$ (also resting potential)

Experiment 1 — increase $[k]^o$

Experiment 2 — increase $[Na]^i$
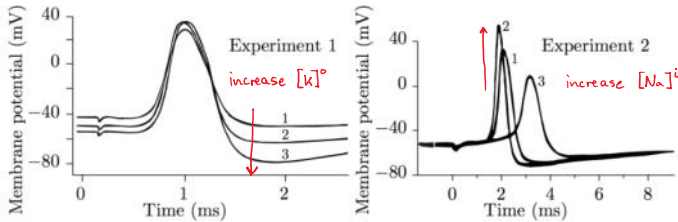
⇒ Concentration differences only create a shift on V_m / J_m

- **Total Current** $J_m = J_{Na} + J_k$

B. Total current ($I_{Na} + I_K$)

Outward current / Inward current / Delayed outward current / Transient inward current

Action Potential in a Neuron

I: Pre-AP threshold

II: AP initiation (Depolarization)

III: AP ending (Repolarization)

IV: Post-AP refractory period (Hyperpolarization)

	A	B	C	D
Stage of action potential	Resting	Depolarization	Repolarization	Hyperpolarization
Voltage-gated Na Channel status	Closed	Open	Closed	Closed
Voltage-gated K Channel status	Closed	Closed	Open	Open
Na Flow through Na Channel	Zero	Flows into Axon	Zero	Zero
K Flow through K Channel	Zero	Zero	Out of Axon	Zero

- **Active Pumps**
  ↪ When blocking the pumps, two things happen
  1. Na influx → ↑$[Na]^i$, ↓$[Na]^o$ ⇒ ↓$V_{m,peak}$
  2. K efflux → ↓$[k]^i$, ↑$[k]^o$ ⇒ ↑$V_{m,rest}$
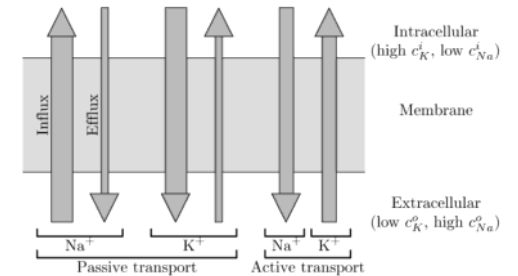
→ Properties

3. Accomodation → Na-channels inactivate over small ramp-up currents ($h \sim 0$)

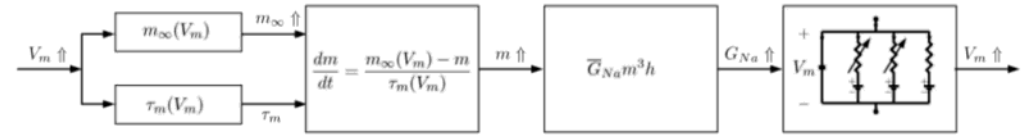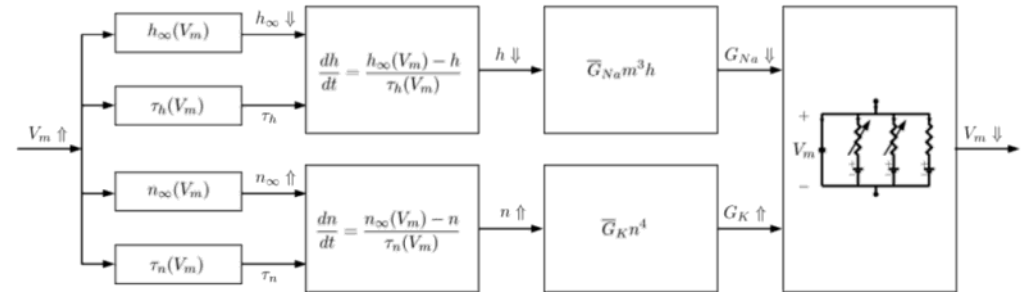4. Refractoriness → Na-channels still inactive ($h \sim 0$)

6. Anode Break

Less inactive Na$^+$ channels ⎫ upon immediate return to old
Less open K$^+$ channels ⎭ resting potential

⇒ These cannot response fast enough ⇒ lower threshold for AP

Depolarization mechanism – positive feedback (early on during AP)

Repolarization mechanisms – negative feedback (later during AP)

Original Hodgkin Huxley Model

4 state variables: $\boxed{V_m(t), n(t), m(t), h(t)}$

4 Initial conditions: $\boxed{\begin{aligned} V(t=0) &= V_m^o \\ n(t=0) &= n_\infty(V_m^o) \\ m(t=0) &= m_\infty(V_m^o) \\ h(t=0) &= h_\infty(V_m^o) \end{aligned}}$

Inject external current J_e

4 state update equations :

$$\tau_n(V_m)\frac{dn(V_m,t)}{dt} + n(V_m,t) = n_\infty(V_m)$$
$$\tau_m(V_m)\frac{dm(V_m,t)}{dt} + m(V_m,t) = m_\infty(V_m)$$
$$\tau_h(V_m)\frac{dh(V_m,t)}{dt} + h(V_m,t) = h_\infty(V_m)$$

$$G_K(V_m,t) = \overline{G}_K n^4(V_m,t)$$
$$G_{Na}(V_m,t) = \overline{G}_{Na} m^3(V_m,t) h(V_m,t)$$

$$
\begin{aligned}
J_m(t) &= J_C + J_{Na} + J_K + J_L + J_{ext} \\
&= C_m \frac{dV_m(t)}{dt} + G_{Na}(V_m,t)\cdot(V_m(t)-V_{Na}) \\
&\quad + G_K(V_m,t)\cdot(V_m(t)-V_K) + G_L\cdot(V_m(t)-V_L) + J_{ext} \\
&= 0
\end{aligned}
$$

$$
\begin{aligned}
\tau_m &= \frac{1}{\alpha_m + \beta_m} \quad \text{and} \quad m_\infty = \frac{\alpha_m}{\alpha_m + \beta_m}, \\
\tau_h &= \frac{1}{\alpha_h + \beta_h} \quad \text{and} \quad h_\infty = \frac{\alpha_h}{\alpha_h + \beta_h}, \\
\tau_n &= \frac{1}{\alpha_n + \beta_n} \quad \text{and} \quad n_\infty = \frac{\alpha_n}{\alpha_n + \beta_n}.
\end{aligned}
$$

$$
\begin{aligned}
\alpha_m &= \frac{-0.1(V_m+35)}{e^{-0.1(V_m+35)}-1} K_T \\
\beta_m &= 4e^{-(V_m+60)/18} K_T \\
\alpha_h &= 0.07 e^{-0.05(V_m+60)} K_T \\
\beta_h &= \frac{1}{1+e^{-0.1(V_m+30)}} K_T \\
\alpha_n &= \frac{-0.01(V_m+50)}{e^{-0.1(V_m+50)}-1} K_T \\
\beta_n &= 0.125 e^{-0.0125(V_m+60)} K_T
\end{aligned}
$$

where V_m is expressed in mV and all the α's and β's are expressed in 1/ms.