BIOSENOSRS

BIO 580

Optical Biosensors- theory part 4

Sources of Biological Recognition Elements: Part 1

WEEK-10

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

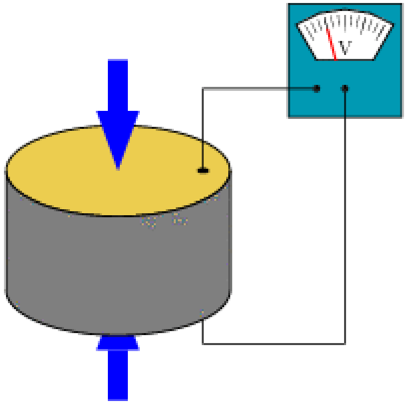
[Click here]

Piezoelectric transducers

Piezoelectricity is the ability of some materials (notably crystals and certain ceramics) to generate an electric field or electric potential in response to applied mechanical stress.

The word *piezo* is derived from the Greek, which means to squeeze or press

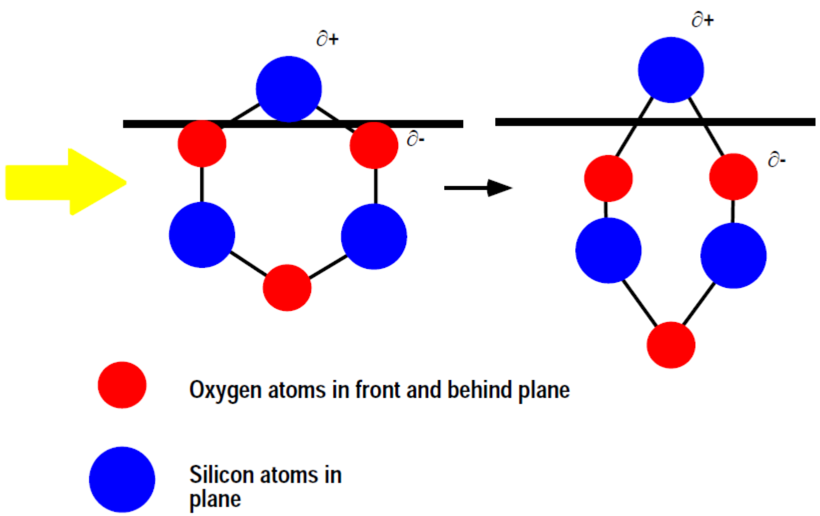
Piezoelectric crystals are one of many small scale energy sources. Whenever piezoelectric crystals are mechanically deformed or subject to vibration they generate a small voltage, commonly know as piezoelectricity. This form of renewable energy is not ideally suited to an industrial situation.



Electric polarization produced by mechanical strain in crystals belong to certain classes, the polarization being proportional to the strain and changing sign with it.

Quartz plates were used to send and receive ultrasonic pulses underwater - SONAR

How does mechanical deformation result in an electrical potential being developed?



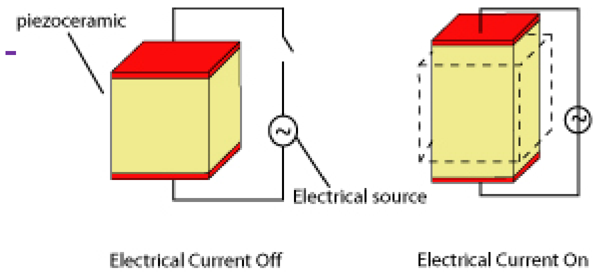
Compressional displacement of atoms within quartz yields a surface potential due to increased polarization.

If we consider quartz, which consists of corner-shared tetrahedra with a central Si atom and four O atoms, we can see that compression of the material in one direction results in an increased polarization - or charge separation -between the more electropositive Si atoms and the more electronegative O atoms

It is essential that the crystalline material lack central symmetry to observe these effects

Eg., of piezoelectric materials include QUARTZ, Cadmium sulphide, Lithium niobate, and Lithium tantalate, some others include ZnO, and Zirconium titanate.

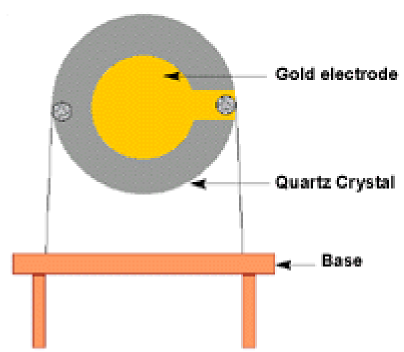
Each crystal has a natural resonant frequency of oscillation - modulated by environments



Usual frequency - 10 MHz range (radio frequency)

The actual frequency -> dependent on the mass of the crystal + any other material coated on it

Change in resonant frequency (Δf) -resulting from adsorption of an analyte on the surface can be measured with a high sensitivity (500-2500 Hz/g) - resulting in sensors with picogram detection limits



Surface mass change, Δm**(g)**, and resonant frequency f, is given by the Sauerbrey equation

$$\Delta f = -2.3 \times 10^6 f^2 \Delta m/A$$

Δm - mass (g) of adsorbed material on an area A (cm^2) of the sensing area

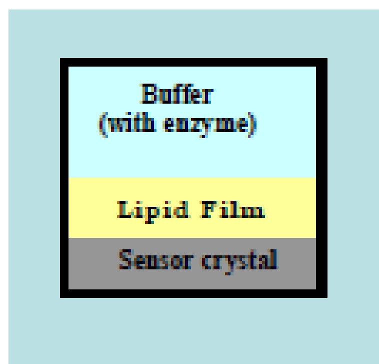
For a 15 kHz crystal a resolution of 2500 Hz/μg is likely, so that a detection limit of 10^{-12} (1 pg) is probable.

Quartz Crystal Microbalance (QCM)

A **quartz crystal microbalance** (QCM) measures a mass per unit area by measuring the change in frequency of a quartz crystal resonator

Sensor crystal

Measurement Chamber

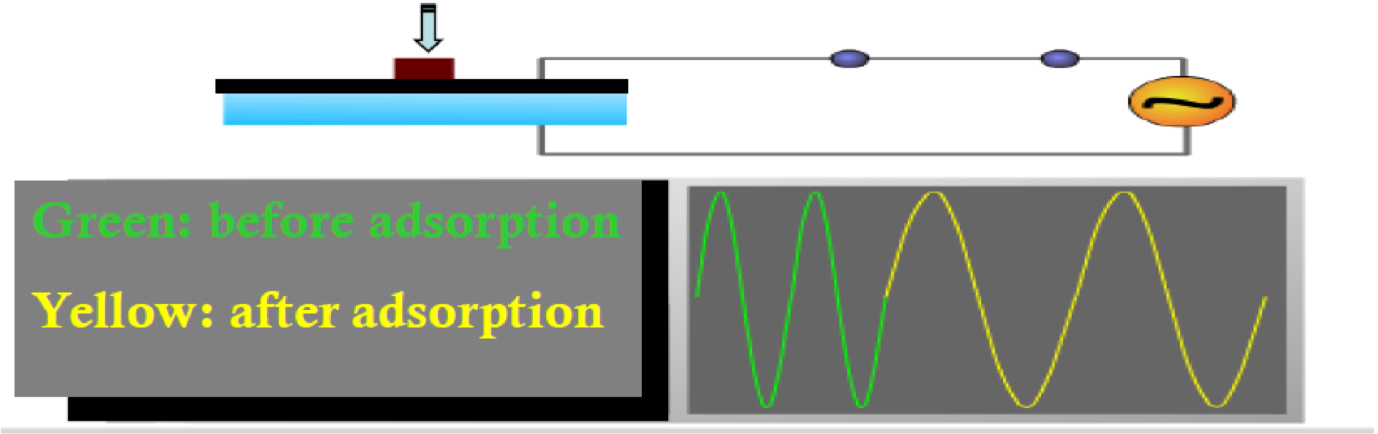


Electronic Interface

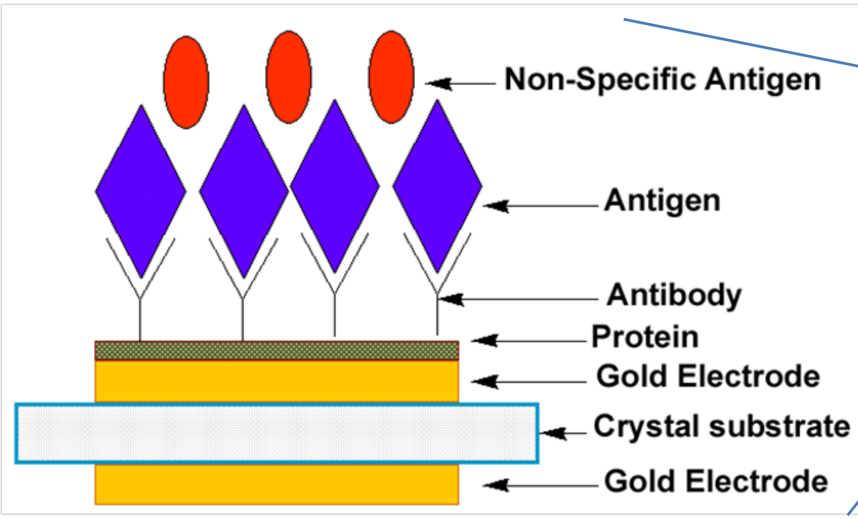
Quratz Crystal Microbalance (QCM) - piezoelectric materials can be used in the devices now include ceramic materials (crystals) such as barium titanate and various zirconium titanates.

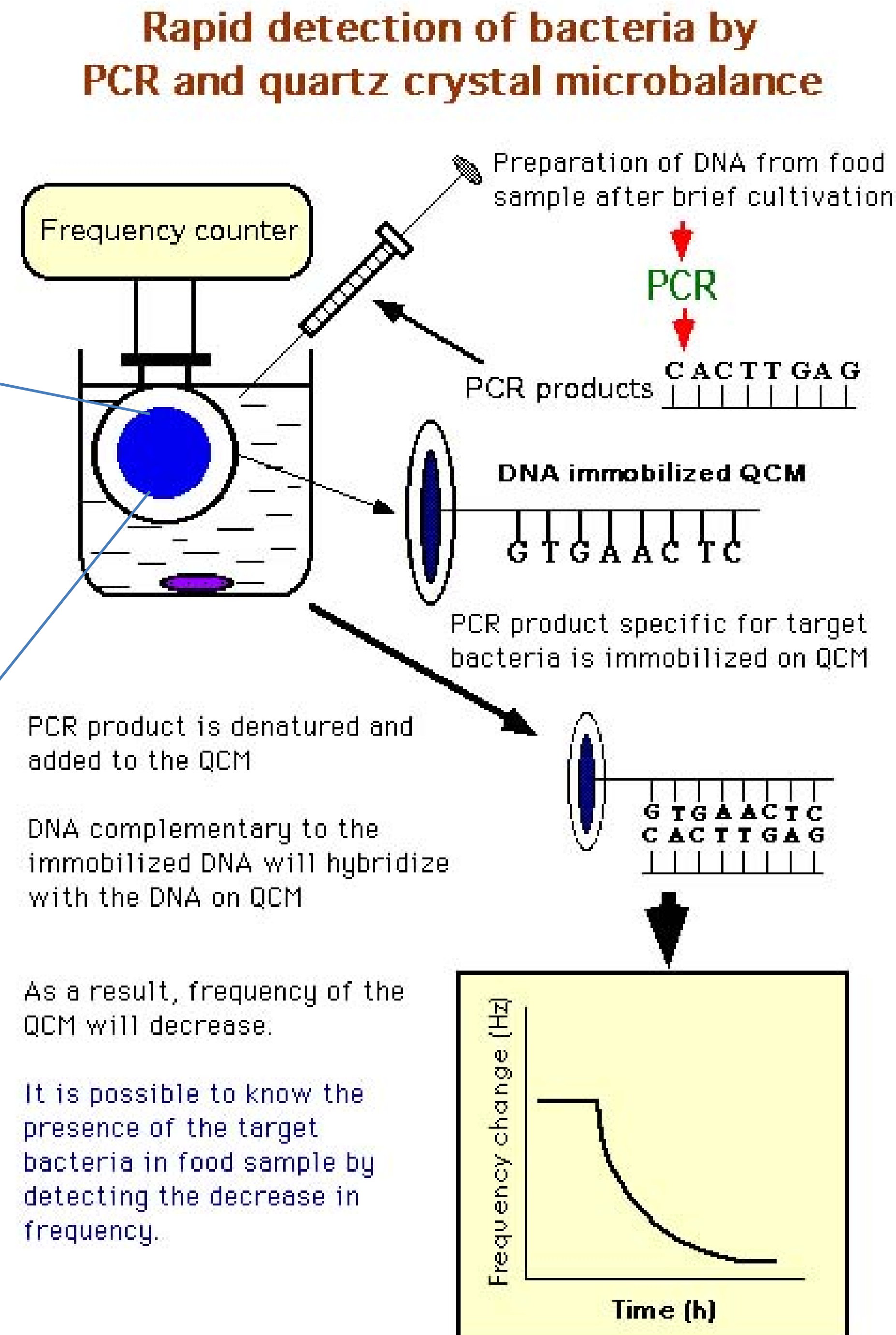
QCM (Frequency)

- A piezo-electric quartz crystal oscillates with a fixed frequency (force = AC-electric field)
- Frequency depends on the mass adsorbed to the crystal surface

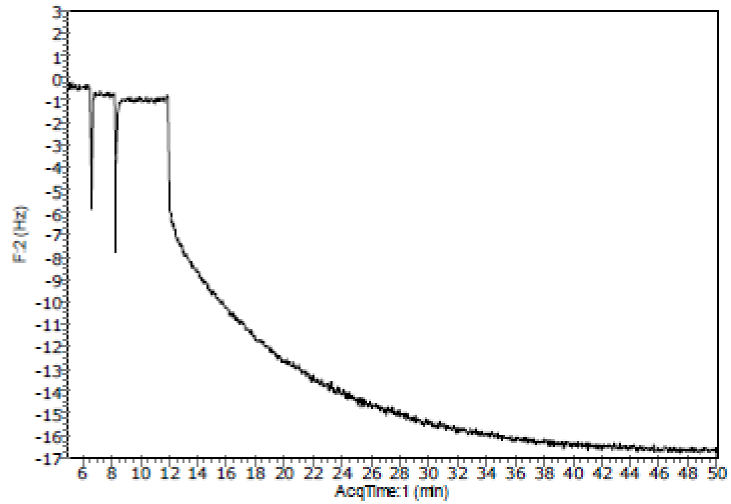


QCM - an example





QCM Sensorgram (Protein Adsorption)

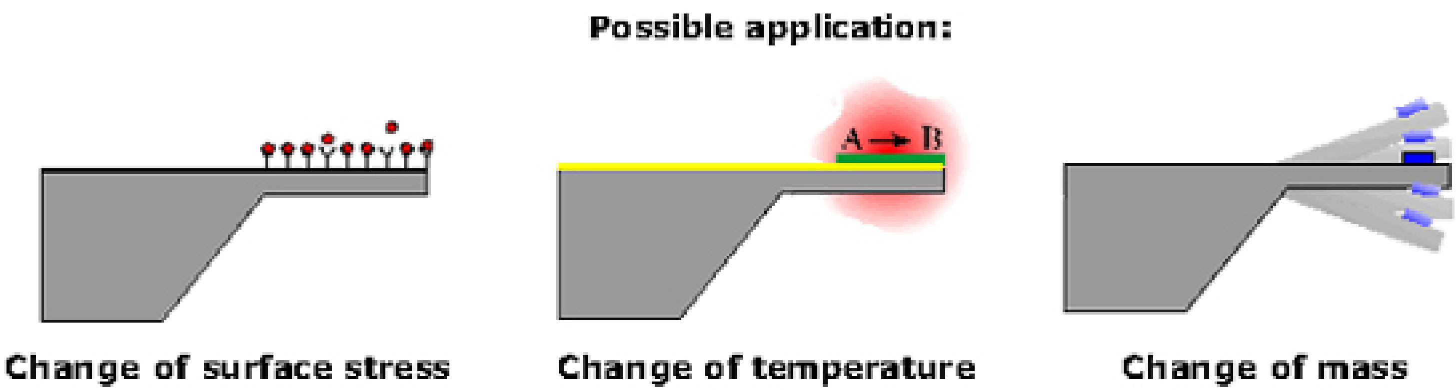


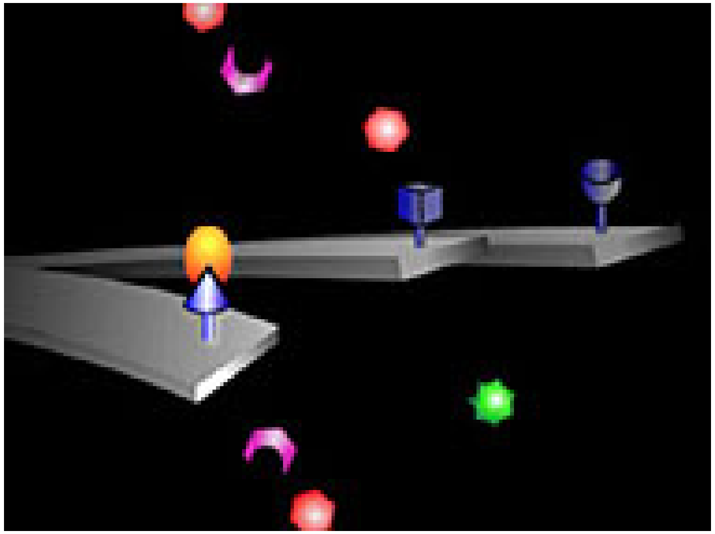
Watch movie here:
http://www.q-sense.com/dbfiles/QCM-D.swf

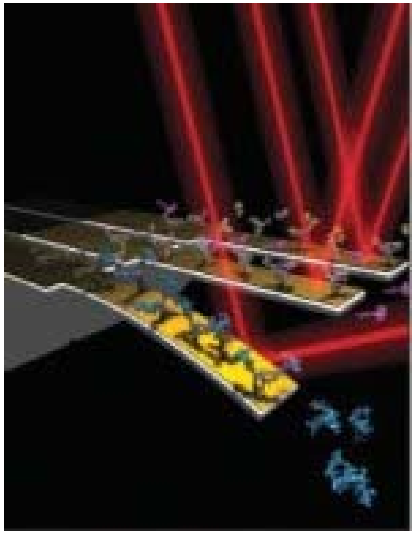
Micro-Cantivlever sensors

Cantilevers as sensors

A **cantilever** is a beam supported on only one end. The beam carries the load to the support where it is resisted by moment and shear stress.







Micro cantilevers

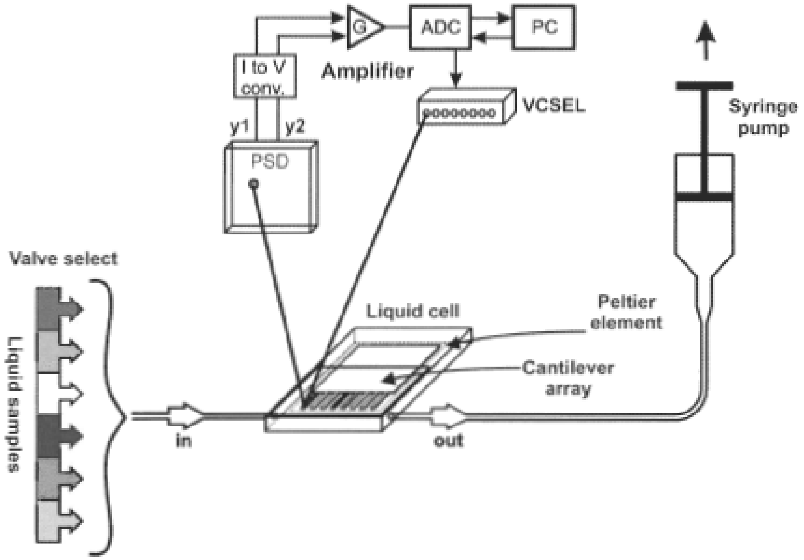
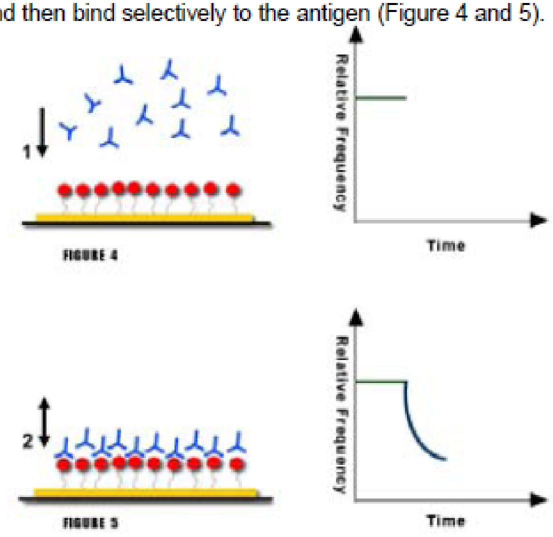


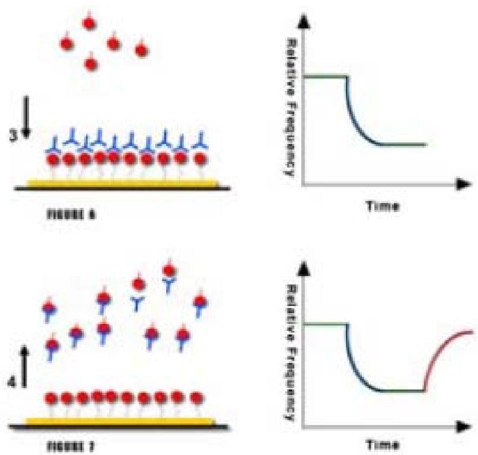
Fig. 10.5 Schematic of the measurement set-up for a liquid environment. The vertical cavity surface emitting laser (VCSEL) light sources are switched on and off in a time-multiplexed manner to facilitate the determination of deflection of each cantilever sensor separately.

The gold surface on the quartz crystal is coated with an antigen. A frequency is obtained from the crystal creating a baseline. Antibodies flow over the surface and then bind selectively to the antigen (Figure 4 and 5).



The antibodies create a weight gain and the frequency decreases. A new baseline is created. (Figure 5.)

Cantilever responses & application

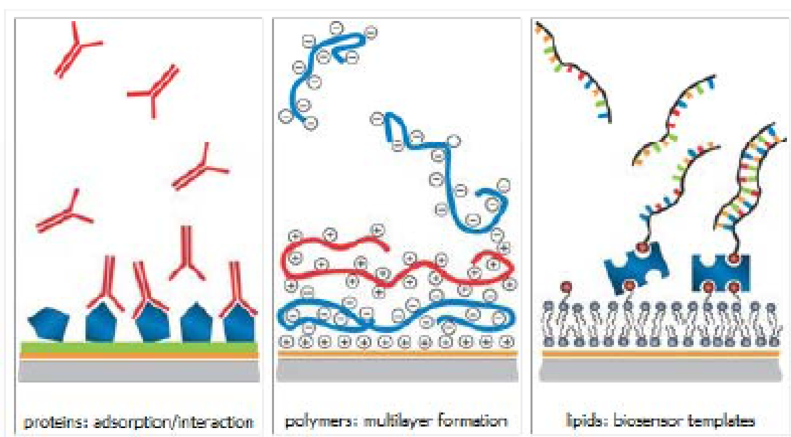


The antibodies move from the surface and bind with the target molecules, which creates a loss of weight and an increase in frequency (Figure 7).

The magnitude of the frequency response of the QCM-electrode is proportional to the weight difference, i.e. the quantity of antigen present in the sample that is presented to the QCM-surface.

APPLICATIONS

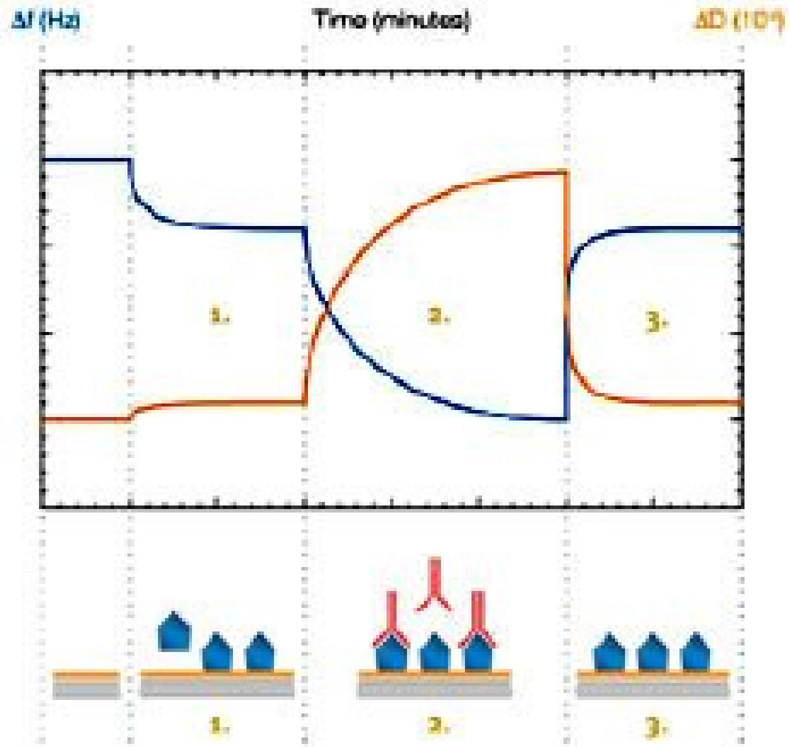
Q-Sense systems are mainly used for characterization of bio-interfaces. Samples range from peptides – proteins - cells.



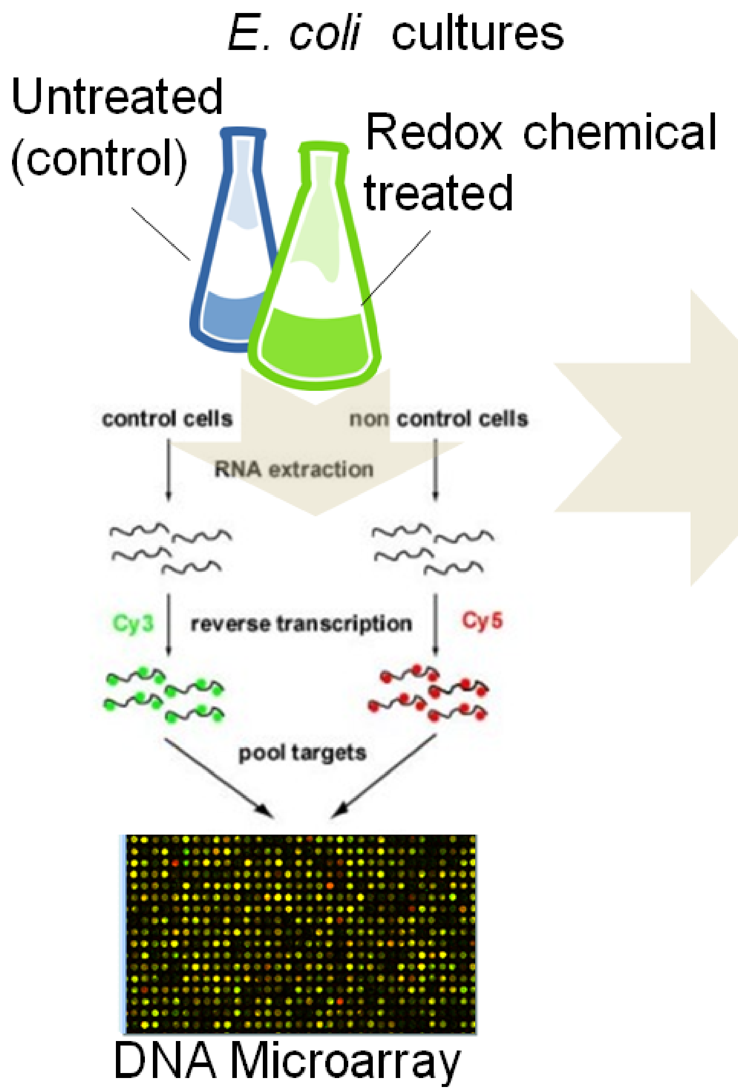
Structural & frequency change

With QCM-D, two parameters - frequency and dissipation - are monitored simul-taneosuly, in real-time, as molecular layers form on the sensor surface. This is what a raw data plot could look like:

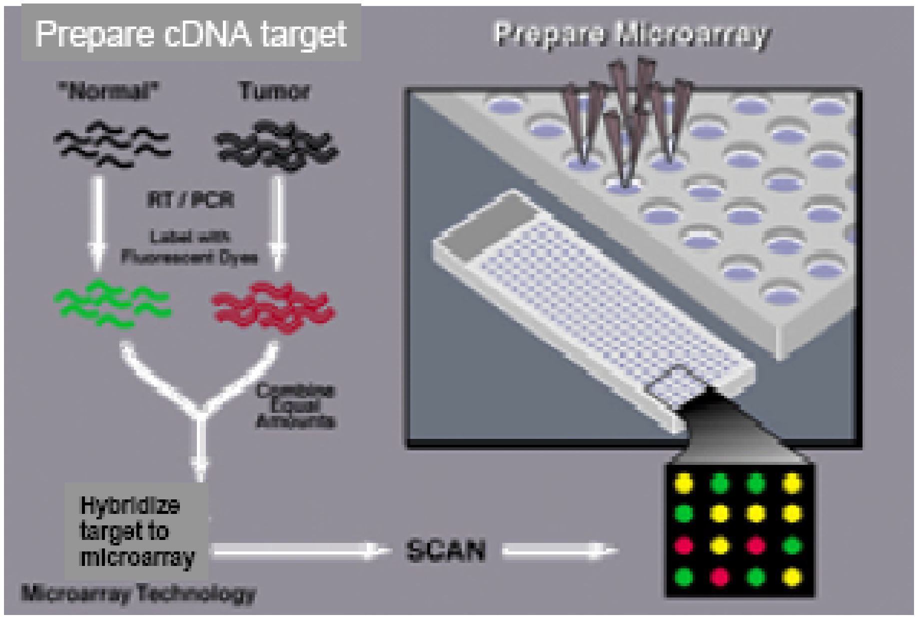
1. **Binding of a small globular molecule**
   Moderate frequency response, Δf (mass change), but low dissipation, ΔD(structural change).
2. **Binding of a large elongated molecule**
   Forms a softer and thicker layer which can be seen by higher Δf and much higher ΔD levels.
3. **Rinsing with buffer**
   The elongated molecule is removed, frequency and dissipation reduce again.



Microarrays



cDNA microarrays



Sources of Biological Recognition Elements

Text Material

- **Primary Text:**
  - *Principles of Physical Biochemistry* (Chapters 1-4)
    - K. E. van Holde, W. C. Johnson, and P. S. Ho
    - Prentice Hall, 1998; ISBN 0-13-720459-0
- **Supplementary Texts:**
  - *Biophysical Chemistry, Parts I and III*
    - C. R. Cantor and P. R. Schimmel
    - W. H. Freeman and Co., 1980; ISBN 0-71 6 7-1189-3.
  - *Principles of Protein Structure*
    - G. E. Schultz and R. H. Schirmer
    - Springer-Verlag, 1979; ISBN 0-387-90334-8.
  - *Introduction to Computational Chemistry* (Ch. 2 and Ch. 16)
    - F. Jensen
    - Wiley, 2001; ISBN 0-471-98425-6

Physical Biochemistry

- Physical Biochemistry –
  - addresses the physical properties of biological macromolecules:
    1. Proteins (polypeptides).
    2. DNA, RNA (polynucleotides).
    3. Sugars (polysaccharides).
  - Here, our main focus is on proteins and polynucleotides.
    - the ‘information-carrying’ molecules of life.
  - However, the techniques we develop will also apply to other biological macromolecules.

Physical Properties & Relationship to Biochemistry

- Physical Properties of biological macromolecules:
  - provide a hierarchical description of molecular structure:
    - atomic level;
    - molecular level;
    - level of large subunit assemblies.
  - measured by observing their interaction with electromagnetic radiation:
    - Ultraviolet (UV) spectroscopy.
    - X-ray crystallography.
    - Nuclear Magnetic Resonance (NMR), etc.
  - An understanding of these properties facilitates structural prediction.
    - Does information about molecule sequence tell us about structure?
      - If so, why??

- We note that...Biochemistry
  - is also concerned with the structure of biological macromolecules.
  - Focus: biologically important molecular mechanisms.
    - *e.g.*, specific details of active-site chemistry.
    - often involves formation/breakage of covalent bonds.
- Biophysical Chemistry has a different focus:
  - A quantitative analysis of structure, and...
  - The physical properties that determine the range of structures which are accessible.
  - concerned primarily with changes in non-covalent interactions

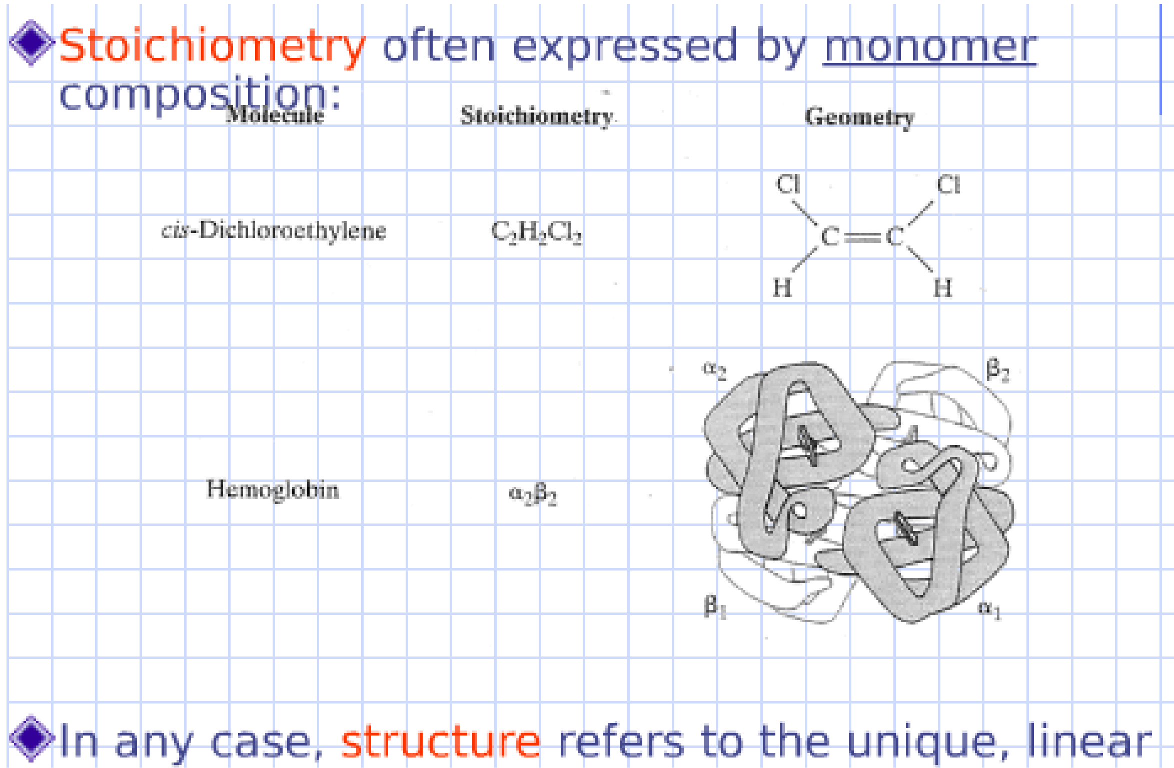
Introduction of Biophysical Chemistry

- 1.1 Basic Terminology.
- 1.2 Review of Monomer Stereochemistry.
- 1.3 Weak Interactions in Macromolecular Structure.

Definition of 'Molecule'

- Chemistry –
  - a molecule…
    - contains 2 or more atoms;
    - atoms covalently (tightly) bonded in specific proportions;
      - *i.e.*, chemical formula (stoichiometry).
    - also has a specific geometry.
- Biochemistry takes a larger view…
  - a molecule:
    - also has well-defined stoichiometry and geometry;
    - not readily dissociated…but, bonds not necessarily covalent.
  - *e.g.*: Hemoglobin has 4 distinct polypeptide subunits:
    - each is a covalently-linked polymer chain.
    - each chain is called a monomer.
    - monomers may be held together by non-covalent interactions.

Basic Definition: Structure



The Biological Macromolecule

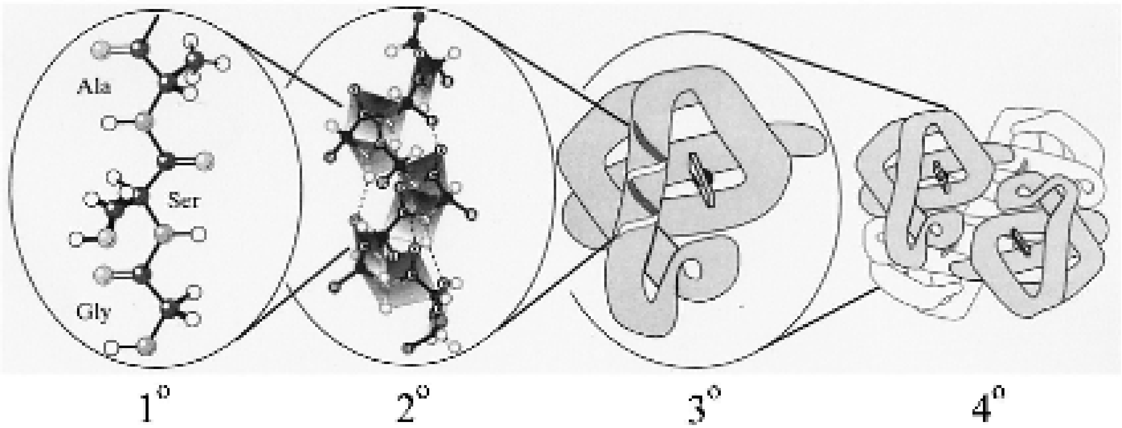
- Simply put…a macromolecule is a large molecule.
  - By ‘large’, we mean large enough to be conveniently divided into distinct subunits.
  - May be several levels of decomposition into ‘monomers’.
- For us, a macromolecule is typically a ‘biopolymer’:
  - *i.e.*, is composed of a string of monomer subunits.
    - Proteins: amino acid residues.
    - RNA and DNA: nucleic acid residues.
    - Polysaccharides: sugar residues.
  - This decomposition admits a useful notion of size:
    - ‘oligomer’: length <= 25 monomer subunits.
    - ‘polymer’: length > 25 monomer subunits.

The Hierarchical Structure of Biopolymers

- Monomers – basic repetitive subunits.
- Primary Structure (1°)
  - linear sequence of monomers...
  - with a specific strand orientation.
- Secondary Structure (2°)
  - the local, regular structure of biomolecules.
  - these are helical structures.
- Tertiary Structure (3°)
  - global, 3-D fold or topology.
  - = native structure, for single-subunit biopolymers.
- Quaternary Structure (4°)
  - spatial arrangement of multiple, covalently distinct subunits.

Illustrative Example

- Hierarchical Structure of Hemoglobin:



- Not all biopolymers have all 4 levels of structure.
  - but, at least 2° structure required for function…
  - Functionality usually requires a correlation:
    - Between sequence and shape (Anfinsen).

The Folding Problems of Biophysical Chemistry

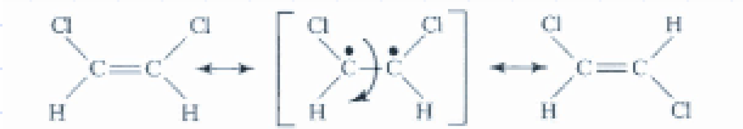
- **Function intimately related to Shape:**
  - *e.g.*: 'Lock and Key' model of enzyme action.
- **A Primary Goal of Biophysical Chemistry:**
  - understanding the <u>rules</u> relating the 4 levels…
    - prediction of 2° and 3° structure from 1° structure.
  - Best-known: the <u>Protein Folding Problem</u>;
    - currently unsolved.
  - A Folding problem exists for <u>each</u> biopolymer class.
- **Before examining biopolymer structure,**
  - let's first review ome general principles…

Configuration Vs. Conformation

- The arrangement of atoms or groups in a molecule is described by two terms:
  - Configuration – refers to the arrangement around:
    - one or more non-rotating bonds, or
      - around a stereocenter (chiral center).
    - Change of configuration requires a chemical change….
      - Breaking one or more covalent bonds.
  - Conformation – arrangement about freely rotating bonds.
    - change of conformation does not require a chemical change.
- Both describe the spatial geometry of biopolymers.
  - However, they are very different terms.

Configuration

- <u>Configuration</u> refers to the position of atoms/groups:
  - around one or more <u>non-rotating</u> bonds.
  - Or, around a <u>stereocenter</u>.
- Change of configuration requires a <u>chemical</u> <u>change</u>:
  - breaking and remaking chemical bonds.
- <u>Example 1</u>: Rotation about a double bond…



cis 1,2-Dichloroethylene sp^2 intermediate   *trans* 1,2-Dichloroethylene

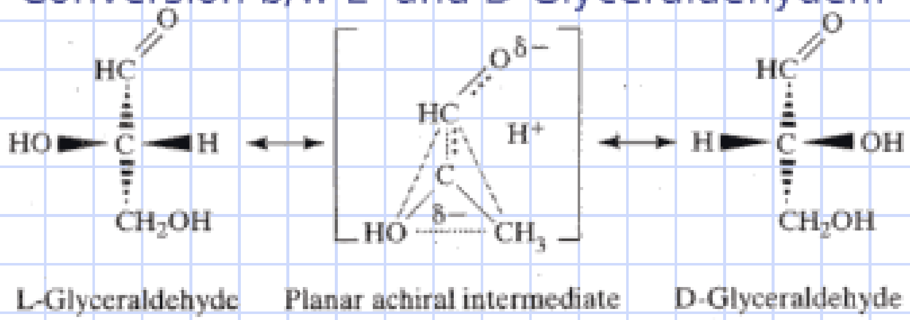
  - requires <u>breakage</u> of a π-bond…
    - with rotation through an sp^3 intermediate.

Configuration (cont.)

◆ Example 2: Conversion b/w Enantiomers.

- *i.e.*, molecules which are non-super-imposable mirror images.

- Conversion b/w L- and D-Glyceraldehyde…



- requires breakage of a single bond;
- formation of a planar, achiral intermediate.

Conformation

- Conformation refers to the spatial arrangement about freely rotating bonds.
  - conformation can be changed by rotations about single bonds;
    - does not require a chemical change.
  - different conformations of the same molecule are called *structural isomers*.
- Example:
  - Rotation about the central bond of 1,2-dicholoroethane.

Eclipsed syn

Staggered anti

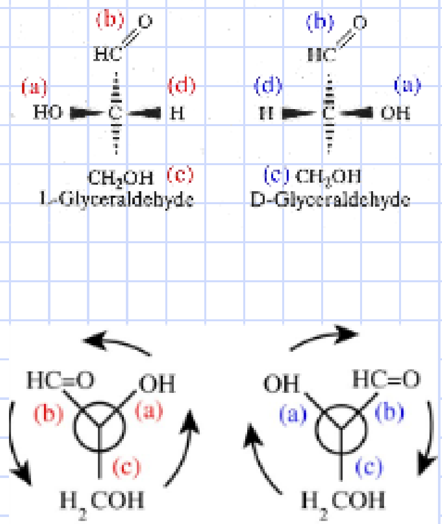
Monomer Stereochemistry

- The monomer building-blocks of biopolymers are almost always <u>chiral</u> molecules.
  - exhibit definite 'handedness'.
  - there are thus, two distinct forms…
    - L-form - 'left-handed'
    - D-form - 'right-handed'.
    - these are mirror images, and are <u>not</u> super-imposable.
      - referred to as <u>enantiomers</u>.
      - Note: these are also called the S and R forms, as well.
  - Enantiomers are <u>distinct</u> molecules.

Example: L Vs. D-Glyceraldehyde

- Each chiral center…
  - has 4 attached groups.

2. Assign group priorities:
   - a (highest) to d (lowest).
   - first basis: atomic mass of directly connected atom.
   - next basis: atomic masses of next closest atoms, etc.
3. Rotate d into the plane.

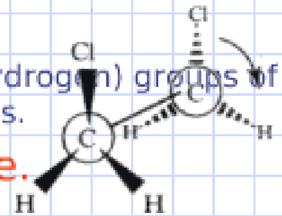


Chirality of Biopolymers

- Biopolymers are generally constructed of only one enantiomer…
  - Each type of monomer units either L- or D-form…
    - Required for formation of regular helices;
    - This facilitates a correlation between 1° and 2° structure.
  - Amino acids in natural proteins are usually L-form.
  - Sugar moiety of the nucleotides which compose DNA (2'-Deoxyribose) is D-form.
- Handedness has biological implications:
  - distinct handedness lends specificity to 3-point contact.
- Handedness also has geometric

Macromolecular Conformation

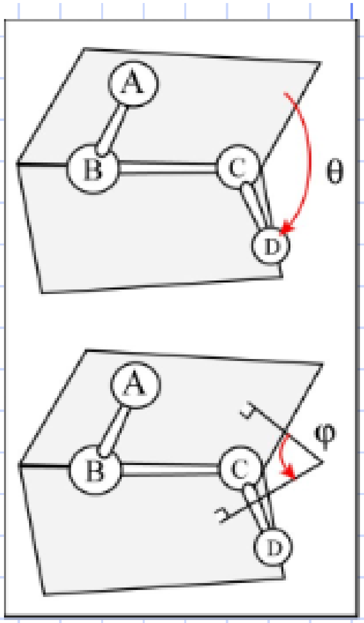
- Macromolecule conformation described by:
  - conformation of each freely rotating bond.
- For a biopolymer, the set of accessible conformations:
  - = the structural isomers generated by these rotations.
- Traditionally, conformation about each single bond:
  - described in terms of a 4-atom center, A-B-C-D defined by the rotating bond, where…
    - B-C is the rotating bond.
    - A and D are the bulky (non-hydrogen) groups of the connected, tetrahedral centers.
- Example: 1,2-Dichoroethane.
  - 4 atom center: Cl-C-C-Cl.



The Torsion and Dihedral Angles

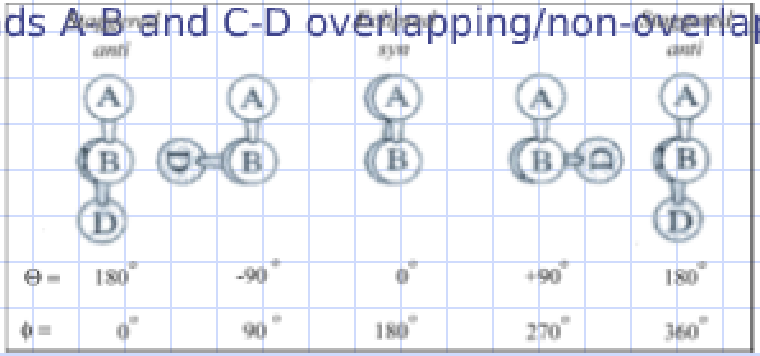
- Conformation of a 4-atom center conveniently described in terms of:
  - the torsion angle, Θ :
    - defined between planes ABC and DCB…
    - …relative to A (looking down BC).
  - $\Theta = 0^{\circ}$ when A and D are in **cis**.
    - (+) Θ defined as CW rotation of D.
    - Standard for polymer chemistry…
- An equivalent description is the dihedral angle, ϕ:
  - In Geometry:
    - Angle b/w normals of planes ABC and BCD.

      $\Theta + \phi = 180^{\circ}$ (see figure)
    - Thus: Θ and φ *supplementary*.



Descriptive Notation

- Conformation also traditionally described in terms of:
  - relative placement of the bulky groups, A and D.
  - Syn/Anti:
    - bulkiest groups on the same/opposite side of a plane through central bond, B-C.
  - Eclipsed/Staggered:
    - bonds A-B and C-D overlapping/non-overlapping.



The Impact of Conformational Changes

- A conformational change in a biopolymer can result in large changes in physical properties.
  - Example: Protein Denaturation
    - The properly folded conformation of a protein…
      - is biologically active.
      - the ‘native state’.
    - In contrast, the unfolded conformation
      - is not biologically active.
      - the ‘denatured state’.
- Thus, Conformation and Configuration
  - Each has important implications for biopolymer shape and function;

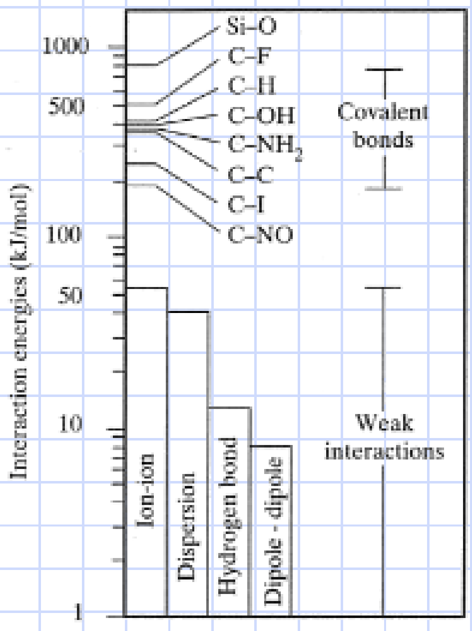
Molecular Interactions in Macromolecular Structures

- For a macromolecule in a cellular environment:
  - configuration is <u>fixed</u> by covalent bonding.
  - conformations, however, are <u>highly variable</u>…
- The sequence-dependent folding of a biopolymer:
  - is no more than a change in conformation.
  - is dependent on a number of interactions:
    - between the atoms within the biopolymer.
    - between the biopolymer and its environment.

Covalent Vs. Weak Interactions

- The configurations of biopolymers are fixed:
  - because covalent bonds require much energy to break…
    - Interaction Energies – 200 – 800 kJ/mol
    - in contrast, thermal energy: RT = 2.58 kJ/mol (37° C).
      - Note 1: 1 mole of a particular molecule = 6.023 x 10^{23} copies
      - Note 2: Joule = a unit of energy equal to 1 Newton-meter
  - at ambient temperatures, can be treated as invariant (fixed).
    - In other words, our molecules do not shake apart at room temperature!
- The conformations of biopolymers:
  - stabilized by weak interactions.
    - 1-2 orders of magnitude smaller than covalent interactions.
    - Only – 1 order of magnitude (10x) greater than RT.
  - These interactions describe how the atoms or groups attract or repel…
    - Together, determine the total energy of a given conformation.
    - Rule: the lower the energy…the more favorable the structure.

The Weak Interactions



- The conformations of biopolymers:
  - determined by weak interactions.
- The 'Weak' Interactions:
  - also called 'non-bonding'.
  - much weaker than covalent interactions.
    - 1 to 10's of kJ/mol.
  - include:
    - Electrostatic (charge-charge).
    - Dipole-dipole, charge-dipole.
    - van der Waals.
    - Hydrogen bonding.

Distance-dependence of the Weak Interactions

- Are all pairwise, distance-dependent interactions.
  - Energy of each ~ $1/r^m$. ; m = 1, 2, 3, 6, 12 (integer).
    - r = separation between a pair of interacting atoms or groups.
- The range of the interaction determined by m.
  - for larger m values, V falls to zero more rapidly, with increasing r.
  - Longest range: Charge-Charge interaction (m = 1).
  - Shortest range: Steric repulsion (m = 12).

Type of Interaction	Distance Relationship
Charge-charge	$1/r$
Charge-dipole	$1/r^2$
Dipole-dipole	$1/r^3$
Charge-induced dipole	$1/r^4$
Dispersion	$1/r^6$

Dependence on the Medium

- The energies of long-range interaction all depend on the intervening medium.
  - Coulombic, charge-dipole, dipole-dipole.
- Example:
  - Interaction b/w 2 charges becomes shielded in a polar or polarizable medium.
    - Example: Water
  - dipoles of the medium line up to oppose the E-field.
  - Result: Interaction is weakened.

The Dielectric Constant

- Long-range interactions all reduced by a factor of $1/\kappa$.
  - the dielectric constant.
  - $\kappa = \varepsilon/\varepsilon_o = E_o/E$
    - $\varepsilon, \varepsilon_o$ = permittivity of our medium, and of free space, respectively.
    - a measure of medium polarizability.
  - a vacuum is the least polarizable medium ($\kappa = 1$).
    - Protein interior: $\kappa \cong 2\text{-}20$.
  - water much more polarizable ($\kappa \cong 80$, for isolated H_2O).
- Thus, the environment is a stabilizing factor for biopolymer structure.
  - long-range interactions greatly weakened in Aq. solution.