BIOSENOSRS

BIO 580

Electrochemical Biosensors - theory part 1

WEEK 1

Fall Semester

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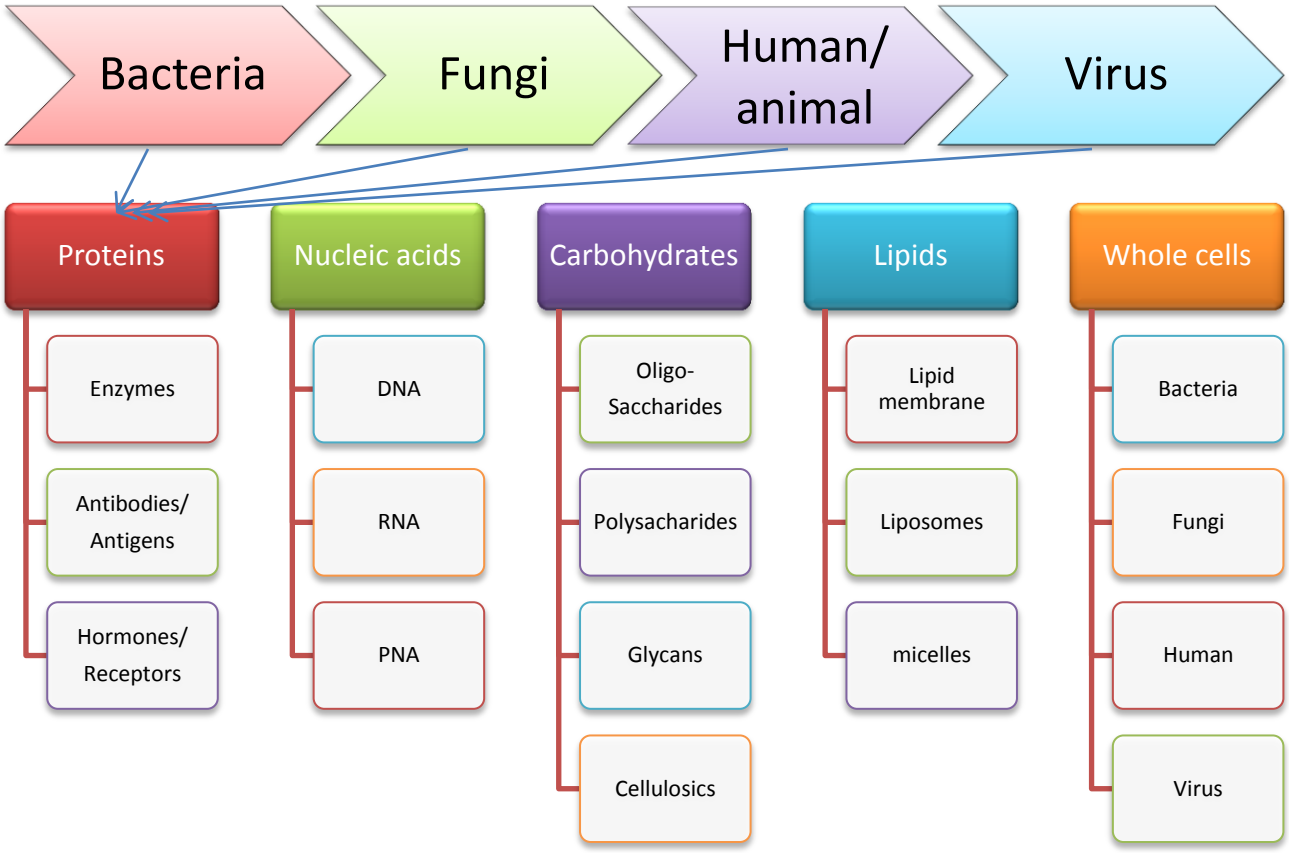
Topics that will be covered in the course

- ❑ History of biosensor development, applications and requirements of biosensors and classification
- ❑ Principles of molecular recognition and transduction signal acquisition
  - ✓Sources of Biological Recognition elements – enzymes/proteins, ssDNAs, antibody and Others
  - ✓Design considerations for use of recognition elements in biosensors
  - ✓Modeling of reactions for various biosensor applications- electrochemical, optical, piezoelectric, colorimetric, fluorometric and others.
- ❑ Modification of sensor surfaces and immobilization techniques
  - ✓Covalent modification of surfaces using surface chemistry
  - ✓Self Assembled Monolayers (SAM) and adsorptions
  - ✓Other ways to immobilize biological macromolecules on various solid surfaces
- ❑ Detection methods and Physical Sensors
  - ✓Electrodes/transducers – electrochemical (amperometric, potentiometric, and conductimetric transductions)
  - ✓Other sensors - for e.g., optical sensors (colorimetric/fluorimetric/luminometric sensors), Surface Plasmon Resonance (SPR) sensors, and piezoelectric resonators.
- ❑ Fabrication of biosensors
  - ✓Miniaturization-application of nano-materials, nanoparticles, carbon nanotubes (CNTs) and others
  - ✓Biocompatibility – stability, reproducibility and repeatability of biomolecules on transducer surfaces
- ❑ Data acquisition, statistical and error analysis
  - ✓Inter and Intra-assays and Coefficient of variation (CV)
  - ✓Signal to noise ratio
  - ✓Normalization/optimization and signal retrieval
- ❑ Examples of commercial biosensors

Sources of Biological recognition elements



Biosensor Recognition Elements

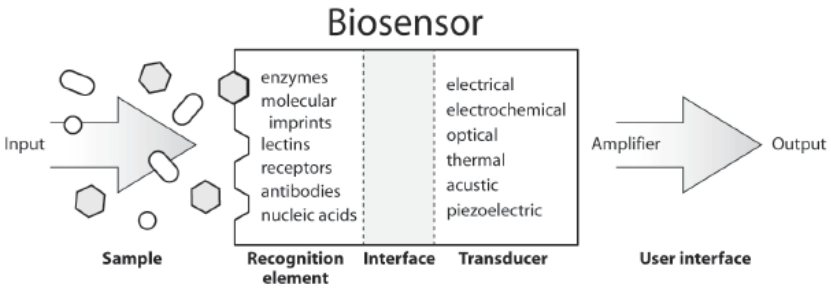
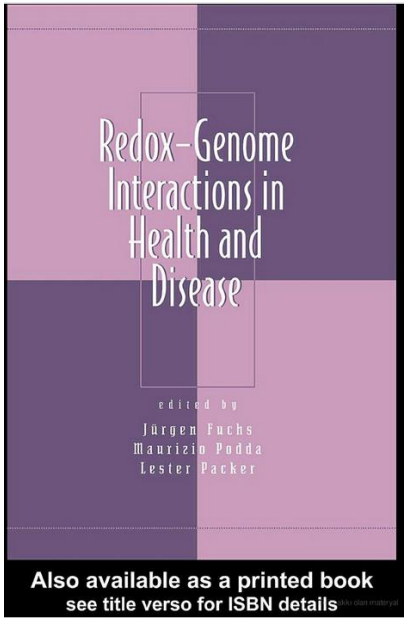
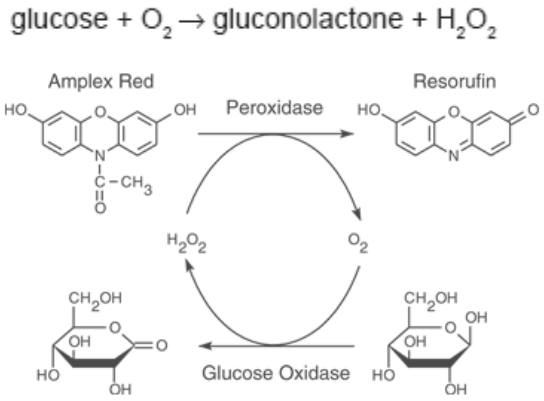
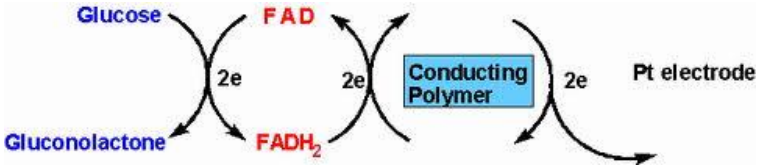


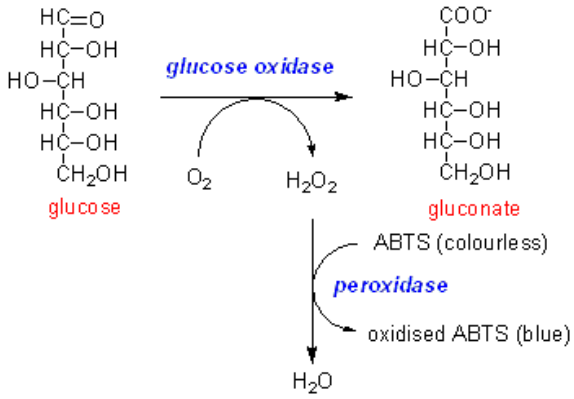
Fig. 1 Configuration of a biosensor showing biorecognition, interface, and transduction elements.



Enzymes: eg., glucose oxidase for detection/measurement of glucose

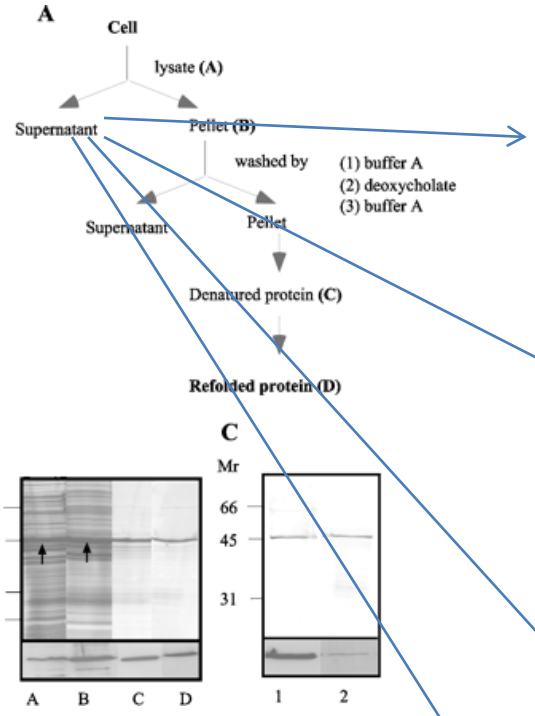


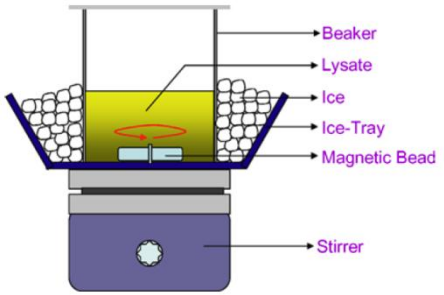


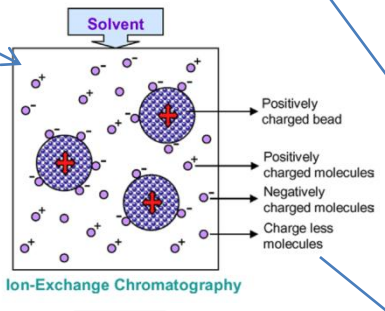


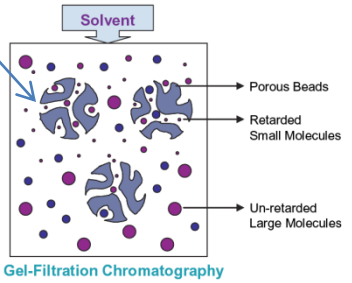
ABTS = 2,2'-azo-di-(3-ethylbenzothiazoyl-sulphonate)

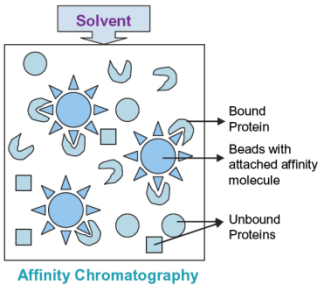
Typical enzyme isolation from cells











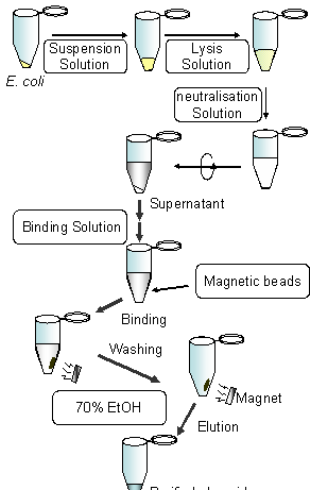
Ammonium Sulfate Precipitation Table:

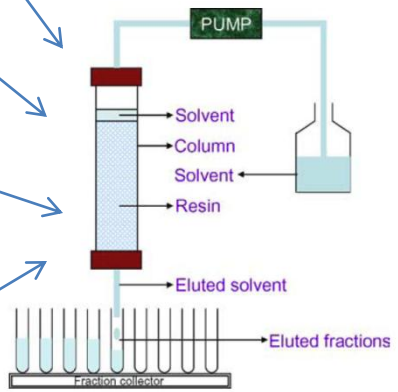
The initial and final concentration of ammonium sulfate, expressed as percent saturation, are on the vertical and horizontal scales, respectively. The point of intersection of two lines drawn from these points indicates the number of grams of salt to be added to each littler of solution at the initial concentration to lead to the final concentration required.

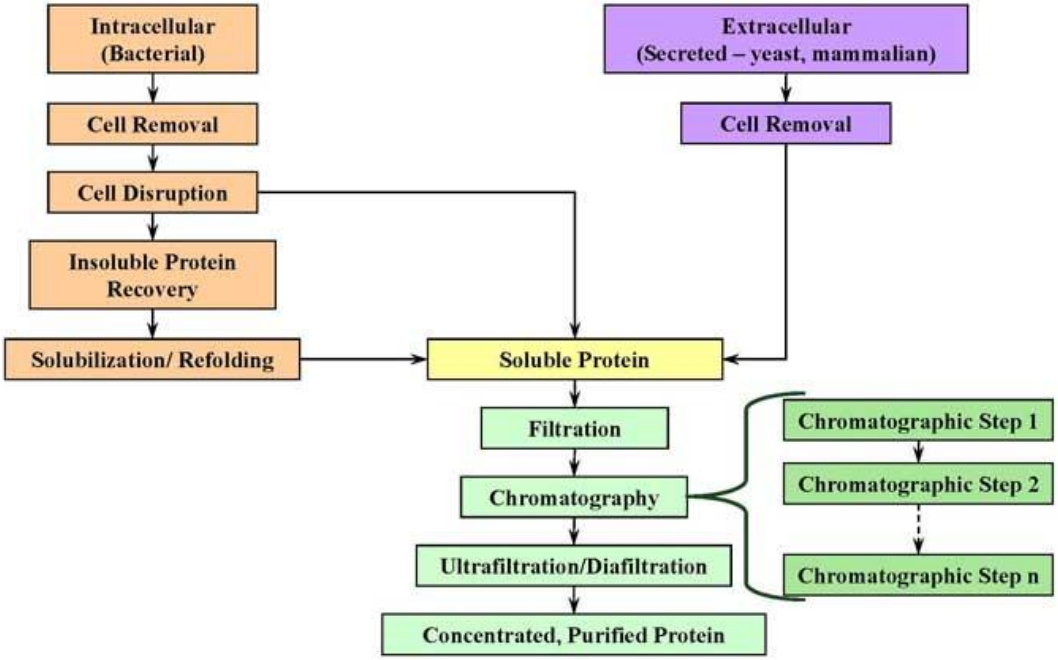
Initial concentration of ammonium sulfate	Solid ammonium sulfate (grams) to be added to 1 liter of solution																
0	106	134	164	194	226	258	291	326	361	398	436	476	516	559	603	650	697
5	79	108	137	166	197	229	262	296	331	368	405	444	484	526	570	615	662
10	53	81	109	139	169	200	233	266	301	337	374	412	452	493	536	581	627
15	26	54	82	111	141	172	204	237	271	306	343	381	420	460	503	547	592
20	0	27	55	83	113	143	175	207	241	276	312	349	387	427	469	512	557
25		0	27	56	84	115	146	179	211	245	280	317	355	395	436	478	522
30			0	28	56	86	117	148	181	214	249	285	323	362	402	445	488
35				0	28	57	87	118	151	184	218	254	291	329	369	410	453
40					0	29	58	89	120	153	187	222	258	296	335	376	418
45						0	29	59	90	123	156	190	226	263	302	342	383
50							0	30	60	92	125	159	194	230	268	308	348
55								0	30	61	93	127	161	197	235	273	313
60									0	31	62	95	129	164	201	239	279
65										0	31	63	97	132	168	205	244
70											0	32	65	99	134	171	209
75												0	32	66	101	137	174
80													0	33	67	103	139
85														0	34	68	105
90															0	34	70
95																0	35
100																	0

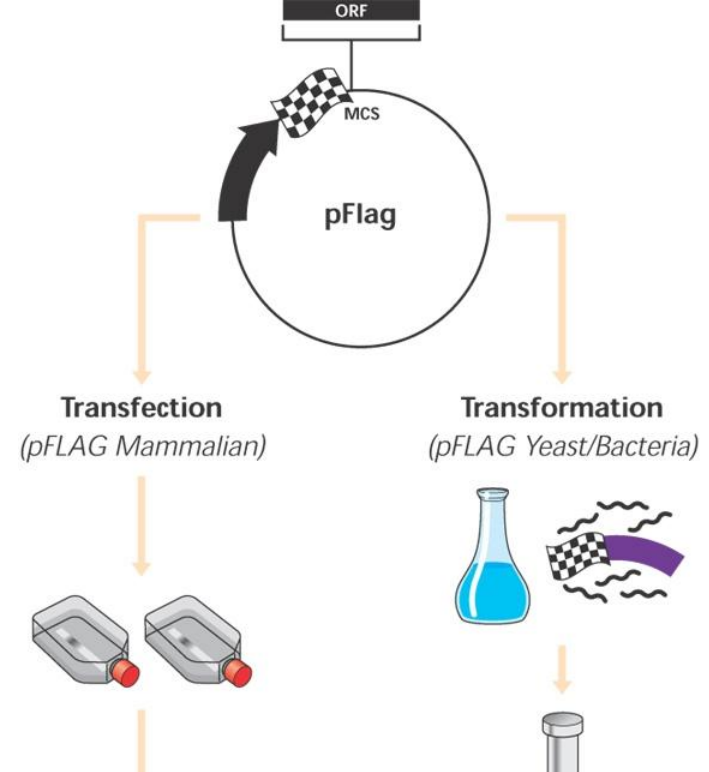
Adapted from "Data for Biochemical Research" (R.M.C. Dawson, D.C. Elliott, and K.M. Jones, eds.), 2nd Ed. Oxford Univ. Press, London, 1969.

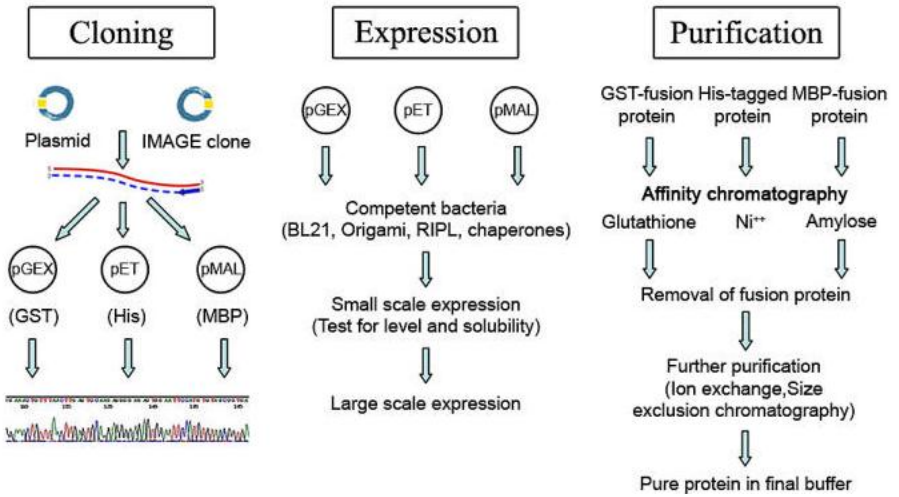
J. Biol. Chem.-2000-Askarian-Amiri-17241-8.pdf

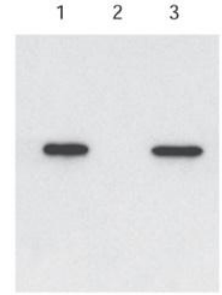












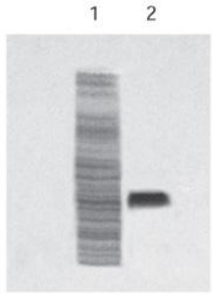
Detection of FLAG fusion protein with ANTI-FLAG® M2 Antibody

Lane 1. FLAG-BAP™* control protein

Lane 2. COS-7 Cell extract control

Lane 3. pFLAG™-CMV-2-BAP* transfected COS-7 cell extract

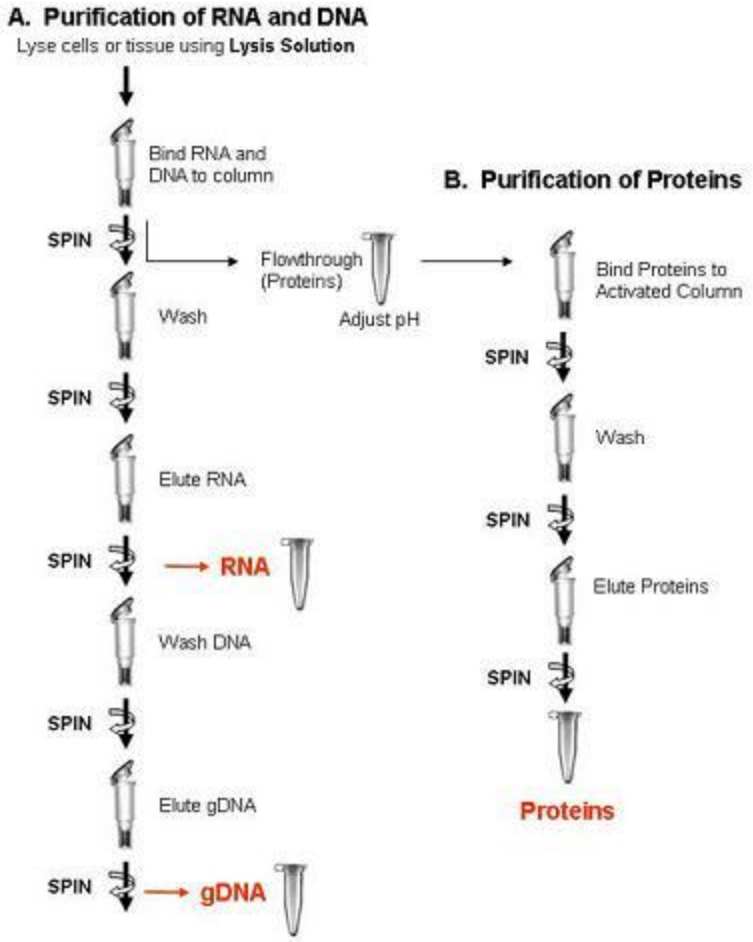
*BAP: Bacterial alkaline phospatase

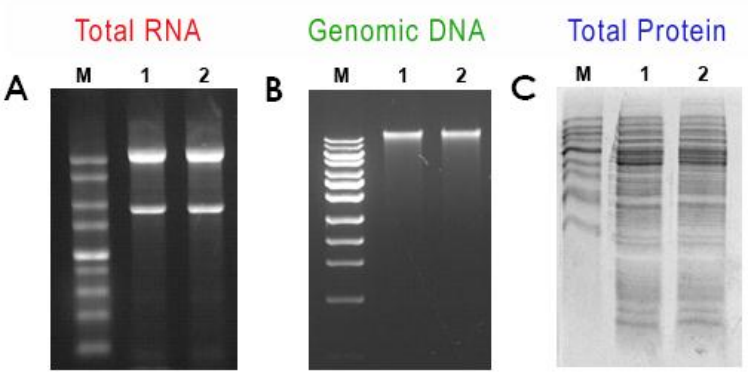


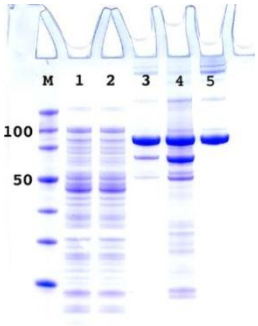
SDS-PAGE of cell lysates form *E. coli* transfected with pFLAG-ATS-BAP purified with ANTI-FLAG M2 affinity gel

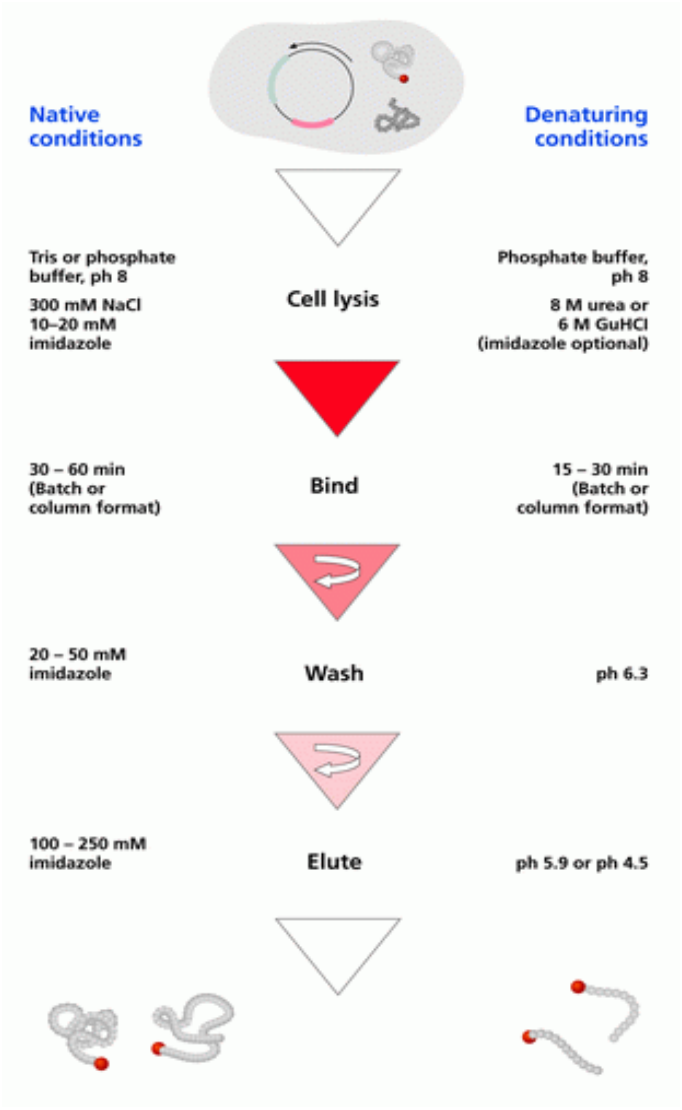
Lane 1. Cell extract prior to purification

Lane 2. Affinity purified FLAG-BAP* fusion protein









6000-7000 different proteins in a cell

Avogadro's number and the mole

Avogadro's number = 6.02×10^{23} atoms/molecules/particles

Name of Substance	Formula	Formula Weight (amu)	Molar Mass (g/mol)	Number and Kind of Particles in One Mole
Atomic nitrogen	N	14.0	14.0	6.02×10^{23} N atoms
Molecular nitrogen	N_2	28.0	28.0	6.02×10^{23} N_2 molecules $2(6.02 \times 10^{23})$ N atoms
Silver	Ag	107.9	107.9	6.02×10^{23} Ag atoms
Silver ions	Ag^+	107.9[a]	107.9	6.02×10^{23} Ag^+ ions
Barium chloride	$BaCl_2$	208.2	208.2	6.02×10^{23} $BaCl_2$ units 6.02×10^{23} Ba^{2+} ions $2(6.02 \times 10^{23})$ Cl^- ions

[a] Recall that the electron has negligible mass; thus, ions and atoms have essentially the same mass.

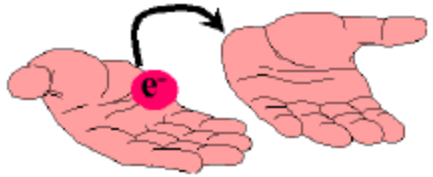
- One mole of atoms, ions, or molecules contains Avogadro's number of those particles.
- One mole of molecules or formula units contains Avogadro's number times the number of atoms or ions of each element in the compound

Electrochemical Biosensor

Electrochemistry -

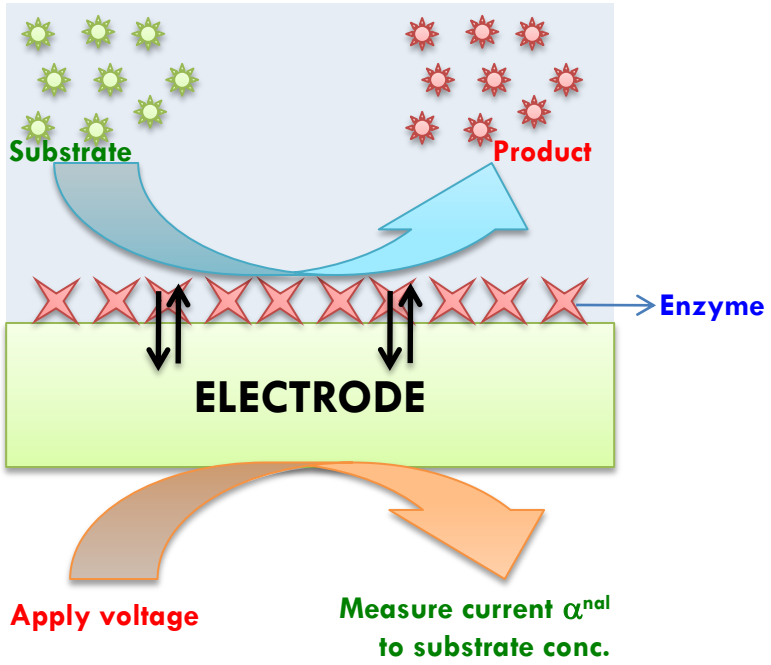
a. branch of chemistry that studies chemical reactions and processes in which electric charges are involved
b. Transfer of an electron from a species in solution to an electrode, or vice versa
c. Common in the analytical field and has resulted in the development of:
   - ¤ Potentiometry
   - ¤ Voltammetry (amperometry)
   - ¤ Coulometry - an electrolysis reaction by measuring the amount of electricity (in coulombs) consumed or produced

Where there is oxidation, there is reduction



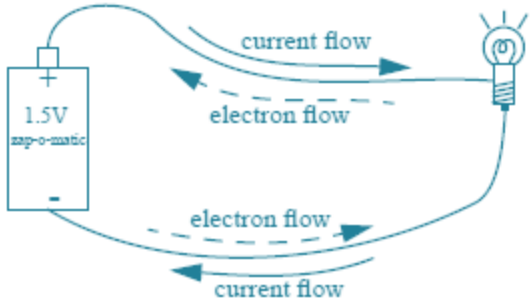
Substance oxidized loses electron(s)

Substance reduced gains electron(s)



Conventional current flow is opposite to electron flow

eecs.oregonstate.edu/~traylor/ece112/.../elect_flow_vs_conv_l.pdf



Electrochemical Biosensor

There are three basic electrochemical processes - useful in transducers for biosensors:

i. **Potentiometry** - the measurement of a cell potential at zero current

ii. **Voltammetry (amperometry)** - an oxidizing (or reducing) potential is applied between the cell electrodes and the cell current is measured

iii. **Conductimetry** - the conductance (reciprocal of resistance) of the cell is measured by an alternating current bridge method.

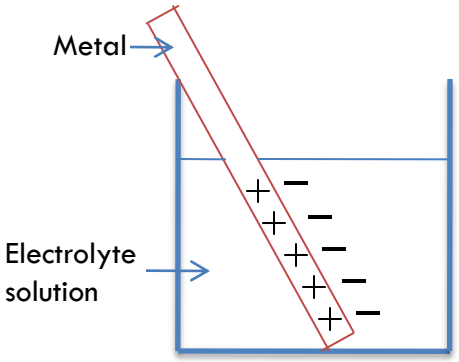
Electrical measurements on a solution of the analyte using two or more electrodes (electronic conductors)

POTENTIOMETRY	AMPEROMETRY	VOLTAMMETRY
✦ Measure potential difference (E) at zero current ✦ Two electrodes are used – indicator and reference ✦ Carried out under equilibrium conditions ✦ Quantitative analysis – E is related to the concentration of ions in the sample	✦ Measure current at fixed potential ✦ Two or three electrodes are used ✦ Coulometry – when the current is integrated to give total charge	✦Measure current as a function of scanned potential ✦ Three electrodes are used – working, reference and counter (auxiliary) ✦ Non-equilibrium measurement – gives kinetic information ✦ Qualitative and quantitative analysis ✦ Polarography – use of a dropping Hg electrode

i. Potentiometry

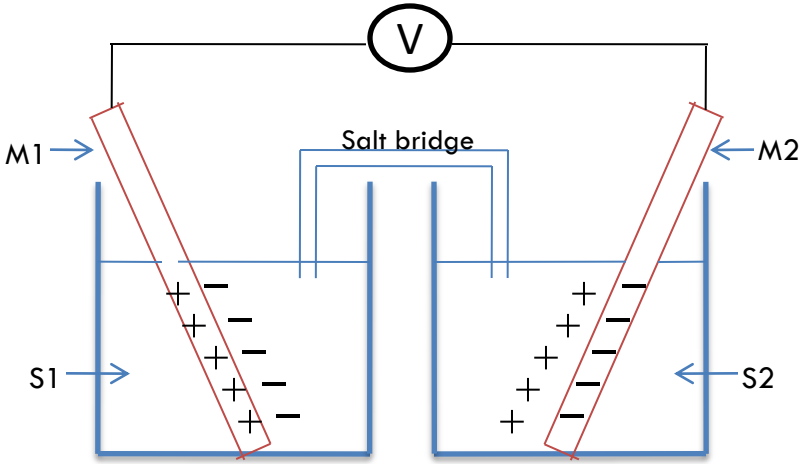
1. Potentiometry - principle

A metal electrode dipped in electrolyte solution (one half cell)



- ✓ If a piece of metal is placed in an electrolyte solution, there is charge separation b/w metal (electrode) and the solution
- ✓ Sets up an electron pressure, usually called a **potential**.
- ✓ It cannot be measured directly - requires a combination of two such electrode-electrolyte solution combinations.
- ✓ Each is called a **half-cell**.

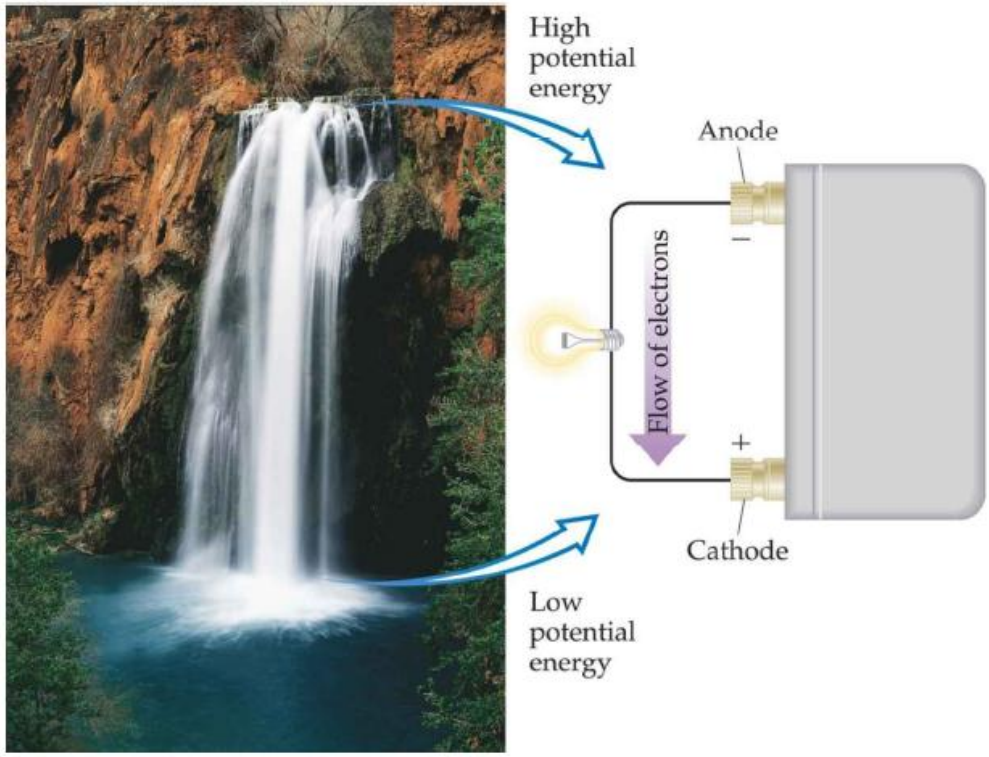
Two half-cell electrodes combined, making a complete cell



- ✓ Two half cells -connected by means of an electrically **conducting bridge or membrane**
- ✓ Two electrodes are connected externally by a potential measuring device (digital voltmeter, DVM).
- ✓ DVM has a very high internal impedance ($\sim 10^{12}\Omega$) - such that very little current will flow through it.
- ✓ If the voltage to be measured is 1V, then the Ohm's law (V=IR), current $I = 10^{-12}$ A (1pA)
- ✓ The electrical circuit is now complete and the e.m.f. of the cell can be measured.
- ✓ This value is the difference between the electrode potentials of the two half-cells.

Electromotive force (e.m.f)

- Water only spontaneously flows one way in a waterfall.
- Likewise, electrons only spontaneously flow one way in a redox reaction : from higher to lower potential energy.



The potential difference between the anode and cathode in a cell is called the electromotive force (emf).

It is also called the cell potential, and is designated E_{cell}.

Ohm's law

To make a current flow through a resistance there must be a voltage across that resistance. Ohm's Law shows the relationship between the voltage (V), current (I) and resistance (R). It can be written in three ways:

$$V=IxR \quad \text{(or)} \quad I=V/R \quad \text{(or)} \quad R=V/I$$

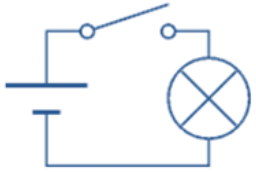
where: **V** = voltage in volts (V)
I = current in amps (A)
R = resistance in ohms (Ω)

Voltage can be thought of as the pressure pushing charges along a conductor, while the electrical resistance of a conductor is a measure of how difficult it is to push the charges along.

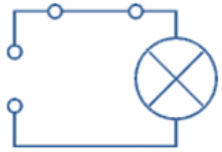
Voltage is the Cause, Current is the Effect

Voltage attempts to make a current flow, and current will flow if the circuit is complete. Voltage is sometimes described as the 'push' or 'force' of the electricity, it isn't really a force but this may help you to imagine what is happening. It is possible to have voltage without current, but current cannot flow without voltage.

Voltage and Current
The switch is closed making a complete circuit so current can flow.



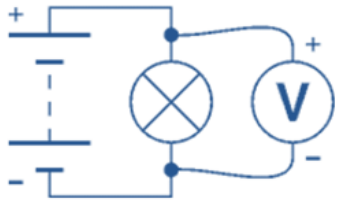
Voltage but No Current
The switch is open so the circuit is broken and current cannot flow.



No Voltage and No Current
Without the cell there is no source of voltage so current cannot flow.

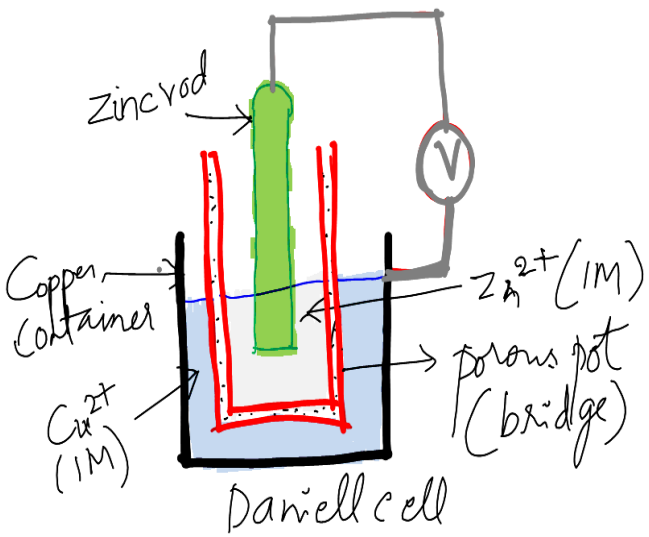
Voltage, V

- Voltage is a measure of the **energy carried by the charge**.
  Strictly: voltage is the "energy per unit charge".
- The proper name for voltage is **potential difference** or p.d. for short, but this term is rarely used in electronics.
- Voltage is **supplied by the battery** (or power supply).
- Voltage is **used up in components**, but not in wires.
- We say **voltage across** a component.
- Voltage is measured in **volts**, **V**.
- Voltage is measured with a **voltmeter**, connected in **parallel**.
- The symbol **V** is used for voltage in equations.



Connecting a voltmeter in parallel

Daniell cell - an example for potentiometry



Observed electrode volt

E_{obs}= 1.10 V

Gibbs free energy, the energy that can be converted into work at a uniform temperature and pressure throughout a system

✓If we consider each half-cell ->reaction for each half-cell is:

$Cu^{2+} + 2e^- = Cu$ (half-cell electrode reaction 1)

$Zn^{2+} + 2e^- = Zn$ (another reaction 2)

If we subtract reaction equation 2 from 1 we obtain

$$Cu^{2+} + Zn = Cu + Zn^{2+}$$

✓The Gibbs free energy (ΔG) for this reaction is negative (spontaneous in the direction shown).

✓The ΔG is simply related to the e.m.f of the cell:

$$\Delta G = -nFE$$

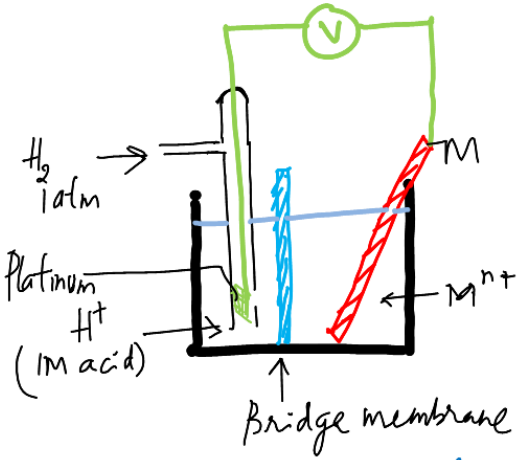
n-No. of electrons transferred (here n = 2)

F - is Faraday constant = 96,487 C/mol

E - is the e.m.f of the cell

What the ΔG values are for reactions 1 and 2 separately ?

Hydrogen electrode (separate measurement of std. **electrode potential** of one half-cell)



Hydrogen electrode connected to another half-cell

- If ΔG_1 and ΔG_2, we could find E_1 and E_2 separately.
- Hydrogen electrode provides separation of E_1 and E_2.

Hydrogen is not a metal but it can be oxidized to H^+ by the removal of an electron:

$$H - e^- = H^+$$

Also written as : $H^+ + e^- = \frac{1}{2}H_2$

ΔG for this reaction is ZERO

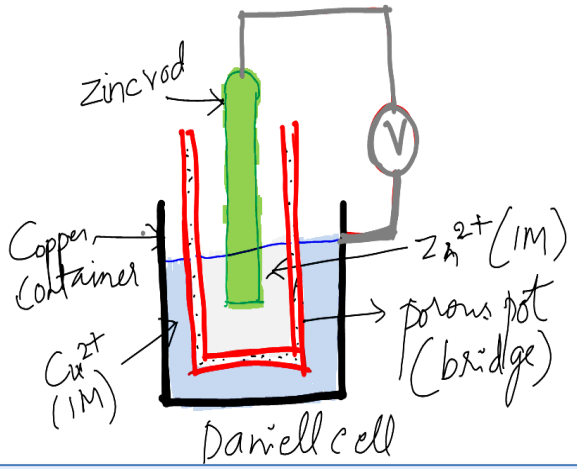
The std. state being with $[H^+]$ - 1M, partial pressure of H_2=1 and temp = 298 K (25 °C).

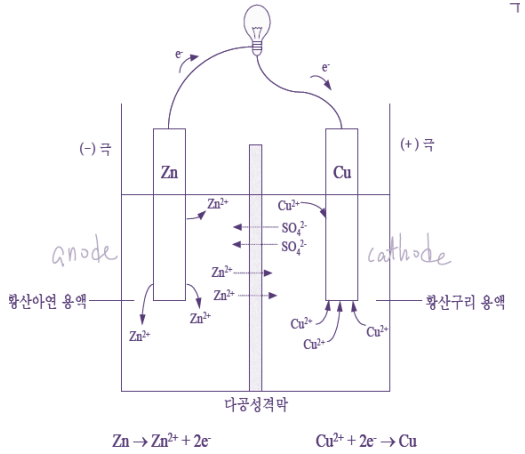
The Gibbs free energy is designated ΔG^0

The std. electrode potential for hydrogen is therefore:

$$E^0_{H^+/H2} = 0$$

Half-cell Potential (single electrode)





Practical- half-cell of **hydrogen** electrode: *this can be combined with any other half-cell electrode*

Eg., $Cu_2^+ + 2e^- = Cu$ E_1.....3

$2H^+ + 2e^- = H_2$ $E_H (=0)$ 4

Subtracting eqn. 4 from eqn. 3:

$Cu_2^+ + H_2 = Cu + 2H^+$

Thus $E_{cell} = E_1 - E_H = +0.34$ V

Therefore, $E^0_{Cu} = +0.34$ V (one half)

The other half of the Daniel Cell, the **zinc** electrode:

$Zn_2^+ + 2e^- = Zn$ E_{Zn}.....5

$2H^+ + 2e^- = H_2$ $E_H (=0)$ 6

Subtracting eqn. 6 from eqn. 5:

$Zn_2^+ + H_2 = Zn + 2H^+$

Thus $E_{cell} = E_{Zn} - E_H = -0.76$ V

Therefore, $E^0_{Zn} = -0.76$ V (other half)

Combining two half-cell e.m.f.s for copper and zinc gives the cell e.m.f. for the Daniel cell

$E_{cell} = +0.34 - (-0.76) =$ **1.10 V**

Cell Potential

Cell potential is measured in volts (V).

$$1\ V = 1\ \frac{J}{C}$$

Standard Cell Potentials - The cell potential at standard conditions can be found through this equation:

$$E^\circ_{cell} = E^\circ_{red}\ (cathode) - E^\circ_{red}\ (anode)$$

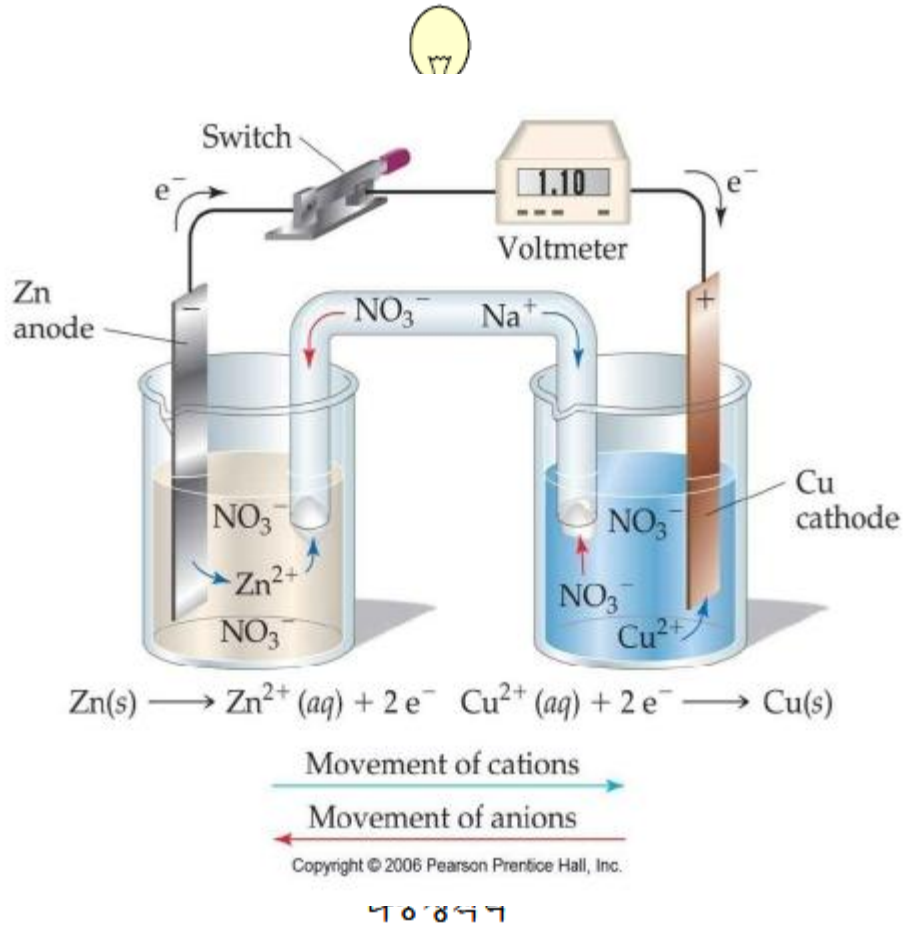
Because cell potential is based on the potential energy per unit of charge, it is an intensive property.

e.m.f (V) = Work/energy (Joule, J)/Charge (Columb, C)

$$E^\circ_{cell} = E^\circ_{red}\ (cathode) - E^\circ_{red}\ (anode)$$
$$= +0.34\ V - (-0.76\ V)$$
$$= +1.10\ V$$

		E°
	$Cu^{2+} + 2e^- \rightleftharpoons Cu(s)$	+0.340
−	$Zn^{2+} + 2e^- \rightleftharpoons Zn(s)$	-0.763
	$Cu^{2+} + Zn(s) \rightleftharpoons Cu(s) + Zn^{2+}$	$E^\circ_{cell} = +1.103\ V$

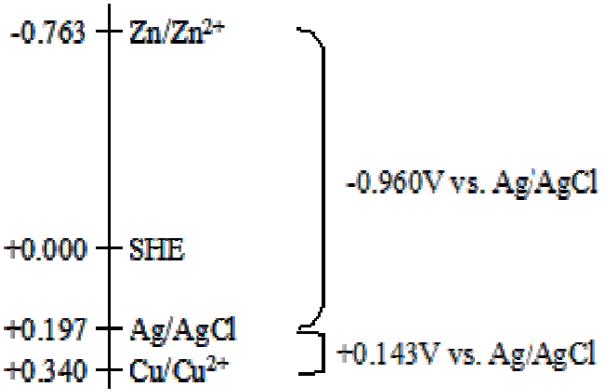
Electron transfer through electrode/solution interface - A schematic representation



Bard

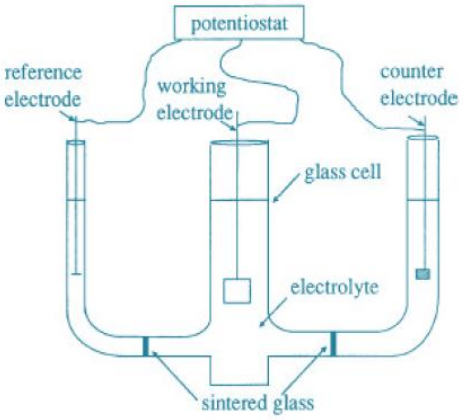
$Zn \rightarrow Zn^{2+} + 2e^-$ $Cu^{2+} + 2e^- \rightarrow Cu$

Standard Electrode Potential (E^0) for few reactions



Half-reaction	E°, V
$Li^+(aq) + e^- \rightarrow Li(s)$	-3.040
$K^+(aq) + e^- \rightarrow K(s)$	-2.924
$Na^+(aq) + e^- \rightarrow Na(s)$	-2.713
$Al^{3+}(aq) + 3e^- \rightarrow Al(s)$	-1.676
$Zn^{2+}(aq) + 2e^- \rightarrow Zn(s)$	-0.763
$Fe^{2+}(aq) + 2e^- \rightarrow Fe(s)$	-0.440
$2H^+(aq) + 2e^- \rightarrow H_2(g)$	**0.000**
$Cu^{2+}(aq) + e^- \rightarrow Cu(s)$	+0.340
$I_2(s) + 2e \rightarrow 2I^-$	+0.535
$Ag^+(aq) + e^- \rightarrow Ag(s)$	+0.800
$Br_2(l) + 2e^- \rightarrow 2Br^-(aq)$	+1.065
$Cl_2(g) + 2e^- \rightarrow 2Cl^-(aq)$	+1.358
$Au^+ + e^- \rightarrow Au(s)$	+1.680
$F_2(g) + 2e^- \rightarrow 2F^-(aq)$	+2.866

Three electrode system



Schematic of an electrochemical cell

Cathode is the working/indicator electrode. (right half-cell). - Anode is the counter/reference electrode. (left half-cell).

Components of Electrochemical cell

Three electrodes:
Working, Counter, and Reference

An electrolyte solution:
Solvent, supporting

Membrane: O or X

Potentiostat: e^- delivery of, or redox reactions
Involves the transmission of ions

The potential difference between the anode and cathode in a cell is called the electromotive force (emf).

It is also called the cell potential, and is designated E_{cell}.

Potentiometer

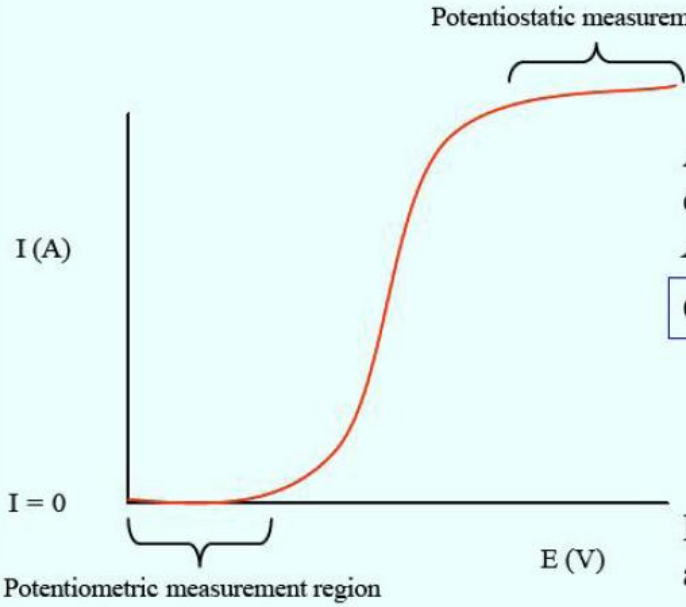
- A device for measuring the potential of an electrochemical cell without drawing a current or altering the cell's composition.

Potentiometric measurements:

Potentiometric measurements are made using a potentiometer to determine the difference in potential between a working (an indicator) electrode and a counter (a reference) electrode.

- Cathode is the working/indicator electrode. (right half-cell)
- Anode is the counter/reference electrode. (left half-cell)

Potentiometric vs. Potentiostatic Methods



Amperometry: Changing current as a function of time - *A variable current condition*

Current *vs.* [analyte]

Potentiometry: Changing voltage as a function of time - *A zero (0) current condition*

Voltage *vs.* [analyte]

Principles of Potentiometry

- The cell voltage, E_{cell}, of a galvanic cell is measured at I = 0, i.e. when the electrochemical system is at equilibrium.
- E_{cell} is governed by the electrode potential of the indicator electrode, E_{IE}
- E_{IE} responds to the activity, a_i, of the analyte
- a_i is related to the concentration, c_i, of an analyte
- E_{cell} and E_{IE} depend on $a_{reactants}$ and $a_{products}$ of the electrode reactions
- The E_{cell} or E_{IE} are related to the activities of the reaction species by the NERNST equation:

$$E = E^{\circ} + \left(\frac{RT}{nF}\right) \ln\left(\frac{a_{ox}}{a_{red}}\right)$$

$$E = E^\circ + \left(\frac{RT}{nF}\right)\ln\left(\frac{a_{ox}}{a_{red}}\right)$$

- **E^o** is the **formal potential** – the potential of the half cell (*vs.* a reference system) when $a_{ox}/a_{red} = 1$ and all other species are present at specified concentrations (eg. specified pH and electrolyte).
- **R** is the molar gas constant, 8.314 J K^{-1} mol^{-1}
- **T** is the absolute temperature (K)
- **n** is the number of electrons transferred
- **F** is Faraday's constant, 96500 C mol^{-1}
- $\mathbf{a_{ox}/a_{red}}$ is the ratio of the activity of the oxidized and reduced forms of the analyte

For infinitely dilute solutions ($C_i < 10^{-1}$ M) $a_i \cong C_i$

POTENTIOMETRIC BIOSENSORS [I]

- In potentiometric sensors, the zero-current potential (relative to a reference) developed at a selective membrane or electrode surface in contact with a sample solution is related to analyte concentration (logarithmic relationship between measured potential and analyte concentration).
- The main use of potentiometric transducers in biosensors: a pH-stat (the reactions consume or produce protons).
- The biosensor consists of an immobilized enzyme membrane surrounding the probe from a pH-meter:
- **Mechanism: pH changes cause changes in enzyme activity; pH maintained at a constant value by the addition of acid or base → the rate of titrant addition proportional to the rate of enzymatic reaction (specially within low buffer concentration!)**
- **Three types of ion-selective electrodes** which are of use in biosensors:

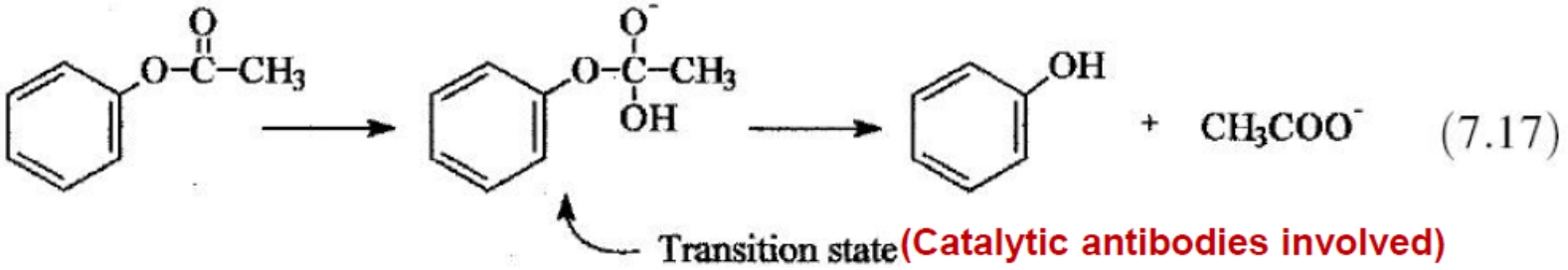
- Glass electrodes for cations (typical potentiometric biosensors)

- Glass pH electrodes coated with a gas-permeable membrane selective for CO_2, NH_3 or H_2S.

- The iodide electrode is useful for the determination of I^- or CN^- in the peroxidase reaction in penicillinase reaction mediated with I^- or CN^- .

POTENTIOMETRIC BIOSENSORS

- Ex) for phenyl acetate as a model for the evaluation of catalytic antibodies:



- **The reaction:** hydrolytic cleavage of the ester linkage of phenyl acatate.
- Ab trapped at the surface of a pH electrode using a dialysis membrane will bind to the transition state of the hydrolysis reaction.

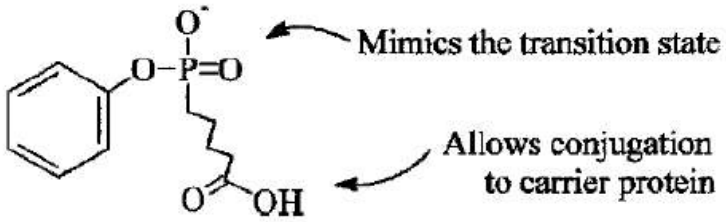
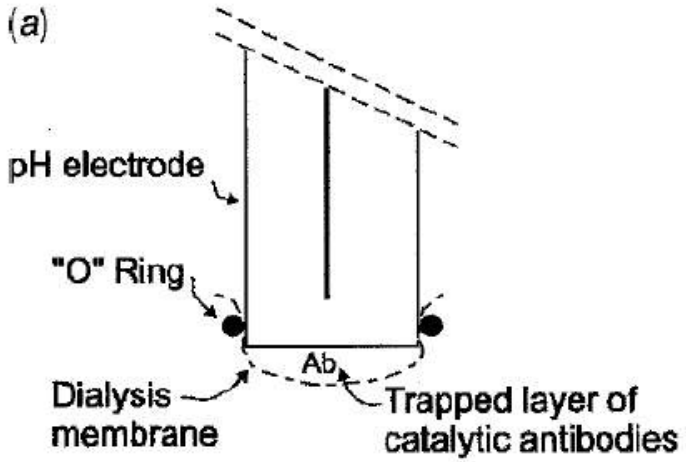


Figure 7.3. Transition state analogue hapten used for the generation of catalytic antibodies for phenyl acetate hydrolysis.

A substance that modifies the transition state to lower the activation energy is termed a catalyst; a biological catalyst is termed an enzyme

- Ex) for phenyl acetate as a model for the evaluation of catalytic antibodies:
- **Mechanism of detection:** Ab trapped at the surface of a pH electrode using a dialysis membrane will bind to the transition state of the hydrolysis reaction → the reaction produce a change in local pH at the surface of the electrode due to an acetic acid as a product → The measured pH decreases as the phenyl acetate concentration increases
- Selectivity: a number of similar compounds produce signal, particularly those containing RCOOC6H5 group but the selectivity similar to the corresponding hydrolytic enzyme.
- A novel use of Abs as chemical recognition agents!



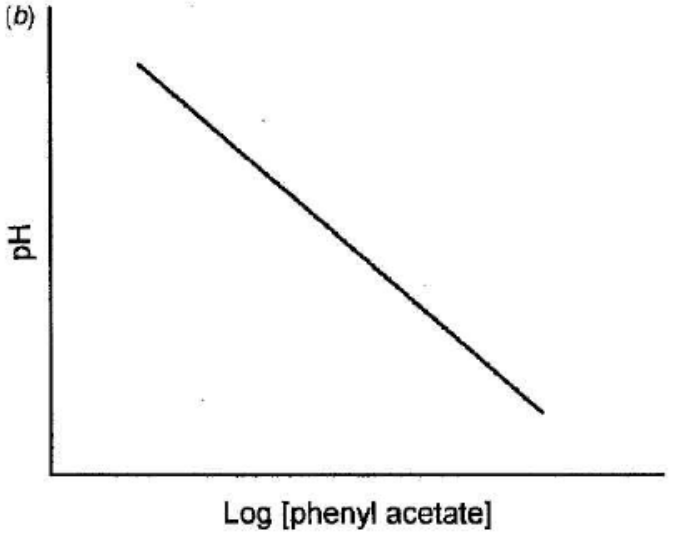


Figure 7.4. (*a*) Diagram of phenyl acetate sensor and (*b*) calibration curve.

POTENTIOMETRIC BIOSENSORS [II]

- The best known potentiometric sensor is the Ion Selective Electrode (ISE).
- ISEs used in conjunction with immobilised enzymes can serve as the basis of electrodes that are selective for specific enzyme substrates.
- The two main ones are for urea and creatinine - These potentiometric enzyme electrodes are produced by entrapment the enzymes urease and creatinase, on the surface of a cation sensitive (NH_4^+) ISE.
- Solvent polymeric membrane electrodes are commercially available and routinely used for the selective detection of several ions such as K^+, Na^+, Ca^{2+}, NH_4^+, H^+, CO_3^{2-}) in complex biological matrices.
- The antibiotics nonactin and valinomycin serve as neutral carriers for the determination of NH_4^+ and K^+, respectively

POTENTIOMETRIC BIOSENSORS

- E = Eo + RT/nF *ln*[analyte]
  - Eo is a constant for the system, R is the universal gas constant, T is the absolute temperature, z is the charge number, F is the Faraday number
  - *ln*[analyte] is the natural logarithm of the analyte activity.
  - E is measured potential (two electrodes system)

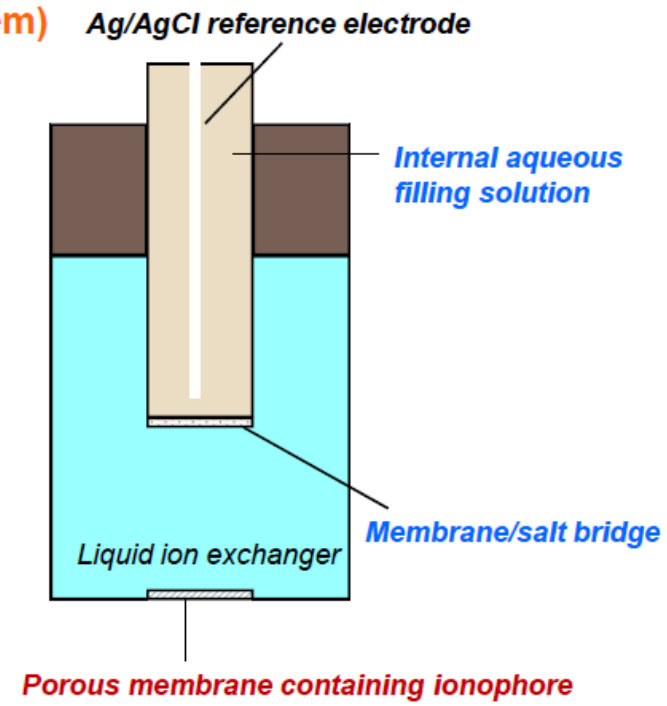
$$\text{Urea} + H_2O + H^+ \xrightleftharpoons{\text{urease}} 2NH_4^+ + HCO_3^-$$

$$\text{Creatinine} + H_2O \xrightarrow{\text{creatininase}} \text{N-methylhydantoin} + NH_4^+$$

$$\text{Penicillin} \xrightarrow{\text{penicillinase}} \text{Penicillonic Acid}$$

↓

In contact with pH electrode.



POTENTIOMETRIC BIOSENSORS - immunosensor

Ex) for digoxin:

- Employs a potassium (K+) ion-selective electrode (ISE) as the transducer:
- Chemical recognition: anti-digoxin Ab and a digoxin-crown ether conjugate.
- PVC [a poly(vinyl chloride)] membrane – separate internal K+ solution from an external layer of digoxin Ab trapped at the sensor surface.
- **Mechanism:** competition between free digoxin (analyte) crossing the dialysis membrane freely and the digoxin-crown conjugate at the PVC-aqueous interface → change in the transport of K+ across the PVC membrane (Ab-digoxin-crown held at the interface!)

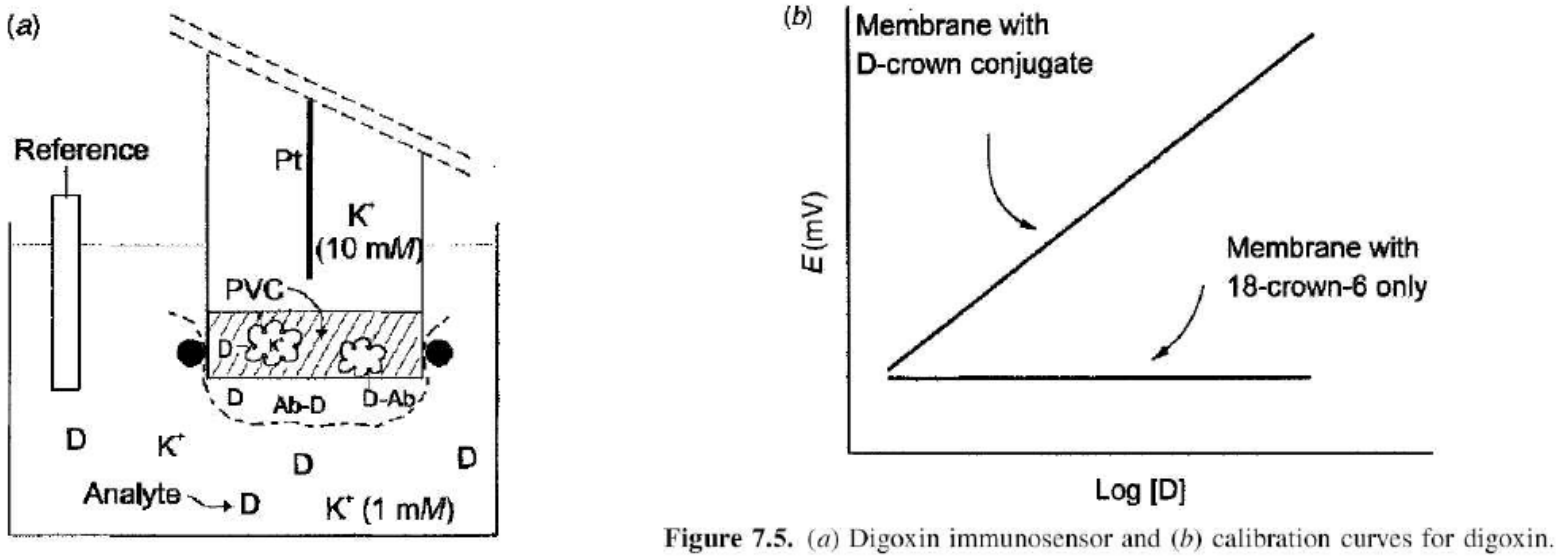


Figure 7.5. (*a*) Digoxin immunosensor and (*b*) calibration curves for digoxin.

Potentiometric Immunosensor

- employs K^+ ion-selective electrode as a transducer
- Chemical recognition involves — anti digoxin Ab & digoxin-crown ether conjugate
- PVC membrane seperates an internal K^+ rich layer of digoxin Ab — trapped at sensor surface with a dialysis membrane.
- The digoxin-crown ether conjugate — selectively binds K^+ present only in the PVC layer → hydrophobic properties
- Competition b/w free digoxin (analyte) that freely crosses the dialysis membrane & the digoxin-crown conjugate at the PVC aqueous interface for antibody in the reaction which causes a change in the transport of K^+ across the membrane
- because the antibody-digoxin-crown conjugate is held at the interface
- ~~An~~ ex.. of competitive immunosensor - Binding Interaction to be monitored.