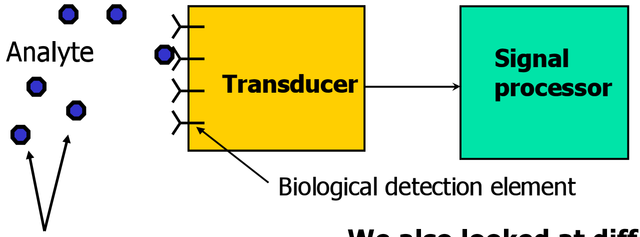
Welcome to Lecture 5

Let's see where we are

Broadly defined "biosensor" is a measuring device that contains a biological sensing element

Biosensor Function: To selectively respond to an analyte through a biochemical reaction/binding and to enable qualitative or quantitative determination of this analyte



Analyte

Transducer

Signal processor

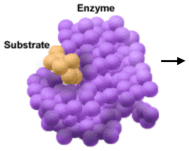
Biological detection element

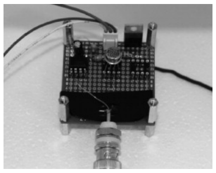
Target substance to be detected/analyzed

We also looked at different biosensing elements

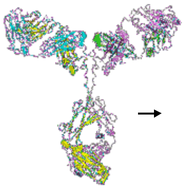
Bioreceptors

Enzymes:

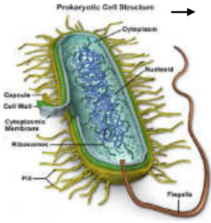

Enzyme
Substrate



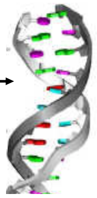
Antibodies:



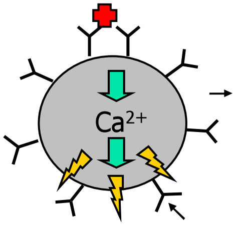
Bacteria:


Prokaryotic Cell Structure
Cytoplasm
Nucleoid
Capsule
Cell Wall
Cytoplasmic Membrane
Ribosomes
Pili
Flagella

Nucleic acids



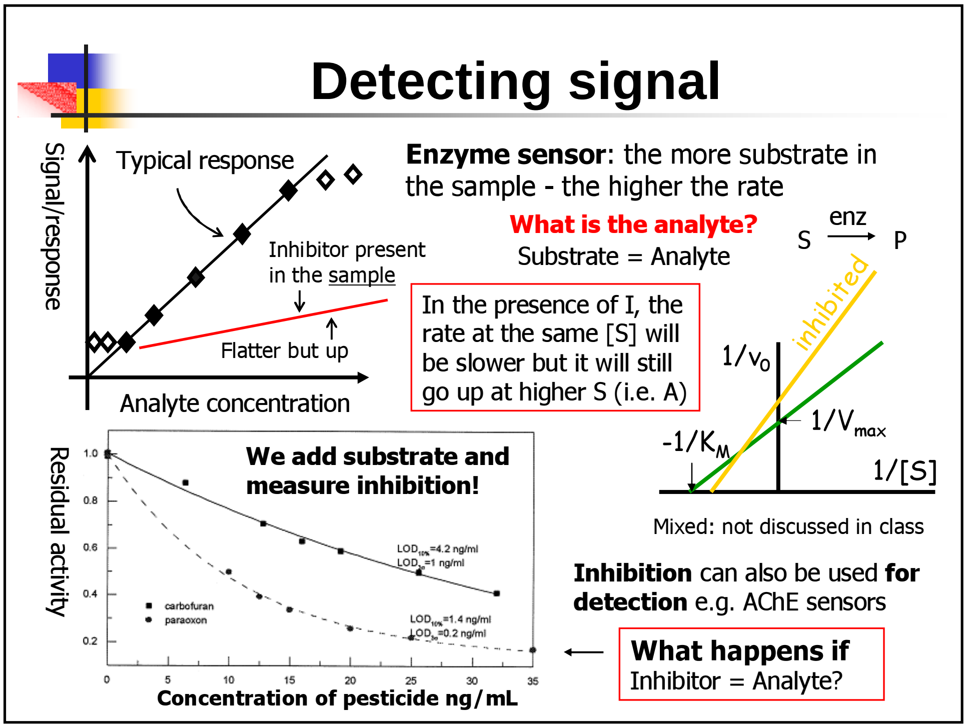
Human cells: CANARY



Pathogen specific Abs

Areas: defense, health care, environment, etc

More about NA later



Detecting signal

Signal/response
Typical response
Inhibitor present in the sample
Flatter but up
Analyte concentration
Residual activity
1.0
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
35
carbofuran
paraoxon
LOD$_{10\%}$=4.2 ng/ml
LOD$_{3\sigma}$=1 ng/ml
LOD$_{10\%}$=1.4 ng/ml
LOD$_{3\sigma}$=0.2 ng/ml
Concentration of pesticide ng/mL
1/v$_0$
inhibited
-1/K$_M$
1/V$_{max}$
1/[S]

We add substrate and measure inhibition!

Enzyme sensor: the more substrate in the sample - the higher the rate

What is the analyte?

Substrate = Analyte

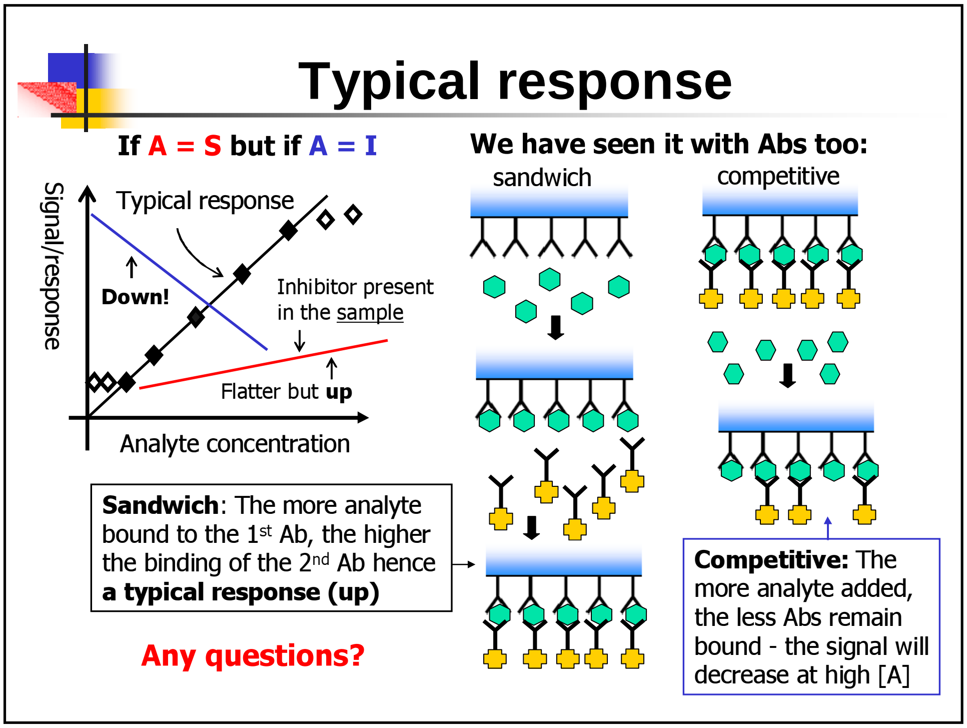
$S \xrightarrow{enz} P$

In the presence of I, the rate at the same [S] will be slower but it will still go up at higher S (i.e. A)

Mixed: not discussed in class

Inhibition can also be used **for detection** e.g. AChE sensors

What happens if Inhibitor = Analyte?



Typical response

If A = S but if A = I

Signal/response
Typical response
Down!
Inhibitor present in the sample
Flatter but up
Analyte concentration
sandwich
competitive

We have seen it with Abs too:

Sandwich: The more analyte bound to the 1st Ab, the higher the binding of the 2nd Ab hence **a typical response (up)**

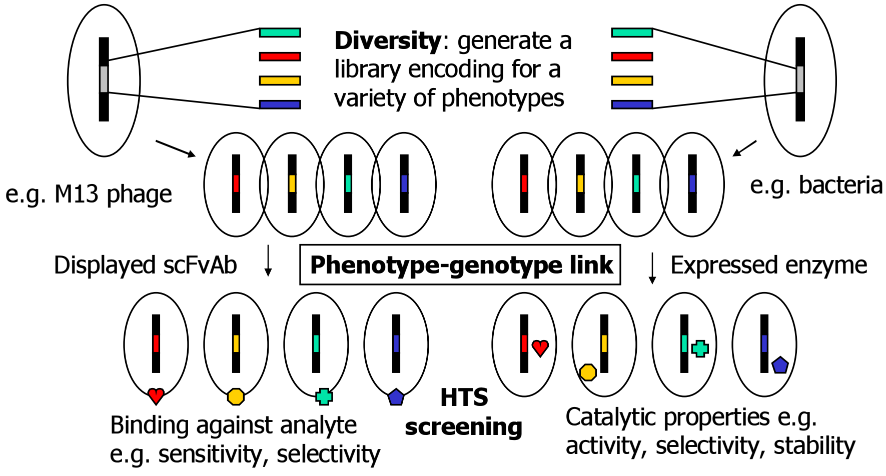
Competitive: The more analyte added, the less Abs remain bound - the signal will decrease at high [A]

Any questions?

Enhancing technologies

Three critical "S" – Selectivity, Sensitivity and Stability

We have seen some great examples and there are technologies available for enhancing the desired properties of Enz and Abs



Diversity: generate a library encoding for a variety of phenotypes

e.g. M13 phage

e.g. bacteria

Displayed scFvAb

Phenotype-genotype link

Expressed enzyme

HTS screening

Binding against analyte e.g. sensitivity, selectivity

Catalytic properties e.g. activity, selectivity, stability

PE vs DE

What is the difference between directed evolution and rational protein engineering?

Protein Engineering:	**Directed evolution**:
•Absolute requirements of on the 3D structure – extensive modeling	•Some structural information is desirable but it's not essential
•Introduce one mutation at the time and hope it's good	•Make a huge library and hope to find a good mutant
•Not requirement for screening	•Smart HTS is essential

How do you know whether you got a good mutant?

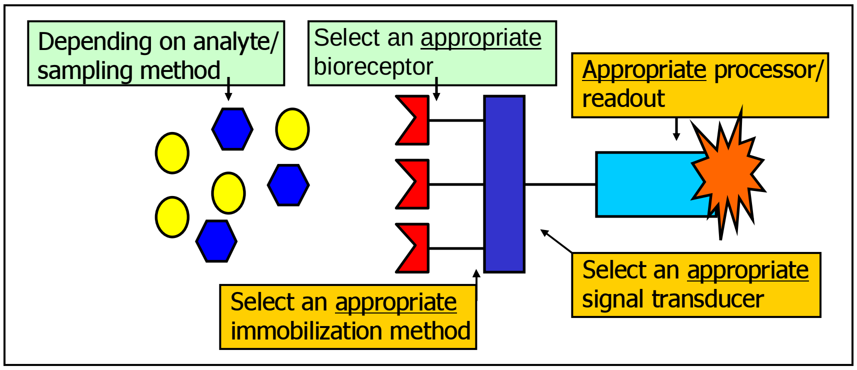
Specificity constant: $\frac{kcat}{Km}$

Allow quantitative comparisons of catalytic efficiency/specificity of different enzymes, and different substrates for the same enzyme

Still a long way go

And you, guys, have a your own cool **bio-**sensor and the structure of your sensing molecule or something similar ☺

What does it take to make it?



Depending on analyte/ sampling method

Select an appropriate bioreceptor

Appropriate processor/ readout

Select an appropriate signal transducer

Select an appropriate immobilization method

We are going to address this next, but first…

Last quiz

What are hydrogen bonds, ionic bonds and what forces hold the protein structure together?

H bonds are formed:

- H-H interactions (or two hydrogen molecules)
- between the H molecule/atoms and another molecules/atoms
- between N-terminus and C terminus of an alfa helix
- between adjacent molecules
- by removal of water molecule
- with the removal of hydrogen, mainly as water
- when water is formed when 2 molecules create a bond
- by H and this bond

Last quiz

What are hydrogen bonds, ionic bonds and what forces hold the protein structure together?

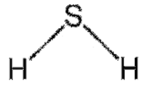
Hydrogen bonds are:

- responsible for stability of the molecule or solubility in water
- helpful in making the hydrolysis of proteins
- are those that enable the structure to accommodate substrate
- bonds that provide an attachment for the protein structure

Plenty of answers - a bond between CO and NH

Hydrogen bonds

What is the difference in physical properties?

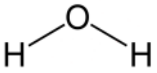


Hydrogen sulfide

H_2S **WHY?** H_2O

Boiling point
-60°C

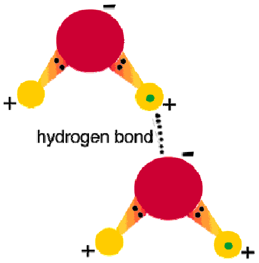
Boiling point
100°C

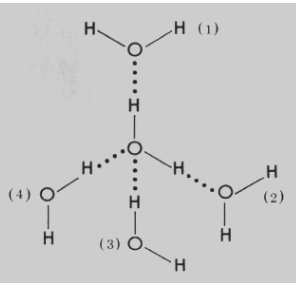


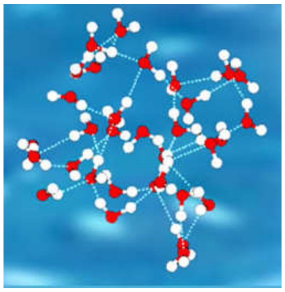
Water

Polar molecules e.g. water have a weak, partial negative charge on one part of the molecule (the oxygen atom in H_2O) and a partial positive charge elsewhere (the hydrogen atoms in H_2O)

Water forms a network of H bonds



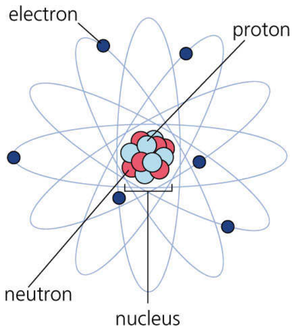




Chemical bonds and atoms

Type of chemical bonds:

- Ionic bonds
- Covalent bond
- Metallic Bonds
- Hydrogen bonds
- Van der Waals forces

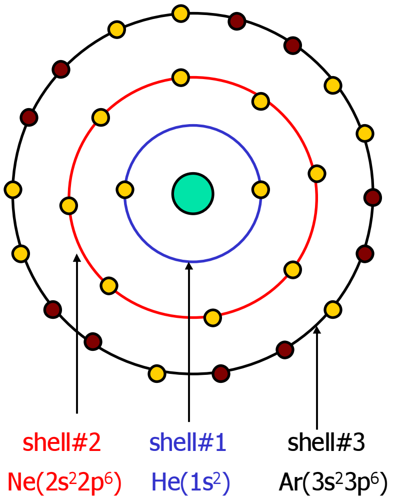

electron
proton
neutron
nucleus

Starting from the beginning

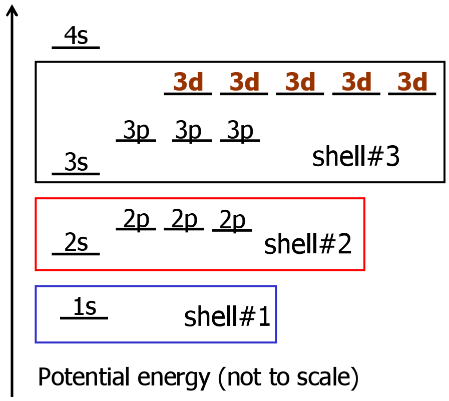
- ➢ One can imagine the atom as a combination of two spheres: one large outer sphere (electrons) and a small one inside (nucleus)
- ➢ The outer sphere comprises negatively charged electrons that move around the nucleus
- ➢ A much denser nucleus comprises of two types of particles: positively charged protons and neutrally-charged neutrons

A biologist view of the atom

Outer shells:


shell#2
Ne($2s^22p^6$)
shell#1
He($1s^2$)
shell#3
Ar($3s^23p^6$)

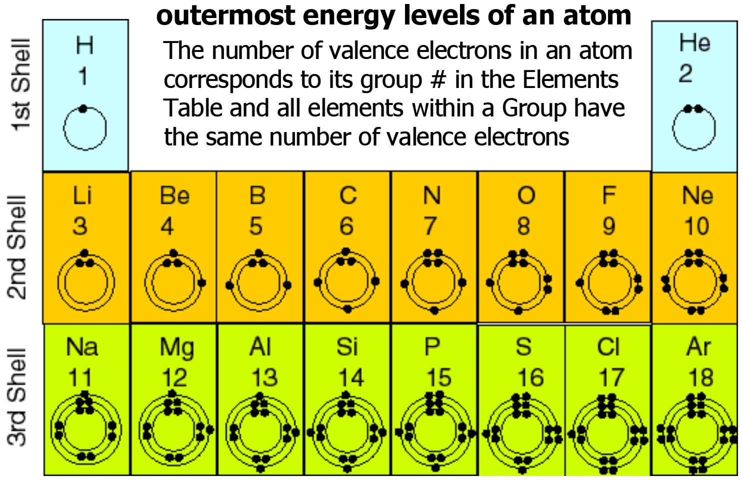
Electron pairs in each shell:


4s
3d 3d 3d 3d 3d
3p 3p 3p
3s
shell#3
2p 2p 2p
2s
shell#2
1s
shell#1
Potential energy (not to scale)

With d electrons it gets more complicated

Valence electrons

Valence electrons are defined as electrons found in the outermost energy levels of an atom


1st Shell
2nd Shell
3rd Shell
H 1
He 2
Li 3
Be 4
B 5
C 6
N 7
O 8
F 9
Ne 10
Na 11
Mg 12
Al 13
Si 14
P 15
S 16
Cl 17
Ar 18

The number of valence electrons in an atom corresponds to its group # in the Elements Table and all elements within a Group have the same number of valence electrons

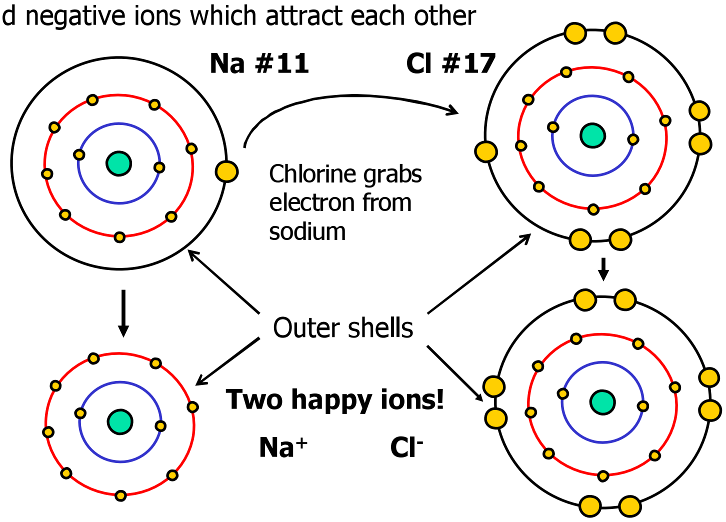
Shell are filled staring from the closest to the nucleus

A few simple principles:

- Atoms are most stable when their outer shells are completely filled
- In search of stability atoms interact with each other e.g. forming chemical bonds
- In most cases it is the valence electrons that make chemical bonds
- The nature of the bond depends on the type of interacting atoms and on their environment (e.g. solvents)

Ionic bonds

Ionic bonds: one or more electrons from one atom are removed and attached to another atom, resulting in positive and negative ions which attract each other



Na #11

Cl #17

Chlorine grabs electron from sodium

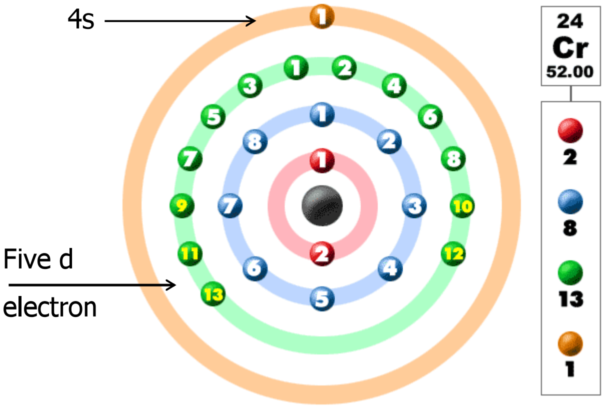
Outer shells

Two happy ions!

Na^{+} **Cl^{-}**

Chromium ions

Which chromium ions are most stable?



4s

Five d electron

Cr^{+}

Cr^{2+}

Cr^{3+}

Cr^{4+}

Cr^{5+}

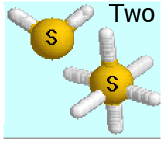
Cr^{+6}

Cr^{3+} most stable

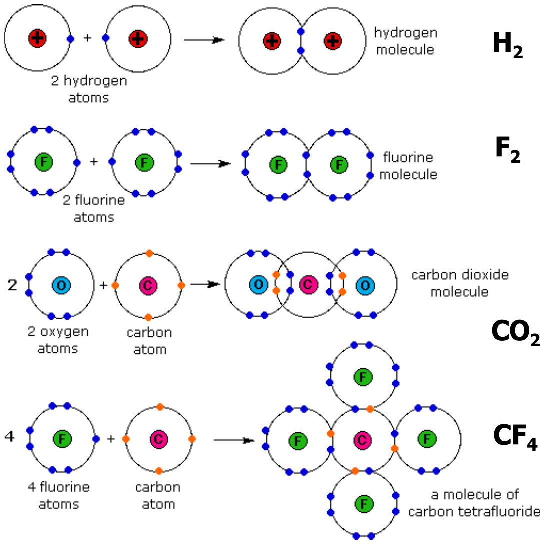
Not that simple with transition metals ☹

Covalent bonds

One or more pairs of electrons are shared by two atoms



Two ways to fill outer shells


2 hydrogen atoms
hydrogen molecule
H_2
2 fluorine atoms
fluorine molecule
F_2
2
2 oxygen atoms
carbon atom
carbon dioxide molecule
CO_2
4
4 fluorine atoms
carbon atom
a molecule of carbon tetrafluoride
CF_4

How is this different from ionic bonds?

Unlike ionic bonds, covalent bonds are formed by sharing of the valence electrons rather than an outright transfer from one atom to another

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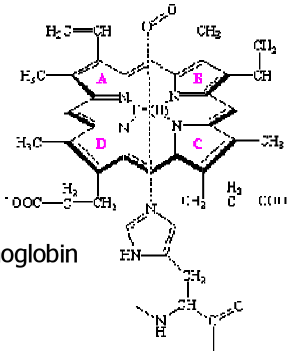
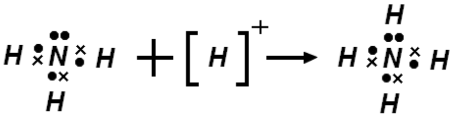
Coordinate covalent bond

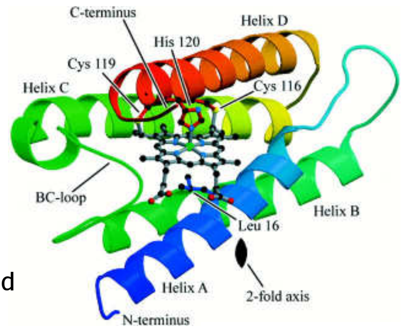
A coordinate bond is a covalent bond between two atoms, in which both shared electrons in the bond come from the same atom


A
B
C
D
Fe(II)
N
N
N
N
H₃C
-OOC
HN

Hemoglobin

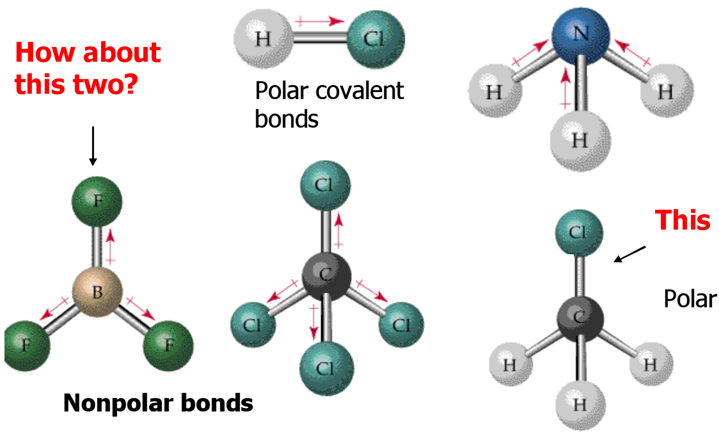

H
N
H
H
+
H
+
H
N
H
H
H

The distinction is artificial - once the bond has been formed, it is no different from any other polar covalent bond


C-terminus
Helix D
His 120
Cys 119
Cys 116
Helix C
BC-loop
Helix B
Leu 16
Helix A
2-fold axis
N-terminus

Cyt C: bound heme

Polarity of the bond



How about this two?

Polar covalent bonds

This one?

Polar bond

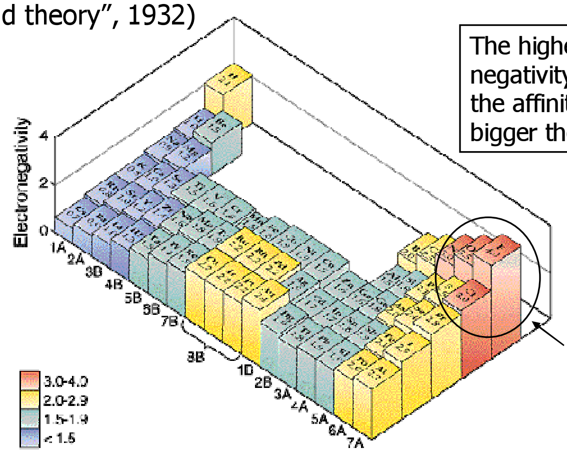
Nonpolar bonds

Polarity of the bond is not the same as polarity of the molecule

How do we know which way electrons move?

Electronegativity

Electronegativity is an intrinsic property of an atom, which describes its power to grab electrons (Pauling in "Valence bond theory", 1932)

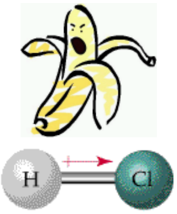


The higher the electro-negativity – the bigger the affinity for e^- i.e. the bigger the grabbing force

Note the red guys here

Electronegativity and polarity

Who is going to get the banana?



- The covalent bond in H-Cl is polar because the bonding electron(s) are attracted to the more electronegative atoms, in this case Cl
- In general the higher the difference in electronegativity, the more polar the bonds are e.g. H-F and H-O bonds are very polar

What would happen if there is even bigger difference in EN?

Let’s summarize

- Covalent bonds are formed as a result of the sharing of one or more pairs of valence electrons, typically each atom donates half of the electrons to be shared
- The polarity of a covalent bond is determined by the electronegativity of the participating atoms (watch out for symmetric arrangements e.g. CCl_4)
- If there is a LARGE difference in electronegativity between the two atoms (>~1.7), then the more electronegative atom can remove electrons from the less electronegative atom - this would result in the formation of an ionic bond

Now we are ready to deal with H-bonds

Hydrogen bond

- It is a bond between one electronegative atom and a hydrogen atom bonded to another electronegative atom. Hydrogen bond always involves H, hence the name
- The electronegative atom to which hydrogen is attached (usually it is O, N, or F) is called a hydrogen bond donor
- Due to bond polarization this atom has a partial negative charge, while the H atom carries a partial positive charge
- A hydrogen bond forms when the positive charge on H is attracted to a lone pair of electrons on another electronegative atom, which is called hydrogen bond acceptor.
- H-bonds can form between different molecules as well as intramolecularly

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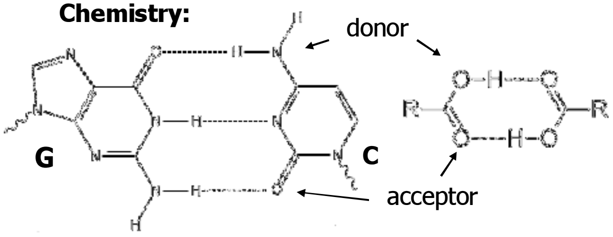
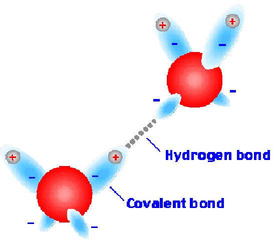
Hydrogen bond

Chemistry:





What bond is this? **And this?**

$A — H^{+} - - - - - - B^{\delta-}$

polar covalent bond **kind of ionic?**

Note the distances!

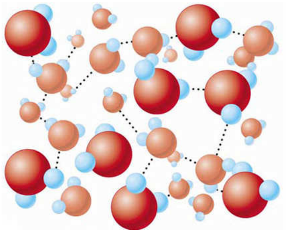
What will happen to the bonds, if H^{+} moves?

$A — H^{+} - - - - - - - B^{-}$

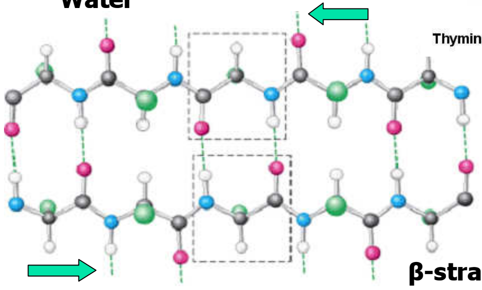
$A^{-} - - - - - - - H^{+} — B$

polar covalent bond

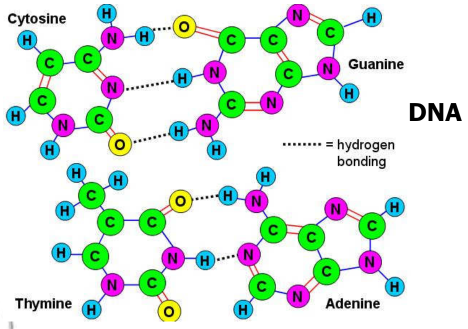
Fundamental to life!



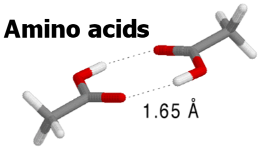
Water



β-strand

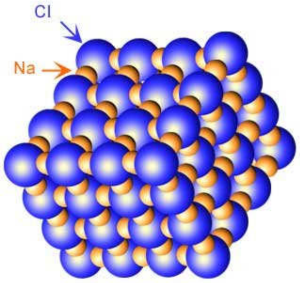

Cytosine
Guanine
DNA
= hydrogen bonding
Thymine
Adenine

Amino acids



Any questions?

Dissolving NaCl



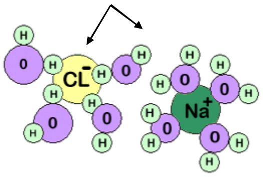
Sodium Chloride Crystal Structure

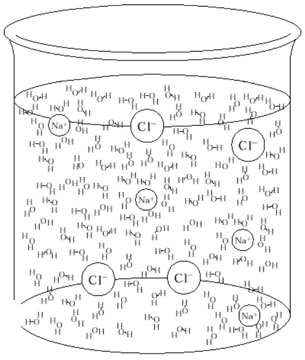
WHY?

water

dissolves

What bonds are these?





Non-polar solvent e.g. octane

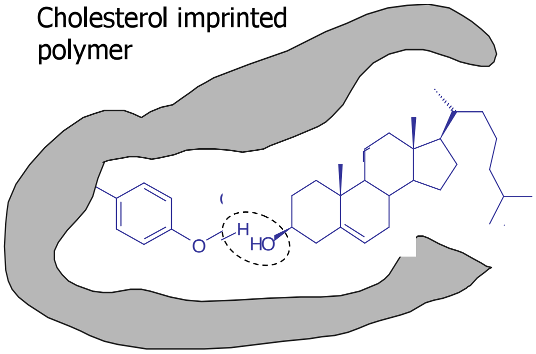
does not dissolve

But proteins also dissolve in water

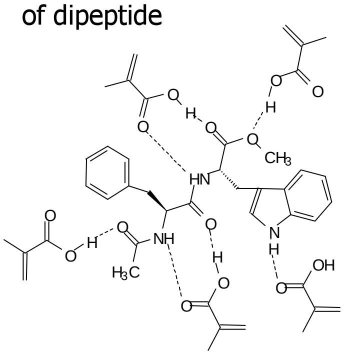
How come ionic bonds do not break in proteins then?

Chemical bonds in imprinting

Cholesterol imprinted polymer



Non-covalent imprinting of dipeptide



Binds cholesterol well but ONLY in non-polar solvents. **Why?**

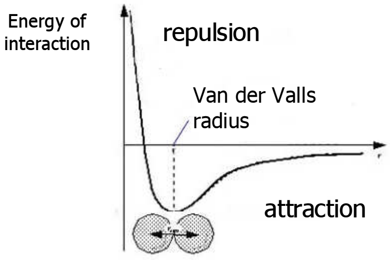
Only one HB: molecules of H-bonding solvent displace cholesterol

What solvent would use to make this complex?

Non-polar: NOT to disrupt hydrogen bonding

Van der Waals forces

Electrically neutral molecules can attract each other at moderate distances and repel each other at close range

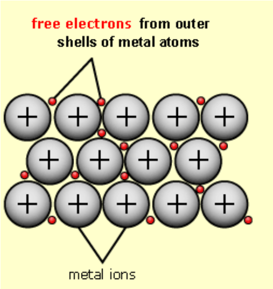


Weak but indispensable for "docking" substrates in binding/catalytic sites

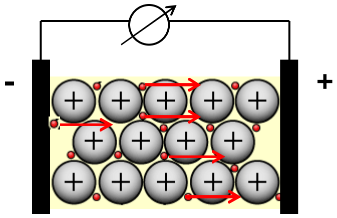
What are Van der Waals forces and why does the graph look this way?

Van der Waals's attraction and repulsion arise from the interactions between positively charged nucleus of one atom with the electron cloud/nucleus of another atom

Metallic bonds:



Metals are conductors



the force that holds atoms together in a metal

- Metals atoms are packed very closely in metallic structures
- In most cases the outermost electron shell of one metal atom overlaps with a large number of neighboring atoms
- As a result the valence electrons are not associated with any specific metal atom but freely move around within the structure
- In essence, the metallic bond is the force of attraction between these free electrons and metal ions

Chemical bonds: summary

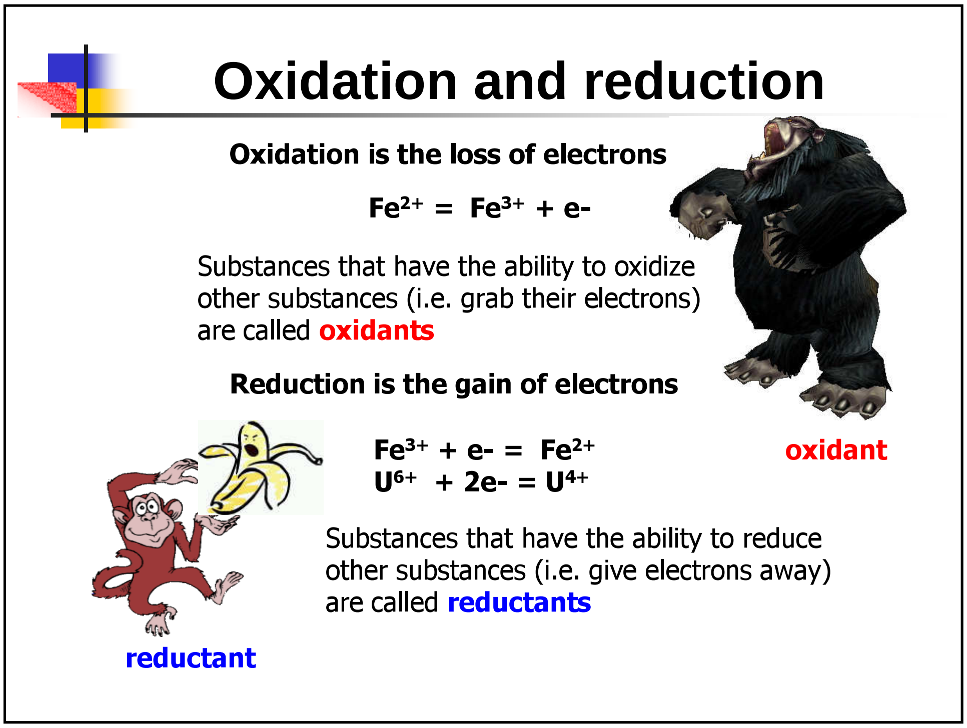
Ionic bonds: one or more electrons from one atom are removed and attached to another atom, resulting in positive and negative ions which attract each other

Covalent bonds: one or more pairs of electrons are shared by two atoms

Hydrogen bonds: is the bond between one electronegative atom and a hydrogen atom bonded to another electronegative atom – “H^+ sharing”

Van der Waals forces: attraction between neutral molecules at moderate distances and repulsion at close range due to the interaction between positively charged nucleus of one atom with the electron cloud/nucleus of another atom

Metallic Bonds: a mobile pool of outer shell electrons that holds metal atoms together in a closely packed metallic structure – “communal electrons”



Oxidation and reduction

Oxidation is the loss of electrons

$$Fe^{2+} = Fe^{3+} + e^-$$

Substances that have the ability to oxidize other substances (i.e. grab their electrons) are called **oxidants**

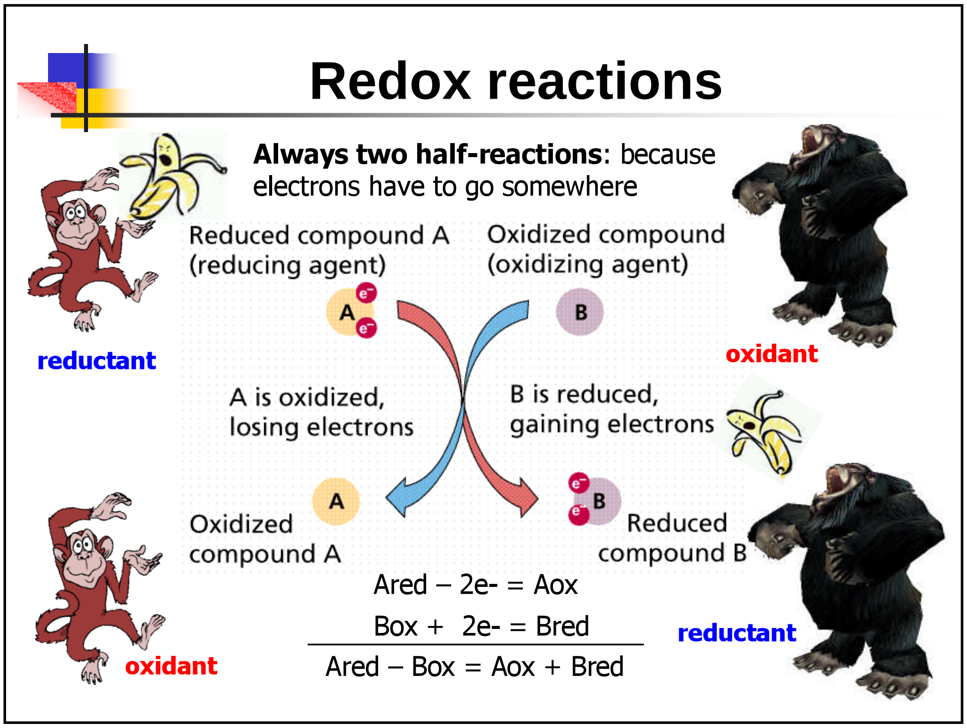
Reduction is the gain of electrons

$$Fe^{3+} + e^- = Fe^{2+}$$
$$U^{6+} + 2e^- = U^{4+}$$

oxidant

Substances that have the ability to reduce other substances (i.e. give electrons away) are called **reductants**

reductant



Redox reactions

Always two half-reactions: because electrons have to go somewhere

Reduced compound A
(reducing agent)
Oxidized compound
(oxidizing agent)
A
e⁻
e⁻
B
A is oxidized,
losing electrons
B is reduced,
gaining electrons
A
Oxidized
compound A
B
e⁻
e⁻
Reduced
compound B

reductant

oxidant

$$Ared - 2e^- = Aox$$
$$Box + 2e^- = Bred$$
$$Ared - Box = Aox + Bred$$

oxidant

reductant

Electrodes

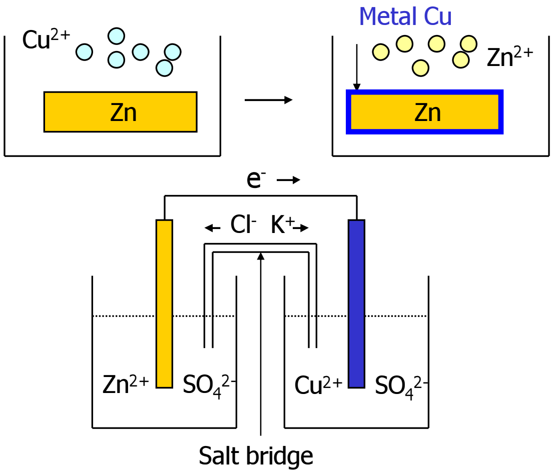
Redox reactions can occur in solution or on solid surfaces - electrodes

Electrodes are electrochemical transducers - interface between chemical and electrical energy

- ✓ Electrodes provide electrons to oxidants (reduction reactions) and remove electron from reductants (oxidation reactions)
- ✓ Electrodes can actively participate in the chemistry or be inert spectators
- ✓ Most electrodes are:
  - Metals, usually platinum, gold, or silver for inert electrodes
  - Metals coated with metal halides or oxides,
  - Graphitic carbon (pressed solid or paste)
  - Semiconductor devices

Electrode reactions

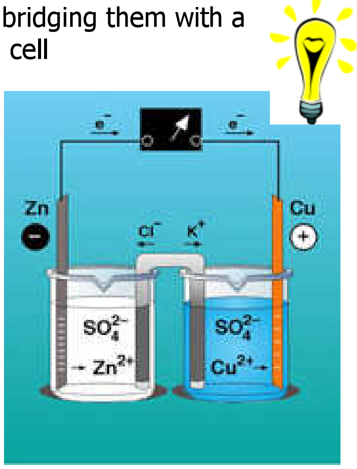
Placing zinc metal in a solution of a copper salt yields copper plating out on the zinc: **$Zn + Cu^{2+} = Zn^{2+} + Cu$**


Metal Cu
Cu^{2+}
Zn
Zn^{2+}
Zn
$e^- \rightarrow$
$\leftarrow Cl^-$ $K^+ \rightarrow$
Zn^{2+}
SO_4^{2-}
Cu^{2+}
SO_4^{2-}
Salt bridge

Electrochemical cell

Placing the metals (e.g. zinc and copper) and their salts in separate containers ("half cells") and bridging them with a salt solution yields an electrochemical cell

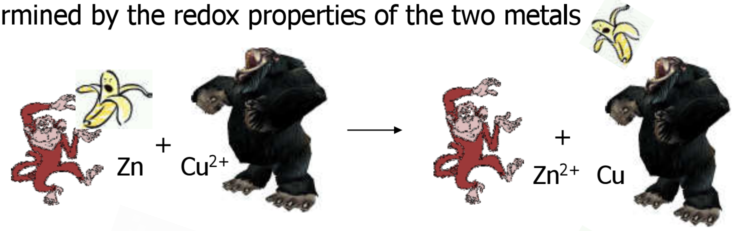
- If a voltmeter is connected to the two metal electrodes, it will show an electric potential difference between the two cells
- Electrical current is flowing from the zinc electrode to the copper electrode
- The zinc electrode gradually disappears and the copper electrode increases
- Negative ions in solution flow toward the zinc electrode (called the anode), and positive ions flow toward the copper (the cathode)



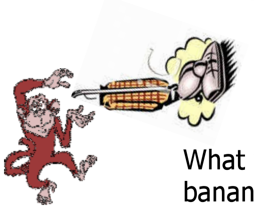
Connecting a light bulb instead will yield glowing light

Electrolytic cells

In an electrochemical cell the direction of electron flow is determined by the redox properties of the two metals



$$Zn + Cu^{2+} \longrightarrow Zn^{2+} + Cu$$



What would happen to the banana if the monkey had a powerful vacuum cleaner?

By applying external energy the electron flow can be reversed

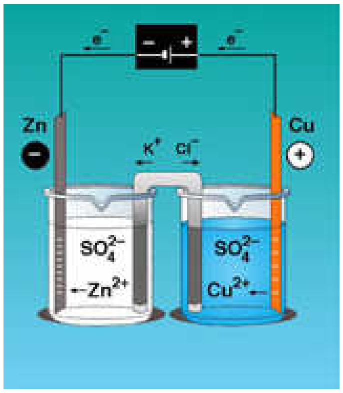
Galvanic and electrolytic cells

Placing a DC power source between the zinc and copper electrodes will reverse the current flow as compared to the previous cell, provided the power supply has a high enough potential

- Now the zinc electrode gains mass, while the copper electrode loses mass
- Positive ions flow to the zinc electrode and negative ions flow to the copper electrode

This cell uses electric power to drive a chemical reaction that requires energy - it is called an **electrolytic cell**

The previous cell uses a chemical reaction to generate electric energy – it is called a **galvanic cell** or a battery ☺

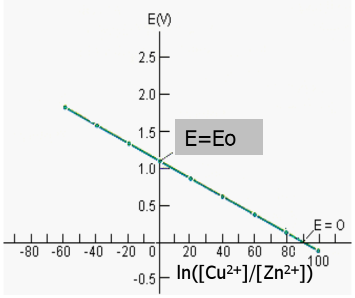


Electrolytic cell uses external power to drive the chemistry

Batteries don’t last forever

The potential difference (E) between the two electrodes in a galvanic cell decreases as chemical energy is converted to electrical energy and reactive species are depleted

- The relationship is quantitatively described by the Nernst equation $E = Eo - (RT/nF)\ ln([Cu^{2+}]/[Zn^{2+}])$
- The potential changes with the changing concentration of Cu and Zn ions in solution
- The initial potential is also different for different initial concentrations of zinc and copper ions
- Under standard conditions the value of the slope is ~59mV/n



The plot of E vs $ln([Cu^{2+}]/[Zn^{2+}])$ yields a linear relationship, where the intercept is at $[Cu^{2+}]=[Zn^{2+}]$, and the slope is a function of T and the number of e^- transferred

Standard electrode

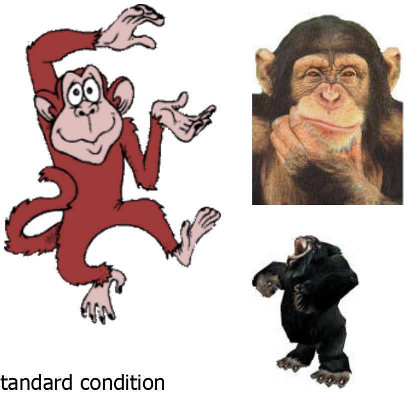
While the potential for a given cell is dependent on concentration, the Eo term is a constant at ionic ratios of 1*

- In the Nernst equation E is a potential difference and the difference cannot be measured for a half cell…
- If there was a reference half cell, all other half cell reactions can be measured against it, and a standard list for different redox couples compiled

The reference half cell selected:

$$\mathbf{2\,H^+ + 2\,e^- = H_2}$$

Who is really bigger?



*Corresponds to 1M ion concentration under standard condition

Reference electrodes

Thus, standard cell potential (Eo) for any redox reaction can be calculated from the standard half cell potentials for the balanced reaction using available values, but…

- The hydrogen/acid electrode, which is the standard reference, is rather inconvenient or safe to use – H_2 gas cylinders would be required for every electrochemical measurement ☹
- There are two half cell reactions that are commonly used as reference cells:

The calomel electrode:	**The silver chloride:**
$Hg_2Cl_2(s) + 2e^- = 2Hg(liq) + 2Cl^-$	$AgCl(s) + e^- = Ag(s) + Cl^-$

both in saturated KCl solution

More on the Nernst equation

It can be applied to both half-cell and full cell

$$\begin{array}{c} A_{red} - ne- \rightarrow A_{ox} \\ \underline{B_{ox} + ne- \rightarrow B_{red}} \\ A_{red} + B_{ox} = A_{ox} + B_{red} \end{array} \qquad \begin{array}{c} Zn - 2e- \rightarrow Zn^{2+} \\ \underline{Cu^{2+} + 2e- \rightarrow Cu} \\ Cu^{2+} + Zn \rightarrow Cu + Zn^{2+} \end{array}$$

Half-cell reduction potential:

$$E_{red} = Eo_{red} - (RT/nF)\ \ln([A_{red}]/[A_{ox}])$$

or half-cell oxidation potential:

$$E_{ox} = Eo_{ox} - (RT/nF)\ \ln([A_{ox}]/[A_{red}])$$

where, $Eo_{red} = - Eo_{ox}$

Whole cell potential:

$$E_{cell} = Eo_{cell} - (RT/nF)\ \ln \frac{[A_{red}] \times [B_{ox}]}{[A_{ox}] \times [B_{red}]}$$

Why did we write the equation for Cu-Zn like this then?

$$E = Eo - (RT/nF)\ \ln \frac{[Cu^{2+}]}{[Zn^{2+}]}$$

Because metal Cu and Zn are solids- their concentrations don't change and are defined to be 1

The Nernst equation

General case - the reactants stoichiometry may vary:

a x A + b x B = c x C + d x D

for 25ºC

$$E = \boxed{Eo} - (RT/nF)\ \ln \boxed{\frac{[C]^c \times [D]^d}{[A]^a \times [B]^b}} = Eo - \overset{\downarrow}{\frac{0.059V}{n}} \ln \frac{[C]^c \times [D]^d}{[A]^a \times [B]^b}$$

The equation has two terms:

Who is bigger

and how many are there

R - the universal gas constant ($R = 8.31\ JK^{-1}\ mol^{-1}$)

T is the abs temp in kelvins (T= T°C + 273.15)

F - the Faraday constant ($F = 9.65{\times}10^4\ Cul\ mol^{-1}$)

n - the number of electrons transferred in the cell (or half-cell) reaction

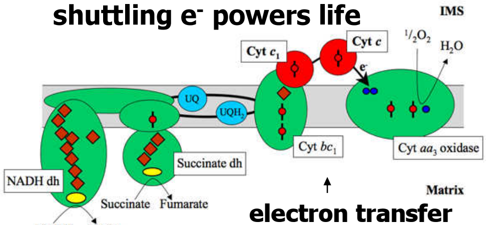
Bio-electrochemistry

Fundamental biochemical processes require electron transfer:

shuttling e$^-$ powers life

Cellular respiration:

$C_6H_{12}O_6 + 6O_2 \rightarrow$
$6CO_2 + 6H_2O$

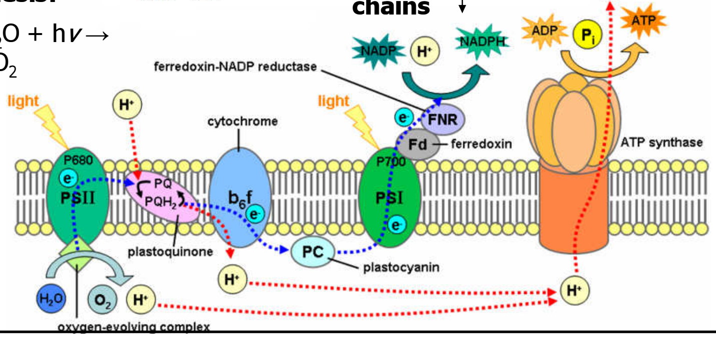

IMS
Cyt c_1
Cyt c
$^1/_2O_2$
H_2O
e$^-$
UQ
UQH$_2$
Cyt bc_1
Cyt aa_3 oxidase
NADH dh
Succinate dh
Succinate
Fumarate
NADH
NAD$^+$
Matrix

Mitochondria

Photosynthesis:

$6\ CO_2 + 6\ H_2O + h\nu \rightarrow$
$C_6H_{12}O_6 + 6O_2$

electron transfer chains


H$^+$
ADP
P$_i$
ATP
NADP
H$^+$
NADPH
ferredoxin-NADP reductase
light
H$^+$
cytochrome
light
e
FNR
Fd
ferredoxin
ATP synthase
P680
e
PSII
PQ
PQH$_2$
b$_6$f
e
P700
PSI
e
plastoquinone
PC
plastocyanin
H$^+$
H$^+$
H$_2$O
O$_2$
H$^+$
oxygen-evolving complex

Chloroplast

Which way electrons flow?

In respiration, animals and plants consume O_2 as they break down sugars - this process provides energy and releases CO_2

Formal charge is an "accounting procedure" created by chemists to work out the flow of electrons in such reactions

Glucose + $O_2 \rightarrow CO_2 + H_2O$ However, let's cut on math and take a simpler example – methane eating bacteria:

The bug takes **$CH_4 + O_2$** and makes **$CO_2 + H_2O$** (as above)

First, we need to balance the equation:

$$CH_4 + 2O_2 = CO_2 + 2H_2O$$

Now, let's pretend that **covalent bonds are ionic** and count the charge on every atom as it was a **whole charge**

Formal charge

In a balanced equation count the charge on every atom as it was a **whole charge**

$CH_4 + 2O_2 = CO_2 + 2H_2O$

On the left: C carbon is more electronegative than H; hence it will be -4, while 4xH will be +4

O_2 is sharing electrons with itself; hence oxygen is 0

C: -4	C:+4
4H:+4	4H:+4
4O: 0	4O: -8

On the right:

In CO_2 carbon has 2 double bonds with more electronegative O; hence C is now +4 (it lost four electrons to oxygen)

Oxygen is more electronegative than both C and H; hence O in CO_2 will be -4 and in H_2O it will also be -4 too (-2x2 in two waters); H is +4 again losing out to oxygen

Outcome: Carbon went from +4 to -4 and Oxygen from O to -8, Hydrogen remained the same at 4

Hence, 8 electrons went from Carbon to Oxygen

Any questions?

Are you clear on this?

Let's check it out during the break

Quiz question:

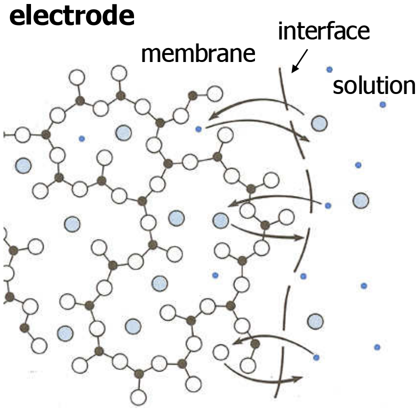
How many electrons and from which atoms are transferred in this reaction?

$$CH_3CH_2OH + O_2 \longrightarrow CH_3COOH$$

Counts for 50% of the quiz

Analytical electrochemistry

Potentiometry: The direct measurement of potential difference and application of the Nernst equation to determine the concentration of a solute **e.g. pH electrode**


interface
membrane
solution

- A thin glass membrane at the tip allows for ion-selective exchange between the glass surface and solution - movement of H^+ across the glass
- The glass in pH electrodes specifically binds protons. This binding is an equilibrium process - the number of H^+ bound on the surface is proportional to the number in solution according to the equilibrium equation:

$H^+(aq) + (glass)^- = H(glass) \quad K = 1/[H^+]$

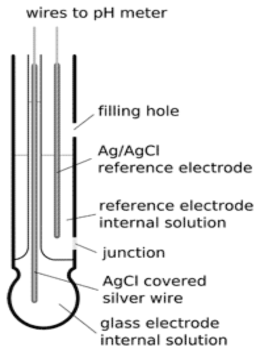
pH electrodes contain a glass ion-selective membrane

---

pH electrode

A reference electrode and a fixed pH solution are placed inside the membrane

- Since the pH is fixed there is a constant amount of protons bound to the inner surface of the membrane
- If the pH of the test solution is different from that inside there will be a different number of protons on the outer surface and, thus, a different charge
- The small potential difference can be accurately measured since there is a reference electrode on both sides of the membrane. The potential follows Nernst's equation

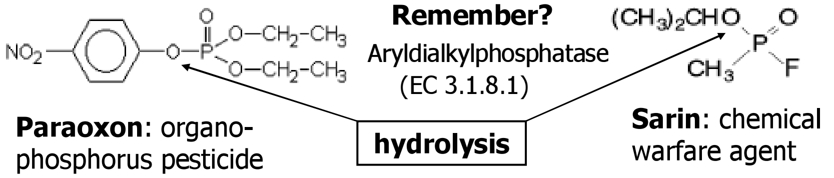

wires to pH meter
filling hole
Ag/AgCl reference electrode
reference electrode internal solution
junction
AgCl covered silver wire
glass electrode internal solution

$pH = -Log[H^+]$

$E = Eo + 0.059\ lg([H^+]_{inside}/\ [H^+]_{outside})$ but inside is constant

Hence, $\mathbf{E = Eo + 0.059\ pH_{outside}}$

Potentiometric biosensors



Remember?
Aryldialkylphosphatase
(EC 3.1.8.1)

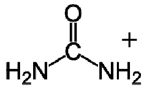
hydrolysis

Paraoxon: organo-
phosphorus pesticide

Sarin: chemical
warfare agent

Operating principle: The hydrolysis products are strong acids - the detection of organophosphates can be done with a pH-sensitive device

Others: Cover the glass membrane with a microporous polymer membrane such as teflon, which allows gases like CO_2 to diffuse through but blocks water and other liquids



$$+ H_2O = 2NH_3 + CO_2 \longrightarrow CO_2 + H_2O = \boxed{H^+} + HCO_3^-$$

(under acidic conditions)

Urease catalyzes the decomposition of the urine - elevated urine in blood is an indication of renal failure

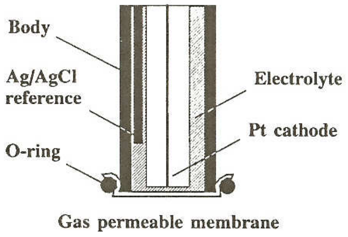
Analytical electrochemistry

Voltammetry is the measurement of the current running through an electrochemical cell as a function of a changing applied voltage

Amperometry is the measure of current holding the potential constant near that for an oxidation or reduction reaction (this current is usually integrated over a time span)

Oxygen electrode is a constant potential amperometric cell

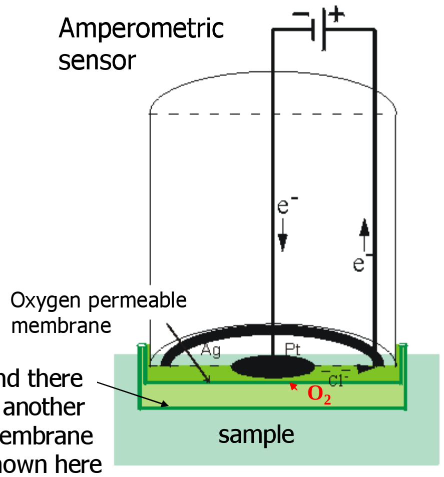
- The Platinum cathode is placed at -0.7 V with respect to the Ag/AgCl reference electrode
- At this potential oxygen is reduced through H_2O_2 to OH^-



$O_2 + 2\ H_2O + 2\ e^- = H_2O_2 + 2\ OH^-$
$H_2O_2 + 2\ e^- = 2\ OH^-$

Clark oxygen electrode

Amperometric sensor


e-
e-
Ag
Pt
Cl-
O_2
sample

Oxygen permeable membrane

And there is another membrane shown here

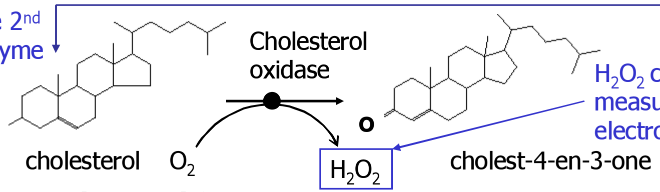
What is it for?

To contain an enzyme ☺

- A potential is applied between the central platinum cathode and the annular silver anode
- This generates electric current which is carried between the electrodes by means saturated KCl
- The current generated by the electrolysis is directly proportional to the amount of dissolved O_2
- The electrode compartment is separated by a thin plastic membrane, which is only permeable to oxygen

Cholesterol biosensor

The 2nd enzyme

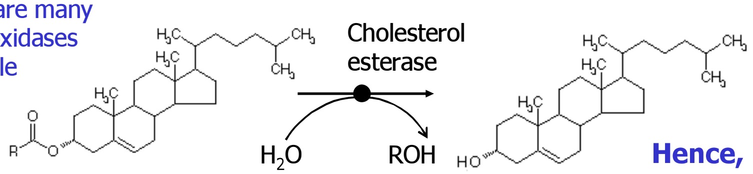

HO
cholesterol
O_2
Cholesterol oxidase
O
H_2O_2
cholest-4-en-3-one

H_2O_2 can also be measured electrochemically

Is this good enough?

Not, really because it doesn't detect esters

There are many other oxidases available


H_3C
CH_3
H_3C
CH_3
H_3C
O
R
O
Cholesterol esterase
H_2O
ROH
H_3C
CH_3
H_3C
CH_3
H_3C
HO

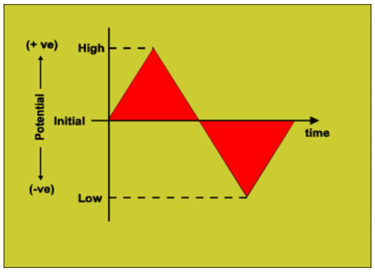
Hence,

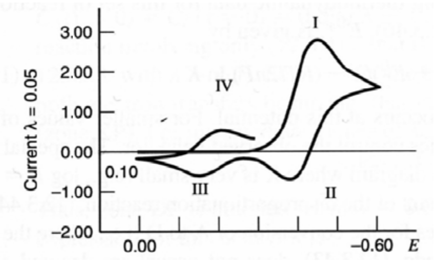
Development of cholesterol biosensor based on **immobilized cholesterol esterase and cholesterol oxidase on oxygen electrode** for the determination of total cholesterol in food samples

Basu et al (2006) Bioelectrochemistry 71, 75–79

Analytical electrochemistry

Cyclic voltammetry is the measure of the current flowing between a reference and working electrode as the potential difference is linearly cycled between a maximum and minimum value


(+ ve)
High
Potential
Initial
time
(-ve)
Low



Note: The analyte solution is not stirred, so the current is due to electrochemical changes very near the working electrode surface

We won't discuss it today – **just a reminder for the final exam**

Semiconductors

Semiconductors had a monumental impact on our society. Anything that's computerized or uses radio waves depends on semiconductors

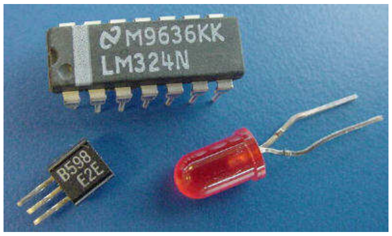
Today most semiconductor chips and transistors are made of silicon, which is at the heart virtually any electronic device

(Note the name "**Silicon valley**")

intel
Core 2 Extreme
quad-core

↑

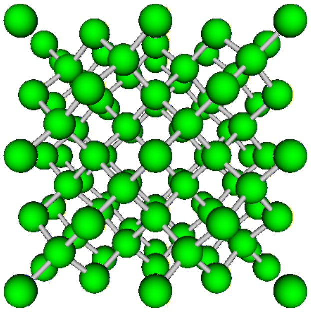
Modern CPU: several hundred million transistors


M9636KK
LM324N
B598
E2E

← **Old stuff**: a chip, a diode (LED) and a transistor

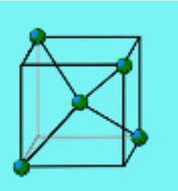
Silicon

- Si (#14) seats just under C(#6) in the Elements Table. It is the 7th or 8th most common element in the universe, and the second most abundant in the earth's crust (as Silicon dioxide, SiO_2)
- At ambient Temp Si exists in amorphous and crystalline; pure silicon is produced by heating sand with carbon to Temp ~ 2,200°C
- Si has four valence e and form very nice crystals, where each Si atom is bonded to 4 other Si atoms

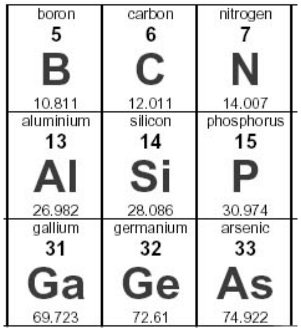


Reaction?

$SiO_2 + C \longrightarrow$
$Si + CO_2$



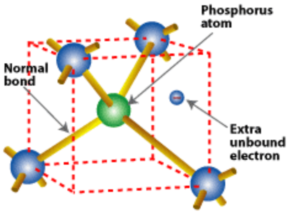
Silicon doping

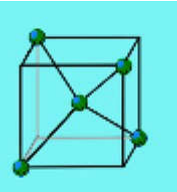


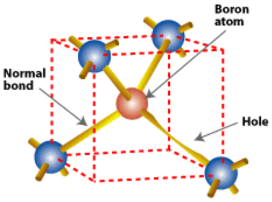
boron 5 B 10.811	carbon 6 C 12.011	nitrogen 7 N 14.007
aluminium 13 Al 26.982	silicon 14 Si 28.086	phosphorus 15 P 30.974
gallium 31 Ga 69.723	germanium 32 Ge 72.61	arsenic 33 As 74.922

- Si is a very weak conductor (nearly an insulator), but this can be changed by the process known as doping
- Doping involves the incorporation of a tiny amount of "impurities" into silicon crystals
- Two type of impurities are used (1) P or As and (2) B and Ga

What will this do to Si?





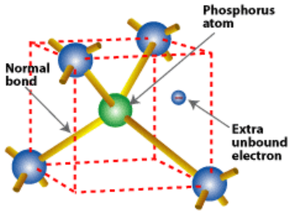


N-type silicon

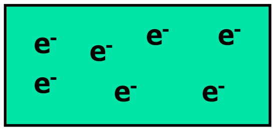
In N-type doping, phosphorus or arsenic is added to the silicon in small quantities. When atoms like P or As with five valence electrons are added to silicon, they do not fit as perfectly as Si. The fifth electron has nothing to bond to and is easily displaced i.e. it freely moves around

Did we see e^- like this?

Small quantity of the impurity is enough to create sufficient number of free electrons for electric current to flow through the silicon. N-type silicon is a good conductor. Electrons have a negative charge, hence the name N-type



N-type



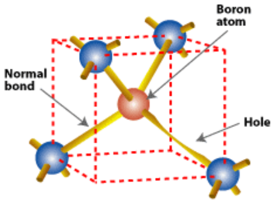
What is the net charge on N-type silicon?

The net charge is **ZERO**

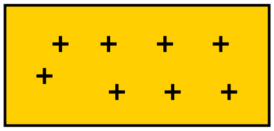
P-type silicon

In P-type doping, boron or gallium are used. They only have three valence electron and are a "bond short." This bond can still form to maintain the perfect symmetry of the crystal but the electron comes from the inner layer. As a result a positive charge develops around the B or Ga atom. These positively charged loci are appropriately called "holes" and such doped silicon is called P-type

Holes conduct current too: holes accept electrons from the neighboring atoms and as a result they move around the material. Thus, P-type silicon is also a conductor



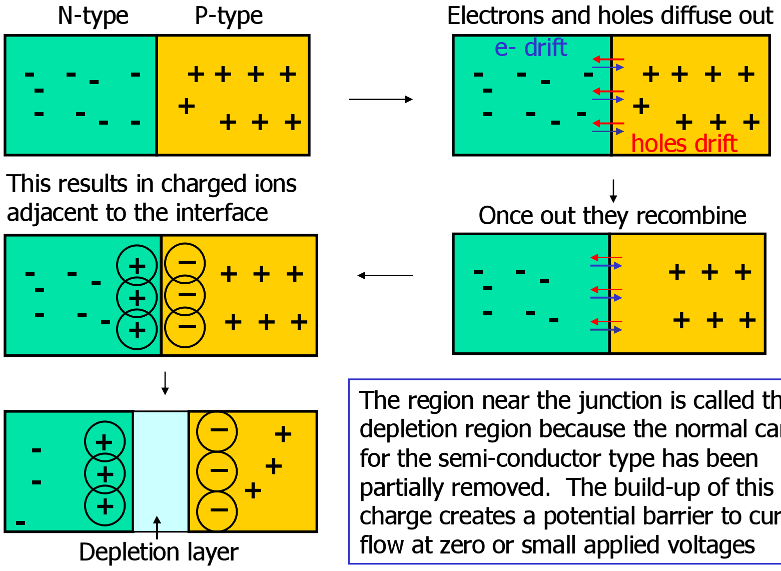
Holes in P-type



What is the net charge on P-type silicon?

Thank you

Putting them together



Electrons and holes diffuse out

Once out they recombine

This results in charged ions adjacent to the interface

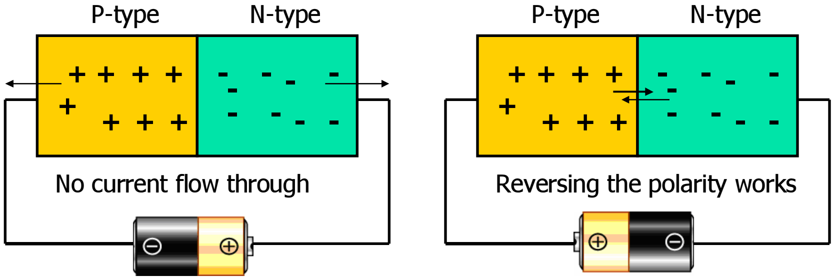
Depletion layer

The region near the junction is called the depletion region because the normal carrier for the semi-conductor type has been partially removed. The build-up of this charge creates a potential barrier to current flow at zero or small applied voltages

---

Putting them together

Doping turns both N-type or P-type silicon crystals from a good insulator into a reasonable (not great) conductor, which is not very exiting in itself. However, when they are put together we get a "junction", and it has pretty interesting properties



No current flow through

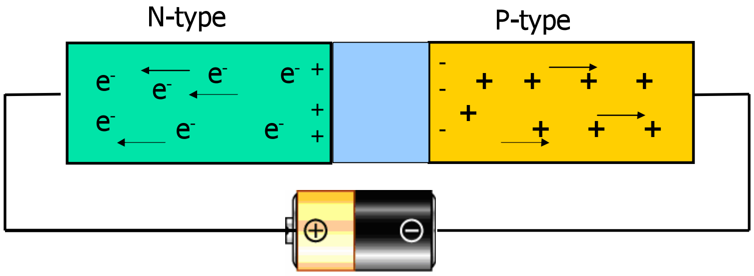
Reversing the polarity works

However, if we flip the battery around it conducts fine :)

Note the name: Semiconductors

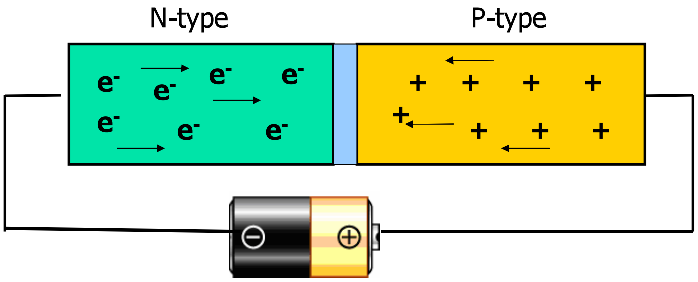
The junction

The electrons in the N-type are attracted to the (+) terminal of the battery and the (+) holes in the P-type to the (-) terminal of the battery. The depletion region grows as electrons move away in N-type and holes move away in P-type. This creates a potential barrier to current flow across the junction



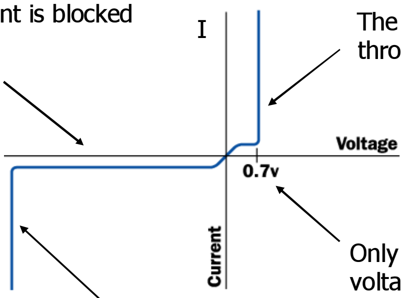
The junction

Electrons are now added to the N-type, which migrate toward the junction filling some of the depletion layer. The same happens with holes in the P-type, so any potential barrier to conduction is lowered and current flows across the junction



Diode

A device that blocks current in one direction, while letting it flow in another direction is called a diode



Current is blocked

Below the threshold potential, there is still little current because the depletion layers is still there

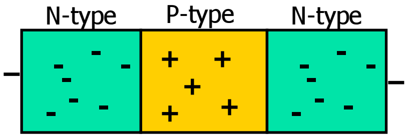
The current flows through

Only a small change in voltage required to get the diode going (0.7V for silicon)

Too much reverse voltage breaks the junction

It gets better still…

By using three layers instead of two (putting two diodes back to back) one can make a transistor

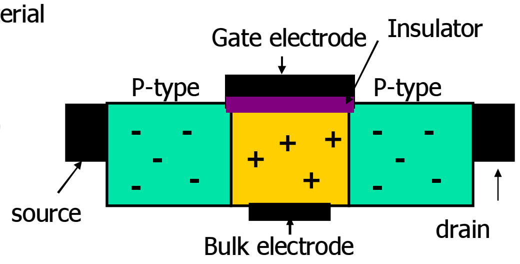


N-P-N sandwich

There will practically no current flow through a transistor because two back-to-back diodes would block it both directions

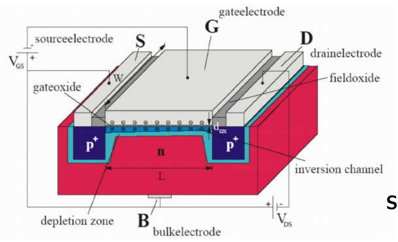
The field-effect transistor (FET) transistor relies on an electric field to control the shape and hence the conductivity of one type of charge carrier in a semiconductor material

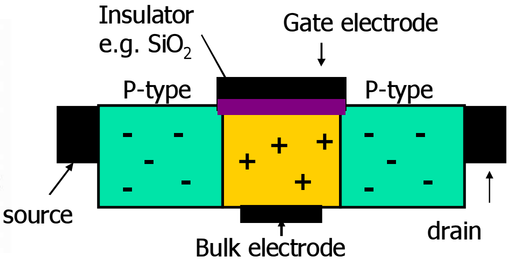
All FETs have a gate, drain, and source terminal and (unlike some other transistors) a fourth terminal called the body or bulk →



Transistors

MOSFET: The metal–oxide–semiconductor field-effect transistor utilizes an insulator (typically SiO_2) between the gate and the body


gateelectrode
G
sourceelectrode
S
D
drainelectrode
V_{GS}
W
fieldoxide
gateoxide
p^+
n
p^+
d_{ox}
inversion channel
L
depletion zone
B
bulkelectrode
V_{DS}


Insulator
e.g. SiO_2
Gate electrode
P-type
P-type
source
Bulk electrode
drain

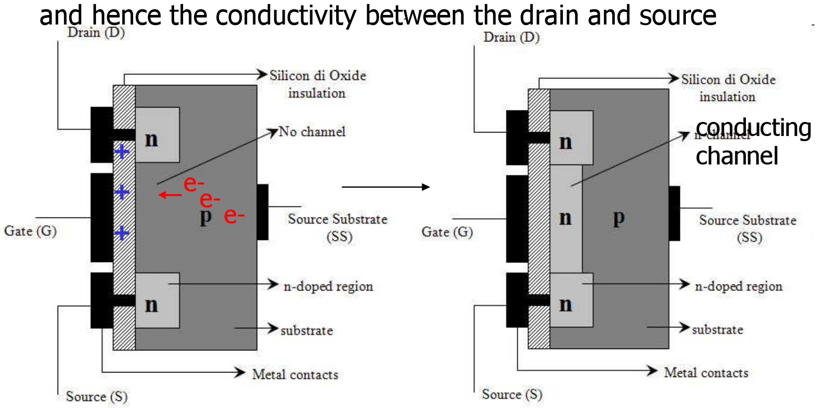
- In FET current flows from the source electrode to the drain electrode along this channel
- The gate controls the flow: a small voltage applied across the center layer of the sandwich, allows a much larger current to flow through the sandwich

---

MOSFET

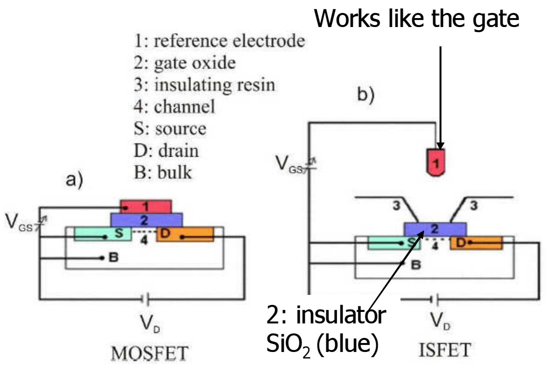
Applying sufficient potential on the gate creates a conducting channel across the structure – current flows from source to drain

A change in the gate potential changes the size of the channe and hence the conductivity between the drain and source


Drain (D)
Silicon di Oxide insulation
n
No channel
e-
e-
p
e-
Gate (G)
Source Substrate (SS)
n
n-doped region
substrate
Metal contacts
Source (S)
Drain (D)
Silicon di Oxide insulation
n
conducting channel
n
p
Gate (G)
Source Substrate (SS)
n
n-doped region
substrate
Metal contacts
Source (S)

Oversimplification but it should give you a flavor…

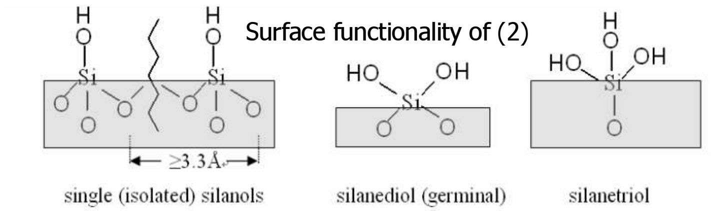
IonSensitive FET


Works like the gate
1: reference electrode
2: gate oxide
3: insulating resin
4: channel
S: source
D: drain
B: bulk
a)
b)
V_{GS}
V_D
MOSFET
2: insulator
SiO_2 (blue)
ISFET

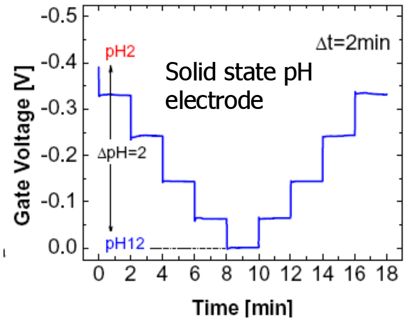
Key modification in ISFET – sample reservoir (3) on top of SiO_2 (gate oxide)

The surface of gate oxide has exchangeable protons, which are associated with the surface oxygen atoms

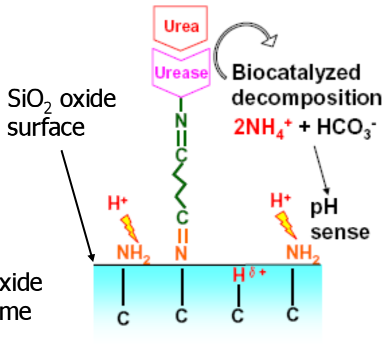
Addition of acid will increase the positive charge at the surface and thus influence the channel conduction. Hence we have a solid state pH electrode


Surface functionality of (2)
H
O
Si
HO
OH
≥3.3Å
single (isolated) silanols
silanediol (germinal)
silanetriol

ENzymeFET



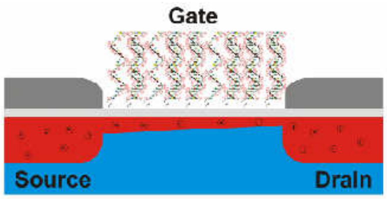
Urease immobilization of on the FET surface


Urea
Urease
Biocatalyzed decomposition
$2NH_4^+ + HCO_3^-$
SiO_2 oxide surface
N
C
H⁺
NH_2
pH sense
H δ+
C

Immobilizing an enzyme on the gate oxide surface allows the analysis of the enzyme reaction

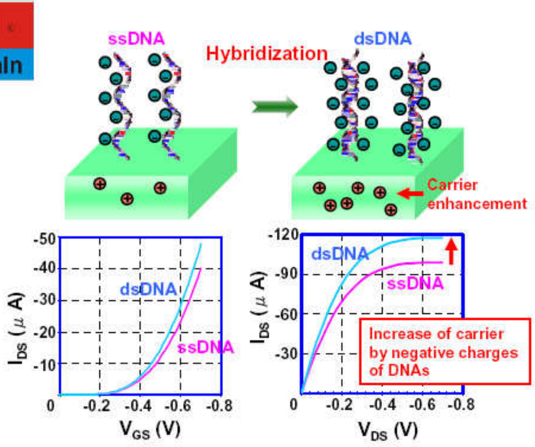
The reaction must generate a change in the number of charged species in solution, or at least generate a change in a species that is acid-base sensitive

DNA binding on FET



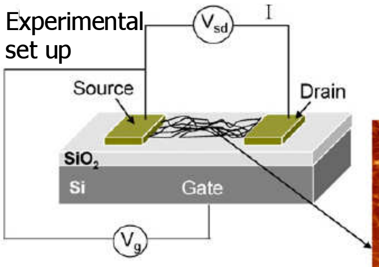
Immobilization of single stranded DNA or antibodies to the surface of the ISFET allows the possibility of detection of specific binding

Since DNA is a charged polymer, interaction of metal oxide surface follows similar patterns of other ISFET devices - binding of the complementary DNA strain would add charge to the bound system and increase the observed current

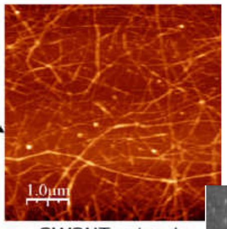


FET-based biosensors

Experimental set up

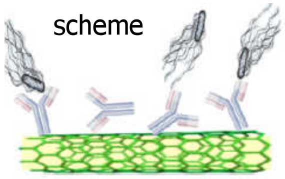


Detection of pathogenic *Salmonella*

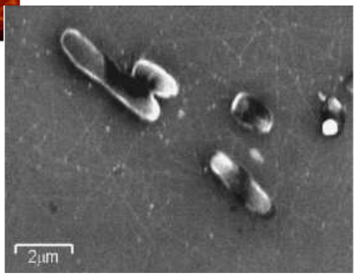


SWCNT network

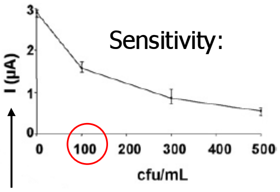
scheme



Antibodies immobilized on carbon nanotubes (bright spots)

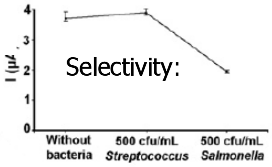


Sensitivity:



Source-drain current →

Selectivity:

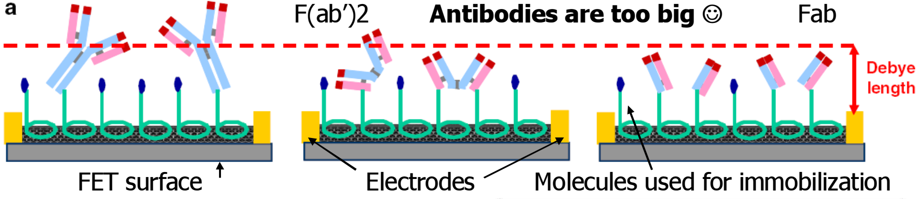


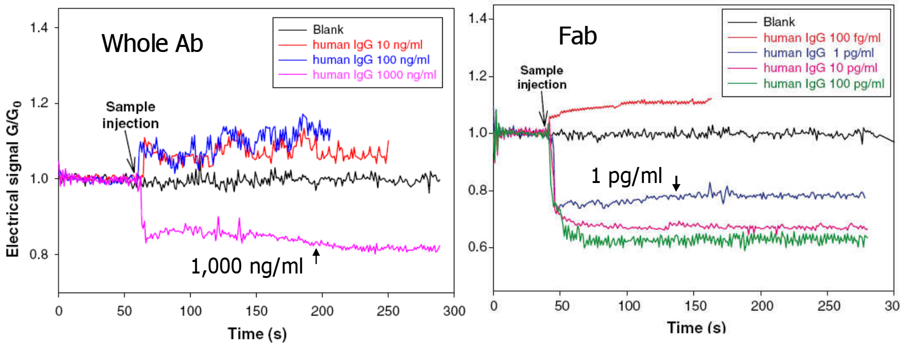
Villamizar et al (2008)
Biosensors & Bioelectronics

FET-based biosensors

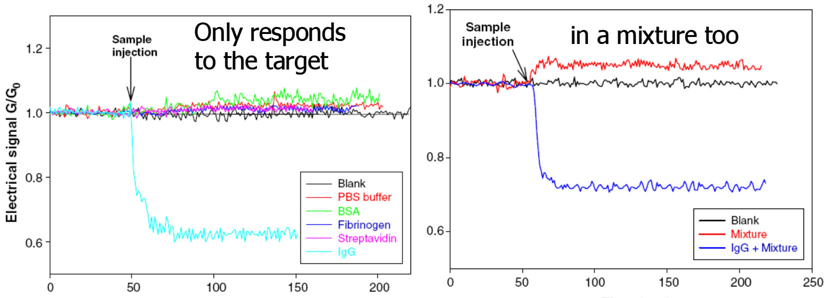
Ultrasensitive carbon nanotube-FET (CNT–FETs)

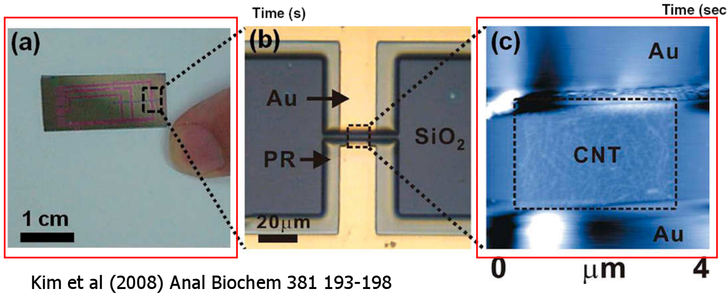
Antibodies are too big ☺


a
Whole Ab
F(ab')2
Fab
Debye length
FET surface
Electrodes
Molecules used for immobilization



Selectivity and the size




(a)
1 cm
(b)
Au
PR
SiO2
20µm
(c)
Au
CNT
Au
0
µm
4

A: Optical image of a CNT–FET chip with patterned gold electrodes

C: AFM image of channel of CNT–FET device

Kim et al (2008) Anal Biochem 381 193-198