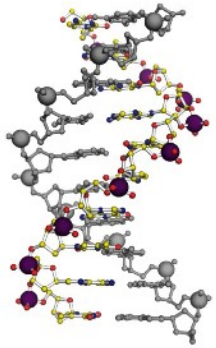
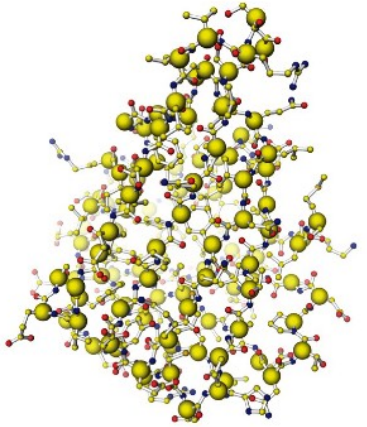
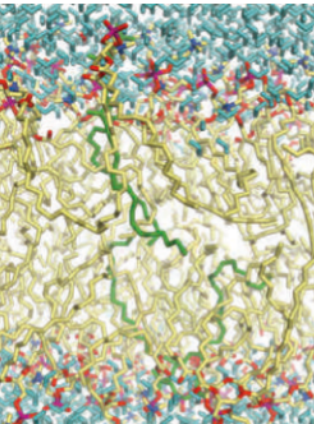


Chimie Biologique I
Biological Chemistry I
BIO-212

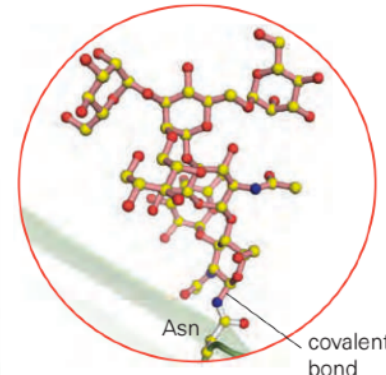


Welcome !!!!!!

Lecture 1
Matteo Dal Peraro, IBI-SV



EPFL



About me …

- **physicist**, studied at University of Padova, Italy

- became a **biophysicist** (PhD at SISSA in Trieste)

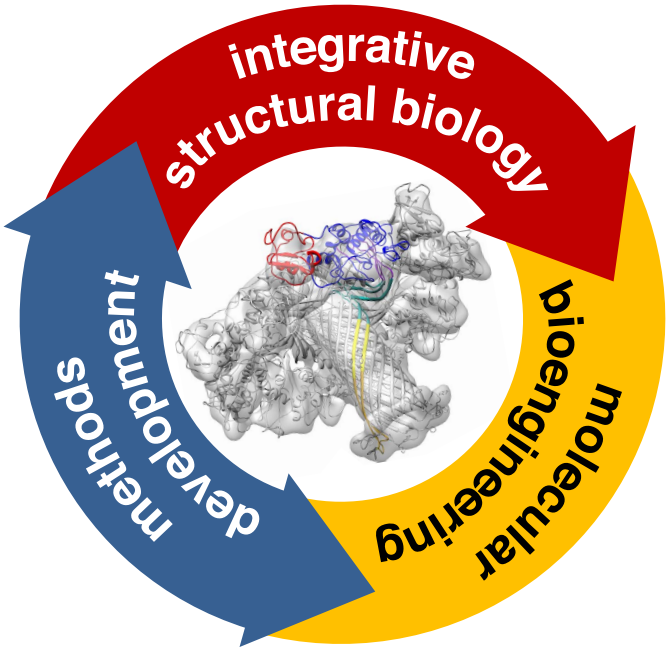
- postdoc at UPenn Chemistry, Philadelphia USA

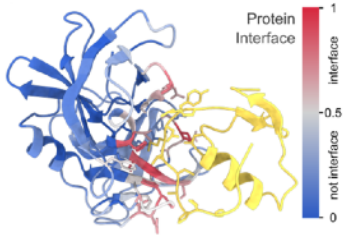
- associate professor at SV, Institute of Bioengineering (IBI)

- associate director of the Institute of Bioengineering (IBI)
- office AAB 048 - matteo.dalperaro@epfl.ch

About my lab …

- **Laboratory of Biomolecular Modeling** (LBM) - AAB 0th floor, AI 2nd floor
- computational and experimental structural biology
- **goal:** understanding the physico-chemical principles of biological function and use them for bioengineering (e.g., drug and protein design, nanopores)

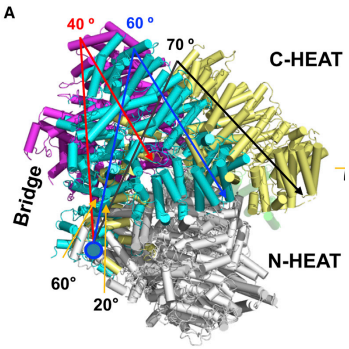




- **deep learning for molecular recognition**

http//pesto.epfl.ch

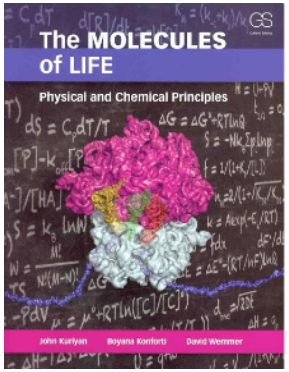
- **large molecular assembly**



- **biological nanopore sensing**

Overview

- Material (slides, exercises, Q&A forum …) on Moodle
- Organization of exercises: 8 TAs:
  - Casper
  - Stephen
  - Jana
  - Fernando
  - Zhidian
  - Evgenia
  - Maddalena
  - Benjamin
  - + Matteo
- Exam: 3h written exam, questions will be provided in both English and French and you may answer in either language. Likely will be like last year: you can bring anything but the book material, 1/4 QCM, 3/4 open exercises
- Textbook:
  Kuriyan et al. ‘The Molecules of Life’



Vision and Rules

- Vision for the course:
  - build an understanding of biology from the molecular level
  - understanding of the energetic principles that govern molecular interactions in biomolecules
  - leverage protein structures to understand biological function
  - learn about experimental and computational methods to analyze proteins

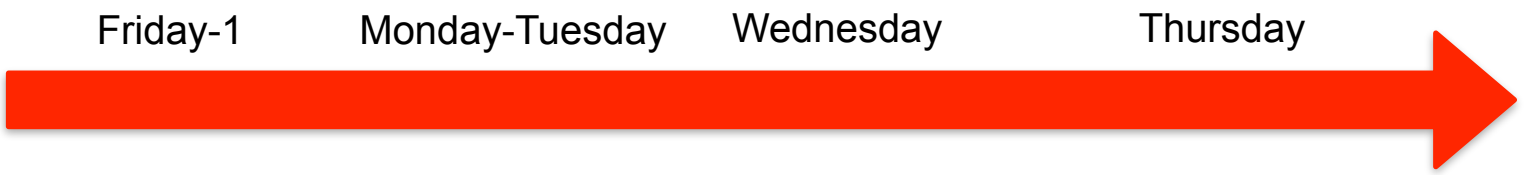
- you are encouraged to ask questions
- Take notes
- Read the book if you can - expand on what we discuss in class
- Attend lecture regularly
- Attend exercises regularly
- on week 4/5 feedback questionnaire (provided by EPFL)

Exercise Session Guidelines

- 4 ECTS this year = 2h exercise session (8-10am Thursdays morning)
- Give a look ahead of time if you can
- pair up with classmates and discuss
- discuss with the TAs - do not be shy, they are there to help
- work on series before checking the solutions

Timetable

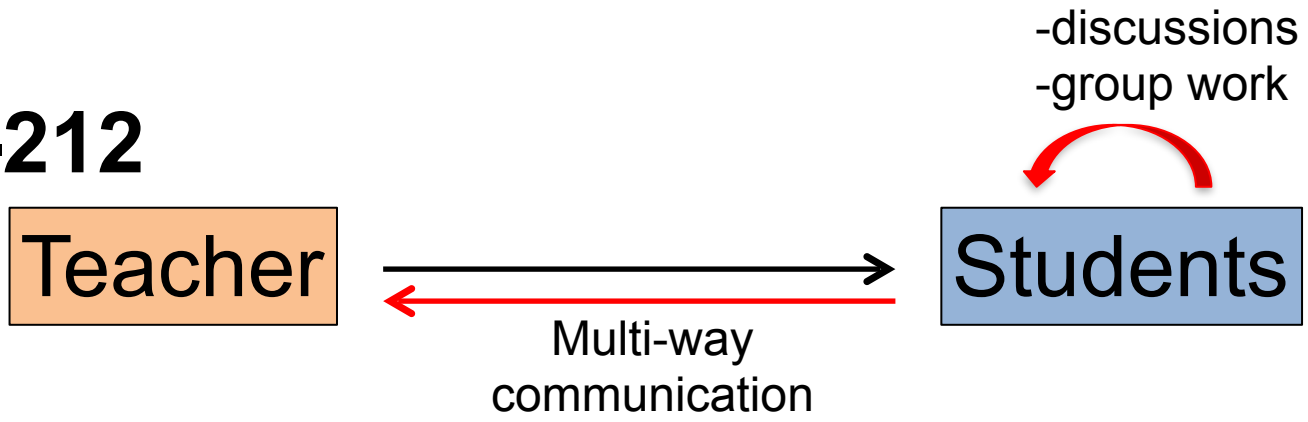
Week 1	Matteo	Intro - Biomolecular interactions - Nucleic acids - Proteins
Week 2	Matteo	Proteins and protein structure
Week 3	Matteo	Structural biology - X-ray crystallography and AlphaFold
Week 4	TAs	Bioinformatics practical
Week 5	Aleks A	Cryo-EM and NMR
Week 6	Giovanni DA	Lipids and membranes
Week 7	Matteo	Protein expression and purification
Week 8	Matteo	Protein expression and purification – cont'd
Week 9	Matteo	Thermodynamics and energetics
Week 10	Matteo	Entropy, free energy, folding
Week 11	Matteo	Recognition and binding
Week 12	Matteo	Methods for protein- and protein-ligand interactions
Week 13	Matteo	Cooperativity and allostery
Week 14	Matteo	Kinetics + lab activities showcase



Teaching Model

Traditional course

BIO-212



-Participation in the classroom and exercises
-Questions and Emails
-In person discussions

Common Problems in this class

- ”I don’t understand the subject, too difficult”
- “The pace is too fast”
- “The slides are not very helpful”
- ”We have heard this subject in other courses”
- “The lectures and the exercises are disconnected”
- “The exercise sessions are too short”
- “The lectures are not well structured”
- “ I do not know what to learn and remember (for the exam)”

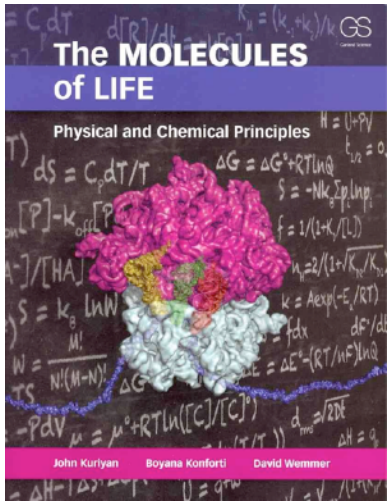
Lecture 1 - Outline

Today:

- The molecules of life
- Energetic principles of molecular interactions
- The building blocks:
  - Nucleic Acids

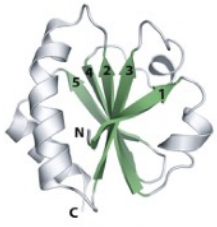
Reading suggestions:

-The Molecules of Life (Chapters 1-2)



The Essentials of Life

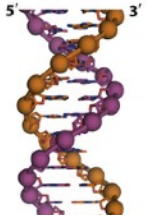
Molecular Machinery



- Proteins

- Metabolites

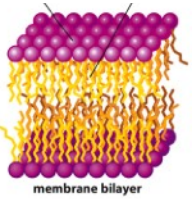
Heritable information



- DNA

- RNA

Boundary



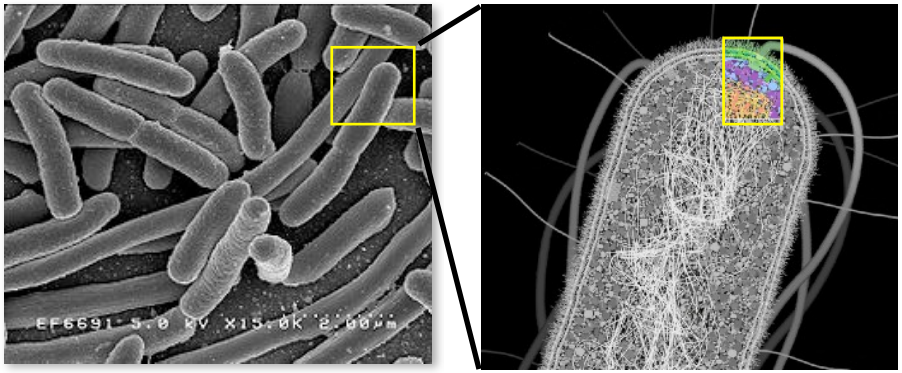
- Lipids

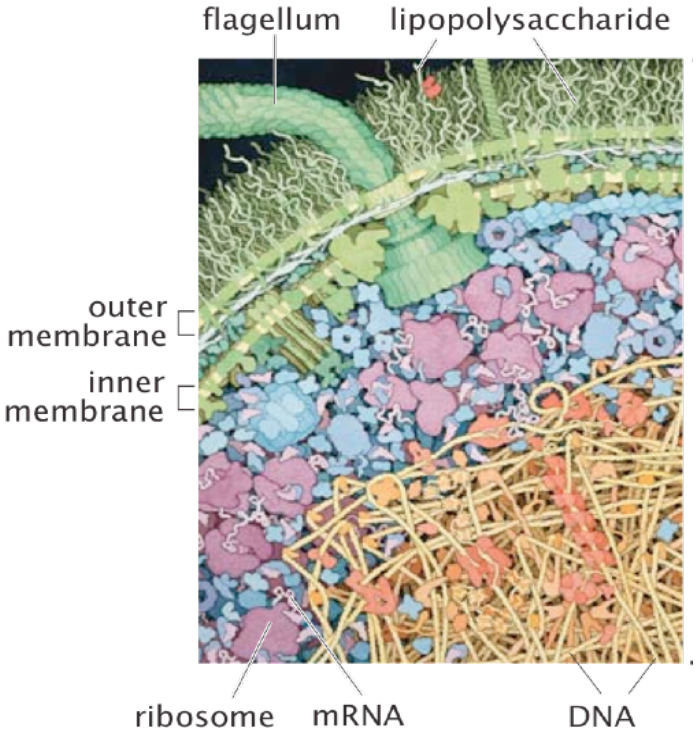
- Cell wall

these ingredients build up all cells

Inspired from Michael Hecht

Anatomy of a *E.coli* cell

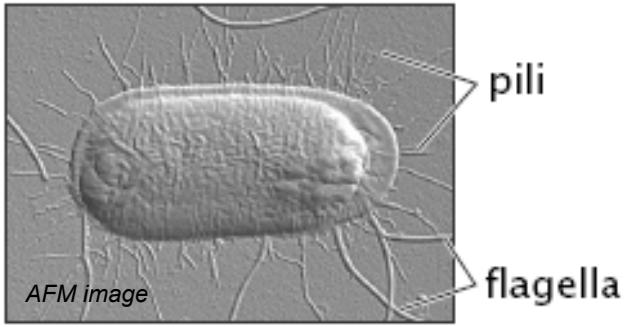




- Gram-negative bacterium
- commonly found in human intestine; certain strains cause food poisoning
- replicates rapidly *in vitro*, easily adjusts to changes in its environment; cell size depends on available nutrients
- routinely used in biology and biotechnology (to produce recombinant proteins)

E.coli as molecular ruler

- it is a good representative of biological cells (e.g. DNA-based genome, transcription machinery, lipid bilayer membranes)
- cell size and molecular population can be used as a biological ruler (≈ 1 μm^3)
- cellular equilibrium and dynamics depends on the concentration inside the cell (*in vivo* conditions)



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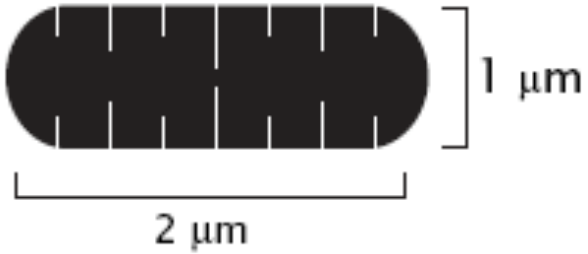

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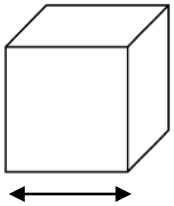


$V_{E.coli}$ ≈ 1 μm^3 = 1 fL

$A_{E.coli}$ ≈ 6 μm^2

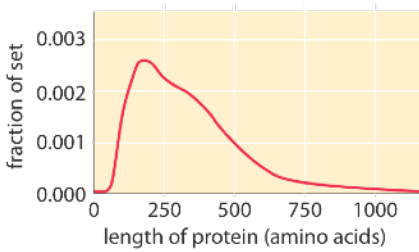
Counting up *E.coli*

Estimate the number of proteins: $N_{protein} = [m_{total\ protein} / m_{per\ protein}]$

$V_{E.coli} \approx 1fL$, assume $\rho_{E.coli} \approx \rho_{H20} = 1$ g/mL => $m_{E.coli} \approx 1$ pg

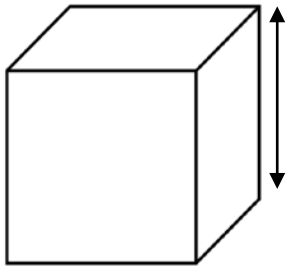
- experimentally is known that dry weight is 30% total weight, and proteins take up to 50% of dry weight, thus $m_{total\ protein} \approx 0.15$ pg

- average protein is ~30 kDa (300 AA, $m_{AA} \approx 100$ Da >> $m_{per\ protein} \sim 30$ kDa); being a Da $\approx 1.66 \times 10^{-24}$ g we obtain that $m_{per\ protein} = 5 \times 10^{-20}$ g, thus

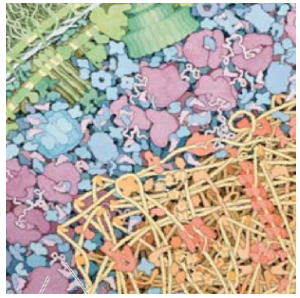


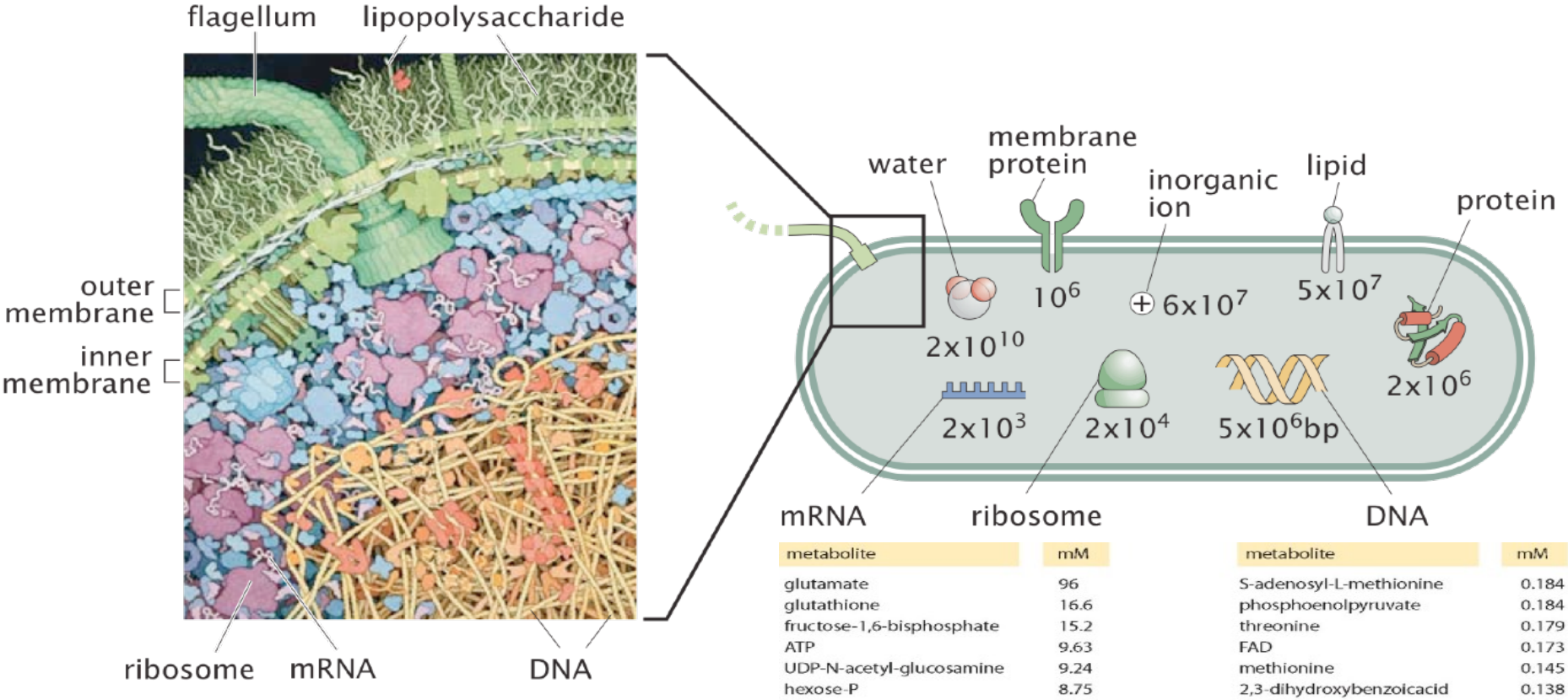
$N_{protein} = m_{total\ protein} / m_{per\ protein} \approx (15 \times 10^{-14}\ g) / (5 \times 10^{-20}\ g) \approx \mathbf{3 \times 10^6}$

of which 1/3 are typically membrane proteins $\mathbf{N_{cytoplasmic} \approx 2 \times 10^6}$, $\mathbf{N_{membrane} \approx 10^6}$



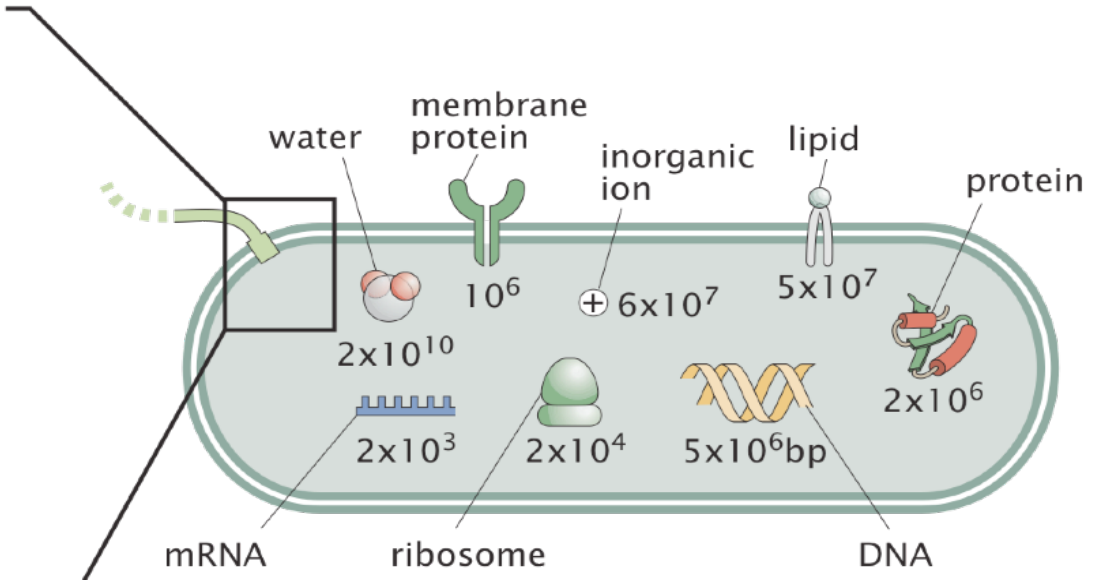
in 1 μm ~ 100 proteins each with 10 nm linear space, given ~2 nm of radius per protein, the space between 2 protein is less than ~ 5 nm





metabolite	mM
glutamate	96
glutathione	16.6
fructose-1,6-bisphosphate	15.2
ATP	9.63
UDP-N-acetyl-glucosamine	9.24
hexose-P	8.75
UTP	8.29
GTP	4.87
dTTP	4.62
aspartate	4.23
valine	4.02
glutamine	3.81
6-phospho-D-gluconate	3.77
CTP	2.73
NAD	2.55
alanine	2.55
UDP-glucose	2.5
glutathionedisulfide	2.37
uridine	2.09
citrate	1.96
UDP	1.79
malate	1.68
3-phosphoglycerate	1.54
glycerate	1.41
coenzyme-A	1.37
citrulline	1.35
pentose-P	1.32
glucosamine-6_phosphate	1.15
acetylphosphate	1.07
gluconolactone	1.04

metabolite	mM
S-adenosyl-L-methionine	0.184
phosphoenolpyruvate	0.184
threonine	0.179
FAD	0.173
methionine	0.145
2,3-dihydroxybenzoicacid	0.138
NADPH	0.121
fumarate	0.115
phenylpyruvate	0.0898
NADH	0.0832
N-acetyl-glucosamine-1P	0.0819
serine	0.068
histidine	0.0676
flavinmononucleotide	0.0537
4-hydroxybenzoate	0.0522
dGMP	0.0507
glycerolphosphate	0.049
N-acetyl-ornithine	0.0433
gluconate	0.0416
malonyl-CoA	0.0354
cyclic-AMP	0.0352
dCTP	0.0345
tyrosine	0.0289
inosine-diphosphate	0.0238
GMP	0.0237
acetoacetyl-CoA	0.0218
riboflavin	0.019
phenylalanine	0.0182
aconitate	0.0161
dATP	0.0155



cells are crowded places

NB: cellular (in vivo) conditions and in vitro (dilute) conditions are very different

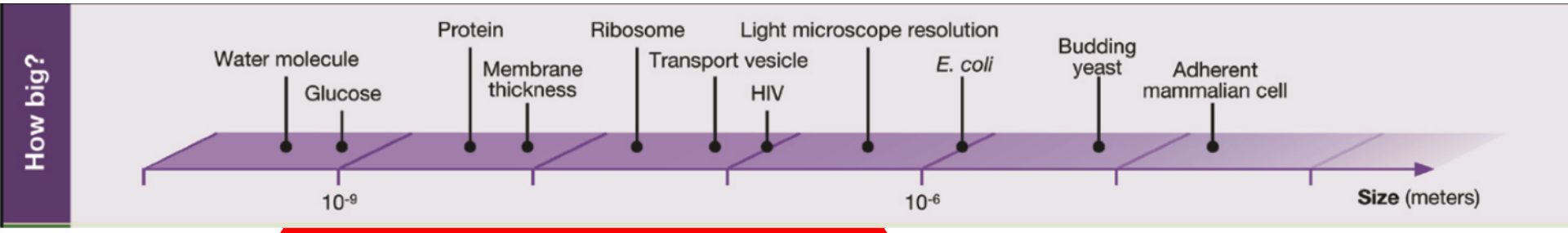
metabolite	mM
glutamate	96
glutathione	16.6
fructose-1,6-bisphosphate	15.2
ATP	9.63
UDP-N-acetyl-glucosamine	9.24
hexose-P	8.75
UTP	8.29
GTP	4.87
dTTP	4.62
aspartate	4.23
valine	4.02
glutamine	3.81
6-phospho-D-gluconate	3.77
CTP	2.73
NAD	2.55
alanine	2.55
UDP-glucose	2.5
glutathionedisulfide	2.37
uridine	2.09
citrate	1.96
UDP	1.79
malate	1.68
3-phosphoglycerate	1.54
glycerate	1.41
coenzyme-A	1.37
citrulline	1.35
pentose-P	1.32
glucosamine-6_phosphate	1.15
acetylphosphate	1.07
gluconolactone	1.04

metabolite	mM
S-adenosyl-L-methionine	0.184
phosphoenolpyruvate	0.184
threonine	0.179
FAD	0.173
methionine	0.145
2,3-dihydroxybenzoicacid	0.138
NADPH	0.121
fumarate	0.115
phenylpyruvate	0.0898
NADH	0.0832
N-acetyl-glucosamine-1P	0.0819
serine	0.068
histidine	0.0676
flavinmononucleotide	0.0537
4-hydroxybenzoate	0.0522
dGMP	0.0507
glycerolphosphate	0.049
N-acetyl-ornithine	0.0433
gluconate	0.0416
malonyl-CoA	0.0354
cyclic-AMP	0.0352
dCTP	0.0345
tyrosine	0.0289
inosine-diphosphate	0.0238
GMP	0.0237
acetoacetyl-CoA	0.0218
riboflavin	0.019
phenylalanine	0.0182
aconitate	0.0161
dATP	0.0155

Numbers of Life

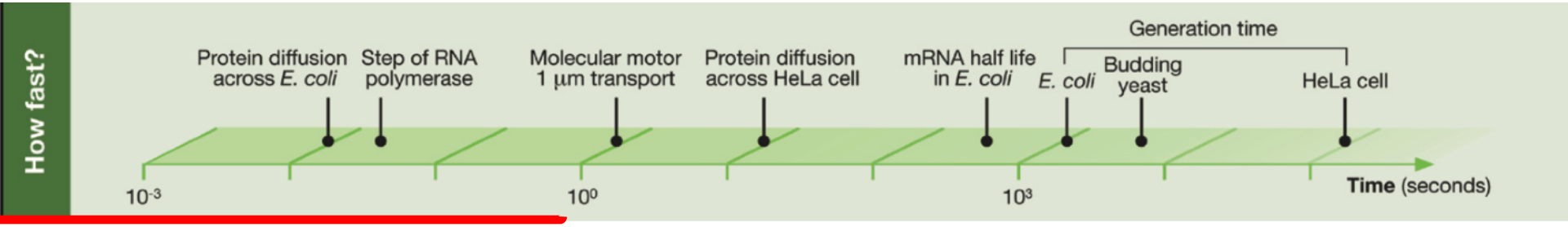
these are the relevant scales important when we talk about biomolecules

size



Å nm

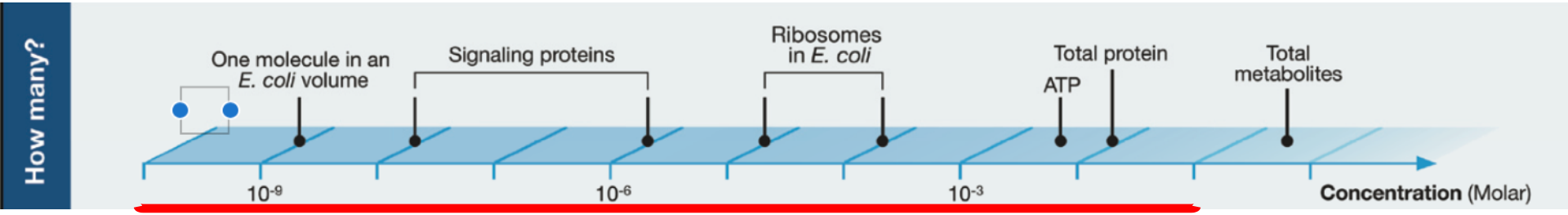
time



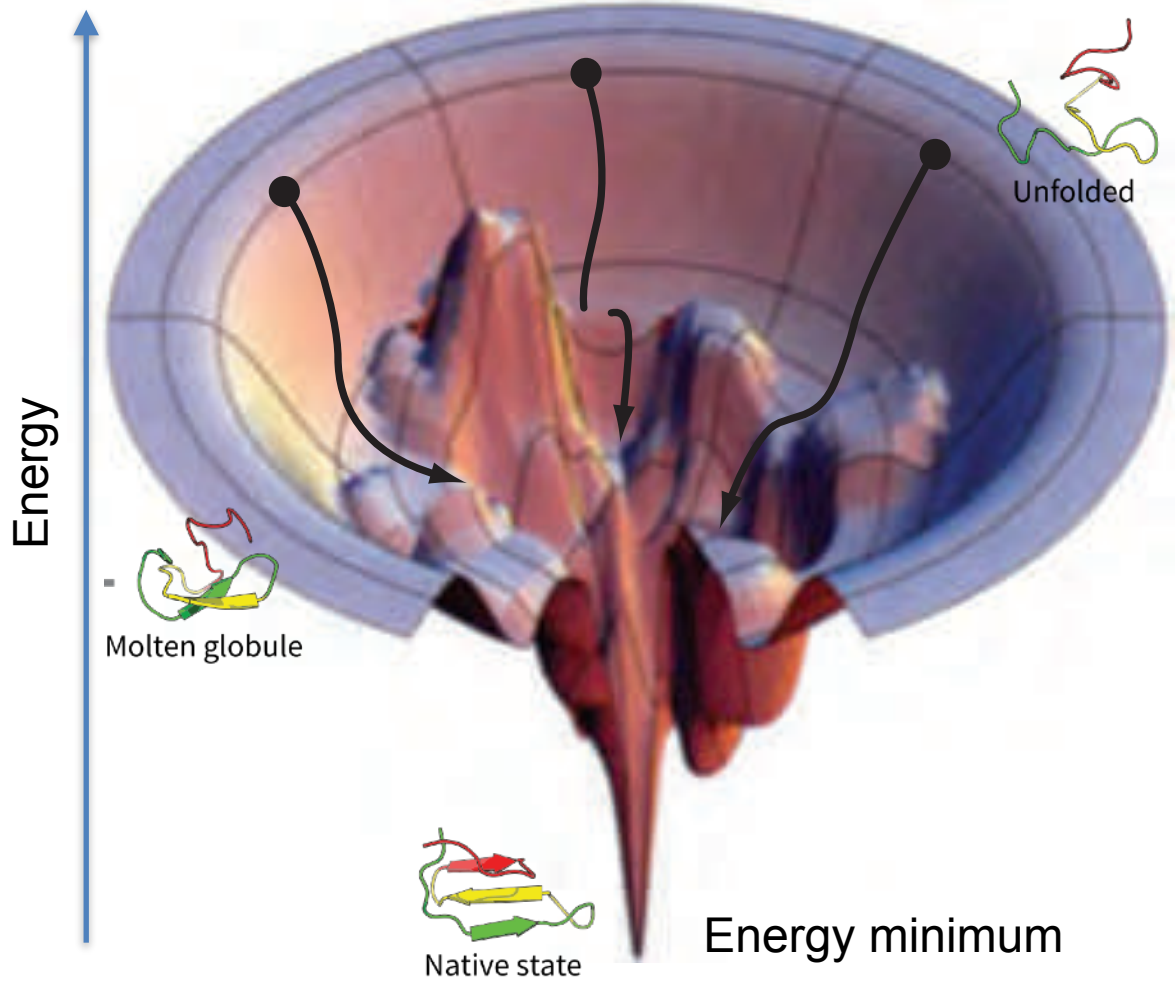
ns — s

protein conformational changes

concentration



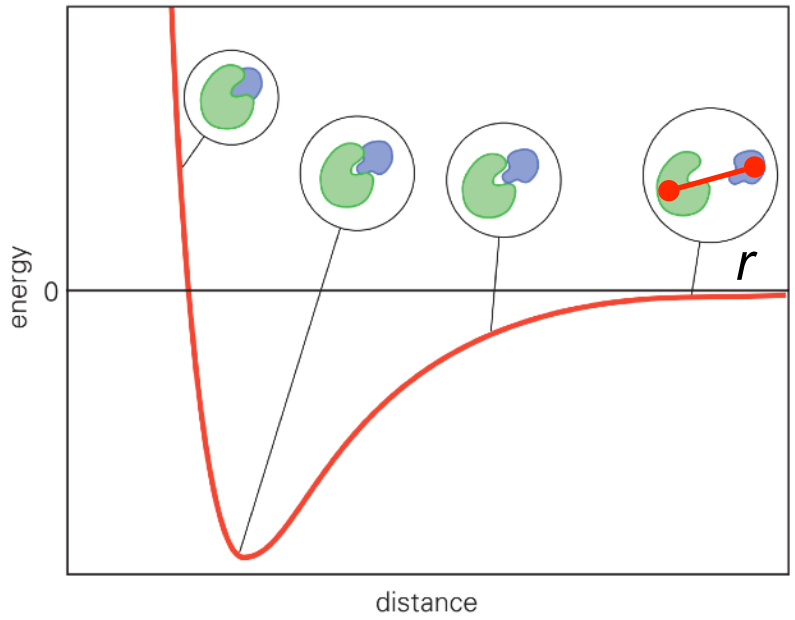
Energy as the main driver of biological processes



Changes that drive a molecular system to lower energies determines what happens at the molecular and higher levels in biology (eg protein folding)

Molecular Interactions in Biomolecules

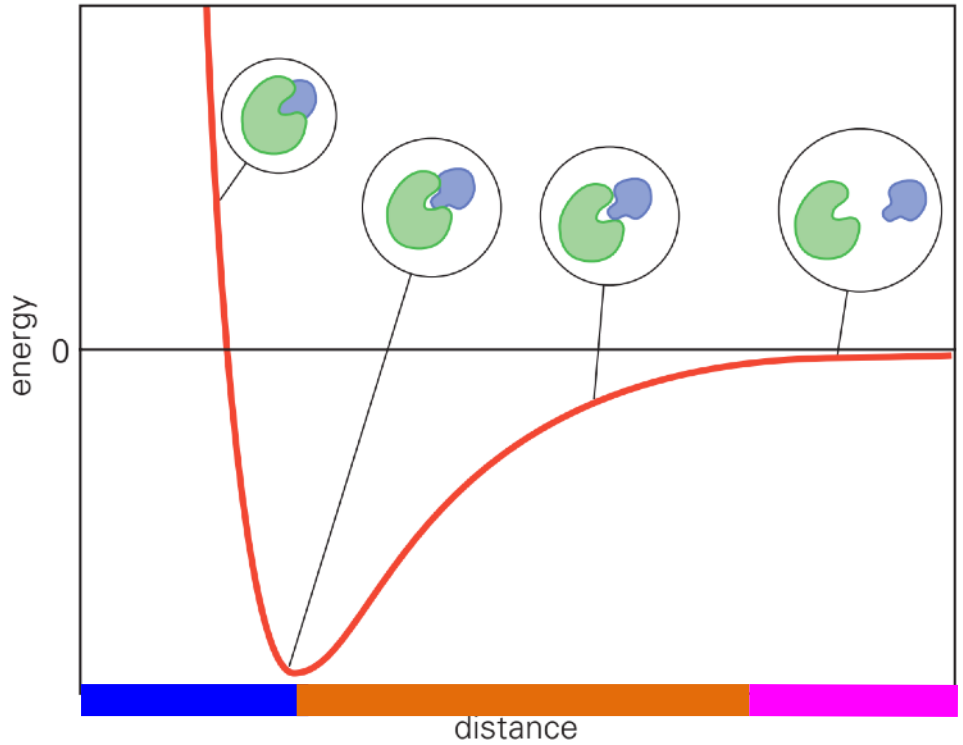
There are two levels:
- interactions that happens within a biomolecule (intramolecular), mostly **covalent** (but not only, as we will see) (covalent interactions are defined by chemical bonds)
- interaction between two different biomolecules (intermolecular) mostly determined by **noncovalent** interactions



The energy of non-covalent interactions is dependent on the **distances** between molecules - ***U(r)***

Molecular Interactions in Biomolecules

The energy of interaction between two molecules is determined by **noncovalent** interactions



r_0 = equilibrium distance

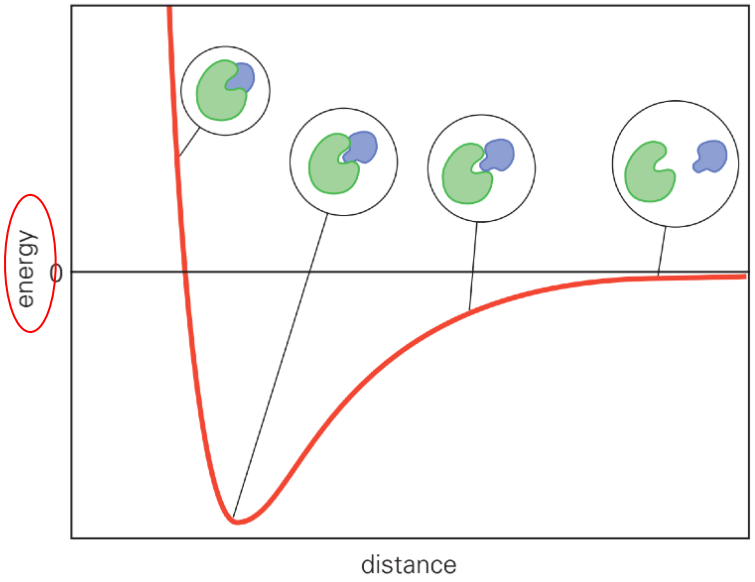
Nature of the Interaction

Repulsive Attractive Non-Interacting

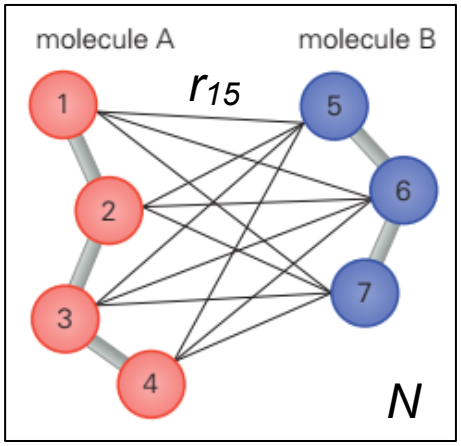
(due to the electronic repulsion
Pauli exclusion principle)

Molecular Interactions in Biomolecules

The energy of interaction between two molecules is determined by **noncovalent** interactions



Atomic pairwise Interactions



$$U(r) \sim \sum_{i<j}^{N} U(r_{ij})$$

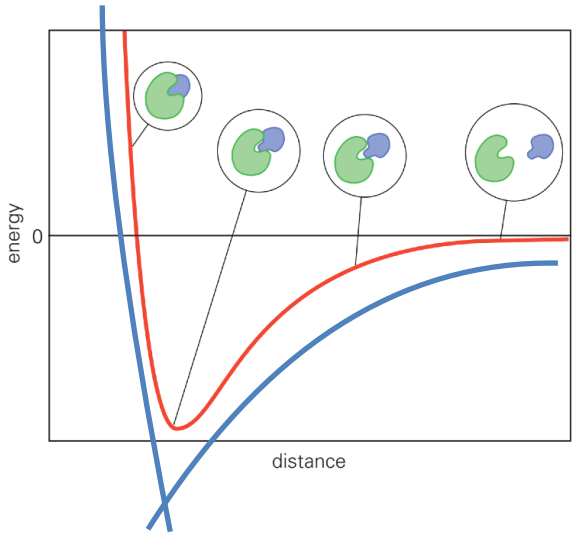
But how do we compute this energy ?

-Given that we can describe our system of interest at the atomic level, we can compute the sum of the **pairwise interactions** between all the different pairs of atoms.

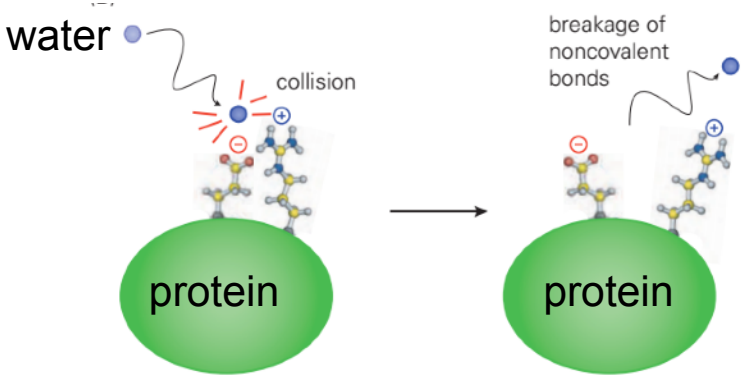
-This approach is applicable to all the building blocks of life (based on classical mechanics - somehow empirical) .

Molecular Interactions in Biomolecules

noncovalent interactions



Noncovalent interactions are transient



Noncovalent interactions are broken and remade simply due to thermal fluctuations (related to the thermal energy of every degree of freedom, k_BT **~ 2.5 kJ/mol** at 300 K)

"everything that living things do can be understood in terms of the jigglings and wigglings of atoms." R. Feynman

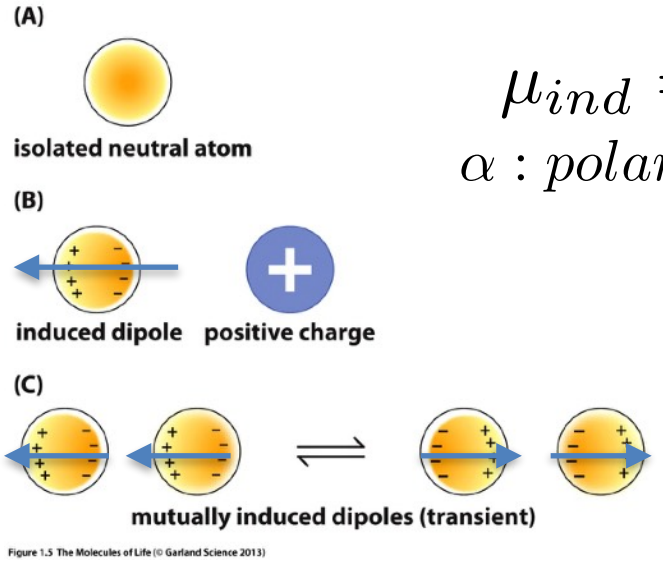
-Important types of noncovalent interactions in biomolecules:

- **van der Waals interactions -**
- **Ionic interactions**
- **Hydrogen bonds**

$$U(r) \sim -\sum_{i<j}^{N} \frac{1}{r_{ij}^{n}}$$

Molecular Interactions in Biomolecules

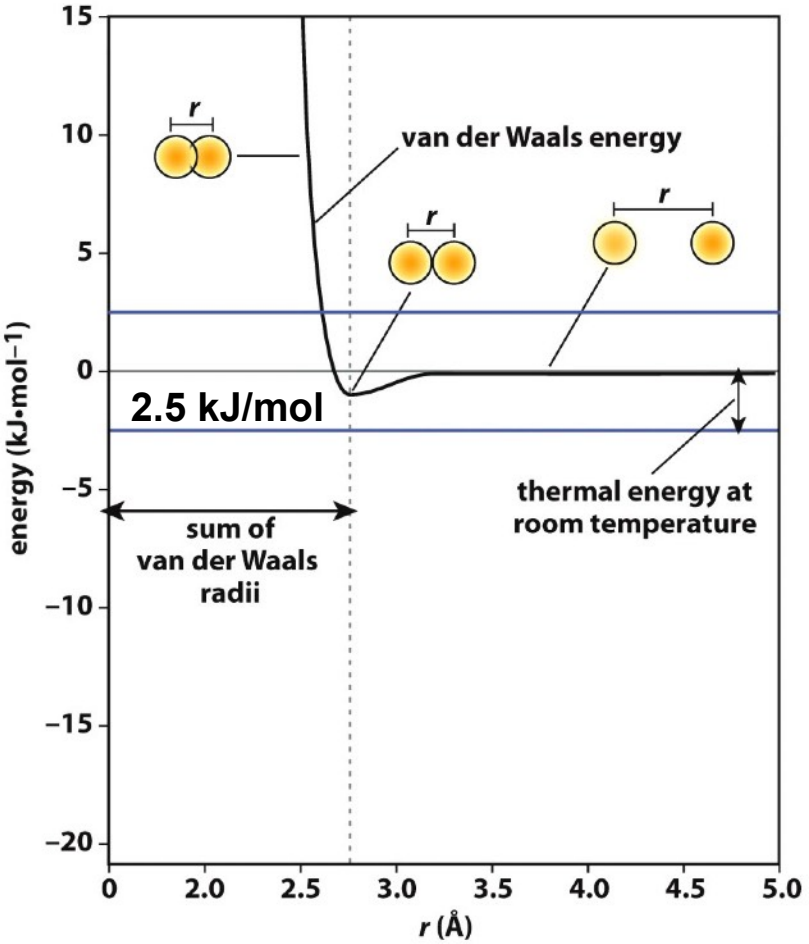
van der Walls interactions (London forces)



$$\mu_{ind} = \alpha \mathbf{E}$$

$$\alpha : polarizability$$

Atom	van der Waals radius (Å)	Electro-negativity (Pauling scale)
O	1.5	3.4
Cl	1.9	3.2
N	1.6	3.0
S	1.8	2.6
C	1.7	2.6
P	1.8	2.2
H	1.2	2.1



- due to induced dipoles in atoms

- Notice the quantitative information of such potential (real energies and distances).

- In this potential there is a repulsive, attractive and non interacting region.

- The radius of each atom determines the distance of minimal energy (sum of vdW radii).

Molecular Interactions in Biomolecules

van der Walls interactions (London forces)

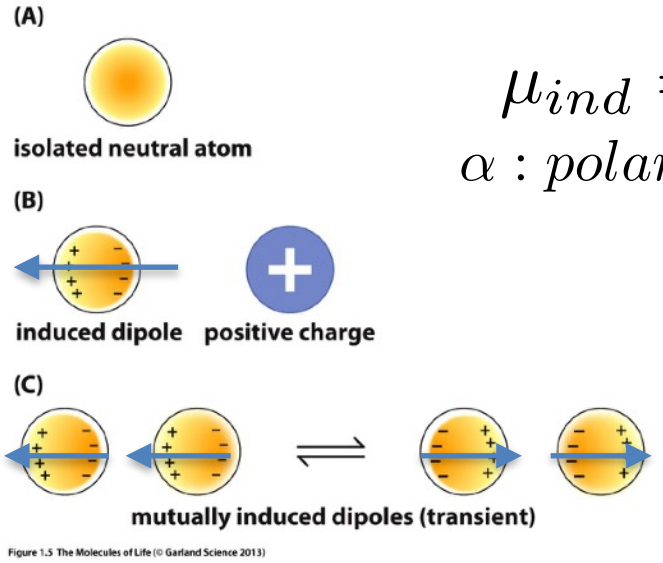


Figure 1.5 The Molecules of Life (© Garland Science 2013)

$$\mu_{ind} = \alpha \mathbf{E}$$

$$\alpha : polarizability$$

Atom	van der Waals radius (Å)	Electro-negativity (Pauling scale)
O	1.5	3.4
Cl	1.9	3.2
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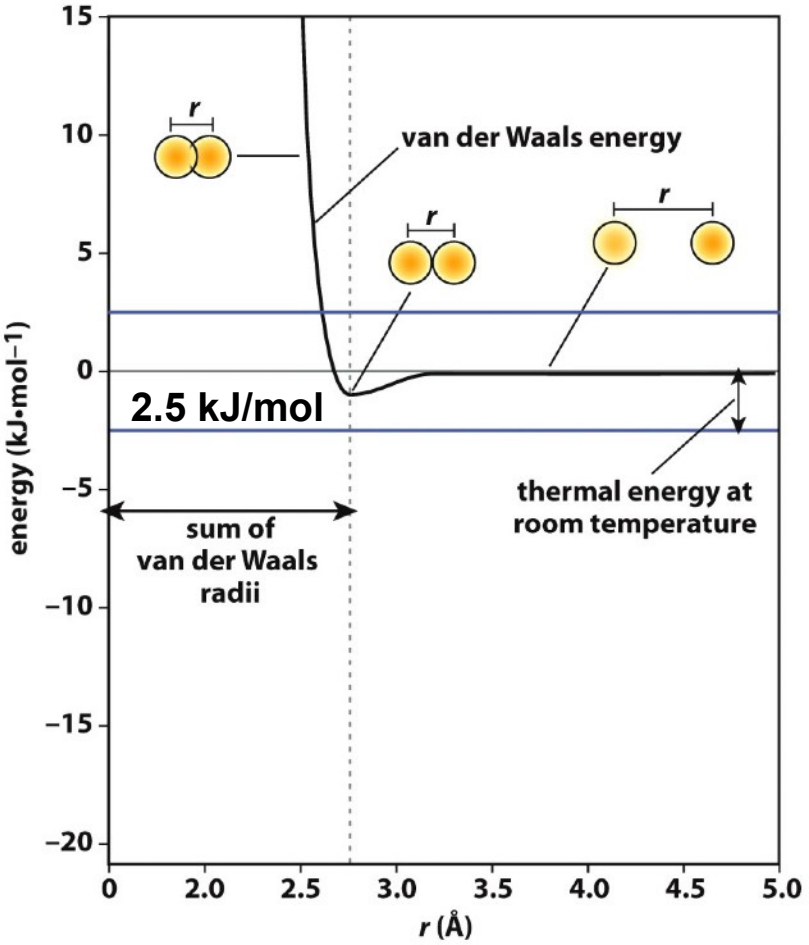


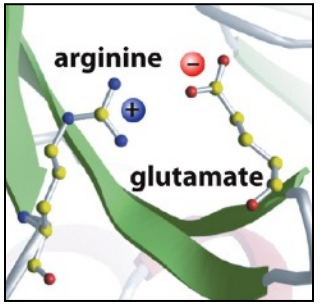
-Individual vdW interactions are very weak - attractive part goes like U(r) ~ -1/r^6.

-Magnitude of the thermal energy is greater than a vdW interaction.

-Many add up to significant energies for the stabilization of biomolecules

Molecular Interactions in Biomolecules

Ionic interactions: Simplest kind of interaction is between two charged atoms (if charges are opposite are called **salt bridges**)



Energy potential for ion pair interactions:

-Similar distance dependency to van der Waals

-Stabilization energy is much greater - ***U(r) ~ -1/r***

-The equilibrium distance is smaller

$$U_{electrostatic}(r) = \frac{1}{4\pi\epsilon_0}\frac{q_i q_j}{r_{ij}}$$

$$= 1391\ kJ \cdot mol^{-1}\frac{q_i q_j}{r_{ij}[\mathring{A}]}$$

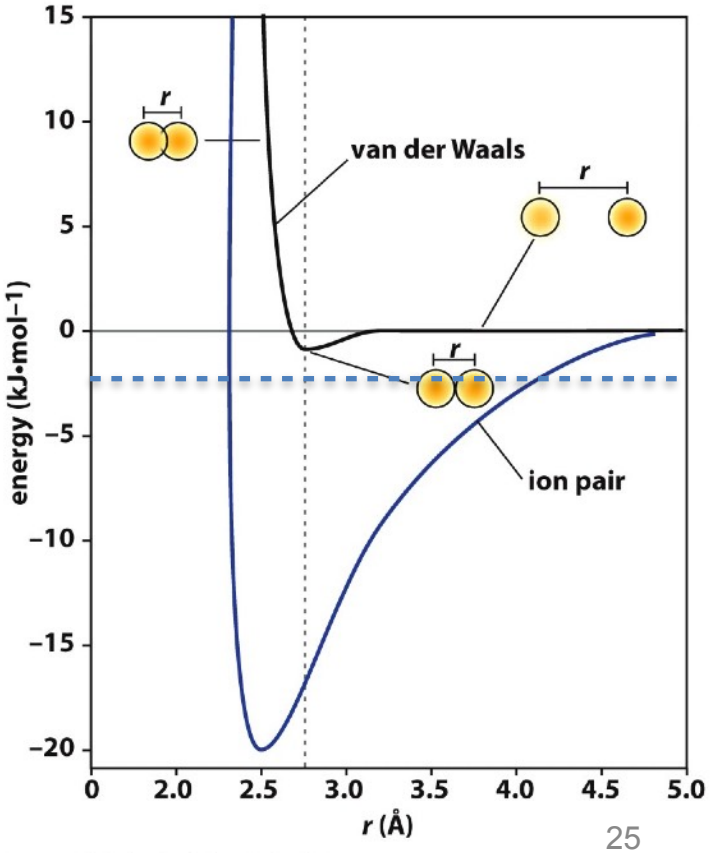


Figure 1.9 The Molecules of Life (© Garland Science 2013)

Molecular Interactions in Biomolecules

Ionic interactions: Simplest kind of interaction is between two charged atoms (if charges are opposite are called **salt bridges**)

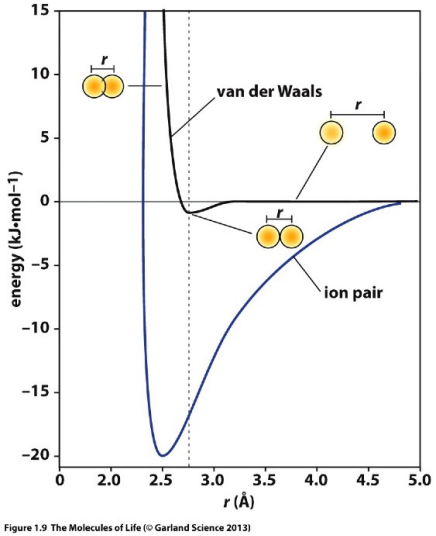


Figure 1.9 The Molecules of Life (© Garland Science 2013)

Energy potential for ion pair interactions:

-Similar distance dependency to van der Waals

-Stabilization energy is much greater

-The equilibrium distance is smaller

Ionic interactions are dependent on the environment:
- Water and ions reduce electrostatic interaction strength

$$U(r) = \frac{1}{4\pi\epsilon_0}\frac{1}{D}\frac{q_1 q_2}{r}$$

$$= 1391\ kJ \cdot mol^{-1}\frac{q_i q_j}{r_{ij}[\mathring{A}]}\frac{1}{D}$$

in water

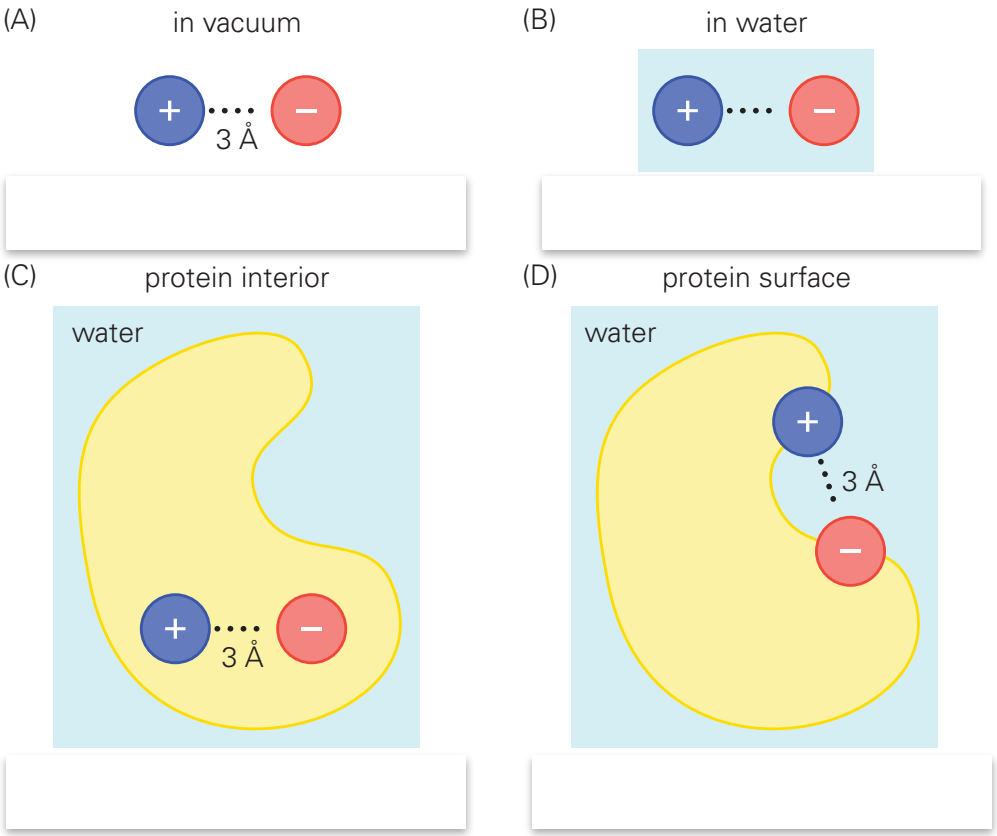
Liquid	T (°C)	D
Heptane	0	1.958
Heptane	30	1.916
Methanol	25	33
Formamide	20	109
Formic acid	16	58
Nitrobenzene	25	35
HCN	0	158
HCN	20	114
Glycol	25	37
Water	0	88.00
Water	25	78.54

D = dielectric constant
[air(T=0°C) D=1.00059]

Discussion

How are salt bridges affected by the environment ?

$$U(r) = 1391\ kJ \cdot mol^{-1} \frac{q_i q_j}{r_{ij}[\mathring{A}]} \frac{1}{D}$$



Discussion

How does the energy potential go if charges are of the same sign?

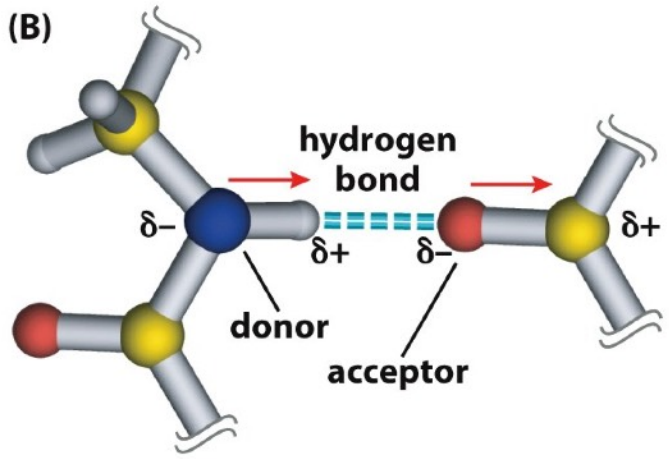
$$U(r) = 1391\ kJ \cdot mol^{-1} \frac{q_i q_j}{r_{ij}[\mathring{A}]} \frac{1}{D}$$

Molecular Interactions in Biomolecules

Hydrogen Bonds

- Interactions between polar groups in which a hydrogen atom with a partial positive charge is close to an atom with a partial negative charge.

H-bond



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P	1.8	2.2
H	1.2	2.1

- H-bonds are distance- and angle-dependent (position of donor and acceptor atoms)

- Typical distances between 2.4-2.7 Å and angles depends nature of donor/acceptor

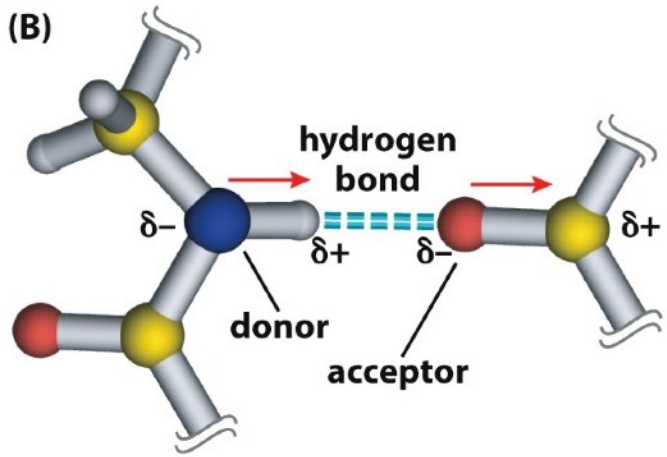
- Hydrogen bonds arise from the polarization of atoms involved in covalent bonds - think in terms of dipole-dipole interactions

Molecular Interactions in Biomolecules

Hydrogen Bonds

- Interactions between polar groups in which a hydrogen atom with a partial positive charge is close to an atom with a partial negative charge.

H-bond



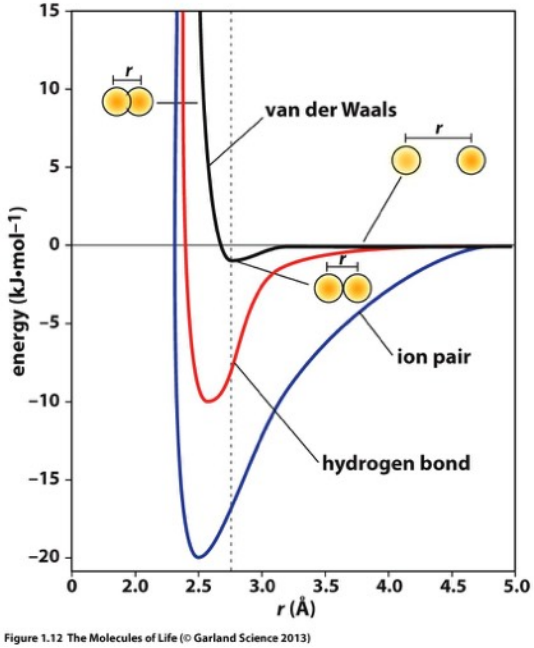


Figure 1.12 The Molecules of Life (© Garland Science 2013)

- H-bonds are distance- and angle-dependent (position of donor and acceptor atoms)

- Typical distances between 2.4-2.7 Å and angles depends nature of donor/acceptor

- Hydrogen bonds arise from the polarization of atoms involved in covalent bonds - think in terms of dipole-dipole interactions

- H-bonds are weaker than salt bridges - they go like $U(r) \sim -1/r^3$

Molecular Interactions in Biomolecules

Hydrogen Bonds

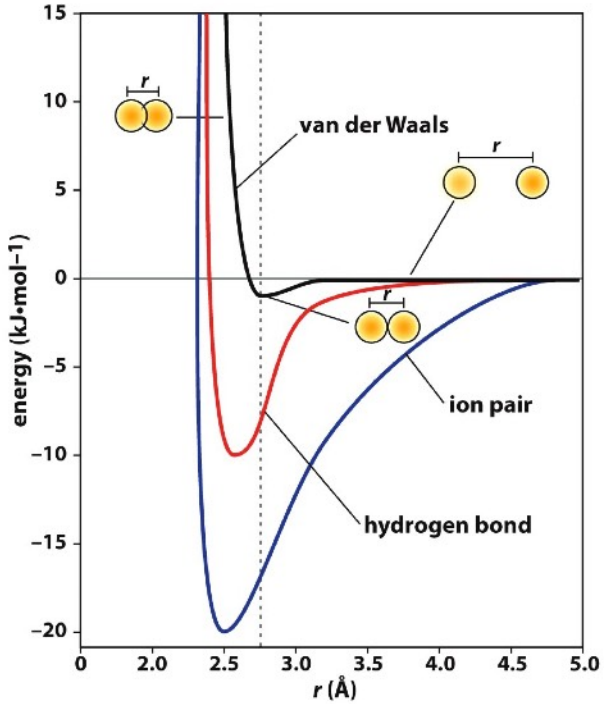


Figure 1.12 The Molecules of Life (© Garland Science 2013)

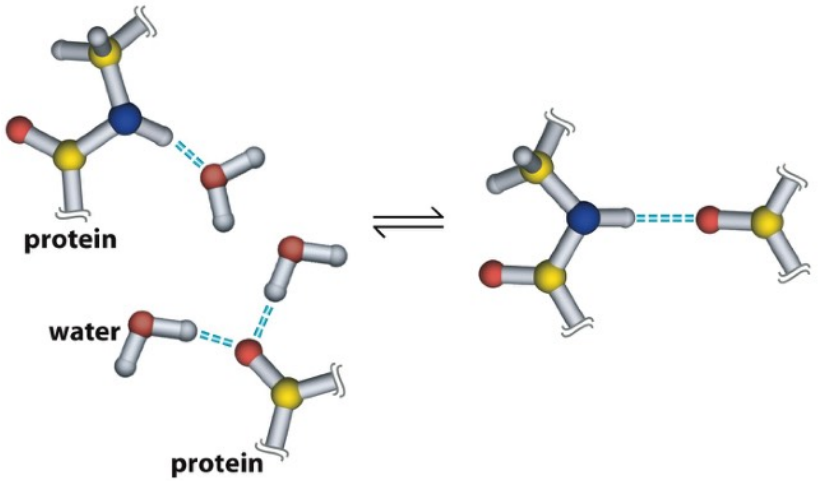


Figure 1.13 The Molecules of Life (© Garland Science 2013)

- H-bonds are also dependent on the environment – water molecules will weaken effective hydrogen bonds - solvation effect

Molecular Interactions in Biomolecules

Atomic Interactions

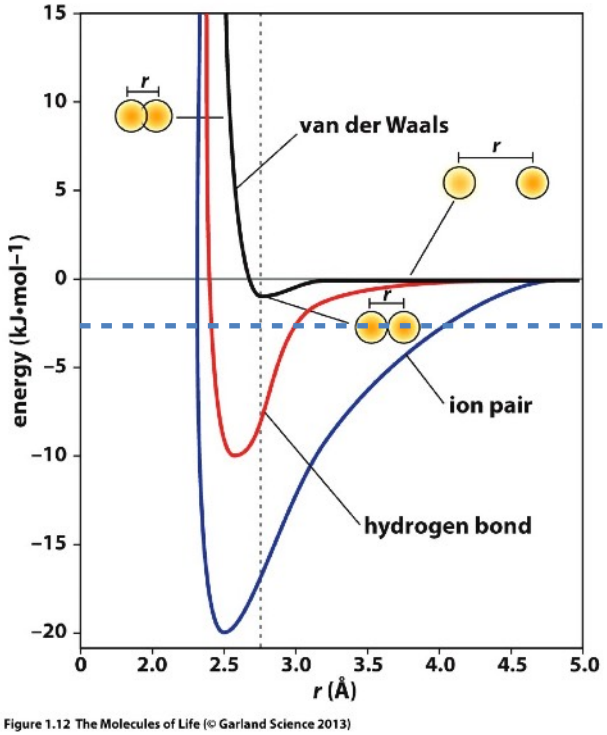
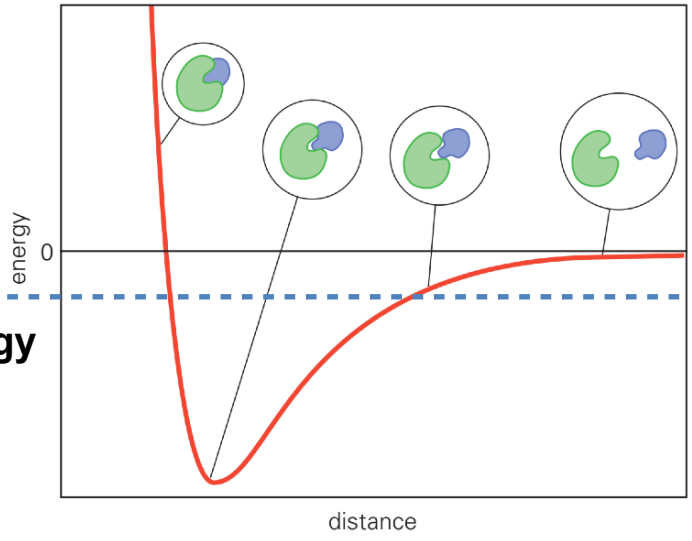


Figure 1.12 The Molecules of Life (© Garland Science 2013)

thermal energy

Macromolecular interactions



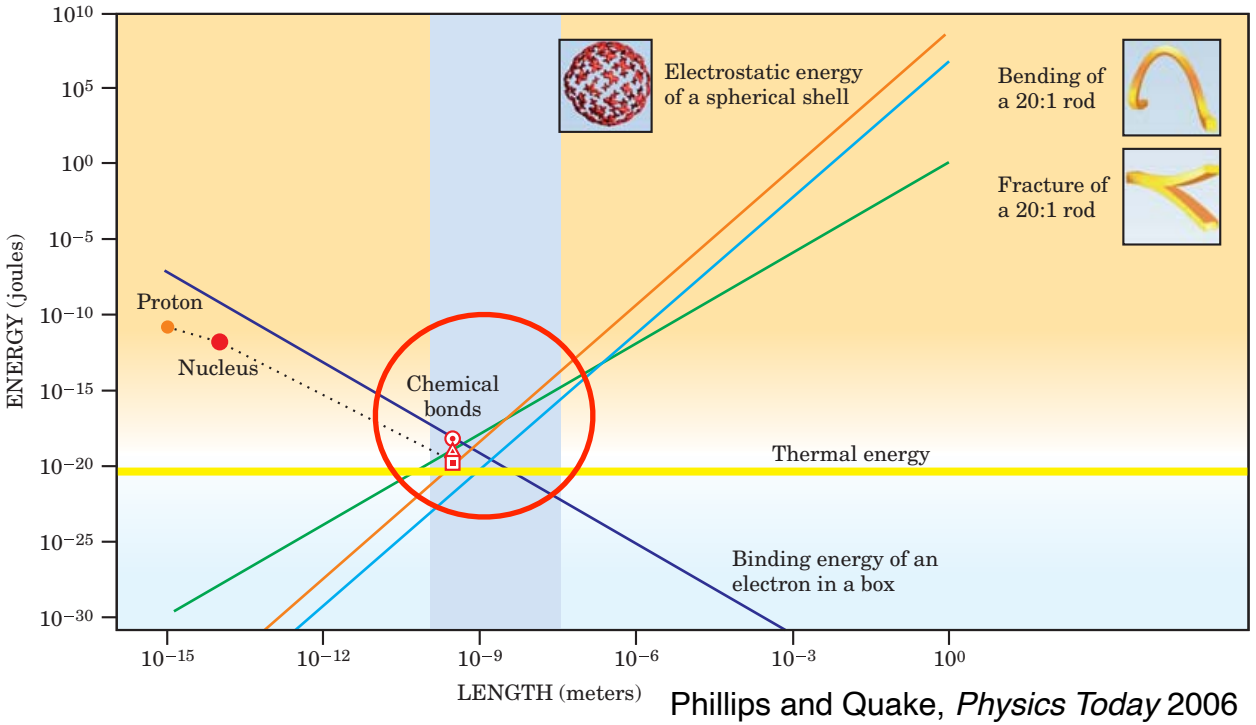
2.5 kJ/mol

$$U(r) \sim \sum_{i<j}^{N} U(r_{ij})$$ (addictive)

We can now understand some of the energetic principles that govern the interactions between macromolecules and determine how complex biological processes occur

The intriguing nature of biological interactions

- biological systems are subjected to deterministic forces (enthalpy) and thermal forces (entropy)
- at the dimension scale of biological systems these are however on the same order of magnitude
- all transformations in cells are thus determined by this subtle interplay, defined by the free energy of the system (**G=H-TS**) (accuracy in a noisy world, and use of thermal fluctuations to deploy biological function)



Phillips and Quake, *Physics Today* 2006

E_{det}/k_BT

k_BT = 4.1 pN*nm
= 0.6 kcal/mol
= 2.5 kJ/mol
= 0.025 eV

at room temperature (300K)

Molecular Interactions in Biomolecules

Some important take-home messages:

-The energy of interaction between different biomolecules is determined by **noncovalent interactions**

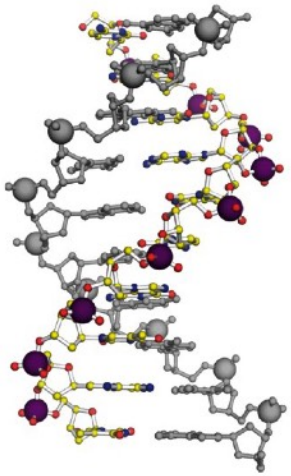
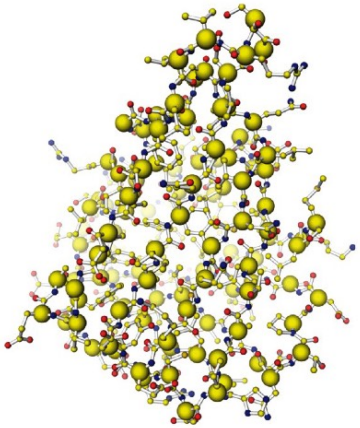
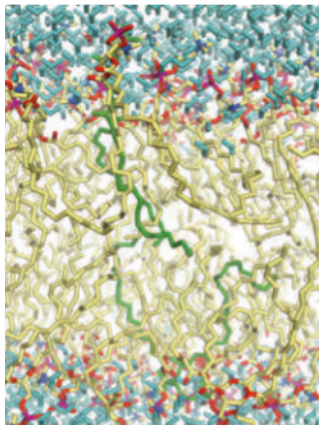
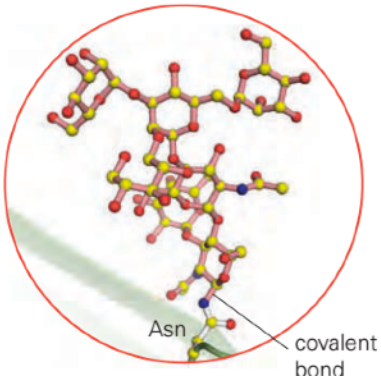
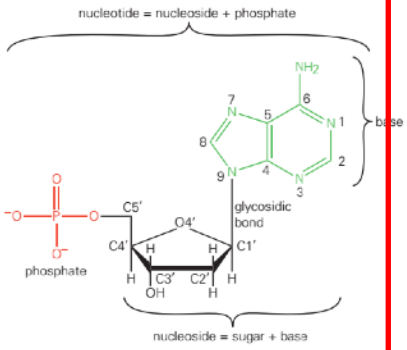
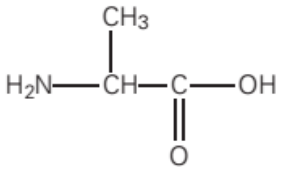
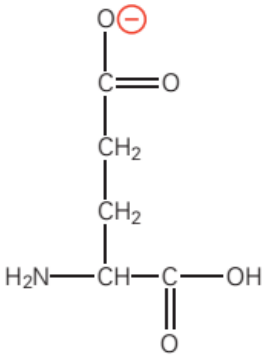
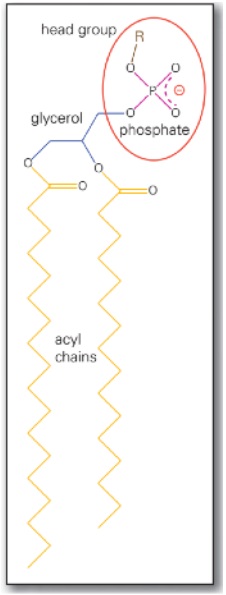
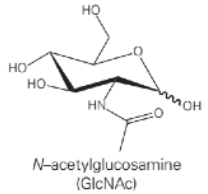
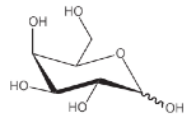
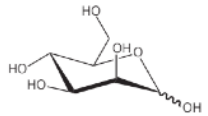
- Neutral atoms attract each other at short distances through **van der Waals interactions**

- **Ionic interactions** between charged molecules can be very strong, but are attenuated by water molecules (screening effect)

- **Hydrogen bonds** are very common in biological macromolecules and are a consequence of polarization of covalent bonds

- Always consider these molecular interactions with respect to the **thermal energy** level

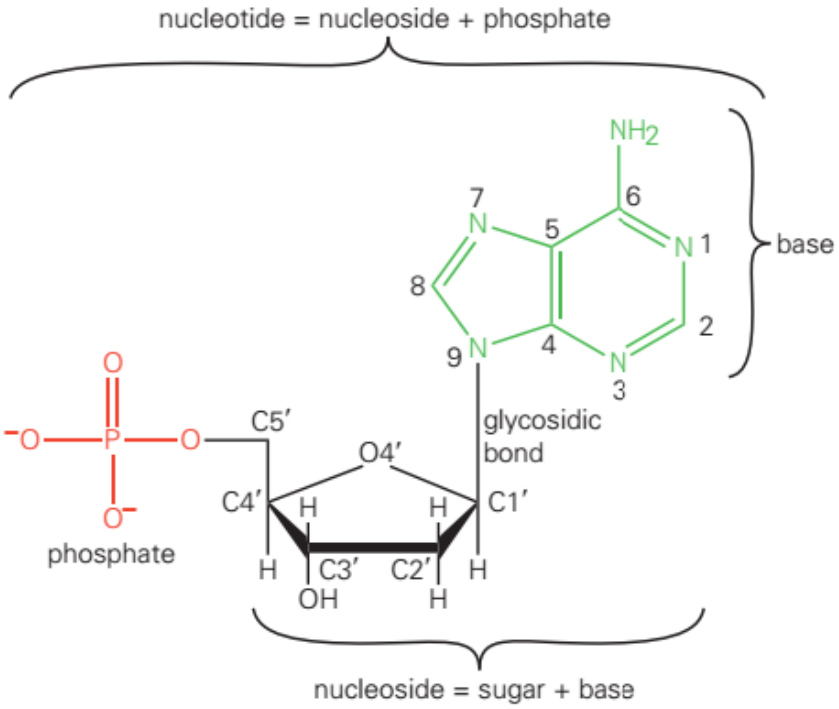
The Molecules of Life

	Nucleic Acids	Proteins	Lipids	Glycans
Macromolecular Structure				
Building Block		 		  

Nucleic Acids

-DNA and RNA are both polymers of **nucleotides**.

Structure of a nucleotide

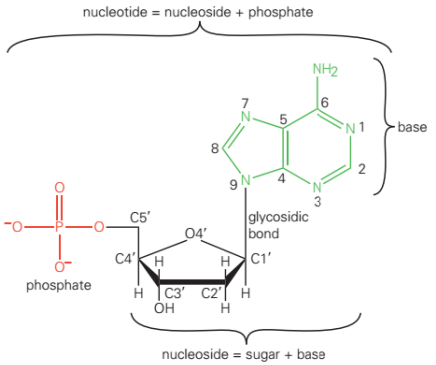


-Key functional groups:

- Five carbon sugar – **pentose** in black
- nitrogen-containing aromatic ring system, i.e. **base** – adenine in green
- **phosphate** group in red (ranging from 1 to 3)

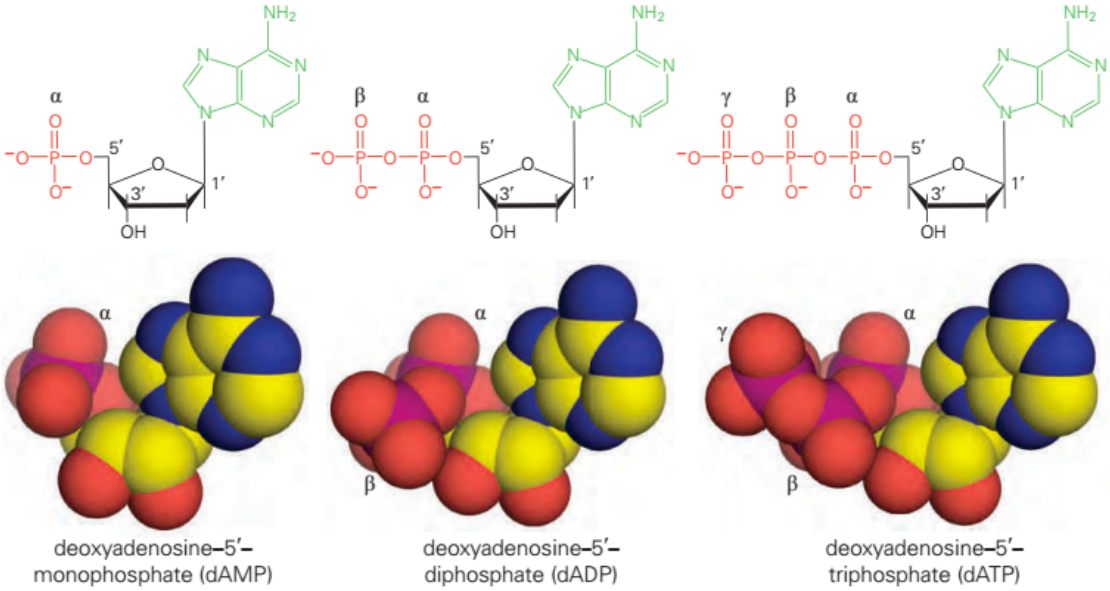
-Notice the linkages between the groups

Nucleic Acids – The phosphate



-Nucleotides with one, two or three phosphate groups are referred to as nucleotide mono, di or triphosphate.

-The three phosphate groups are called alpha, beta and gamma



Nucleic Acids are Polymers

-Nucleotides are joined together in DNA and RNA by the formation of a phosphodiester linkage between the 3’ carbon of one nucleotide and the 5’ of another

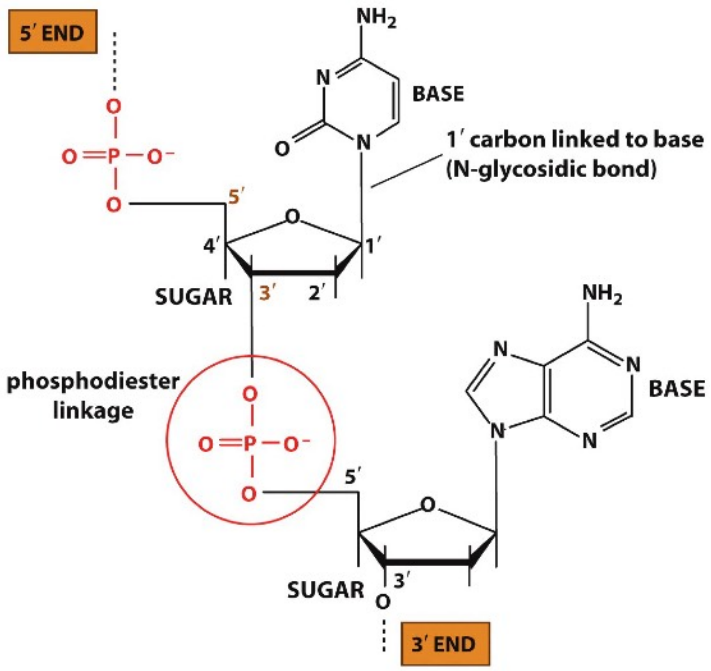


Figure 1.20 The Molecules of Life (© Garland Science 2013)

-The phosphate groups are negatively charged – important determinant for the 3D structure of of DNA and RNA

Nucleic Acids are Polymers

-The synthesis of new molecules of DNA and RNA involves the stepwise addition of nucleotide to one end of the chain.

-The triphosphate group is high in energy and its hydrolysis drives the reaction

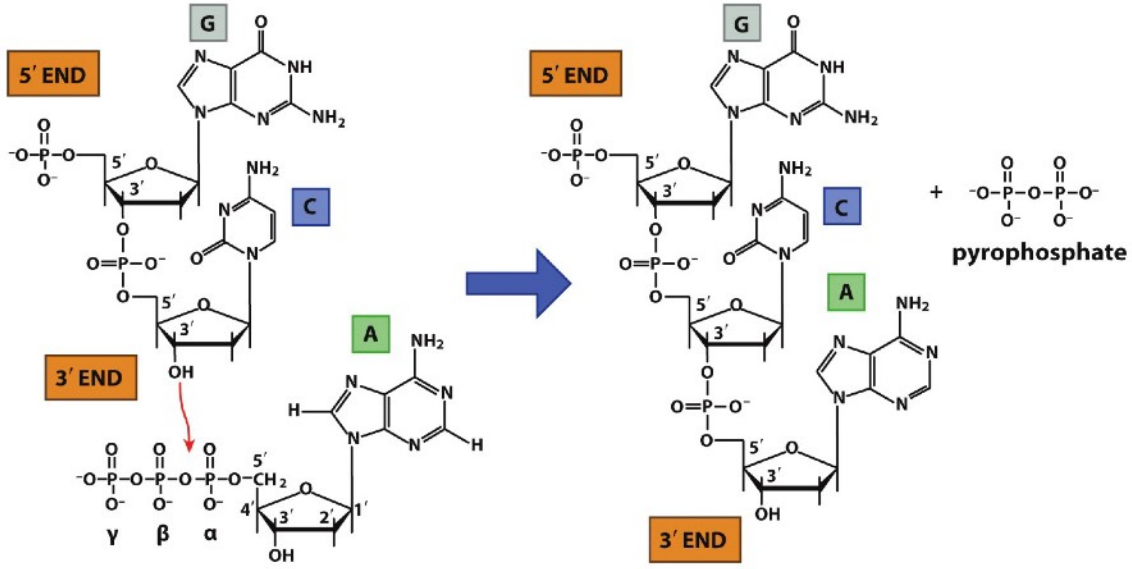


Figure 1.21 The Molecules of Life (© Garland Science 2013)

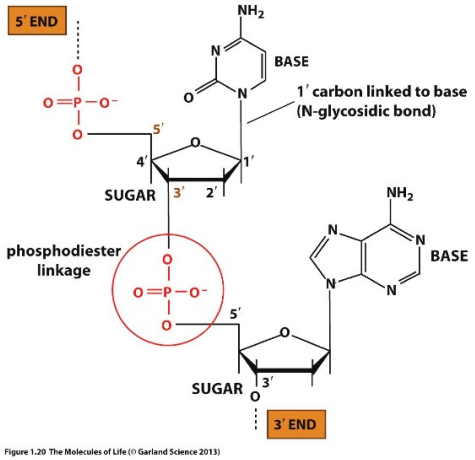
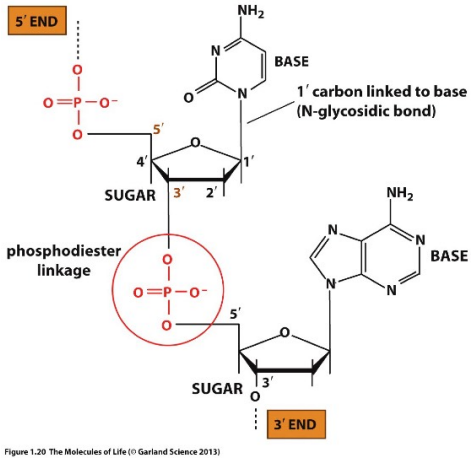


Figure 1.20 The Molecules of Life (© Garland Science 2013)

Nucleic Acids are Polymers



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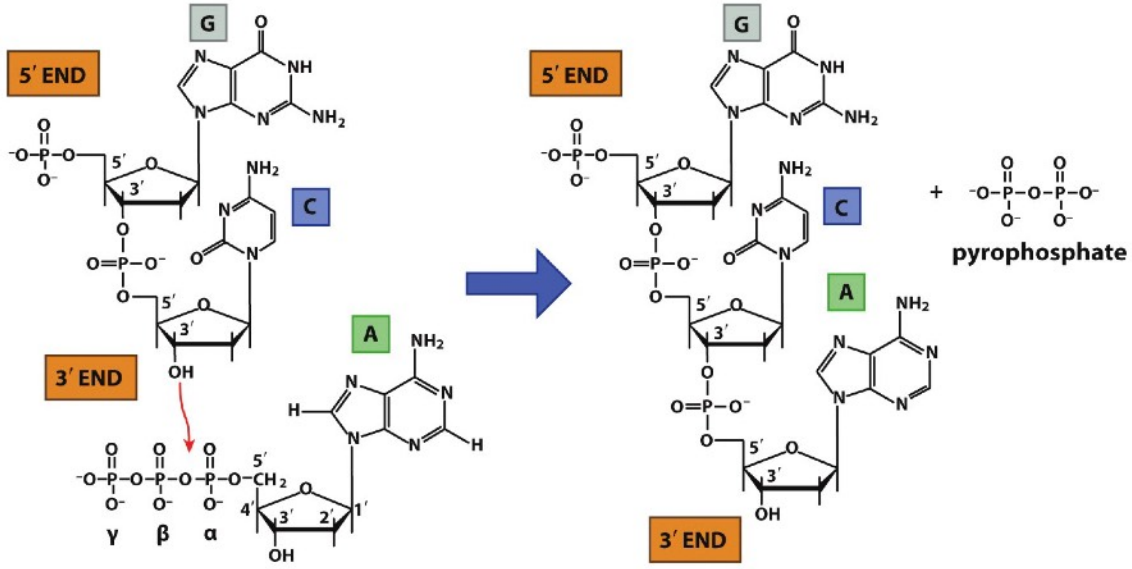


Figure 1.21 The Molecules of Life (© Garland Science 2013)

-DNA and RNA synthesis are template directed – DNA polymerases use a template strand to select each nucleotide to be added to the growing chain

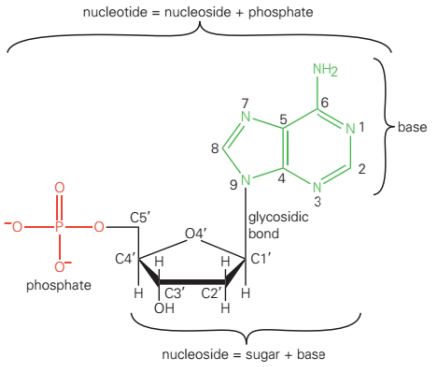
-3’->5’ phosphodiester linkage imposes directionality

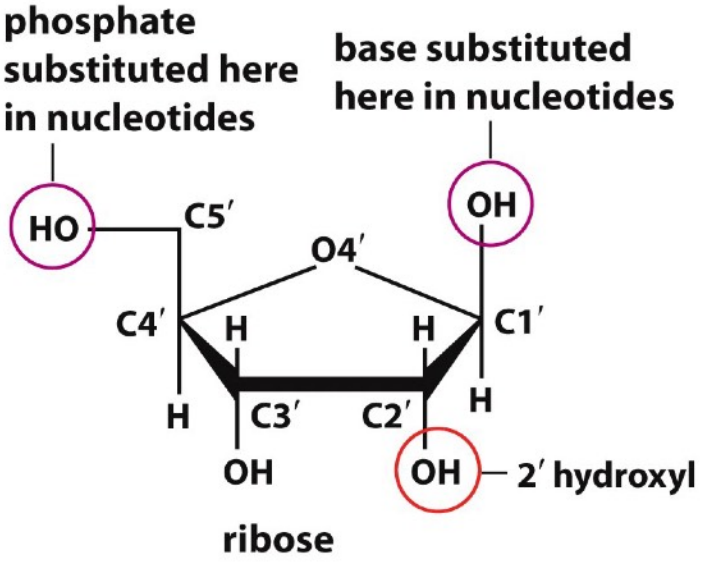
-By convention DNA sequences are written from 5’ to the 3’ end

Nucleic Acids – The pentose

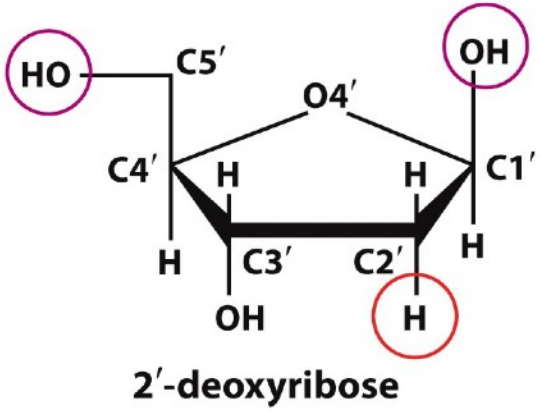
-Sugars used in RNA are derived ribose.

-Sugars used in DNA are derived from 2'-deoxyribose





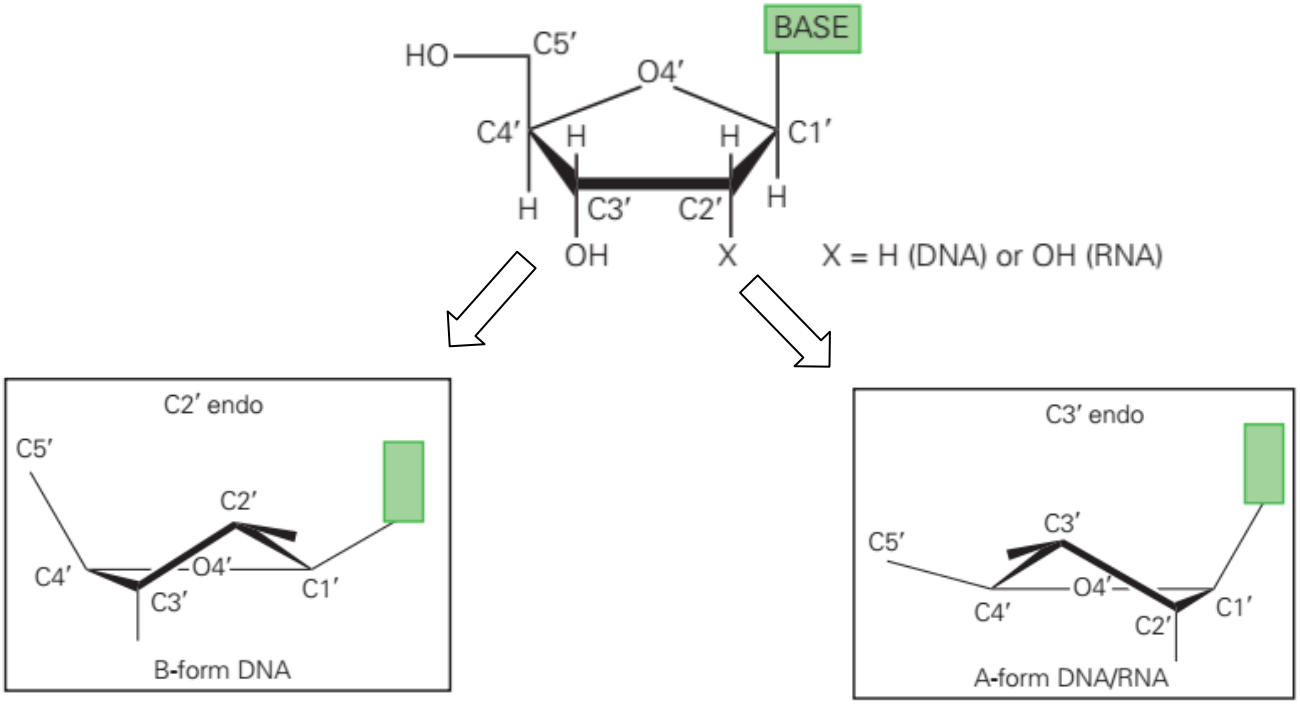
ribonucleotides



2'-deoxyribonucleotides

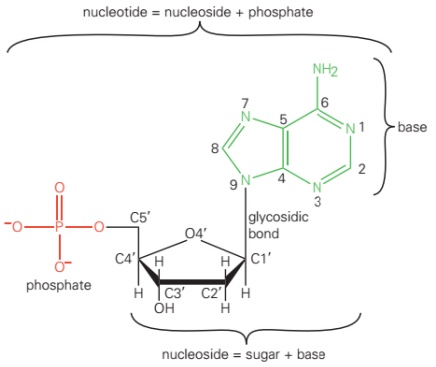
Nucleic Acids – The pentose

-In DNA/RNA molecules the pentose adopts a so-called sugar pucker conformation



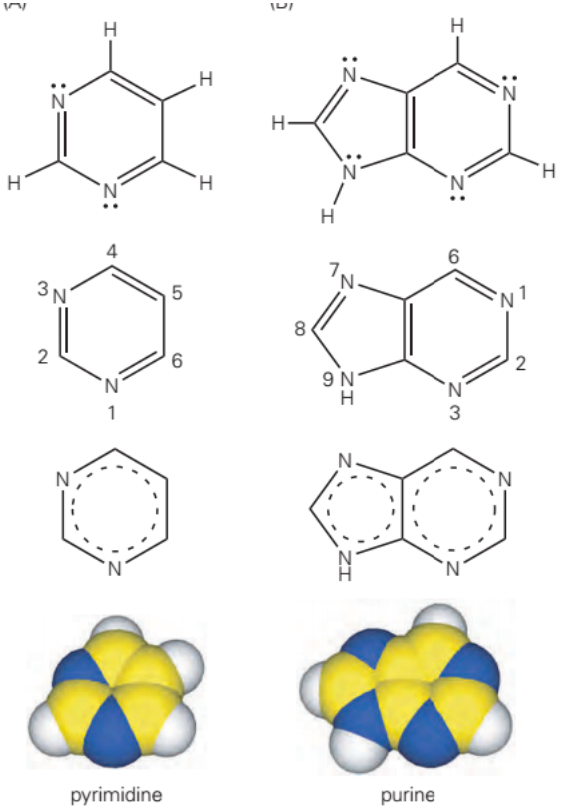
-In energetically favorable conformations four of the atoms of the pentose ring are roughly coplanar and one is out of the plane

Nucleic Acids – The base



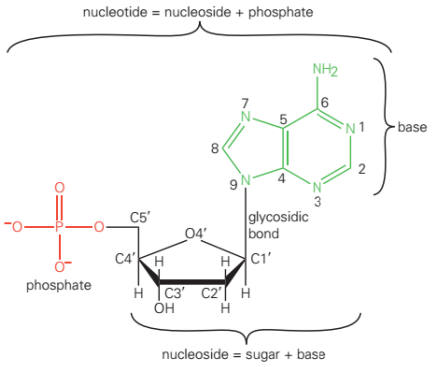
-DNA and RNA are built with 5 different bases

-The name “base” comes from its chemical composition – the ring systems contain lone pairs of electrons in the nitrogens being able to act as electron pair donors – so called Lewis bases



Nucleotide bases in RNA and DNA are substituted forms of two heterocyclic molecules known as **pyrimidine** and **purine**

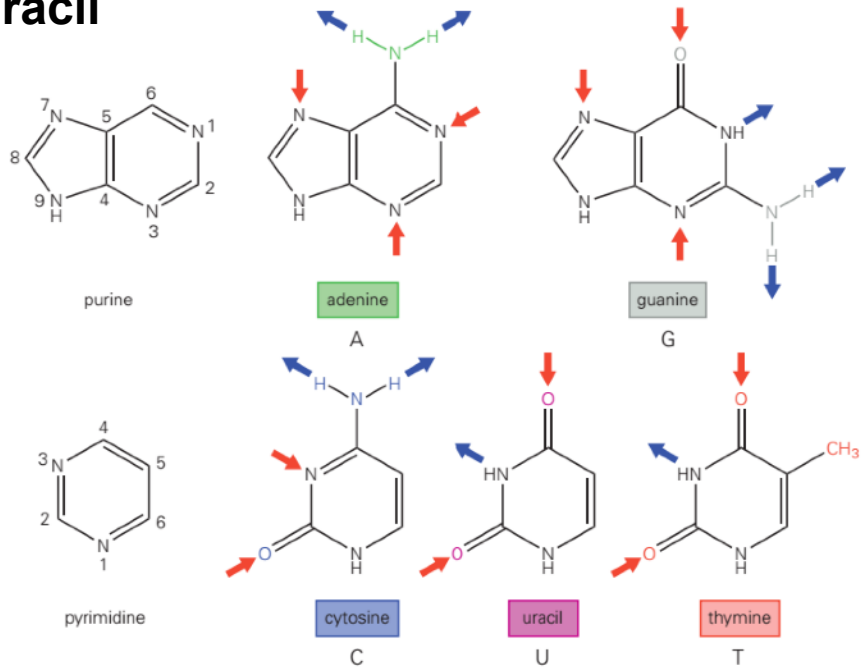
Nucleic Acids – The base



-DNA contains two substituted purines (**adenine** and **guanine**)

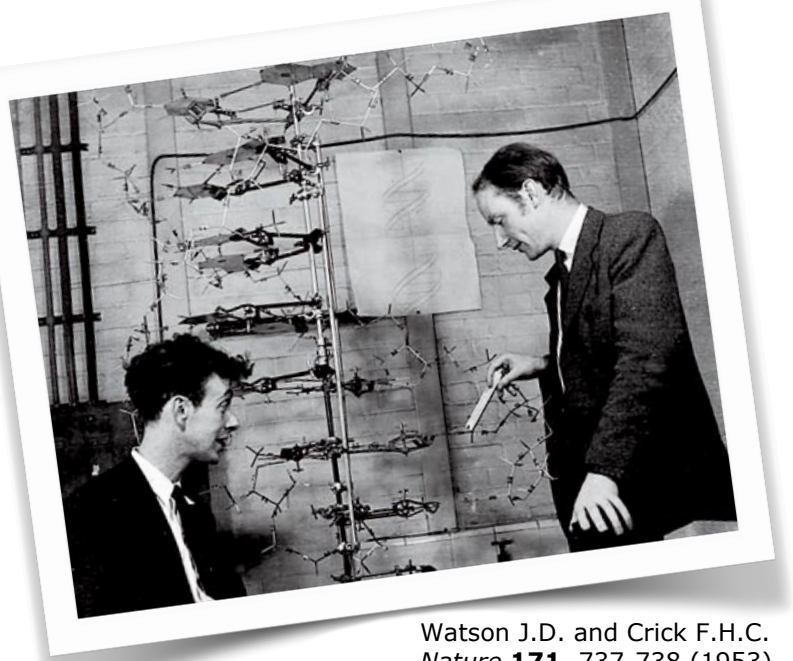
-DNA contains two substituted pyrimidines (**cytosine** and **thymine**)

-In RNA **thymine** is replaced by **uracil**

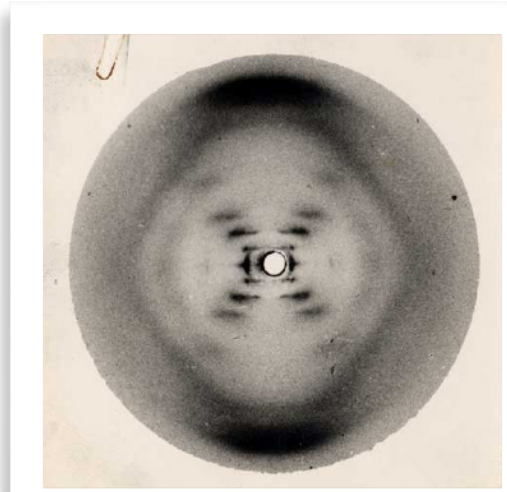


-Blue arrows point to hydrogen bond donor groups
-Red arrows point to hydrogen bond acceptor groups

Key for the ability of RNA and DNA to serve as templates for the transfer of genetic information



Watson J.D. and Crick F.H.C.
Nature **171**, 737-738 (1953)



Rosalind Franklin's X-ray image of DNA

historical detour

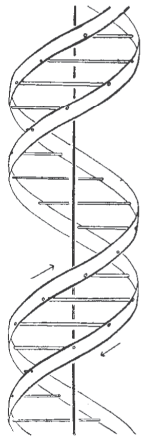
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the *z*-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical *z*-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

James Watson Explains DNA Basepairing

Reprinted from Nature, Vol. 287, No. 5784, pp. 755-758, October 23 1980

Crystal structure analysis of a complete turn of B-DNA

Richard Wing*, Horace Drew, Tsunehiro Takano, Chris Broka, Shoji Tanaka, Keiichi Itakura† & Richard E. Dickerson

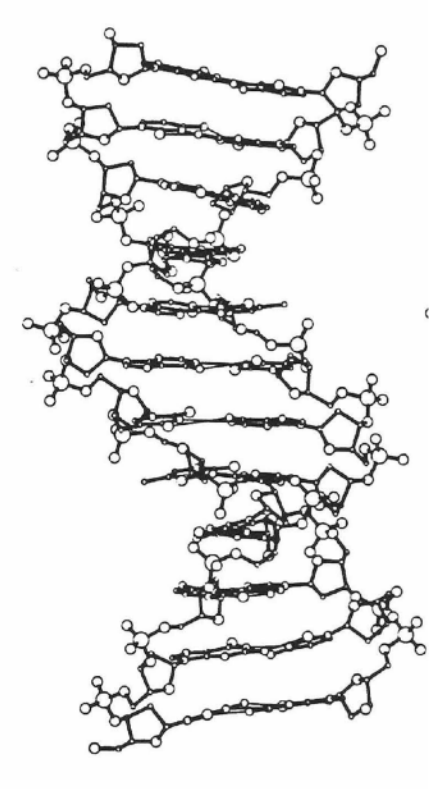
Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

DNA is probably the most discussed and least observed of all biological macromolecules. Although its role in biology is a central one, with many examples such as operators and restriction sites where specific base sequences have control functions or interact with specific enzymes, the structures that DNA can adopt have been based until now only on sequence-averaged fibre diffraction patterns. Recent improvements in triester synthesis methods have made possible the preparation of sufficient homogeneous DNA of predetermined sequence for crystallization and X-ray structure analysis. We report here the first single-crystal structure analysis of more than a complete turn of right-handed B-DNA, with the self-complementary dodecamer sequence d(CpGpCpGpApApTpTpCpGpCpG) or CGCGAATTCGCG.

helix axis. Intensities remain strong in all directions out to 2.9 Å, and then exhibit a rapid decline until essentially no data can be obtained beyond 1.9 Å. Of the 5,691 possible reflections to 1.9 Å resolution, 2,818 were found to have an intensity greater than 2σ and were used in the analysis. Two isomorphous heavy atom derivatives were used: *cis*-dichlorodiamino platinum (II) obtained by diffusion, and a 3-Br derivative obtained by *de novo* synthesis of the dodecamer with 5-bromocytosine in the third position along each chain. The 1-Br derivative was crystallized but proved not to be isomorphous, and the 9-Br derivative was synthesized but not needed. Isomorphism in the *cis*-Pt derivative began to fail beyond 4-Å resolution, but the 3-Br derivative remains isomorphous to 2.7 Å.

The present report describes the partially refined structure obtained from multiple isomorphous replacement (MIR) analysis at 2.7 Å (mean figure of merit 57%), followed by Jack–Levitt refinement procedures[3] using 2,725 2σ intensities between 8.0 and 1.9 Å. The current residual error or *R* factor is 24.8% for a DNA molecule of 486 atoms and 9 initial water molecules. The structure of the DNA itself is essentially correct and is reported now because of its general interest. Refinement will continue with the addition of more solvent and spermine atoms, and some improvement in local nucleotide conformations.

A skeletal drawing of CGCGAATTCGCG is presented in Fig. 1, and a space-filling version from the same orientation in



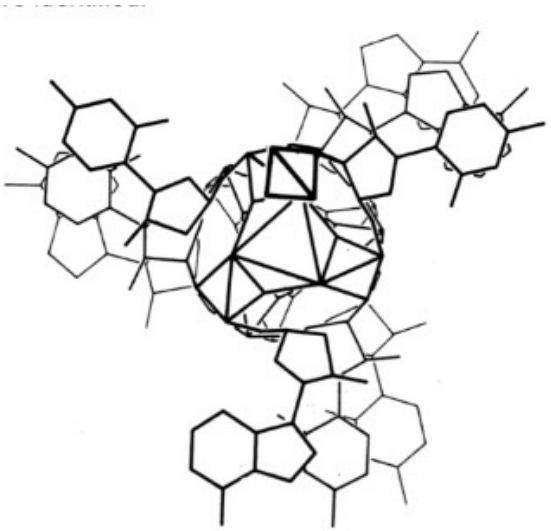


Figure 6, reproduced from: A proposed structure for the nucleic acids. Pauling and Cory (1953) PNAS 39, 84-97.

historical detour

Nucleic Acids - 3D structure

-DNA forms a double helix with antiparallel strands

-Two strands together wind up to form a right-handed double-helix

-Bases are on the inside of the helix and the phosphate backbone group are on the outside. Allowing for interactions with ions and water and minimizing repulsion between phosphates

- Base pairing holds the DNA strands together and is strictly complementary

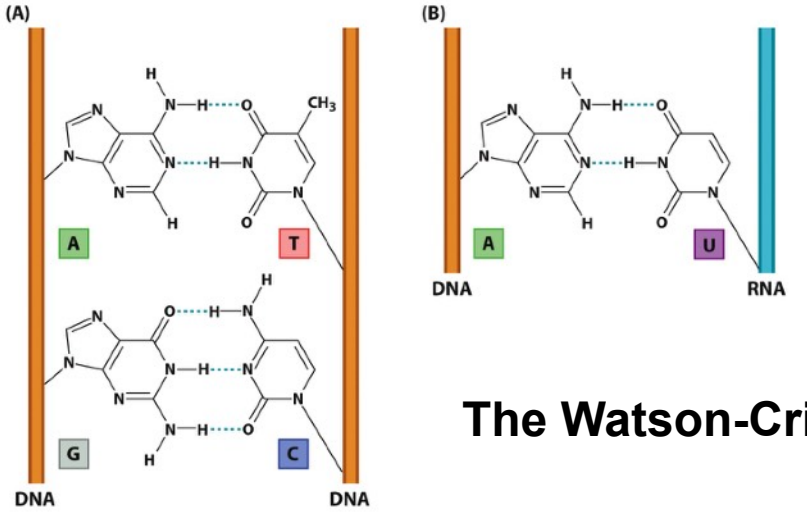


Figure 1.24 The Molecules of Life (© Garland Science 2013)

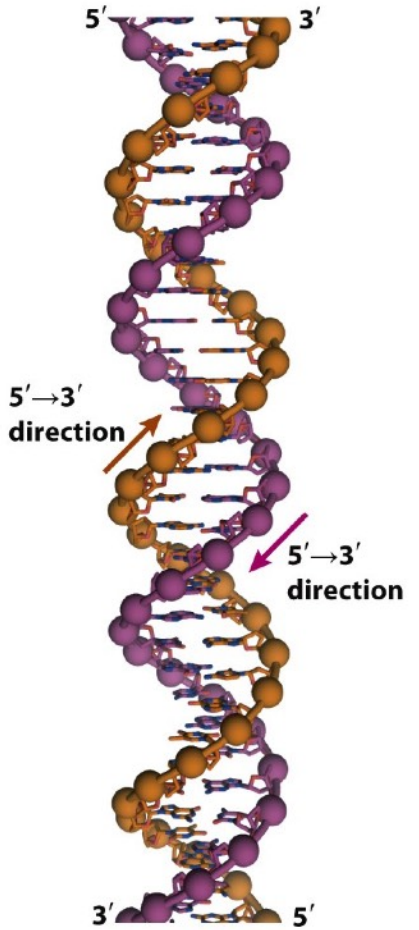


Figure 1.23 The Molecules of Life (© Garland Science 2

Phosphate groups in spheres

The Watson-Crick base pairs: A-T, G-C and A-U

Discussion : DNA 3D structure

Associate the different molecular interactions discussed above to the DNA structure.

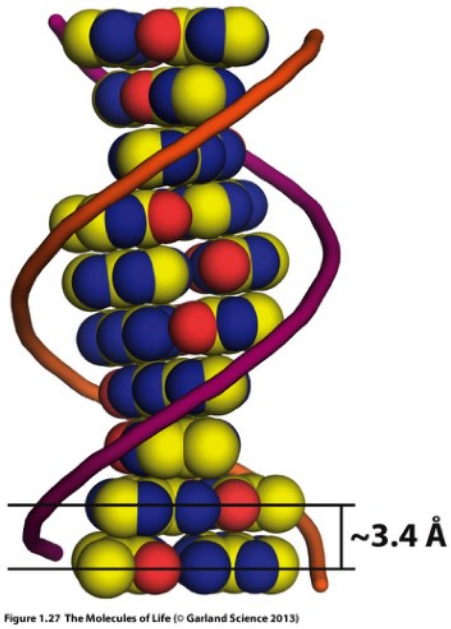
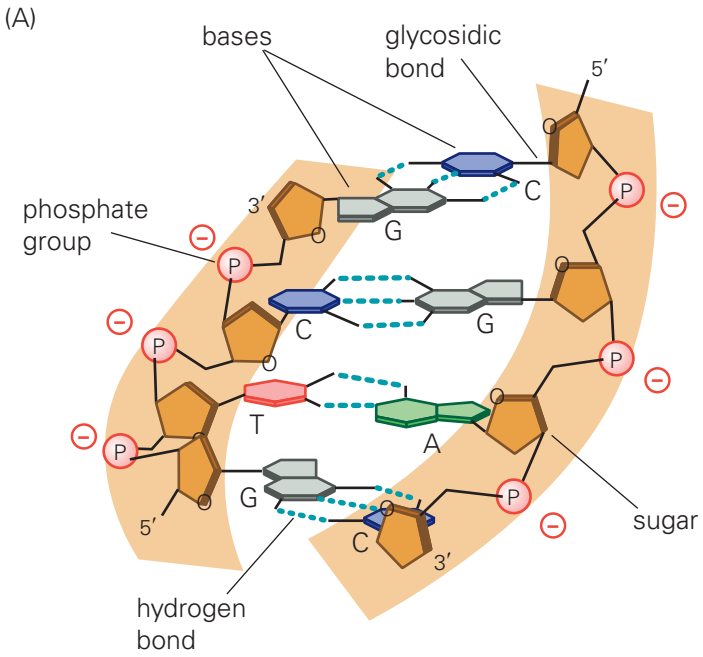


Figure 1.27 The Molecules of Life (© Garland Science 2013)



Nucleic Acids - 3D structure

-In addition to hydrogen bonding the double helix is stabilized by **stacking of base pairs**

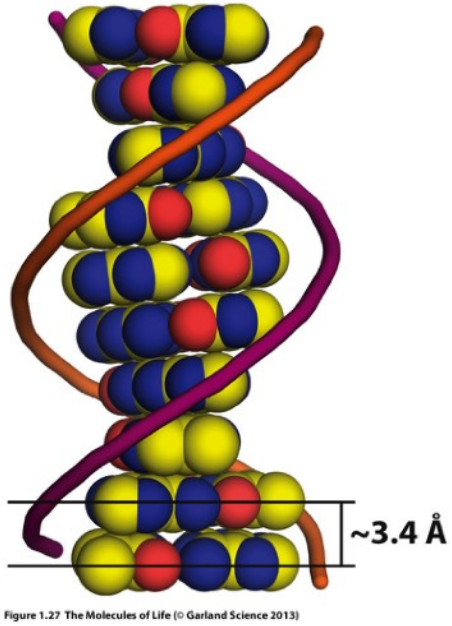


Figure 1.27 The Molecules of Life (© Garland Science 2013)

-There is a combination of electrostatic and van deer Waals interactions

-Beautiful example on van der Waals interactions given that the radius of **carbon and nitrogen are 1.7 and 1.6 Å** respectively – the observed **rise per base-pair is 3.4 Å**.

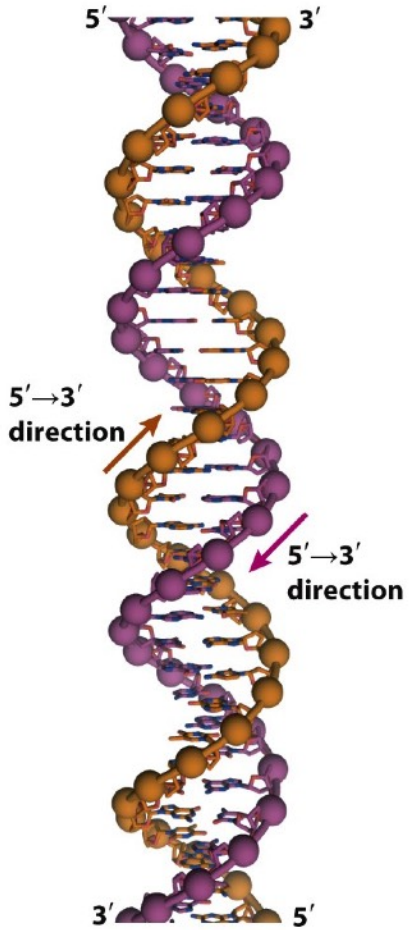


Figure 1.23 The Molecules of Life (© Garland Science 2

Phosphate groups in spheres

Discussion : DNA 3D structure

Associate the different molecular interactions discussed above to the DNA structure.

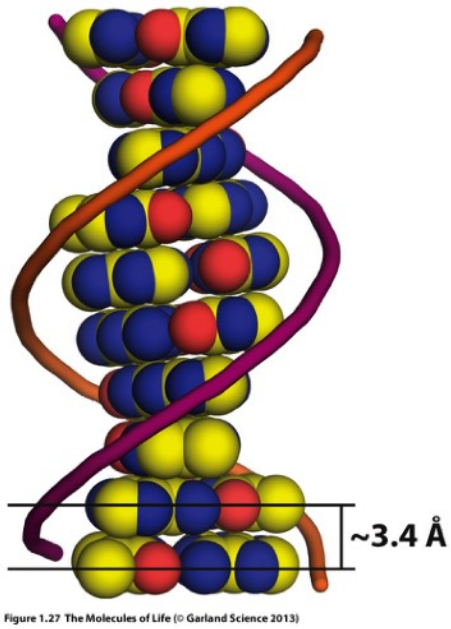
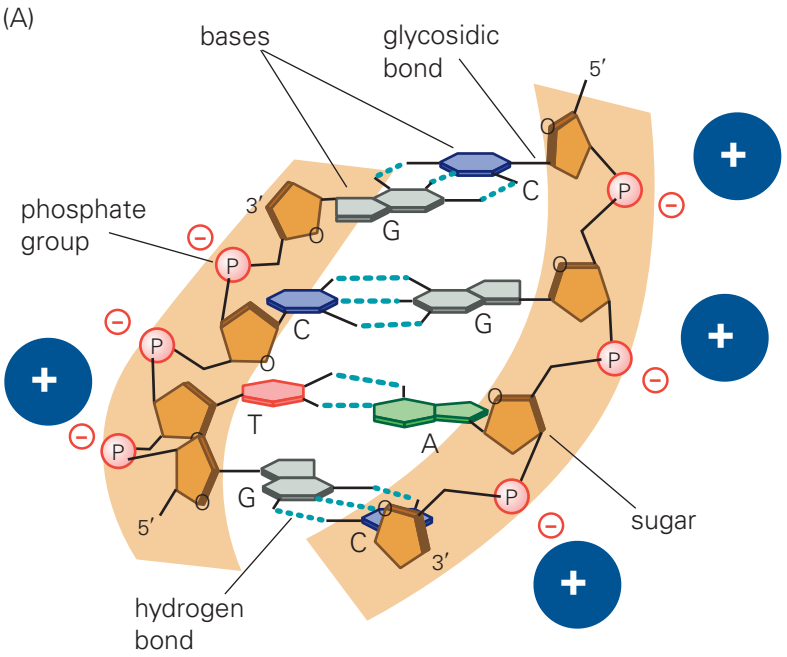


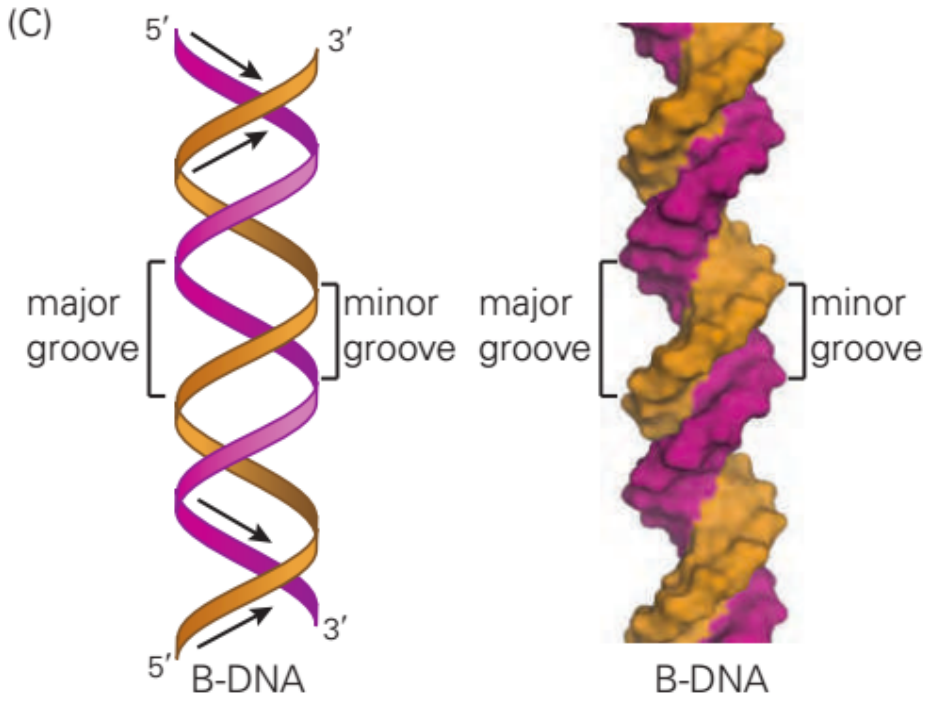
Figure 1.27 The Molecules of Life (© Garland Science 2013)



Nucleic Acids - 3D structure

-DNA can form several types of double helix

-The most common conformation adopted is the B-form

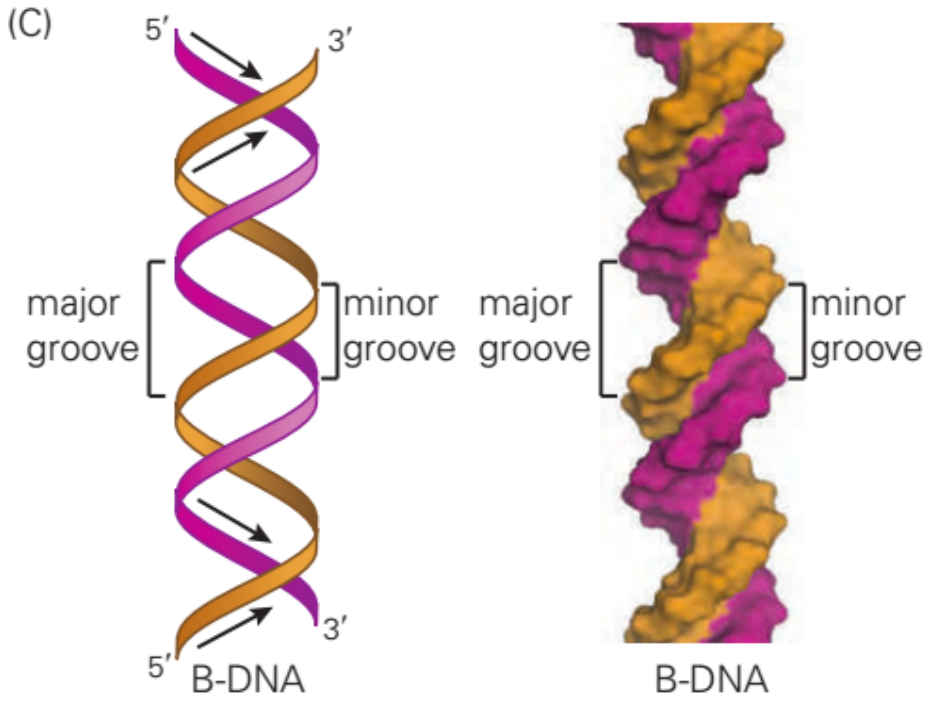


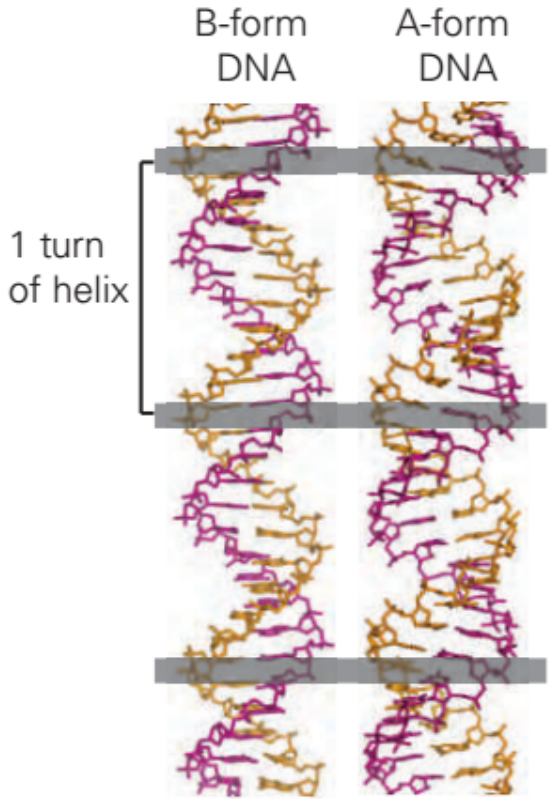
Nucleic Acids - 3D structure

-DNA can form several types of double helix

-The most common conformation adopted is the B-form

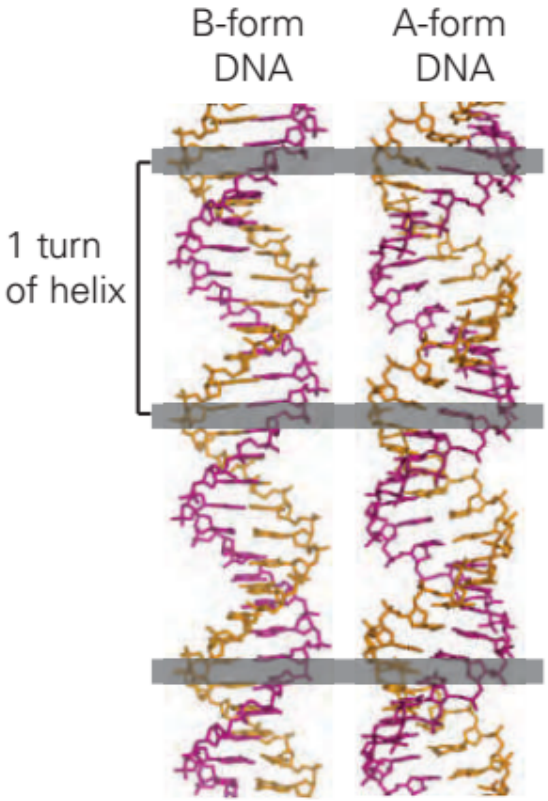
-But DNA and RNA can also adopt the A-form

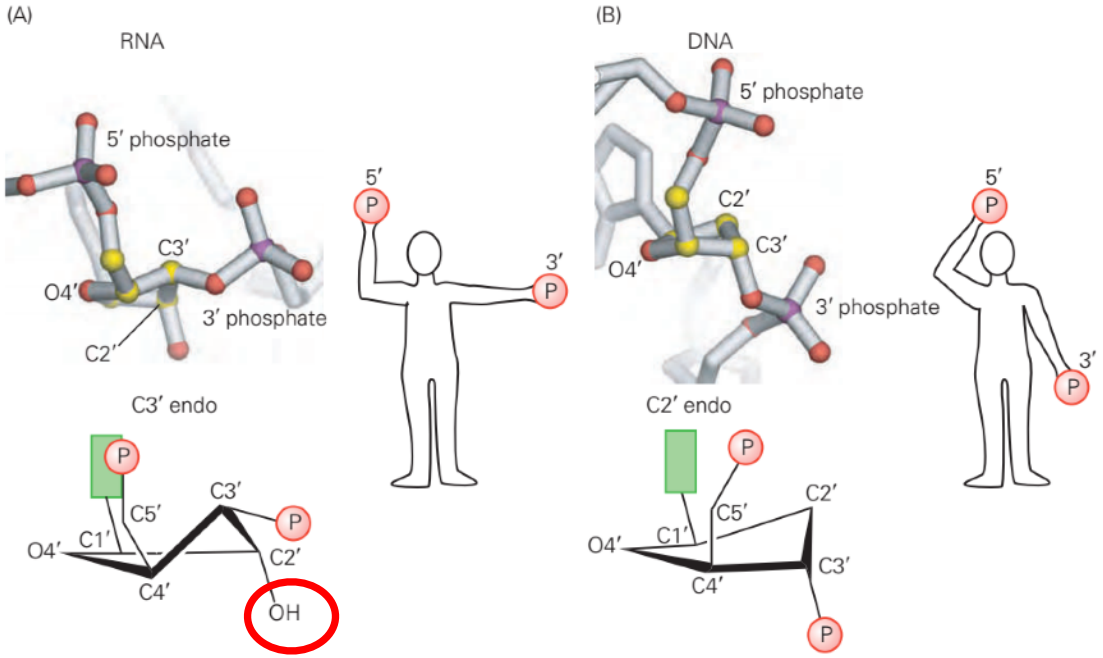




Nucleic Acids - 3D structure

-The difference between the A and B forms arise from the sugar pucker

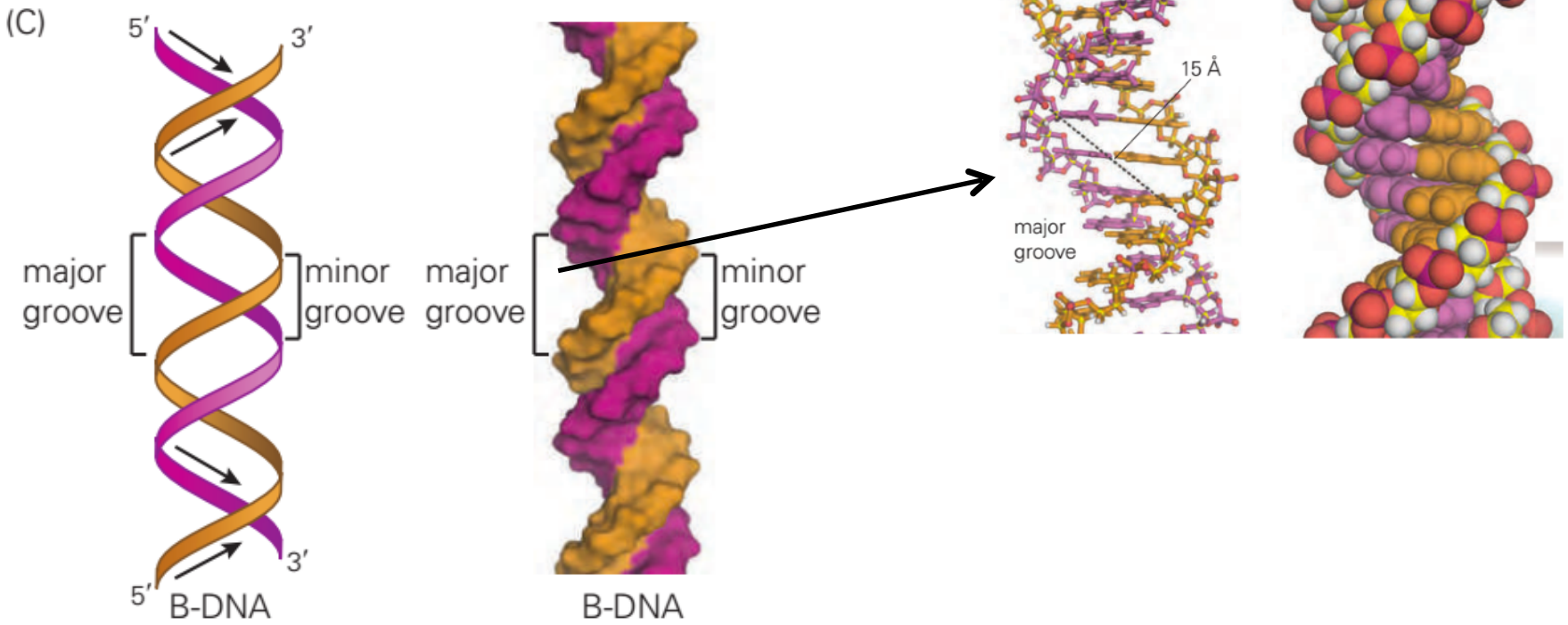


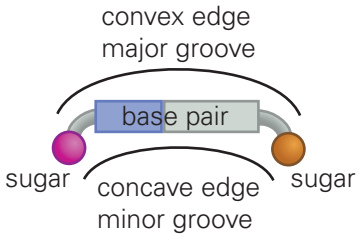


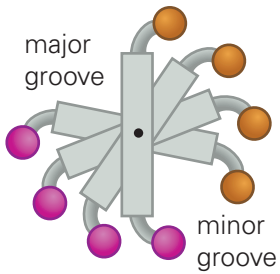
-For RNA the hydroxyl group changes the conformation of the nucleotide conformation with larges impact in the overall structure

Nucleic Acids - 3D structure

-One of the most important features of DNA double helices are the grooves. (we focus on the B-DNA only)

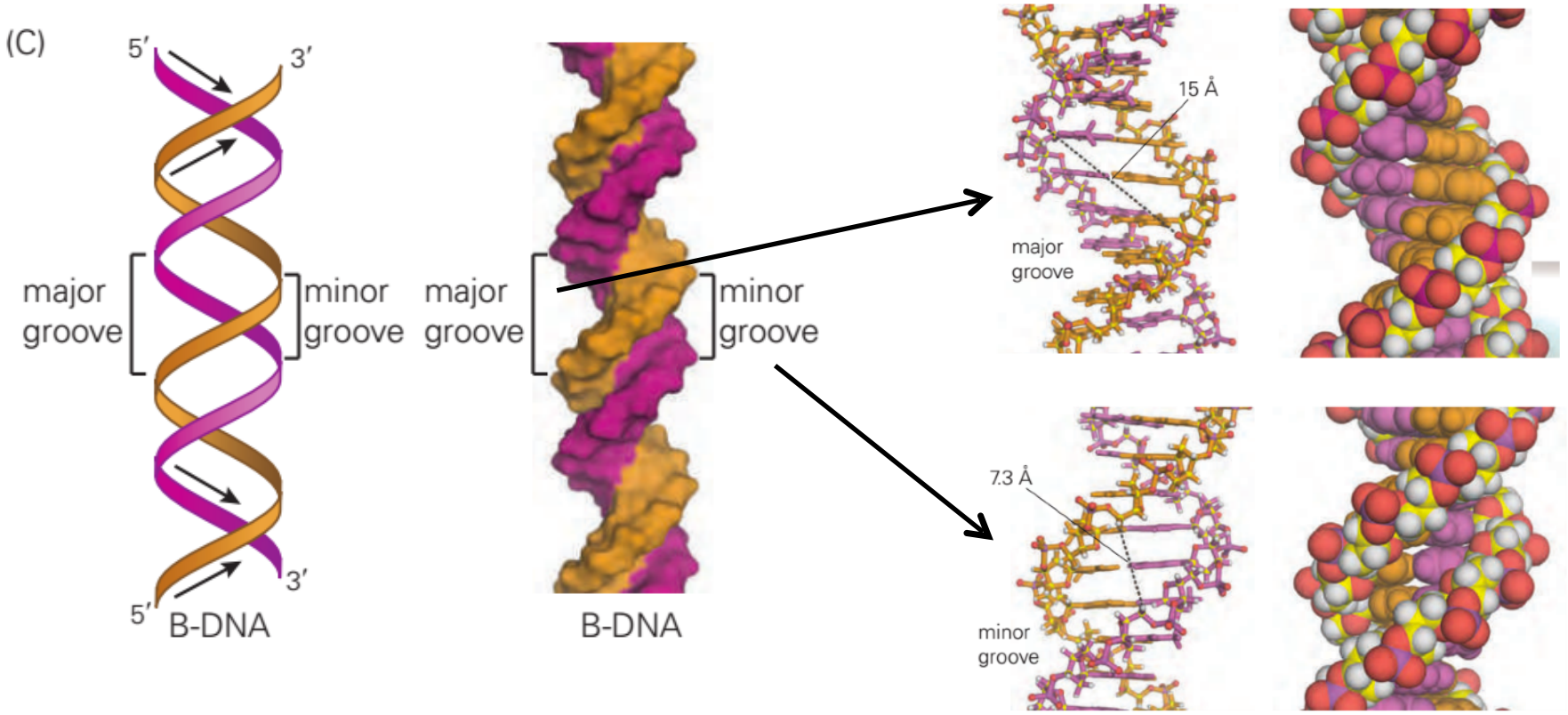






Nucleic Acids - 3D structure

-One of the most important features of DNA double helices are the grooves.



-The major and minor groove present very distinct structural features

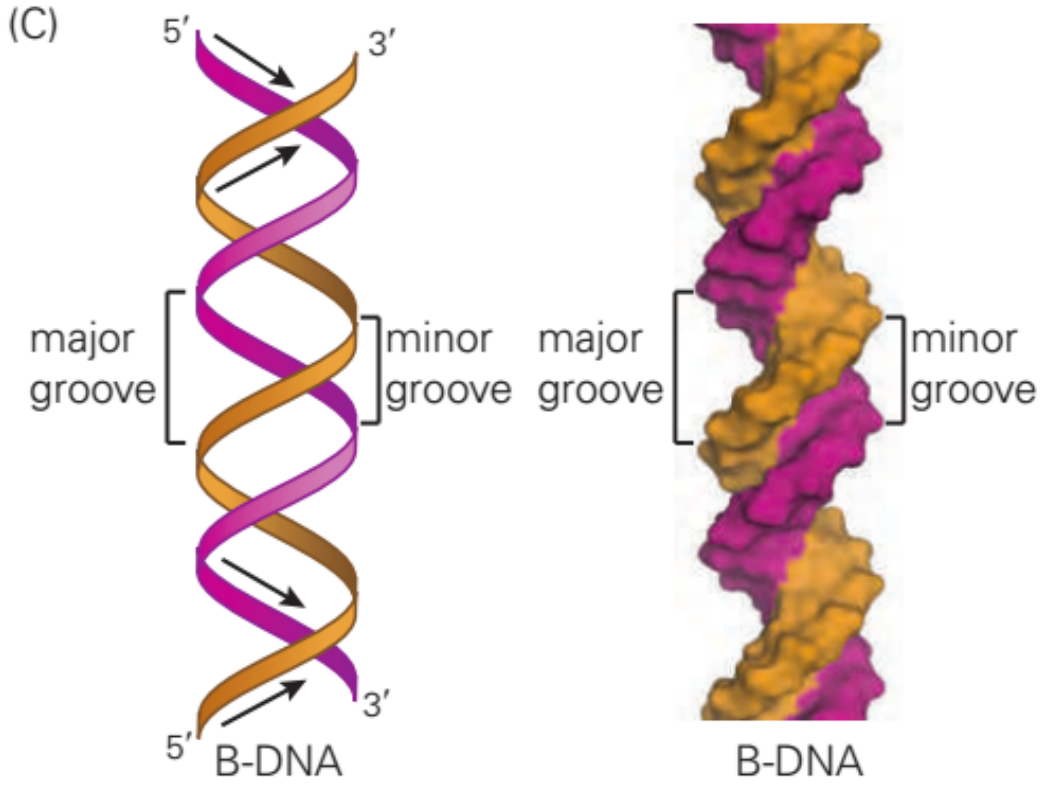
-Very important for the recognition of DNA by proteins.

Statement:

The major groove has a greater information content than the minor groove.

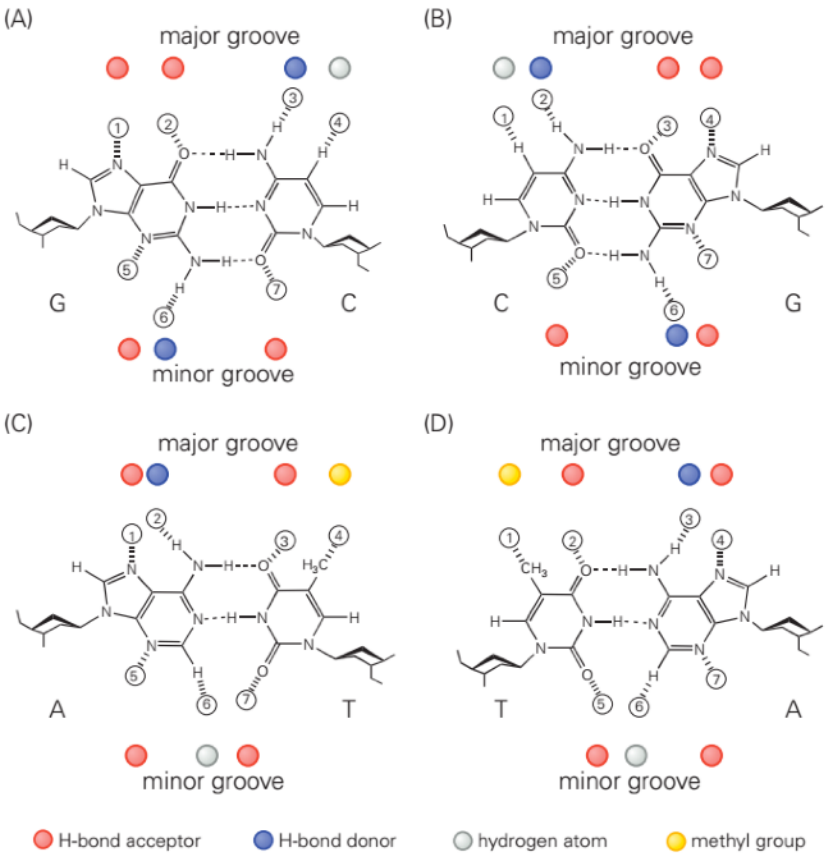
-Yes or No ?

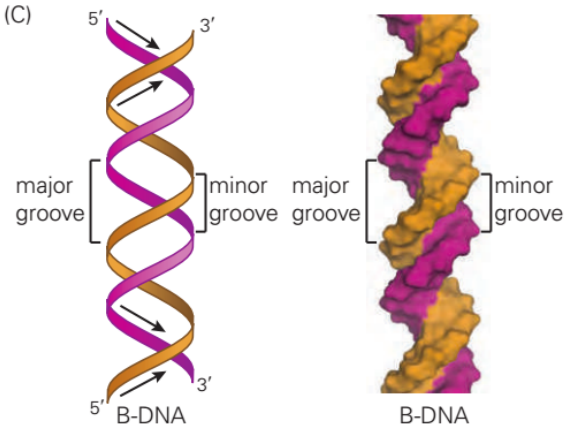
- Why ?

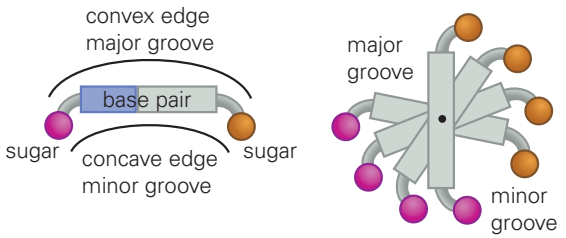


Nucleic Acids - 3D structure

-Potential interaction sites at the edges of Watson-crick base pairs





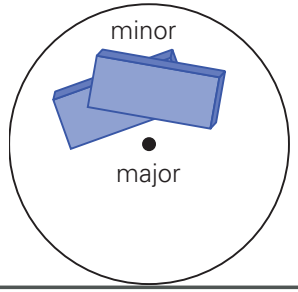
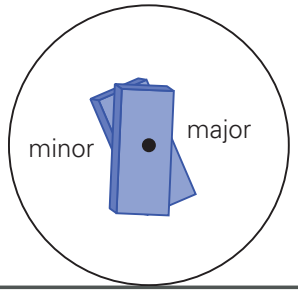
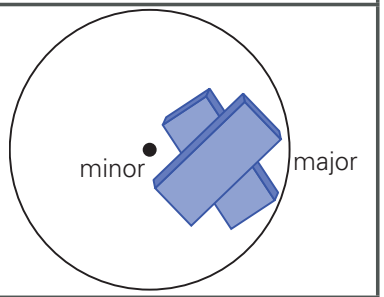


-Four type of interactions are possible.

-The major and minor grooves can be identified by looking at the connections of the base pairs with the sugars. Major groove on the convex edge and the minor groove in on the concave edge.

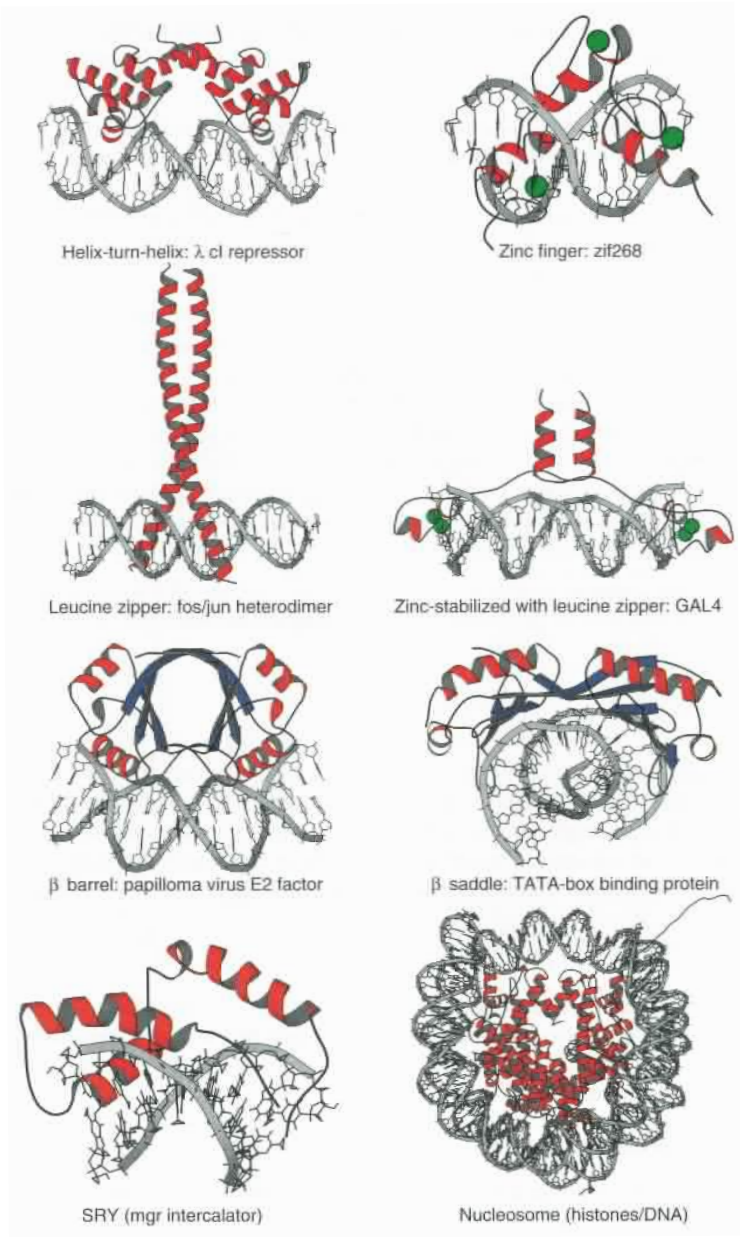
-Notice the chemical diversity of the major grooves vs the minor grooves.

Table 2.1 Structural features of A-, B-, and Z-form helices.

Helical form	A	B	Z
Helical sense	Right	Right	Left
Diameter	~ 26 Å	~ 20 Å	~ 18 Å
Base pairs per turn	~ 11	~ 10	~ 12
Helical twist (rotation per base pair for A and B, per two-base repeat for Z)	~ 34°	~ 36°	~ 60° (CpGp)
Helix pitch (rise per helical turn)	~ 25 Å	~ 33 Å	~ 46 Å
Helix rise (along helix axis; per base pair for A and B, per two-base repeat for Z)	~ 2.3 Å	~ 3.3 Å	~ 7.4 Å (CpGp)
Base tilt (with respect to helix axis)	~ 20°	~ 0°	~ – 9°
Base orientation (with respect to sugar)	Anti	Anti	C anti/G syn
Base pair positions (helix axis indicated by black dot)			
Features of base pair positions	Base pairs displaced from axis; deep major groove, less accessible	Base pairs on axis; both major and minor grooves accessible	Base pairs stick out into the major groove, the minor groove is deep and narrow

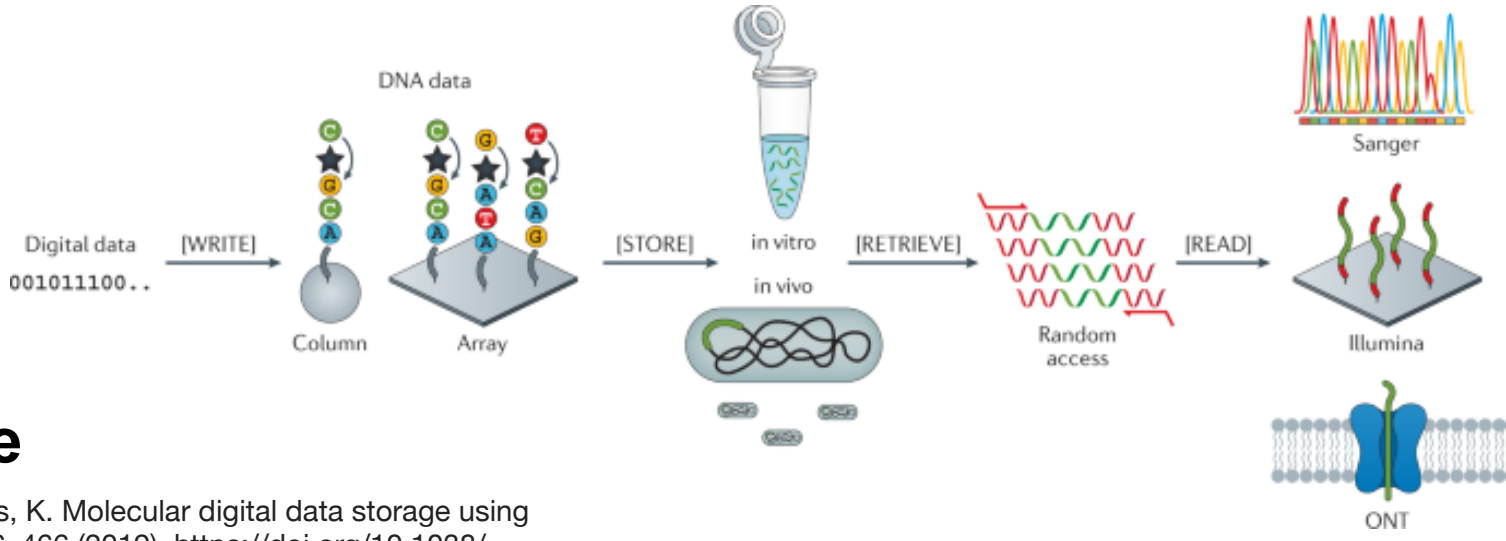
(Adapted from R.E. Dickerson et al., and M.L. Kopka, *Science* 216: 475–482, 1982. With permission from AAAS.)

DNA/protein interactions



Complex	Binding Motif[a]	Binding Groove[b]	Details of Complex
λ repressor	HTH	Mgr	Canonical HTH; homodimers; 2 helices of Cro dimer cradle Mgr, stabilized by direct H-bond and vdW contacts; little DNA distortion.
CAP repressor	HTH	Mgr	About 90° bend.
trp repressor	HTH	Mgr	Indirect, water-mediated base contacts.
Purine rep.	HTH	Mm	α-helices inserted in mgr.
Yeast MATα2	HTH	Mgr	Homeobox domains bind as monomers.
Zif268	Zn	Mgr	Zinc finger subfamily; each Zn finger recognizes 3 bps.
GATA-1	Zn	Mm	Transcription factors subfamily; single domain coordinated by 4 cysteines.
GAL4	Zn	Mgr	Metal binding subfamily; each of two Zn ions, coordinated by 6 cysteines, recognizes 3 bps.
GCN4	Leu/Zip	Mgr	Canonical; basic region/leucine zipper (α helices) motif; slight DNA bending.
fos/jun	Leu/Zip	Mgr	α-helices resemble GCN4; unstructured basic region folds upon DNA binding.
fos/jun/NFAT	Leu/Zip	Mgr	α-helices bend to interact with NFAT.
MetJ	β-ribbon	Mgr	Two anti-parallel β-strands in Mgr; bends each DNA end by 25°.
papillomavirus E2 DNA target	β-barrel	Mgr	Domed β-sheets form an 8-strand β-barrel dimer interface with 2 α-helices in Mgr; strong tailored fit for every base of the recognition element; bent DNA; compressed mgr; DNA target crystallized without protein.
TBP	β-saddle	mgr	Ten-β-strand saddle binds in Mgr; significant distortion, ≈ 90° bend.
p53 tumor supp.	Loop/other	Mm	Binds to DNA via protruding loop and helix anchored to anti-parallel β-barrel.
SRY	Loop/other	mgr	Isoleucine intercalated into mgr.
NFAT	Loop/other	Mm	Flexible binding loop stabilized by DNA.
histones	Loop/other	Mm	Nonspecific PO_4 interactions.
distamycin (drug)		mgr	Selective to AT bps; binds in mgr without distortion.

DNA in biotechnology

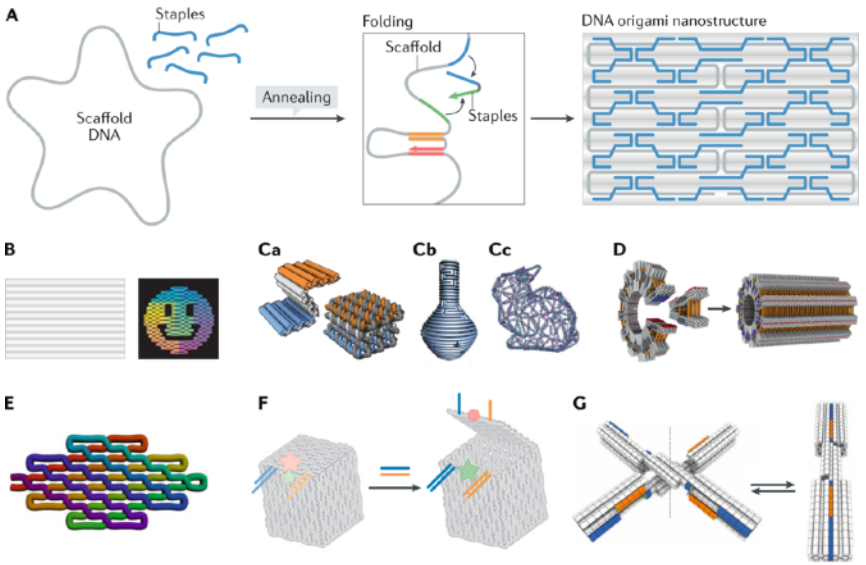


Data storage

Ceze, L., Nivala, J. & Strauss, K. Molecular digital data storage using DNA. *Nat Rev Genet* **20**, 456–466 (2019). https://doi.org/10.1038/s41576-019-0125-3

DNA origami

Dey, S., Fan, C., Gothelf, K.V. *et al.* DNA origami. *Nat Rev Methods Primers* **1**, 13 (2021). https://doi.org/10.1038/s43586-020-00009-8



Nucleic Acids – Take Home Messages

-DNA and RNA are the informational polymers in the cell – encode genetic information in a way that can be read by macromolecular machines, to direct the synthesis of other molecules.

-Nucleotides have pentose sugars attached to nitrogenous bases and phosphate groups.

-The nucleotide bases in DNA and RNA are substituted pyrimidines or purines.

-4 deoxyribonucleotides in DNA (A,T,G,C) and four ribonucleotides in RNA (A,U,C,G)

-DNA and RNA are synthesized in 5' to 3' direction by sequential reactions that are driven by hydrolysis of nucleotide triphosphates

-DNA forms a double helix with antiparallel strands

-Double helix involves complementary base pairing (A-T and C-G) and is stabilized by, hydrogen bonds, base pair stacking and electrostatic interactions

-B-form DNA allows sequence specific recognition of the major groove by proteins. Each base pair has a unique set of interacting elements in the major groove but not in the minor groove.

Questions ?????

- now
- Moodle forum
- @ matteo.dalperaro@epfl.ch
- all the TAs

Next week - Lecture 2

Protein structure - Chapter 4