EPFL

EDMI Microsystems and Microelectronics

MICRO-614: Electrochemical Nano-Bio-Sensing and Bio/CMOS interfaces

Lecture #3

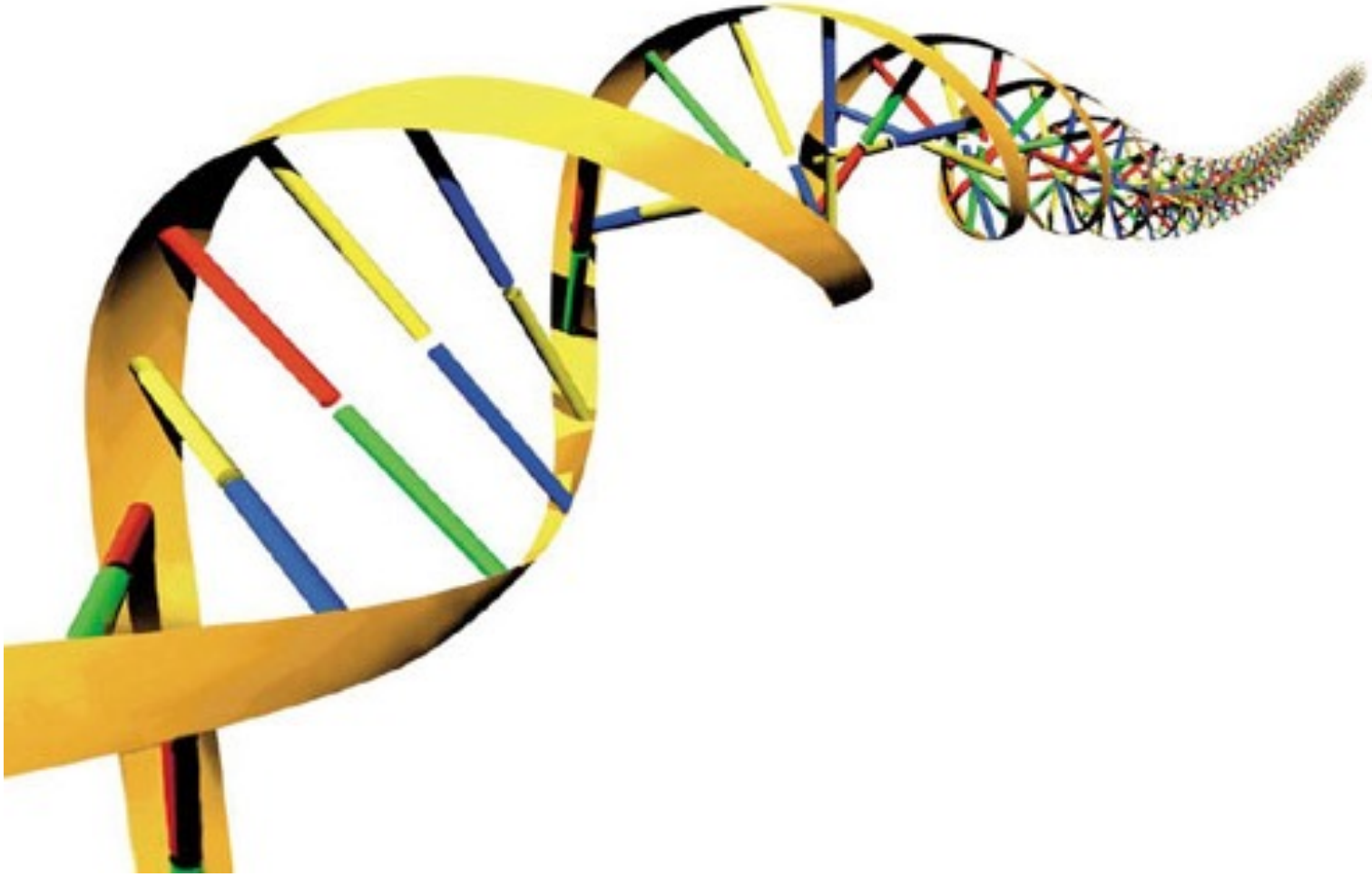
Probe/Target interactions (DNA/Antibodies/Enzymes)

Lecture Outline

(Book Bio/CMOS: Chapter' paragraphs **§** 4.4-16)

- DNA/RNA pairing & Bond energy
- Antibody/Antigen affinity & Bond energy
- Enzymes/substrates & Reaction Energy
- Atypical Enzymatic Kinetics

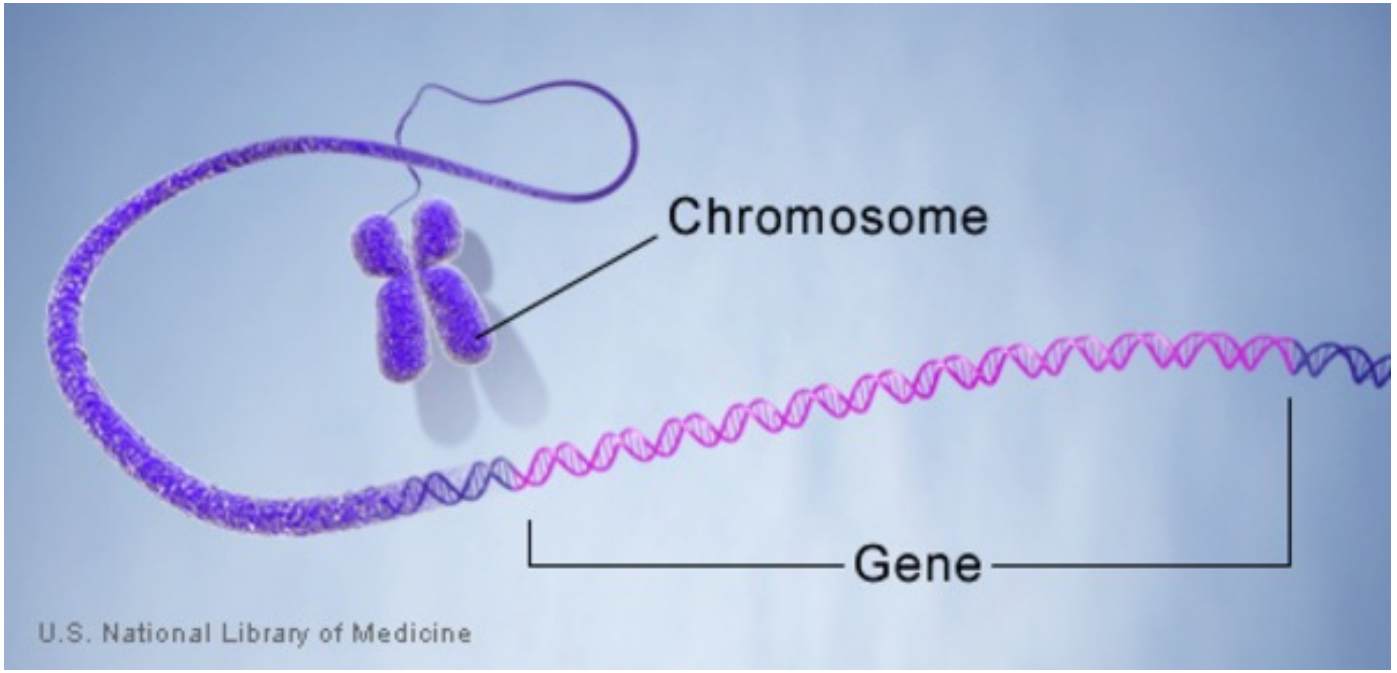
DNA



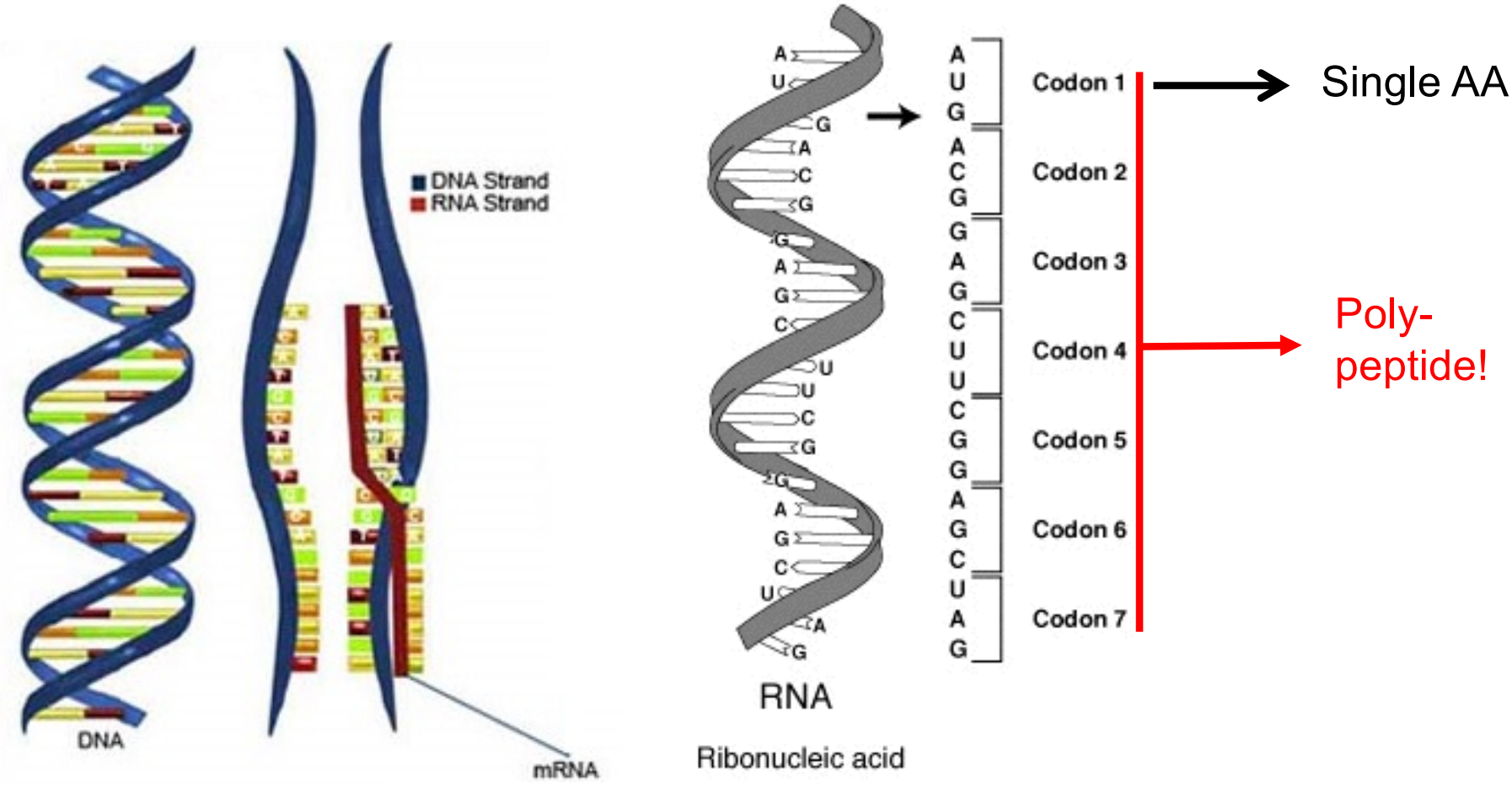
Another fundamental molecules in Biochemistry is the DNA

DNA codes the genes

The function of DNA is to store all of the genetic information that organisms need to develop and self-reproduce

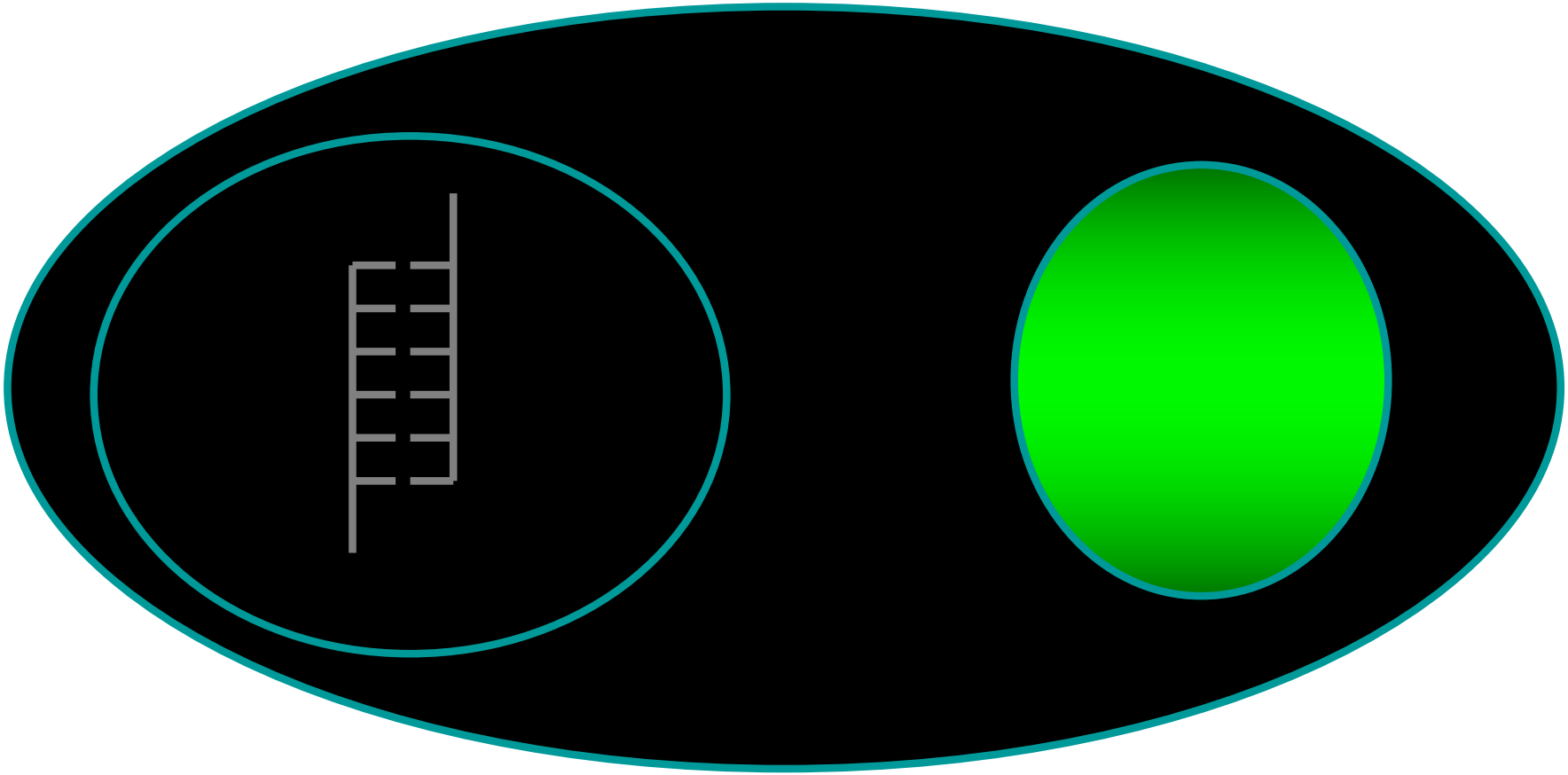


RNA decodes the genes



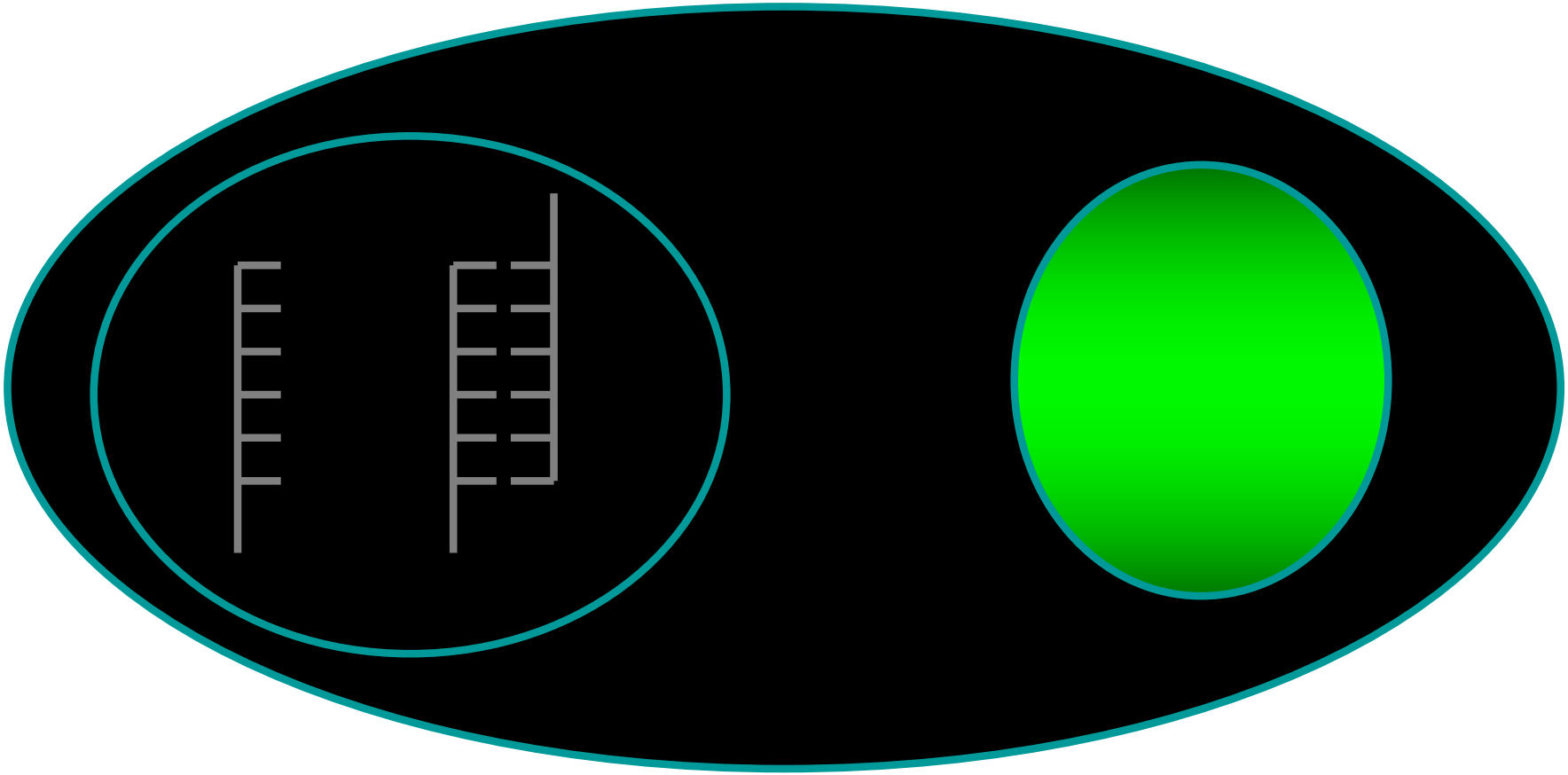
The RNA is another similar molecule useful to decode the genetic code

DNA/RNA functions in cells



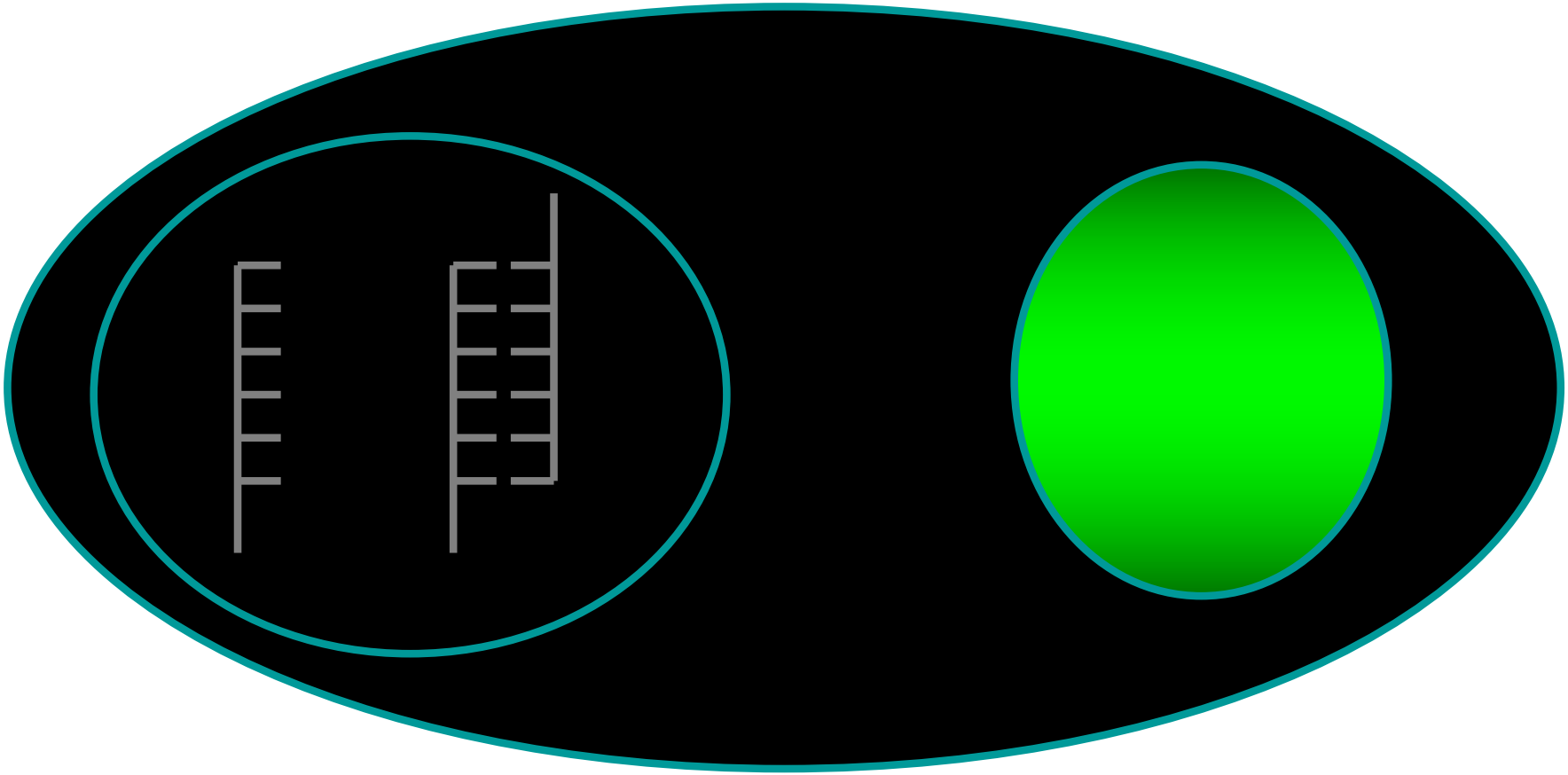
The DNA double helix is opened

DNA/RNA functions in cells



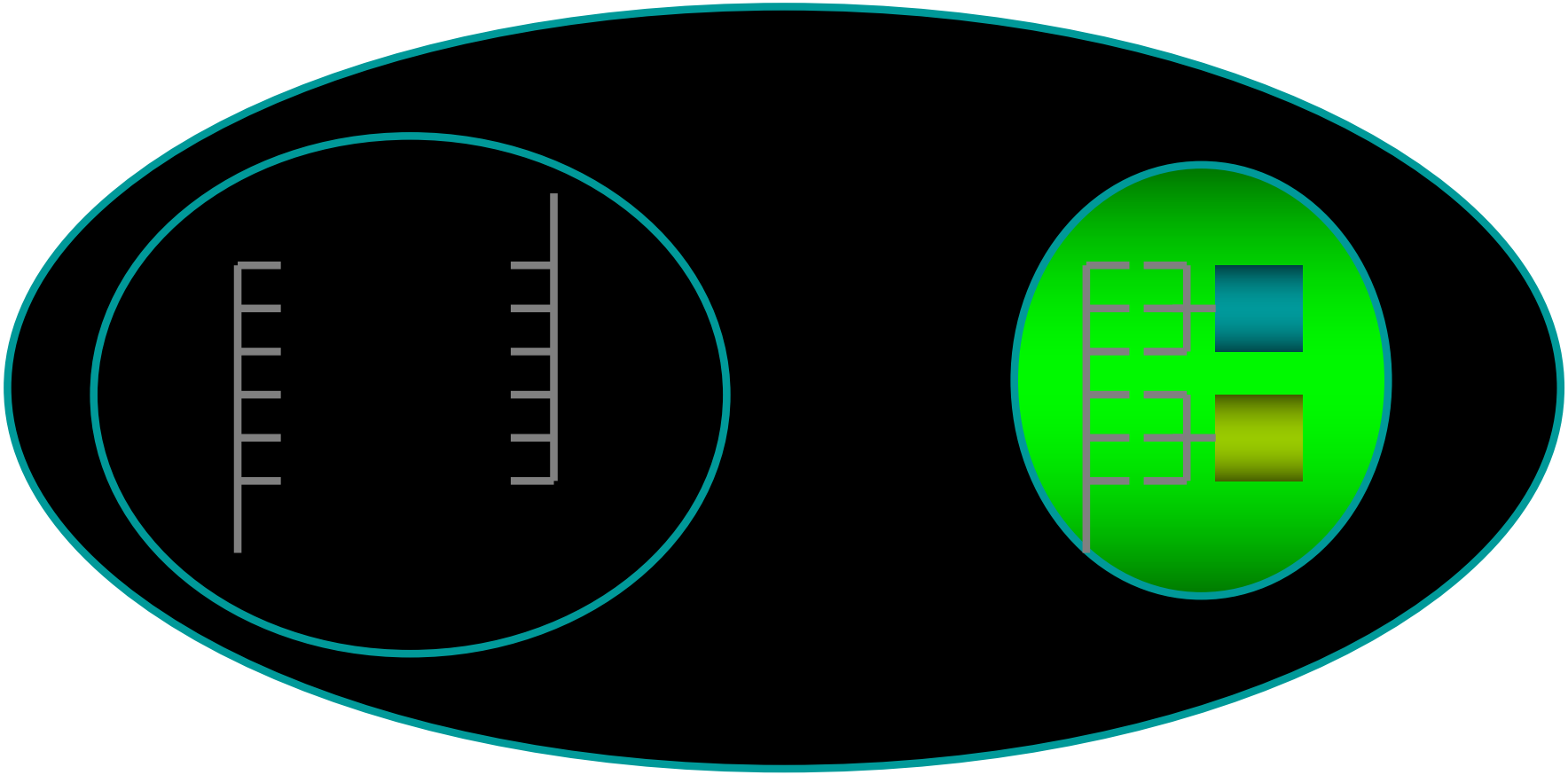
The genetic encoded in DNA is replicated in the messenger RNA molecule

DNA/RNA functions in cells



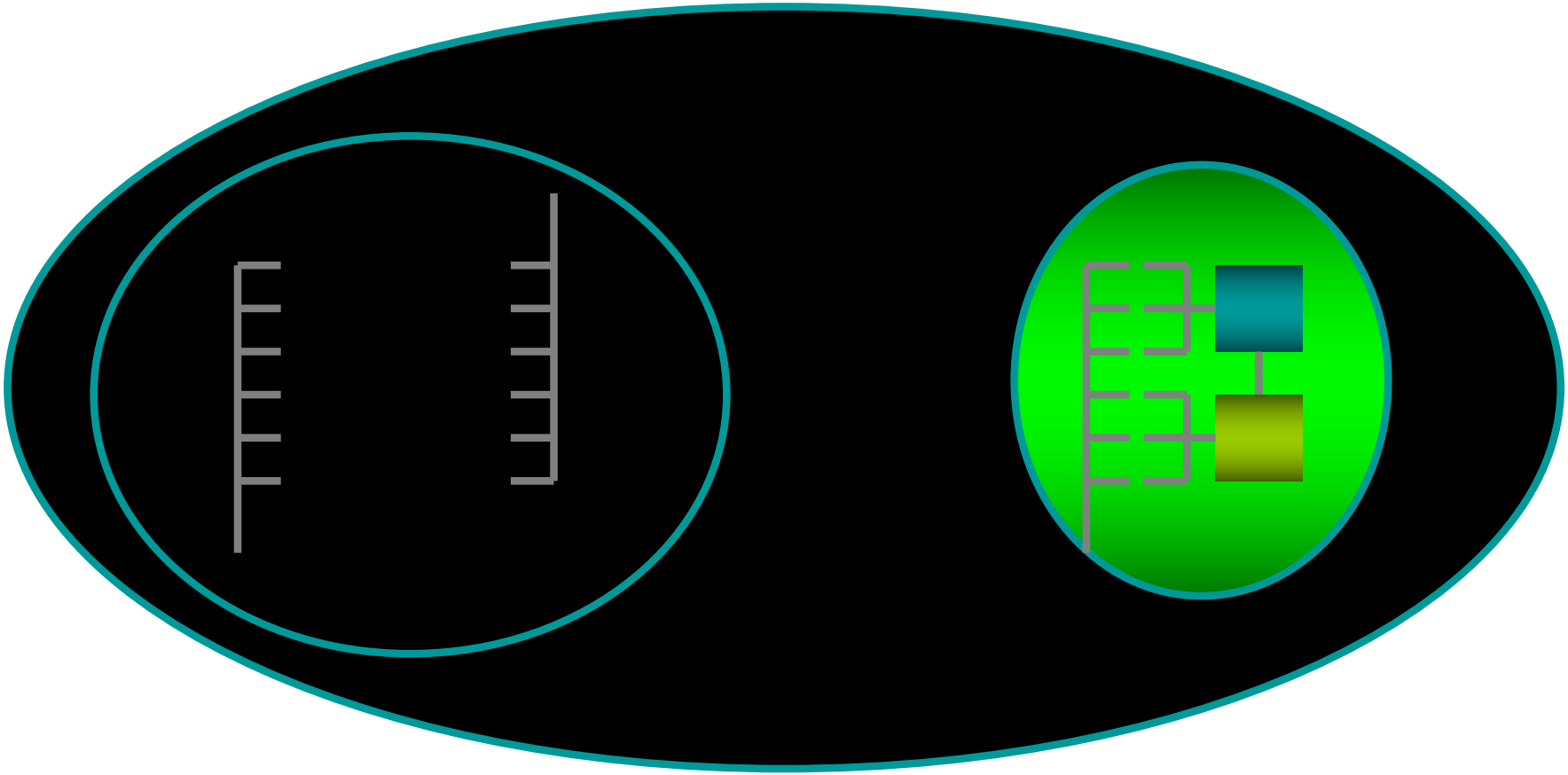
The mRNA is transferred into the ribosome

DNA/RNA functions in cells



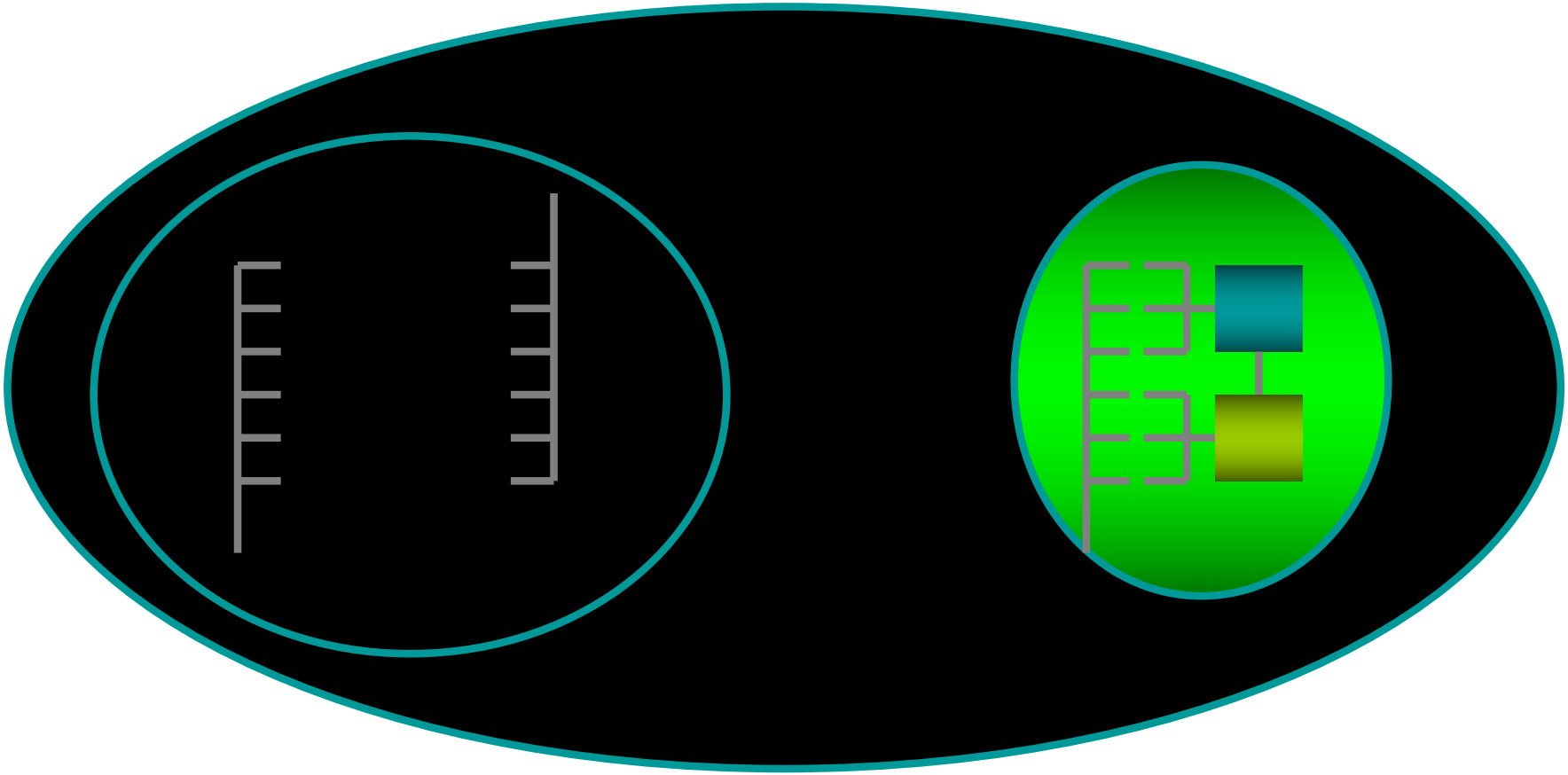
In the Ribosome, the transfer-RNA decode in poly-peptides the genetic code carried by mRNA

DNA/RNA functions in cells



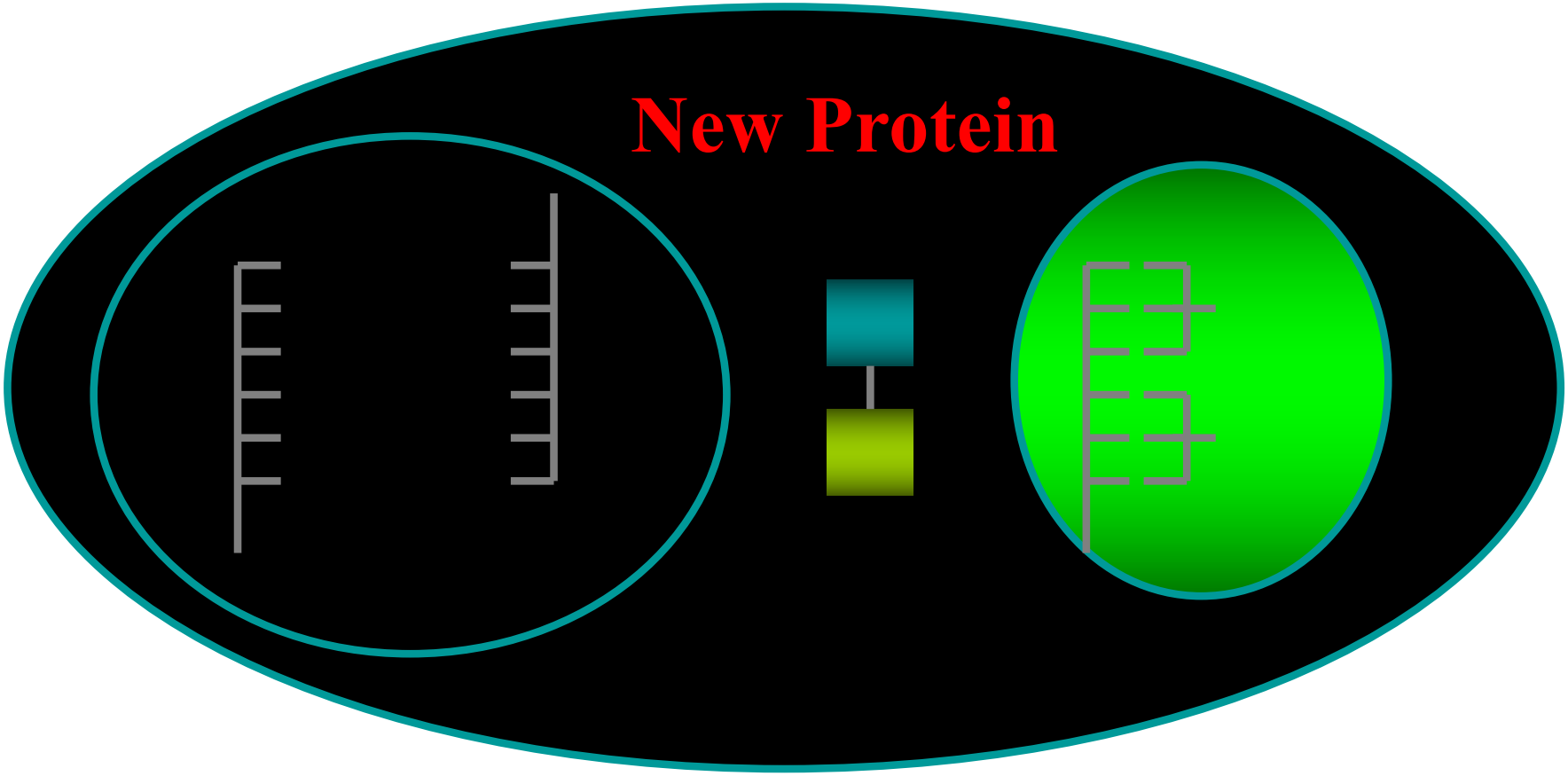
The poly-peptides form new proteins

DNA/RNA functions in cells



New proteins are, then, released into the cell cytoplasm

RNA function in the cell

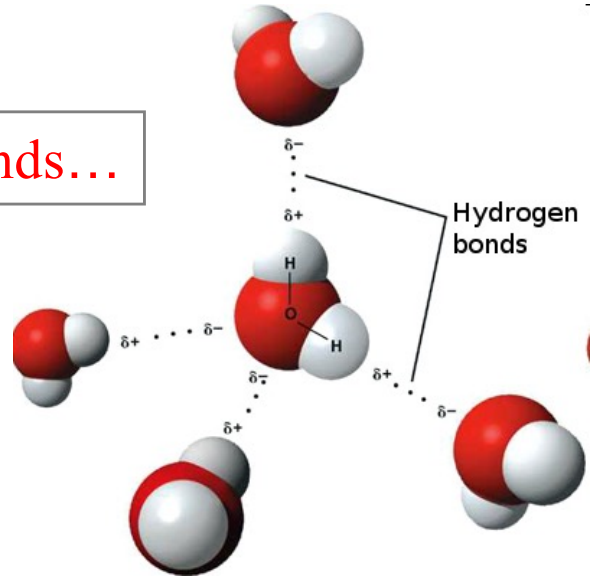


New proteins into the cell cytoplasm enable the cell metabolism

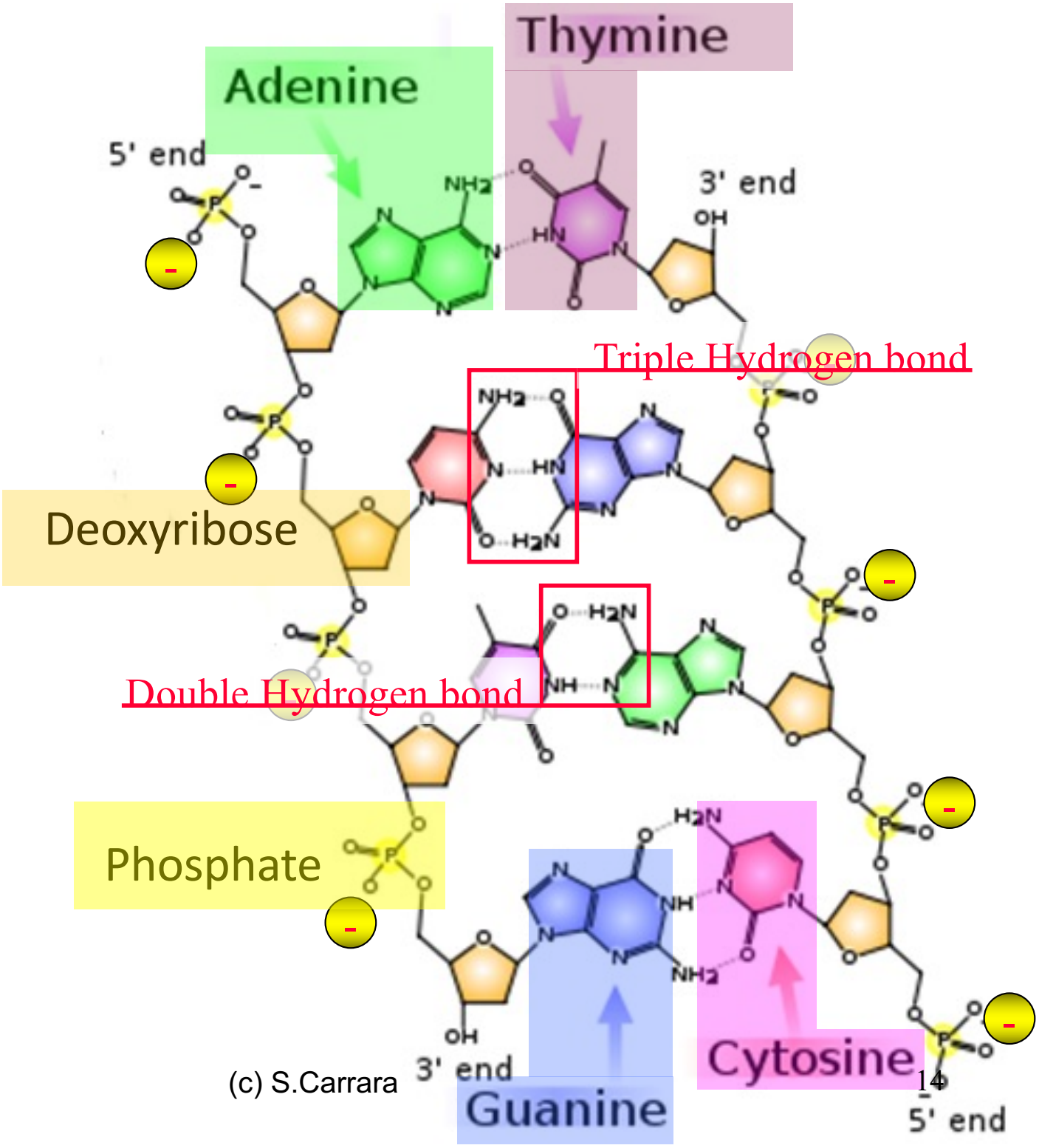
DNA Base pairing

Each type of base on one strand forms a bond with just one type of base on the other strand. This is called **complementary base pairing**.

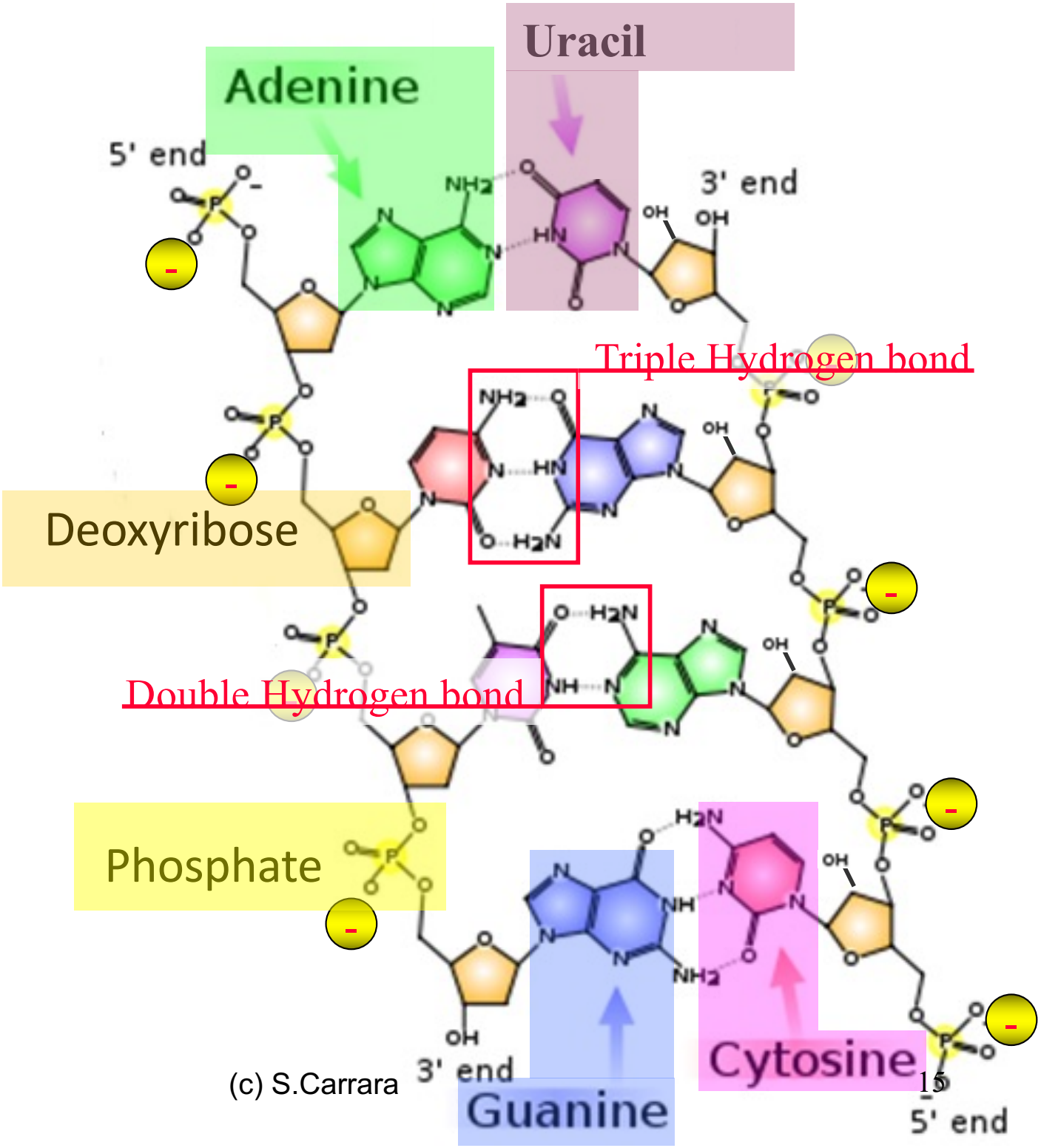
Thanks to Hydrogen Bonds…



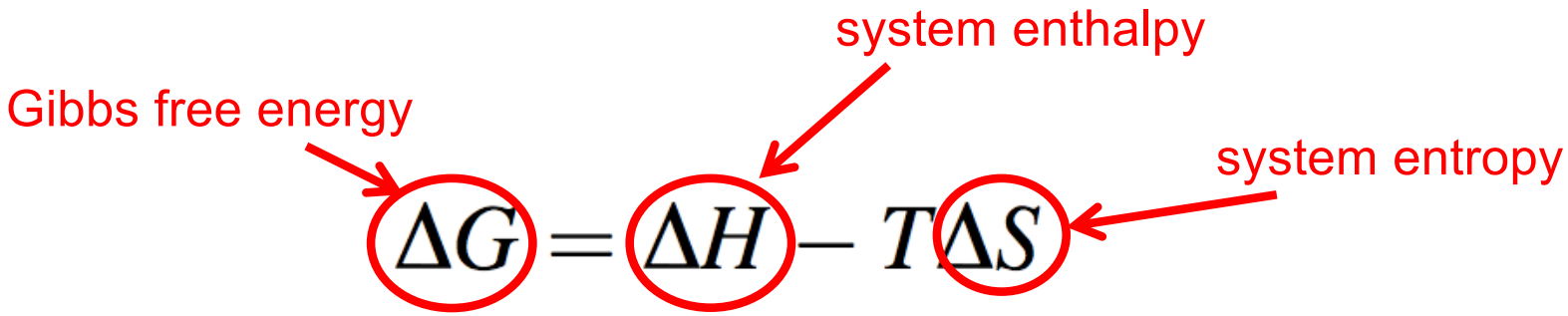
DNA-DNA Interaction



DNA-RNA Interaction



Gibbs free energy

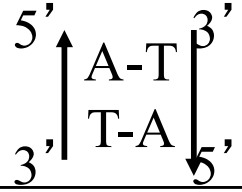
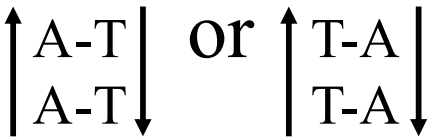
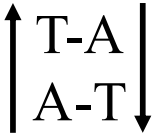
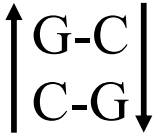
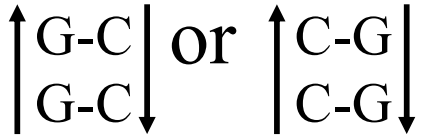
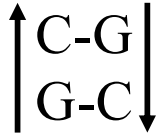


The **Gibb free energy** is the upper limit (some times called thermodynamic potential) of the total work obtainable by the system in processes at constant temperature and pressure

The **enthalpy** is related to variations in **stacking energy** related to bond strength, which includes but is not limited to **hydrogen bonds** strength

The **entropy** is kind of measure of the system disorder and ***TΔS*** is the energy generated/adsorbed by the system in the conformational change

Enthalpy & entropy for different DNA dimers

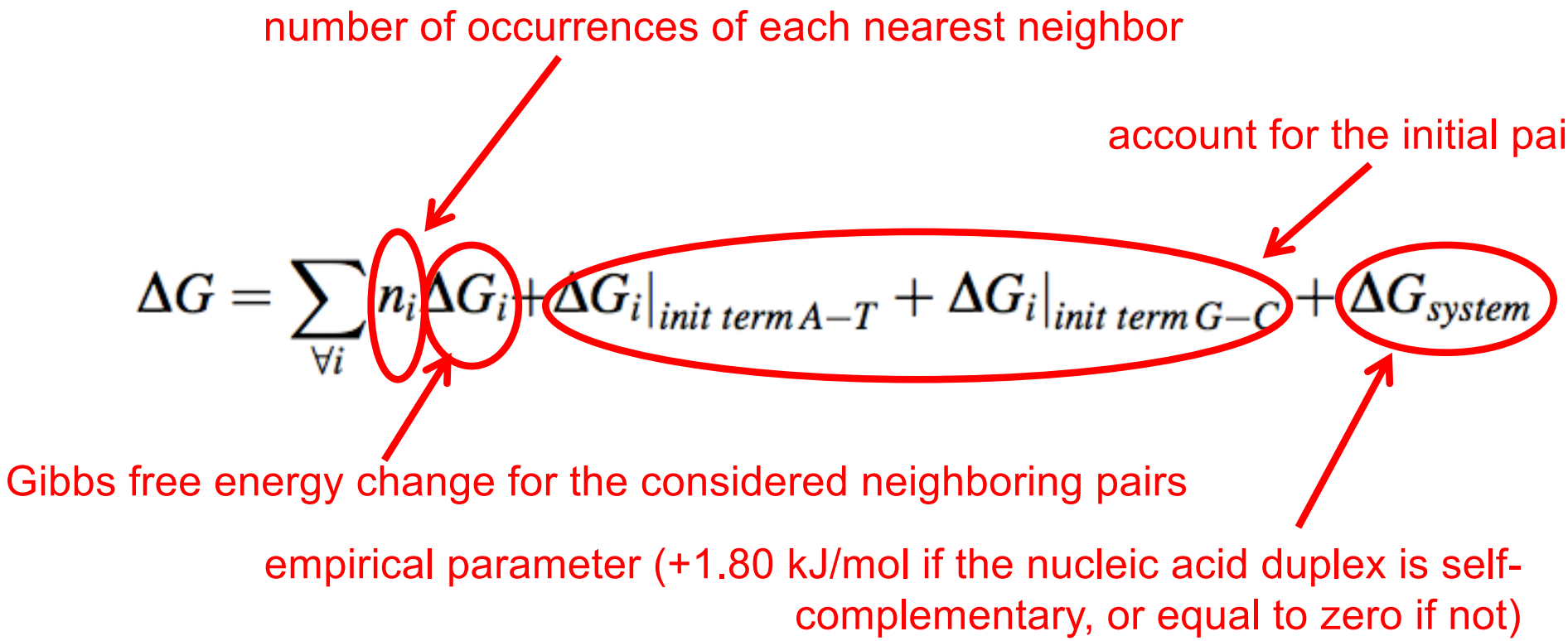
DNA dimer	ΔH [kJ/mol]	ΔS [kJ/(mol K)]
	-30.2	-85.4
	-33.1	-92.9
	-30.2	-89.2
	-41.0	-102.1
	-33.5	-83.3
	-41.9	-113.9

Enthalpy & entropy for different DNA dimers

DNA dimer	ΔH [kJ/mol]	ΔS [kJ/(mol K)]
5' A-T 3' / 3' T-A 5'	-30.2	-85.4
A-T / A-T or T-A / T-A	-33.1	-92.9
T-A / A-T		
G-C / C-G	-33.5	-83.3
C-G / G-C	-41.9	-113.9

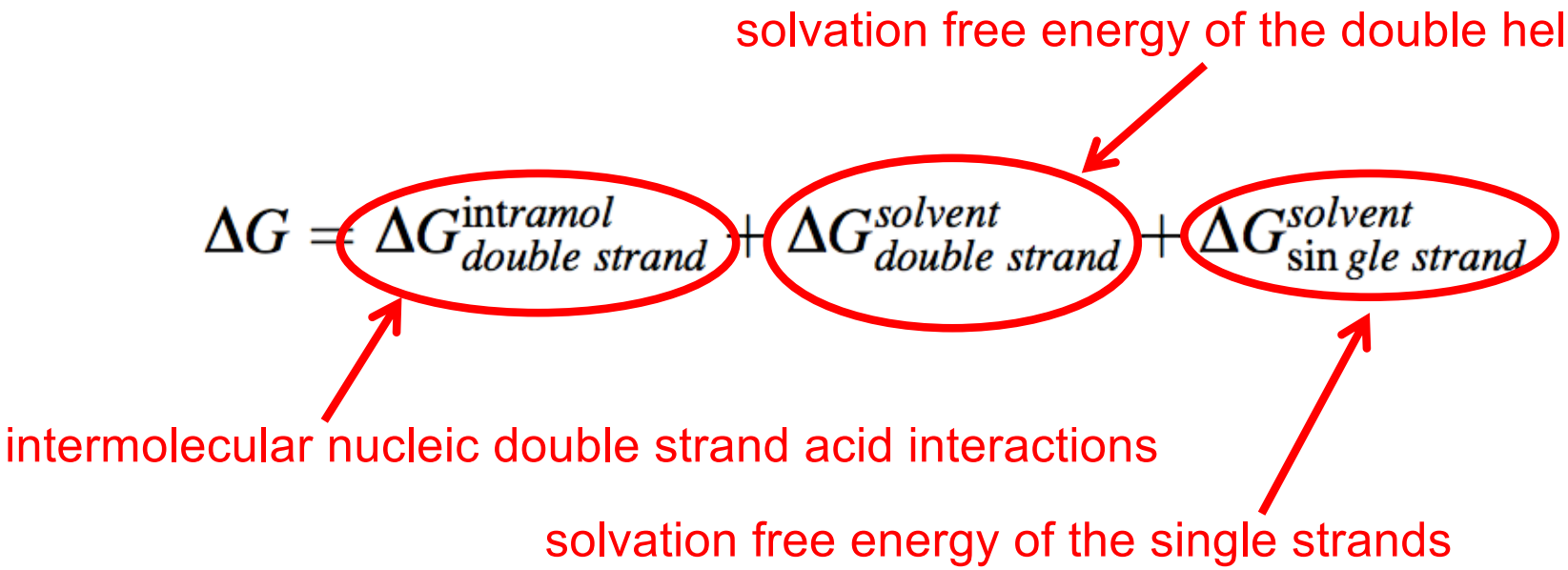
So, Sequence matters in the interaction strenght!

The Nearest-Neighbor Model



It introduces a computation for the total Gibbs free energy that takes into account the interactions from neighbours

Role of Solvent



The solvent also plays a role in DNA/DNA and DNA/RNA pairing, thus, it contributes to the Gibbs free energy too

Role of Solvent

$$\Delta G = \Delta G^{intramol}_{double\ strand} + \Delta G^{solvent}_{double\ strand} + \Delta G^{solvent}_{single\ strand}$$

DNA/RNA hydrogen bonds interactions

$$\Delta G^{intramol}_{double\ strand} = \Delta G^{hydrogen\ bonds}_{DNA-RNA} + \Delta G^{Coulomb}_{DNA-RNA} + \Delta G^{van\ der\ Waals}_{DNA-RNA}$$

Coulomb interactions between the phosphate groups of the backbone and the other ions in the solution

van der Waals interactions between the rings on the two sides of the duplex.

$$\Delta G^{solvent}_{single\ strand} = \Delta G^{solvent}_{DNA} + \Delta G^{solvent}_{RNA}$$

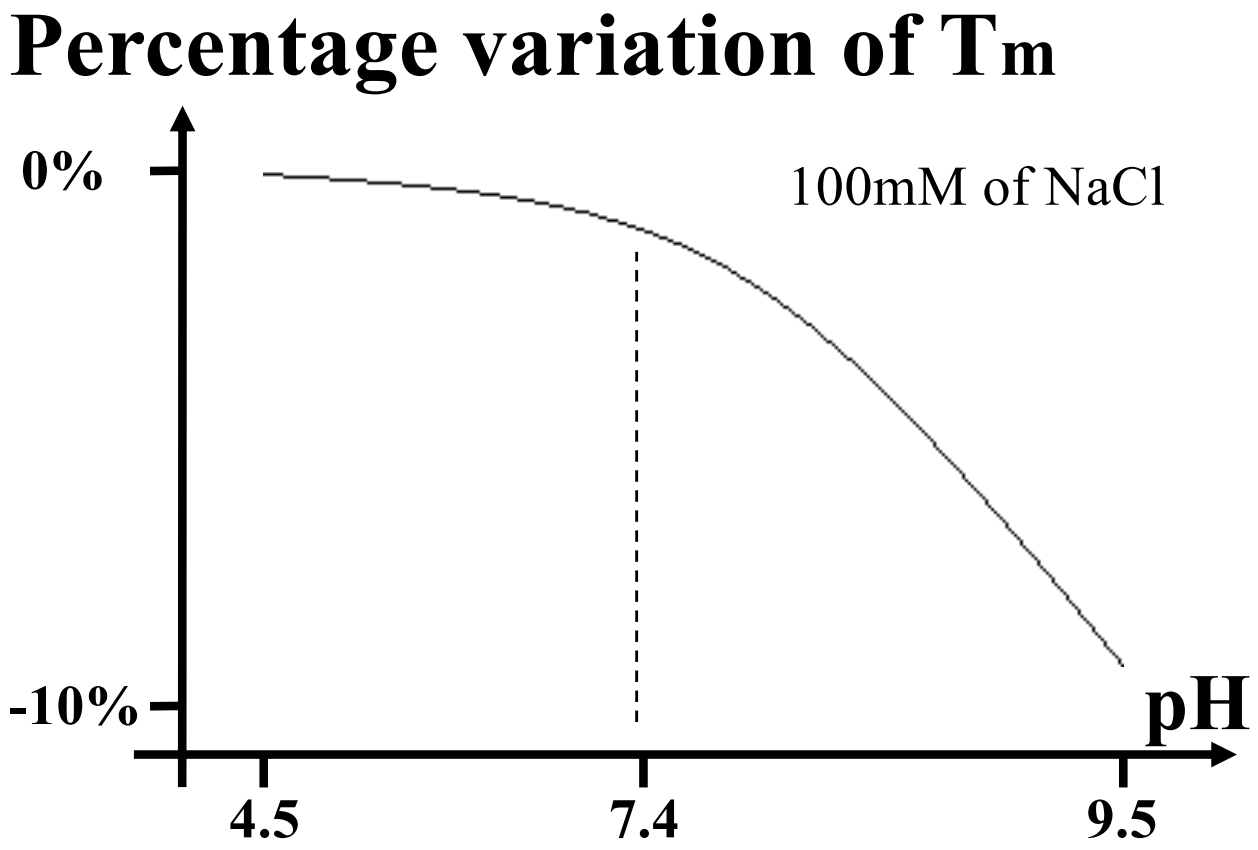
interactions between the different single strands and the solvent

DNA Denaturation

Melting of the DNA helix is the thermodynamic process that unwind the double helix and interrupts the non-covalent and sequence-dependent interactions between two single strands. The denaturation of DNA is another name for the same process.

The term **melting** is usually accomplished by heating the solutions containing the DNA. We can than follow the process by the **Melting Temperature**, and also have here another proof of the role of the solvent

Melting temperature cahanges by pH



Dependence by other solution ions

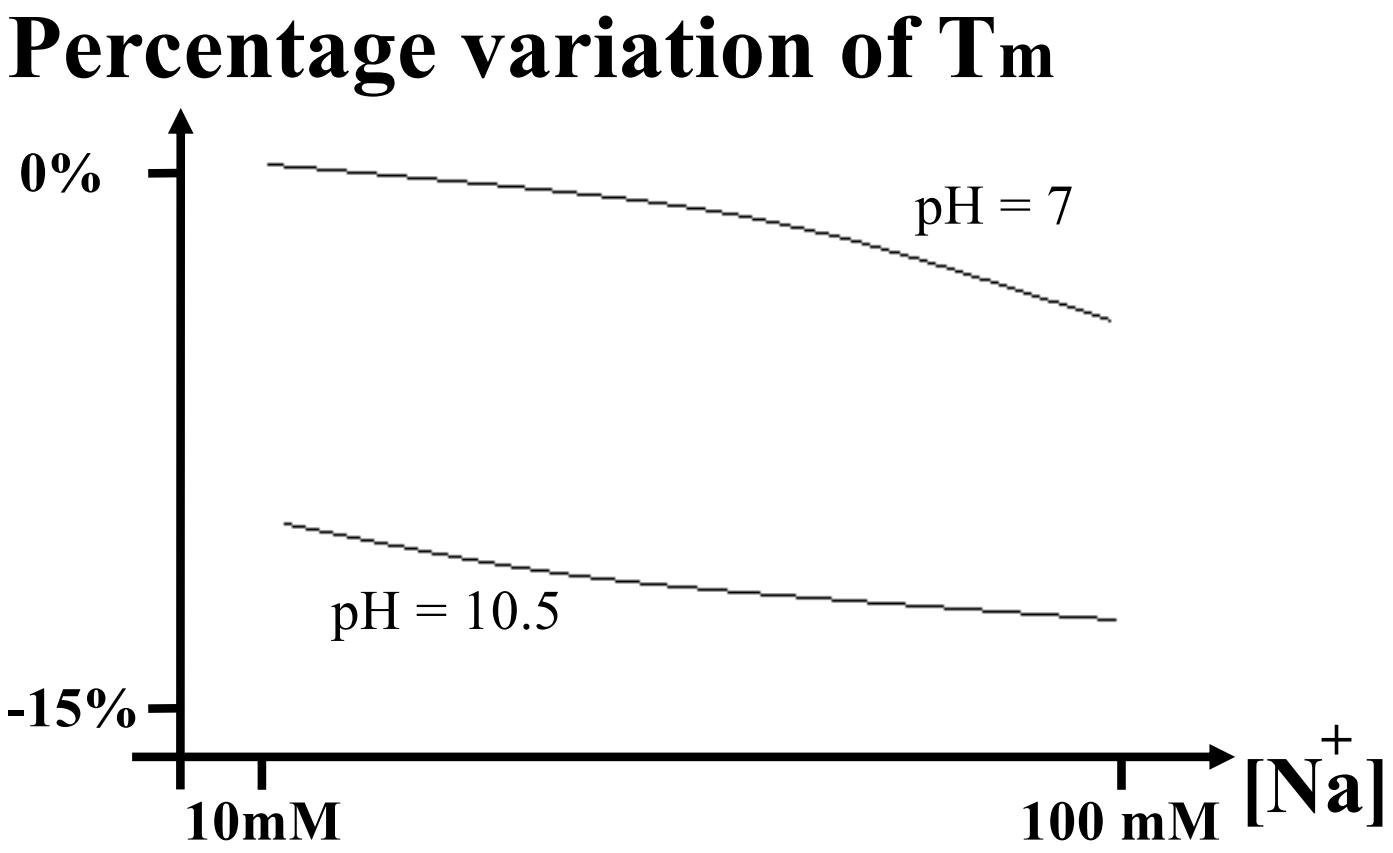
$$T_m = \frac{\Delta H}{\Delta S - R \ln C_{DNA}}$$

The enthalpy is usually considered independent of ions concentration, while Gibbs free energy and entropy are not.

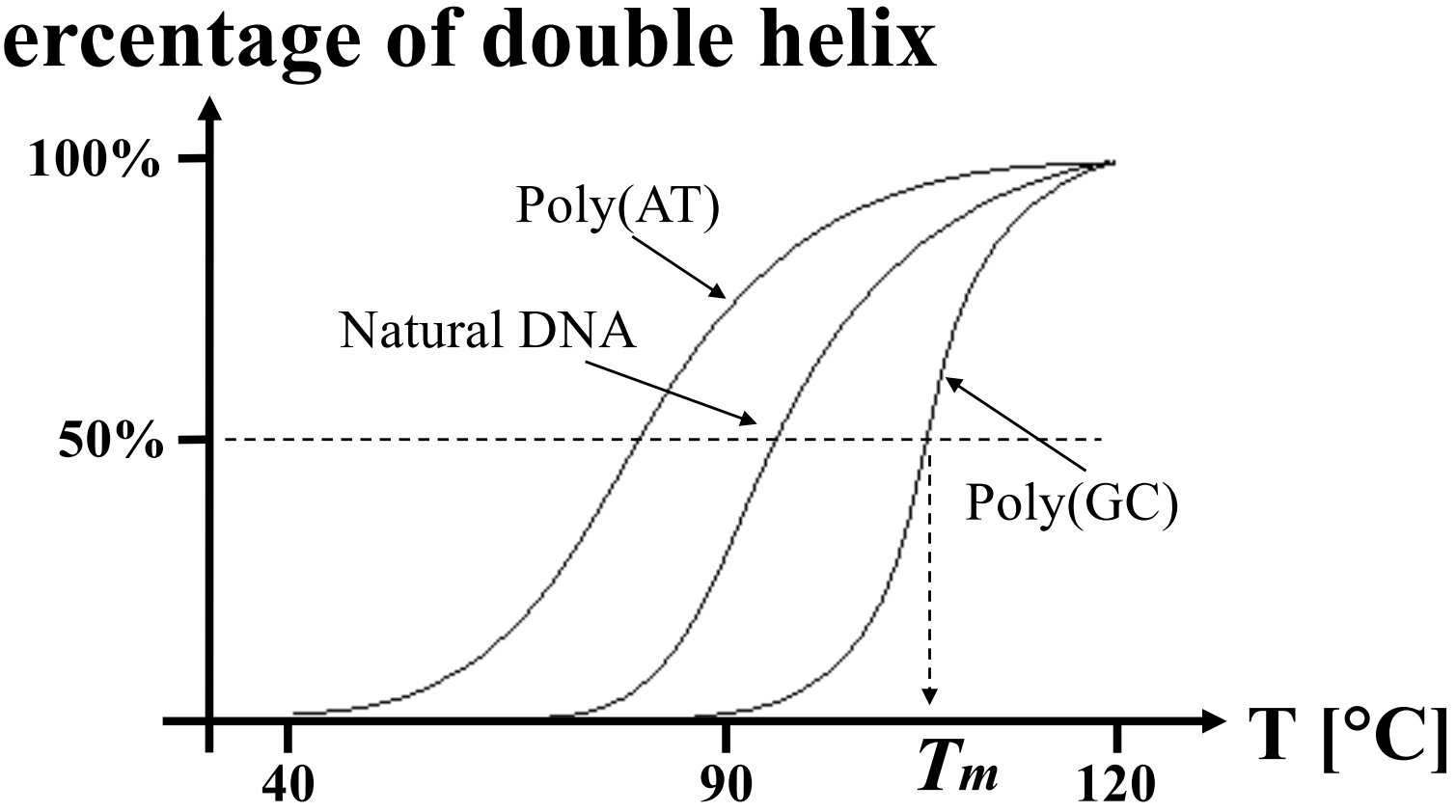
$$\begin{cases} \Delta G_{37^\circ}^{[Na^+]} = \Delta G_{37^\circ}^{[1\,Mole\ \ of\,Na^+]} + \alpha N \ln[Na^+] \\ \Delta S_{37^\circ}^{[Na^+]} = \Delta S_{37^\circ}^{[1\,Mole\ \ of\,Na^+]} + \beta N \ln[Na^+] \end{cases} \qquad \begin{cases} \alpha = -0.114 \\ \beta = +0.368 \end{cases}$$

The pH also affects the hydrogen bonds strength by changing the value of the Gibbs free energy of the interactions between single or double strands and the solvent

Melting temperature of DNA versus sodium concentration



Melting processes for different DNA sequences



NN-model versus Measurements

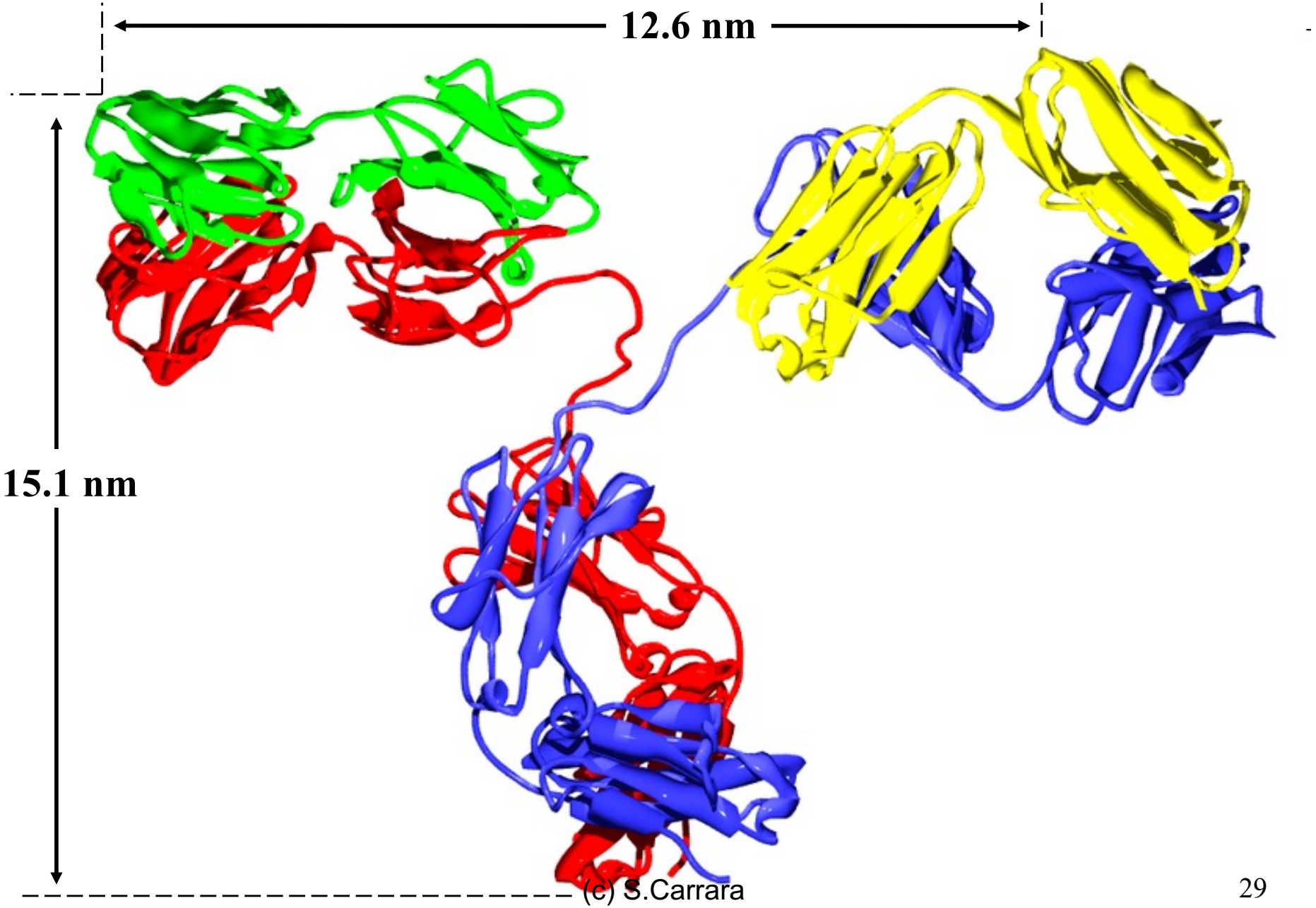
duplex	*NN-model* ΔG [kJ/mol]	*Experimental* ΔG [kJ/mol]
GGTTATTGG CCAATAACC	-25.4	-26.8
GGTTCTTGG CCAAGAACC	-30.1	-31.4
GGTTTTTGG CCAAAAACC	-27.6	-29.5
GGTTATTGG CCAAAAACC	-13.8	-12.0
GGTTCTTGG CCAATAACC	-12.1	-12.4
GGTTTTTGG CCAAGAACC	-17.5	-17.5

Antibody/Antigen interactions

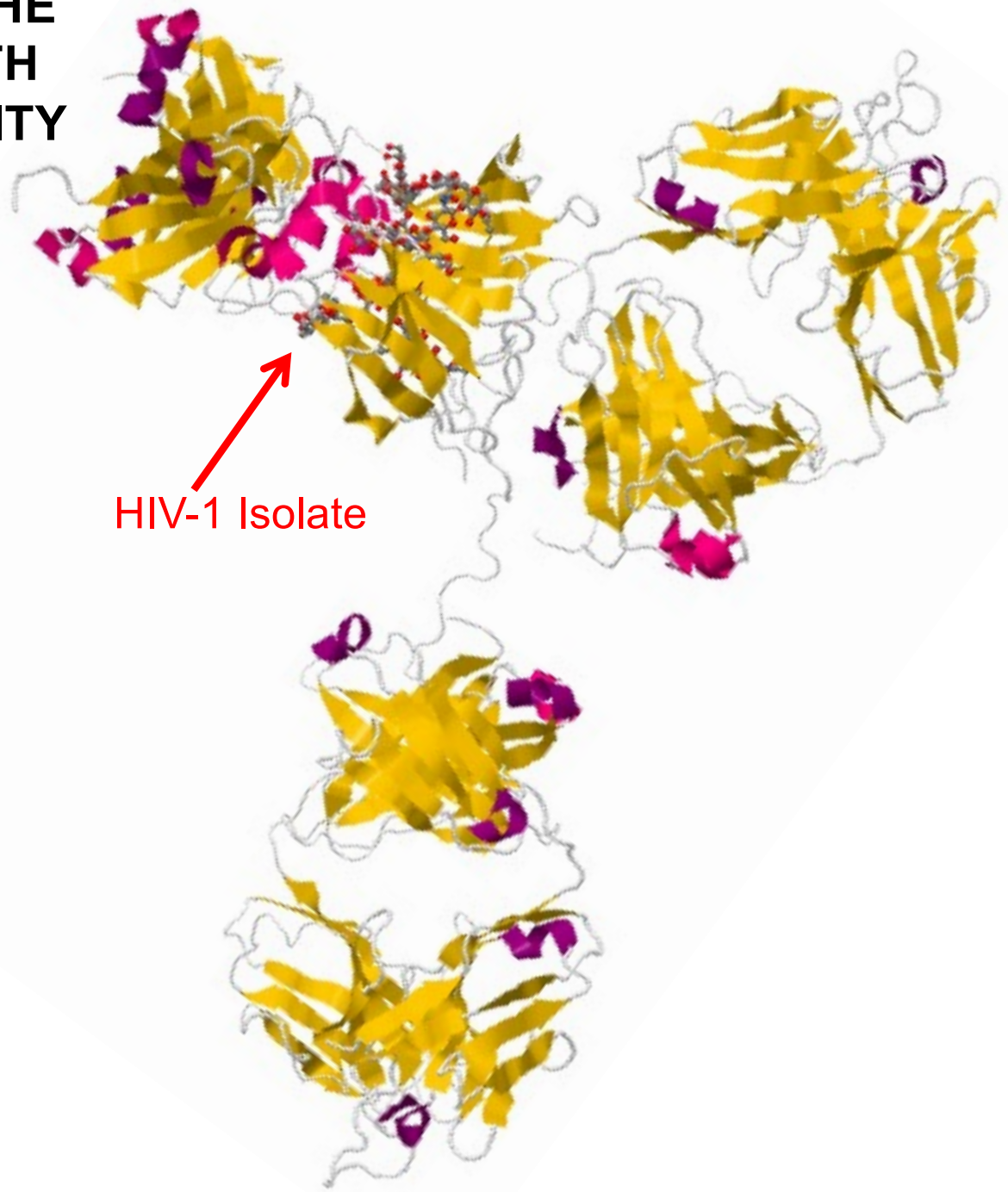
Antibodies are a large class of y-shaped **proteins** called immunoglobulins. An antibody shows a Y-structure with three main protein regions: a constant region and two variable regions. The variable regions are suitable for blocking the antigens. The protein blocks the antigen by closing the b-sheets in the variable region like fingers of a human hand when grasping

An efficient trapping is assured by the perfect match between the steric shape of the antigen and the 3D conformation of the antibody. The right part of the antigen in direct contact with the antibody is called the **epitope**, while the right part of the antibody in contact with the antigen is called the **paratope**

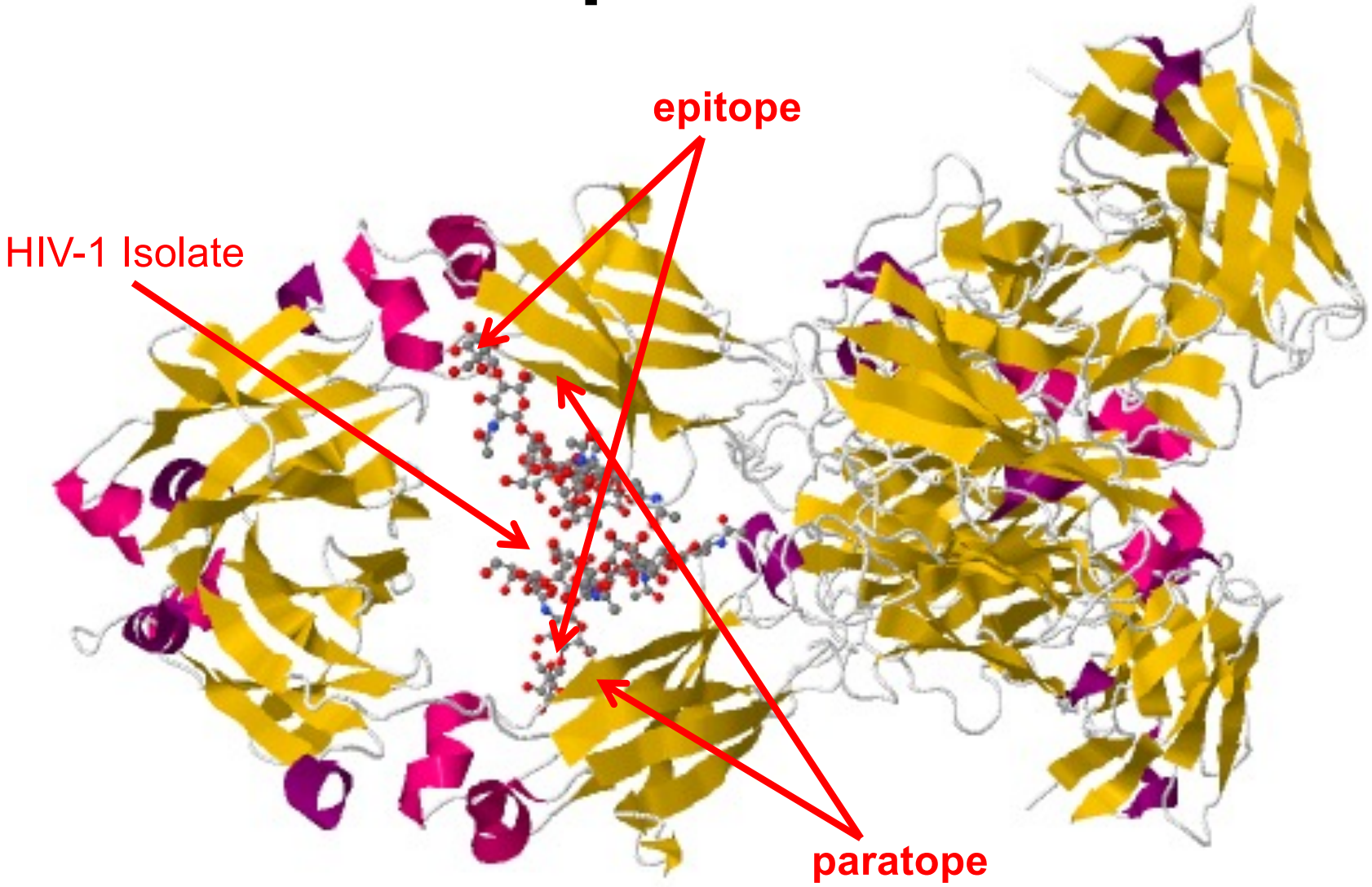
Antibody



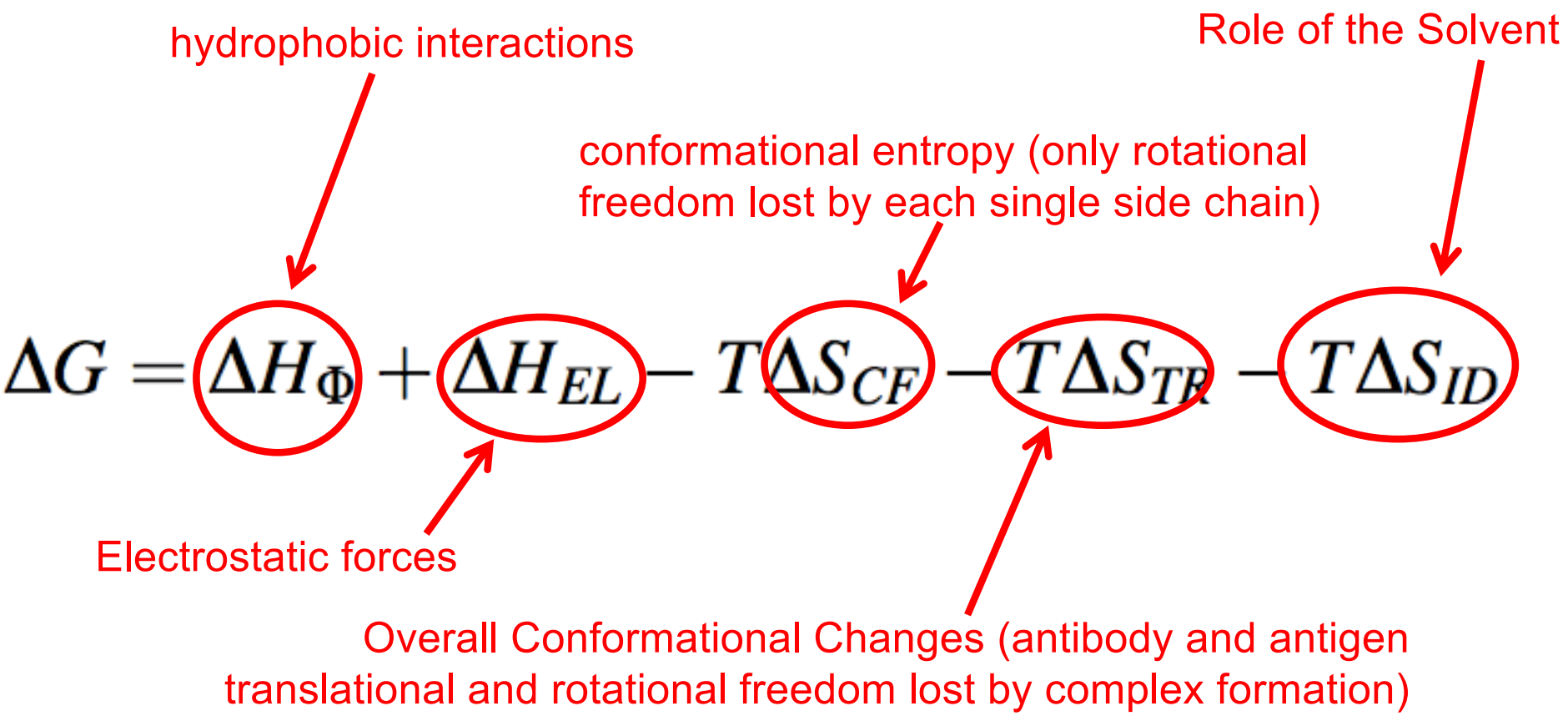
CRYSTAL STRUCTURE OF THE INTACT HUMAN IGG B12 WITH BROAD AND POTENT ACTIVITY AGAINST PRIMARY HIV-1 ISOLATES



Top View

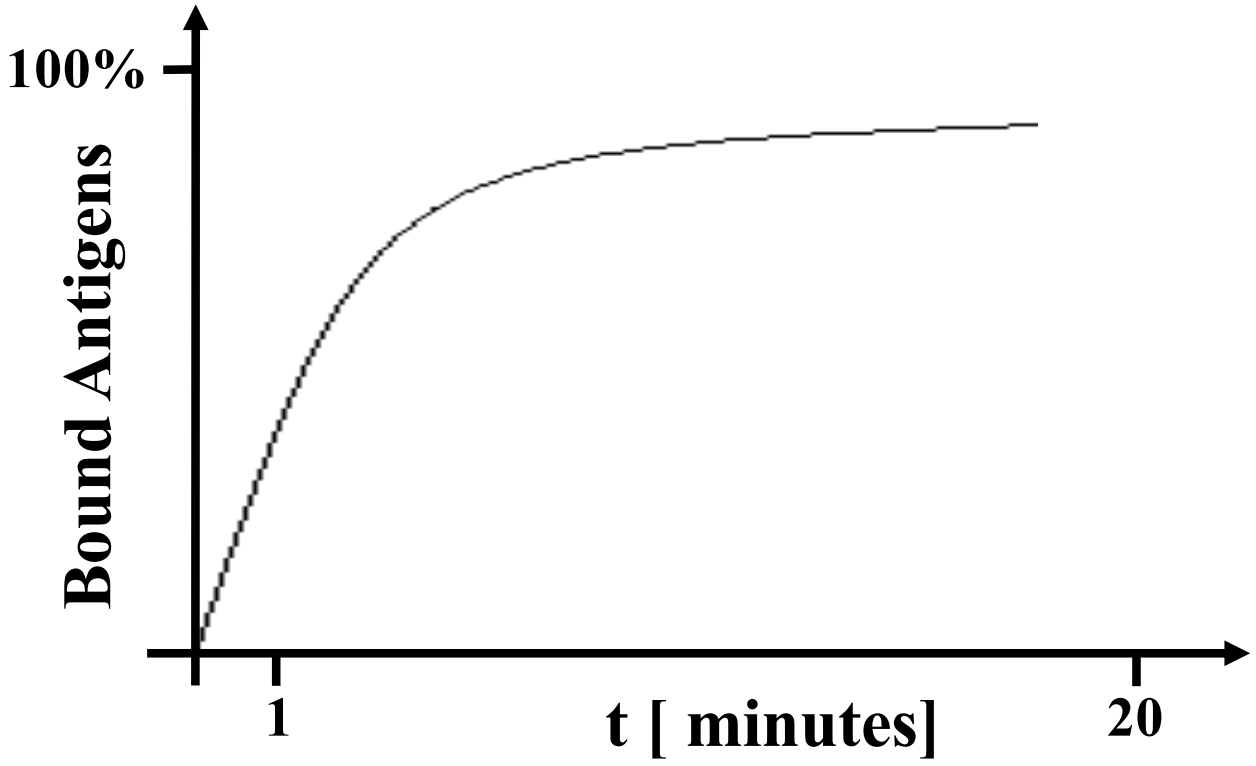


Gibbs free energy contributions



The total Gibbs free energy is related to various sources of molecular interactions

Typical trend of antigen binding versus time

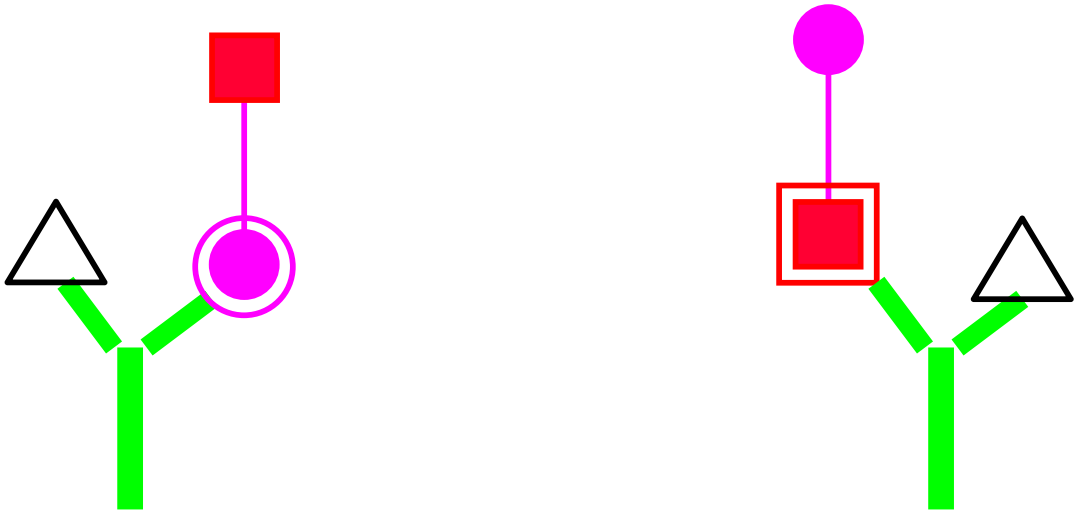


Different Kinds of Antibody

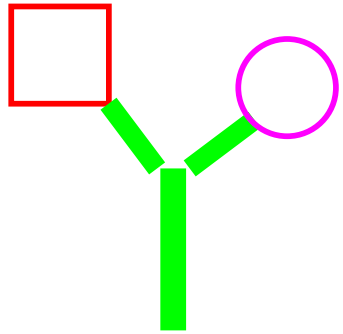
Dealing with real cases results in a bit more complex situation than just adding antigens to antibodies with a unique perfect match. **Monoclonal antibodies** are, then, all antibodies that have exactly the same specificity because they are from the same cloned single cell. However, antibodies are in general secreted in blood plasma by cells that are from different cell lines. Therefore, it is easy to obtain antibodies that are all against the same antigen but that do not have exactly the same specificity: these are **polyclonal antibodies**. Different kinds of antibodies means different kinetics on the same antigen.

We may also obtain different kinetics by involving the same antibody. It happens when the secreted antibody possesses two different paratopes to address two different epitopes of the same antigen

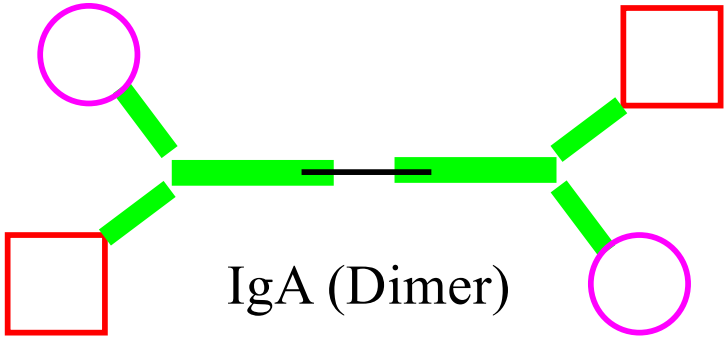
Polyclonal Antibodies to the same Antigen



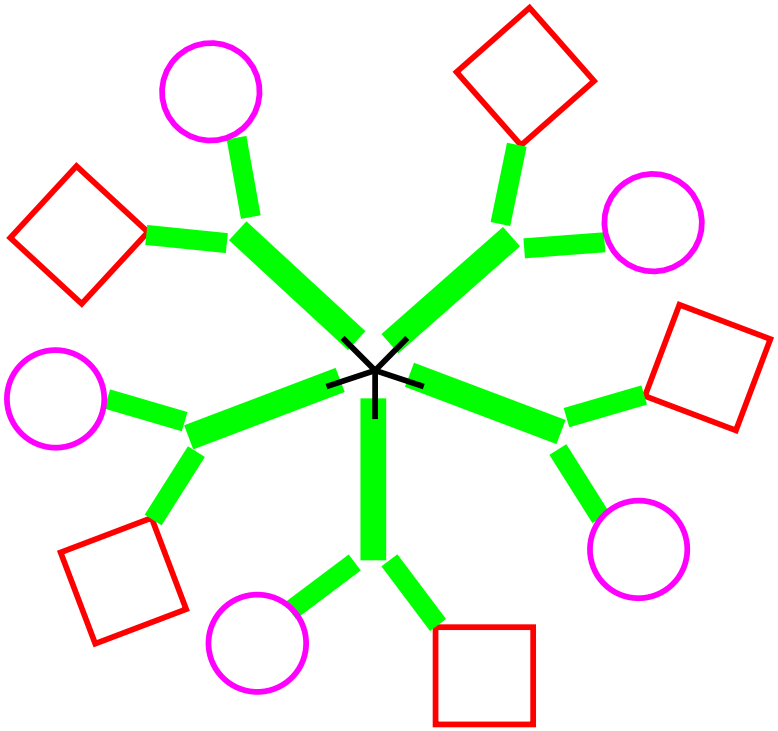
Classes of Immunoglobulins



IgG, IgE, and IgD (Monomer)



IgA (Dimer)



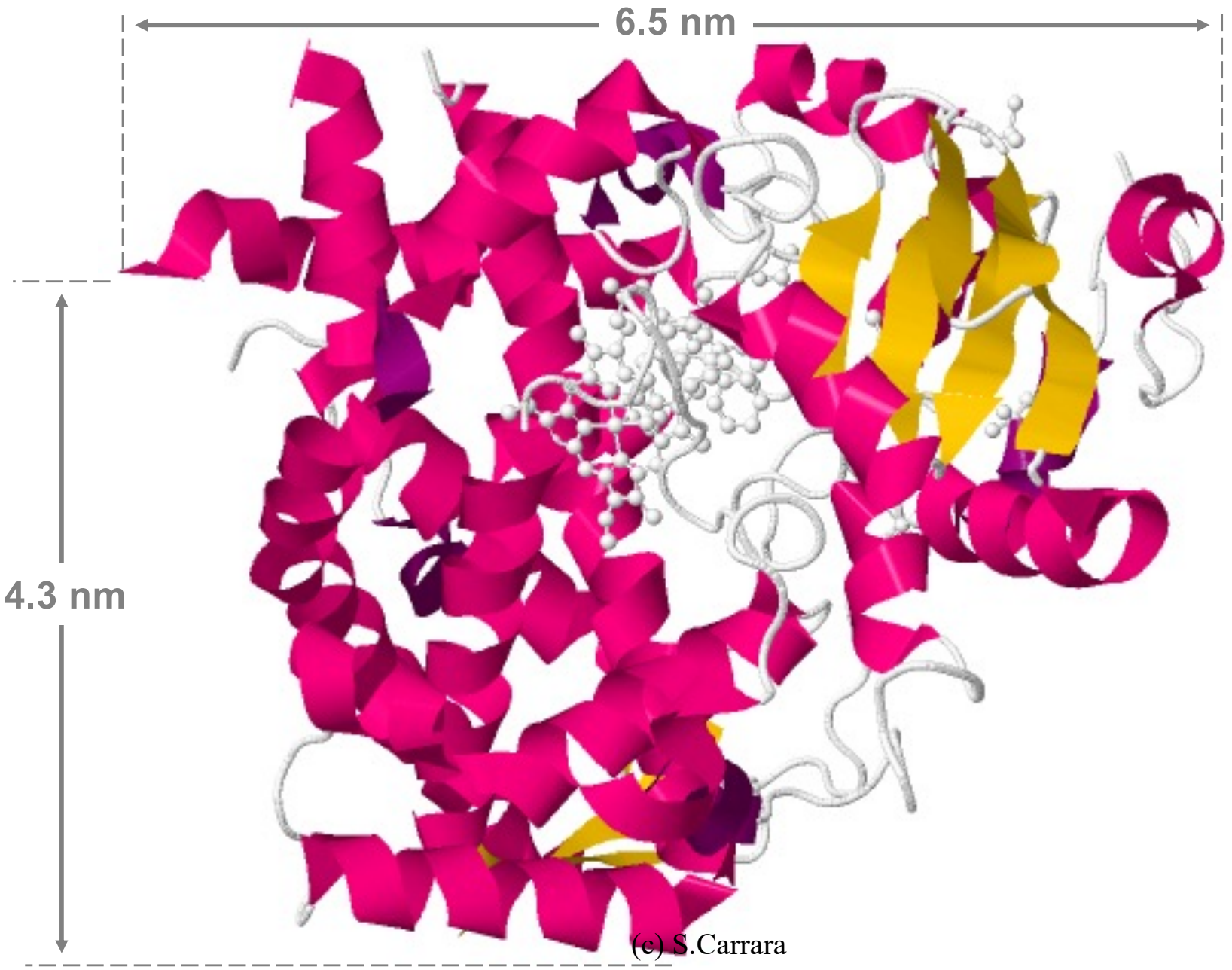
IgM (Pentamer)

Enzyme/Substrate interactions

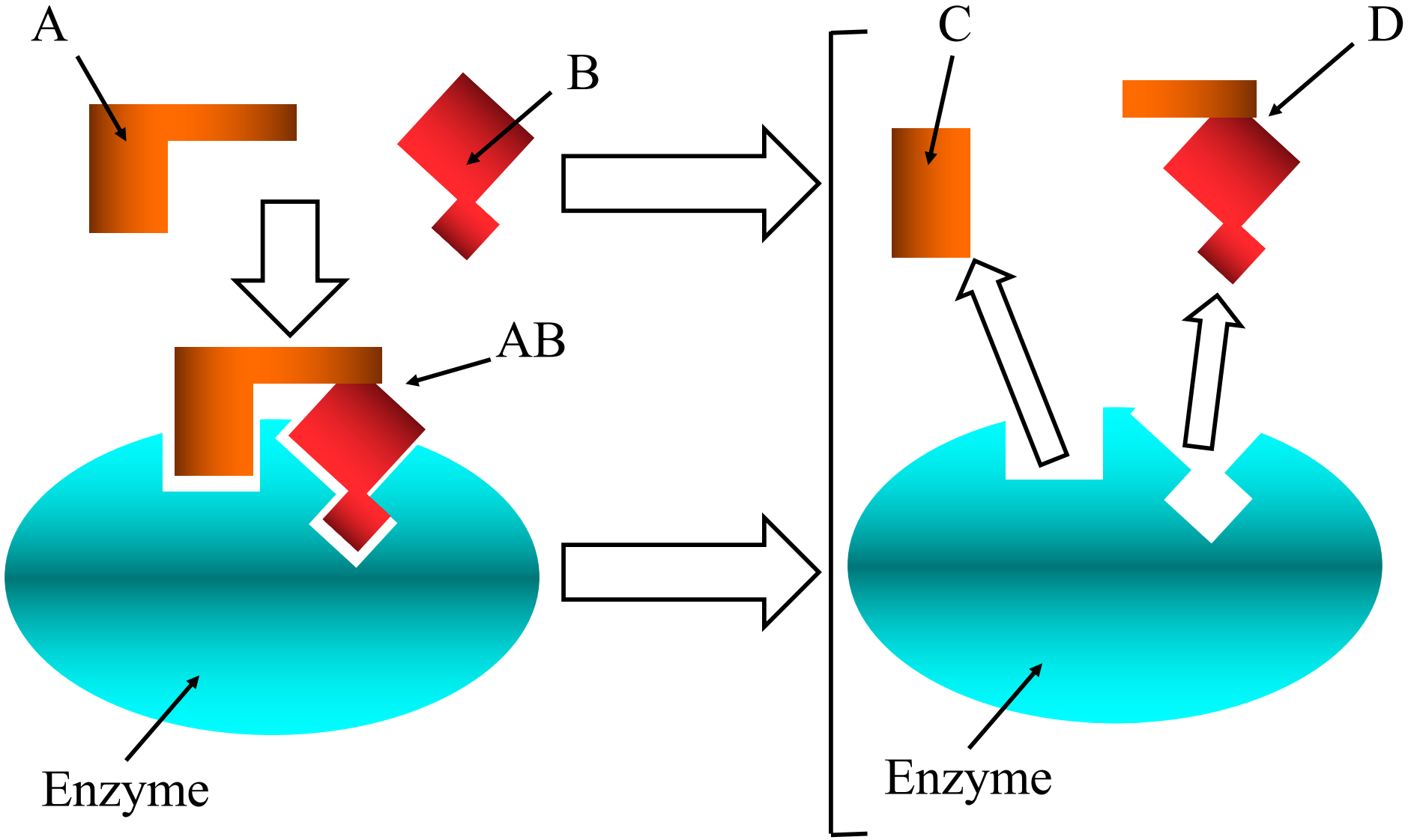
Enzymes are highly selective catalysts, accelerating both the rate and specificity of metabolic reactions. Almost all chemical reactions in a biological cell need enzymes in order to occur at a sufficient rates compatible with life. Most enzymes are proteins, although some catalytic RNA molecules have been identified.

At the beginning of an enzymatic reactions, is called **substrates** the molecules which transformation is catalysed. This molecules are converted into different ones, called **products**.

Cytochromes P450



Mechanism of Enzymatic reactions



Enzymatic Process

$$K = \frac{k_F}{k_B} = \frac{[C][D]}{[A][B]}$$

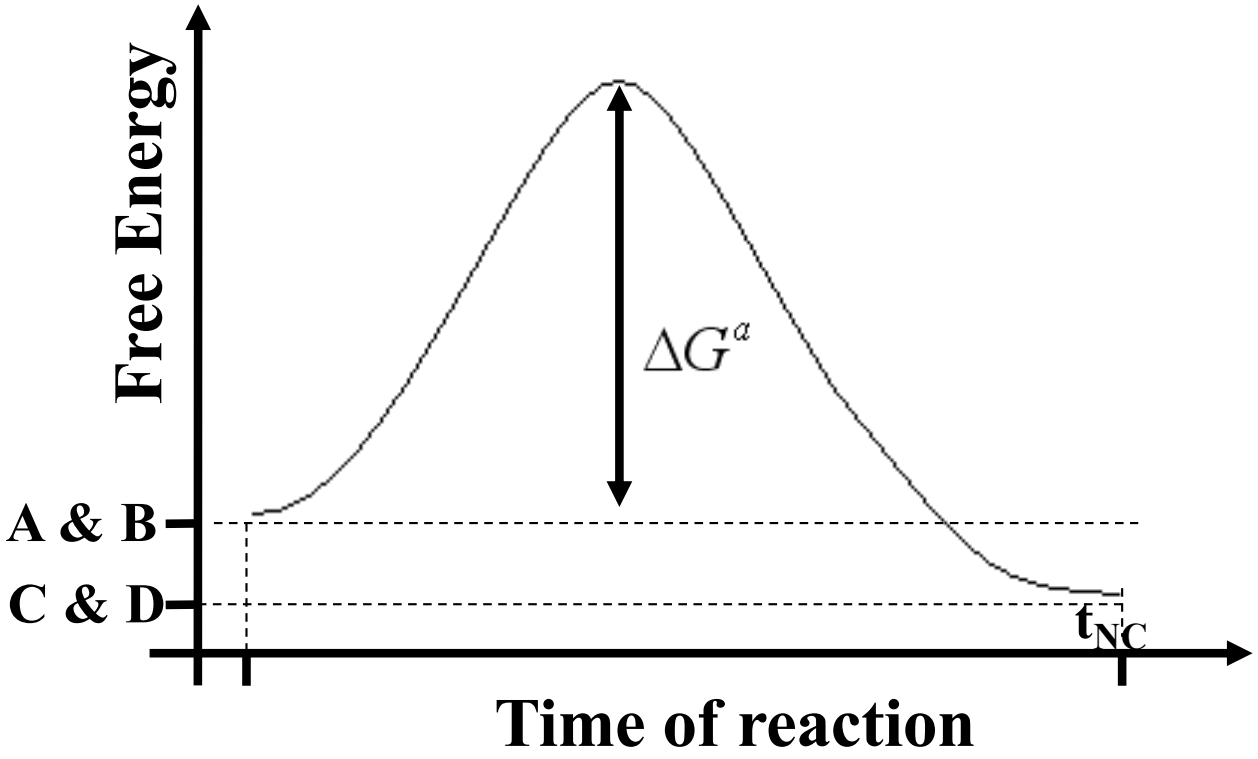
It is worth noting that the presence of an enzyme changes neither the equilibrium constant nor the two forward and backward rates

The enzyme changes the timing of the chemical reaction. If the equilibrium is reached in, let say, 1 h in the absence of enzymes, then it might be reached in few seconds in the presence of the suitable enzyme!

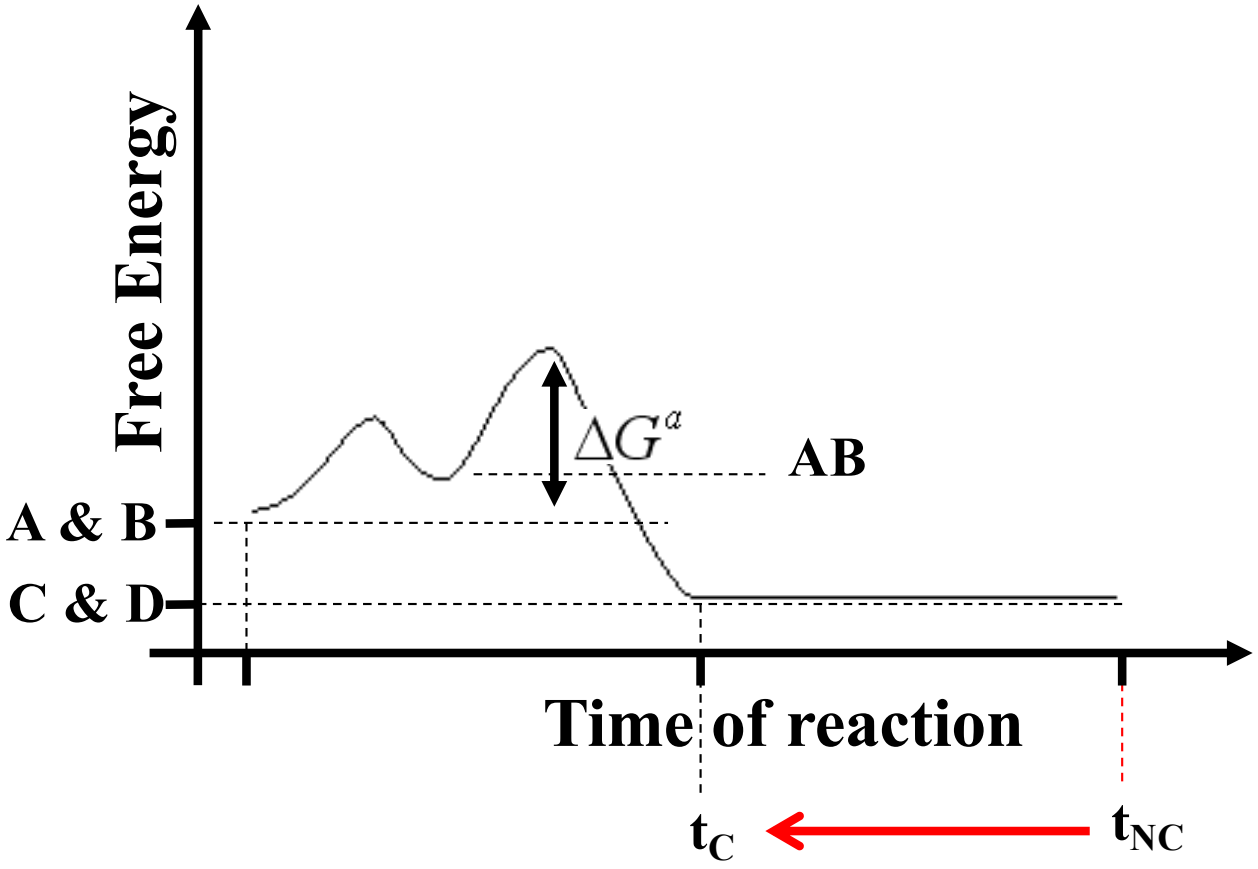
Gibbs free energy

$$\frac{d([C][D])}{dt} = k_p[AB] = k_p[A][B]\, e^{-\frac{\Delta G^a}{RT}}$$

Free energy of a reaction without enzymes



Free energy of a reaction with enzymes



Michaelis-Menten Kinetics

More often, the compound B is a common molecule, such as oxygen, or water, or proton, that is not specific to the enzyme action. Therefore, the enzymatic process is re-written in a form that explicitly introduce the presence of the enzyme:

$$E + S \underset{k_d}{\overset{k_a}{\rightleftharpoons}} ES \xrightarrow{k_p} E + P$$

By neglecting the possibility for E and P to give back the intermediate state AB, we can now write the net rate of product formation as:

$$\frac{d[P]}{dt} = k_p[ES]$$

Michaelis-Menten Kinetics

While the rate of complex formation:

$$\frac{d[ES]}{dt} = k_a[E][S]$$

And the rate of complex breakdown:

$$\frac{d([E][S])}{dt} + \frac{d[P]}{dt} = (k_d + k_p)[ES]$$

Need to be equal at the steady state of the enzymatic reaction:

$$k_a[E][S] = (k_d + k_p)[ES]$$

Michaelis-Menten Kinetics

Thus, the concentration of the complex is:

$$[ES] = \frac{k_a}{(k_d + k_p)}[E][S]$$

Which defines the so-called **Michaelis' constant**:

$$K_M = \left(\frac{k_a}{(k_d + k_p)}\right)^{-1}$$

The Michaelis constant is so important because it shows how efficient is the enzyme in forming the intermediate state that is required to catalyze the reaction.

Michaelis-Menten Kinetics

Usually, there is a certain amount of available enzymes that are still free to accept further substrates:

$$[E] = [E]_0 - [ES]$$

With $[E]_0$ as the initial concentration of total enzymes, which enables to re-write the concentration of the complex:

$$[ES] = \frac{([E]_0 - [ES])[S]}{K_M}$$

Or:

$$[ES] = [E]_0 \frac{[S]}{[S] + K_M}$$

Michaelis-Menten Kinetics

Now, by defining the reaction velocity V and the maximum reaction velocity V_m, respectively, as:

$$V = \frac{d[P]}{dt} \qquad V_{\max} = k_p[E]_0$$

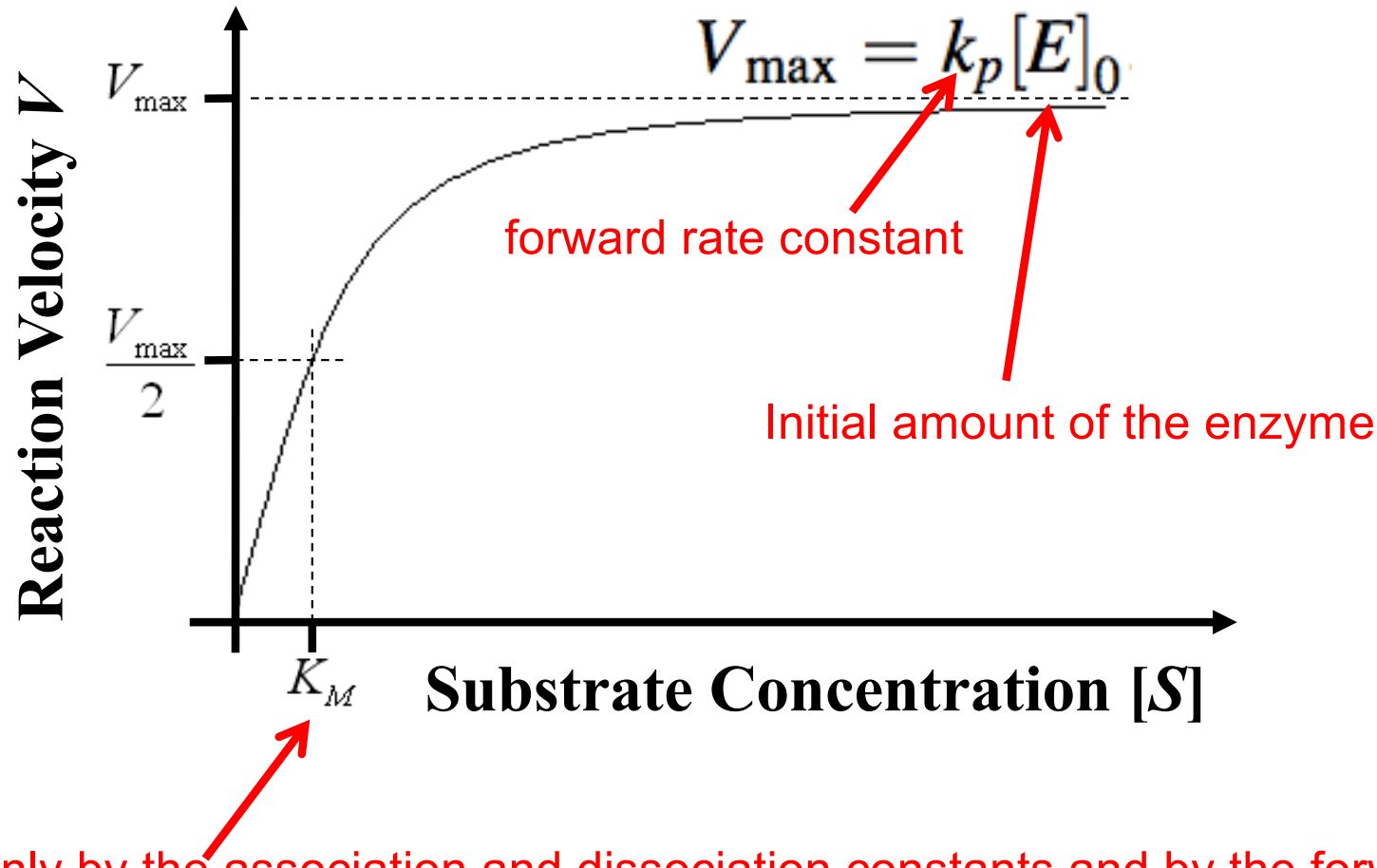
If we, then, go back to couple of previous equations:

$$\frac{d[P]}{dt} = k_p[ES] \qquad [ES] = [E]_0 \frac{[S]}{[S] + K_M}$$

We can finally write the the so-called **Michaelis–Menten equation**:

$$V = V_{\max} \frac{[S]}{[S] + K_M}$$

Michaelis-Menten Kinetics

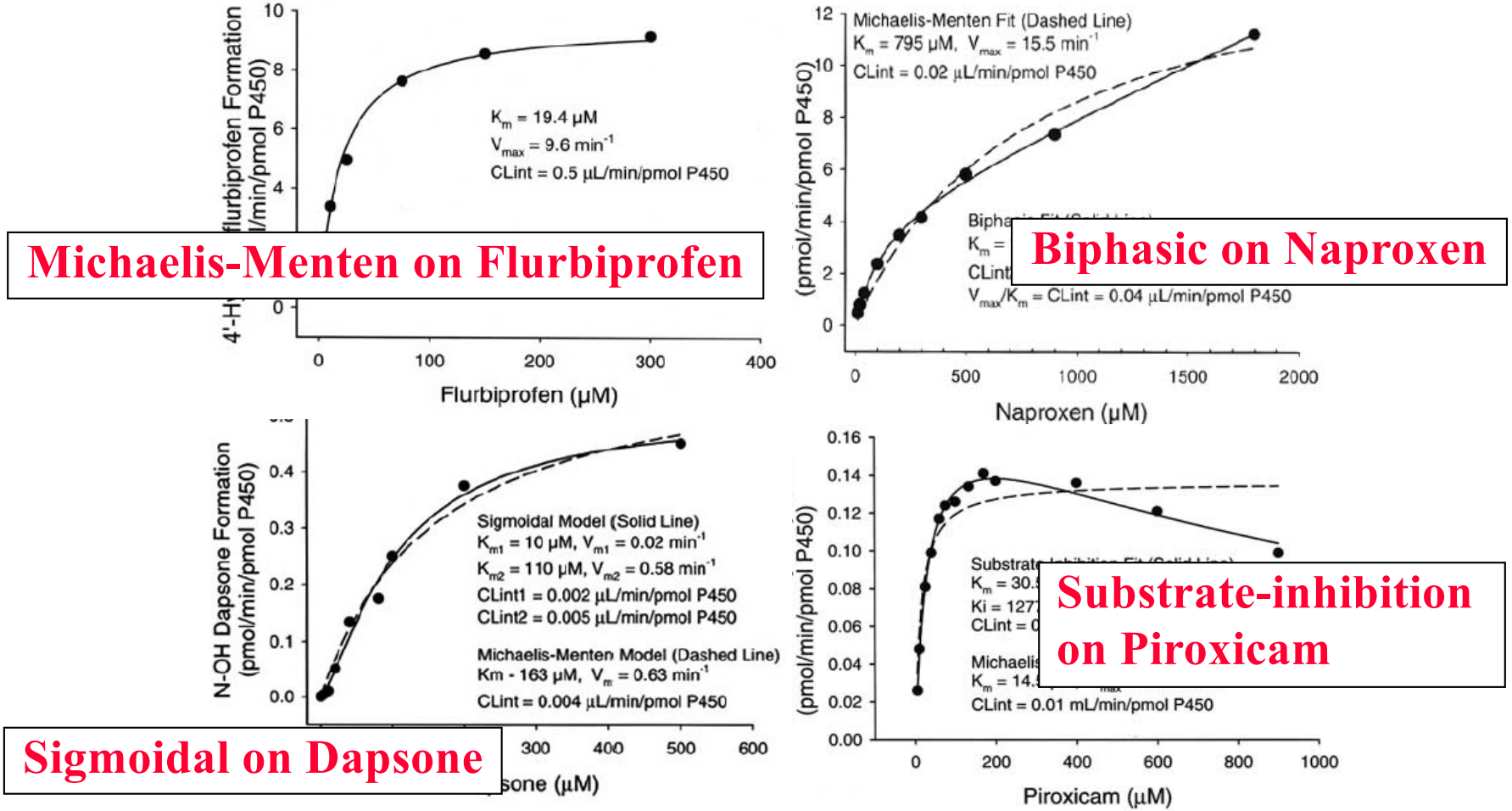


Depends only by the association and dissociation constants and by the forward rate

Atypical Enzymatic Reactions

One example of enzymes that have more than one substrate and that present other kinetics than the Michaelis–Menten is the case of **Cytochromes P450**. They are key enzymes in the metabolism of any mammalian. These proteins catalyze the redox reaction of various compounds and, sometimes, show atypical kinetics.

HOMOTROPIC KINETICS

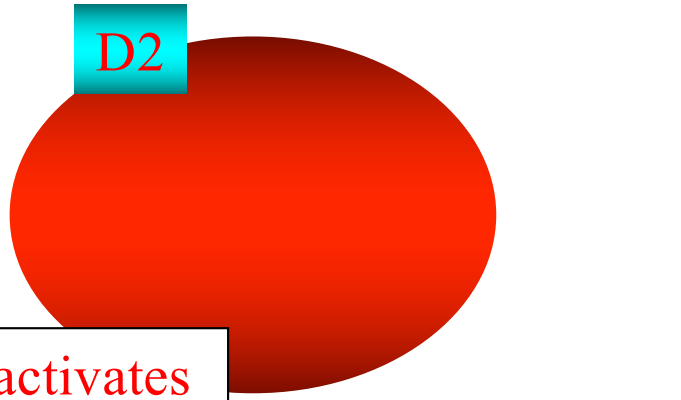


Timothy S. Tracy, Current Drug Metabolism, 2003, Vol. 4, No. 5

The isoform 2C9 of the cytochrome P450 presents all previously mentioned kinetics upon different substrates!

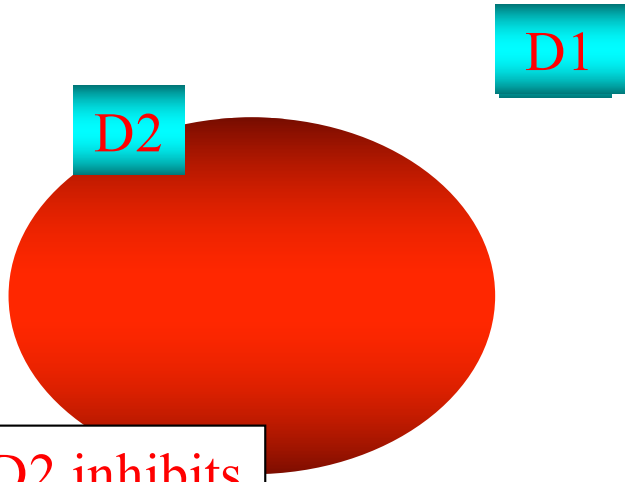
Phenomena of Heterotropic Kinetics

- **HETERO ACTIVATION**



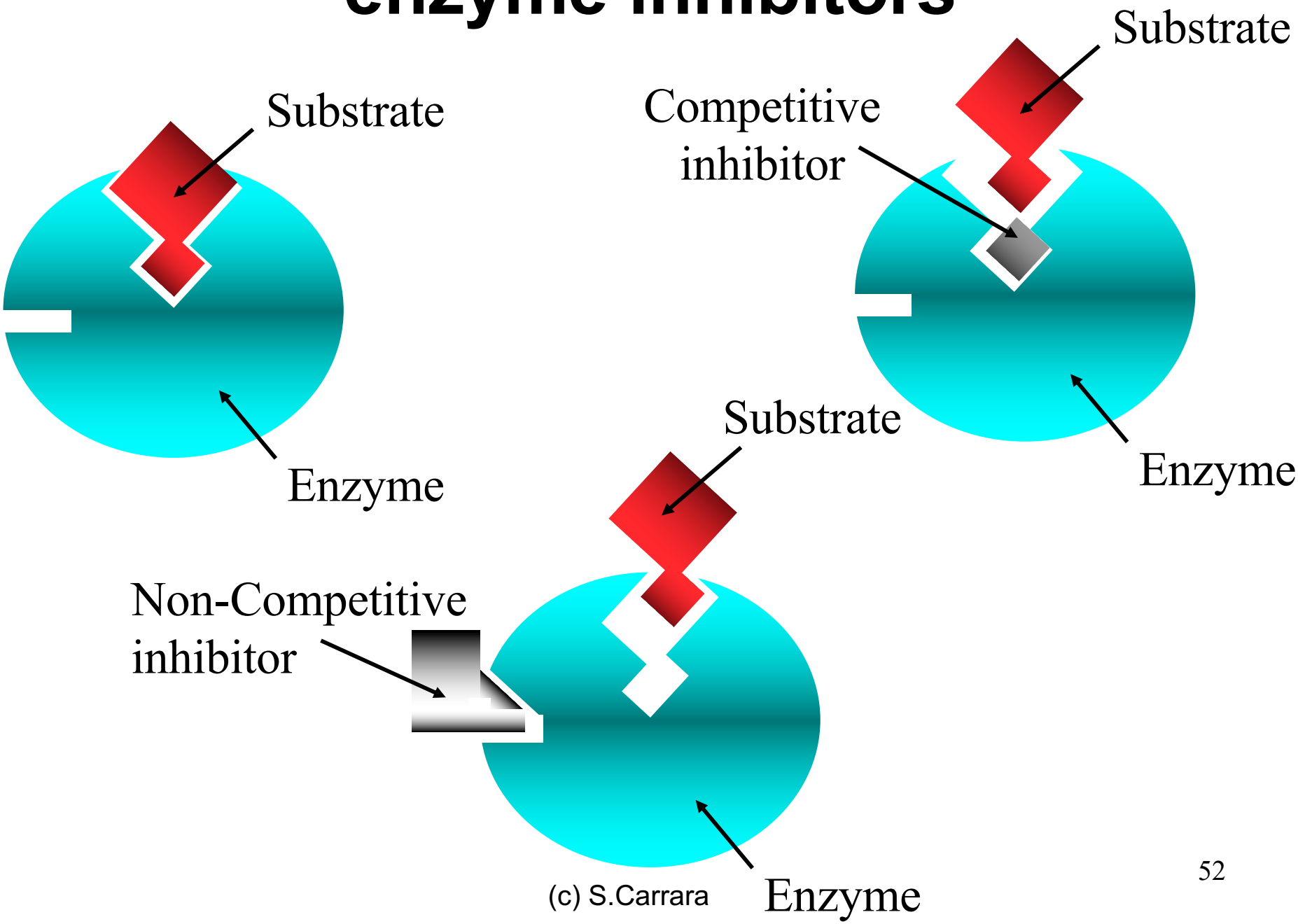
The presence of D2 activates
The catalysis on D1

- **PARTIAL INHIBITION**

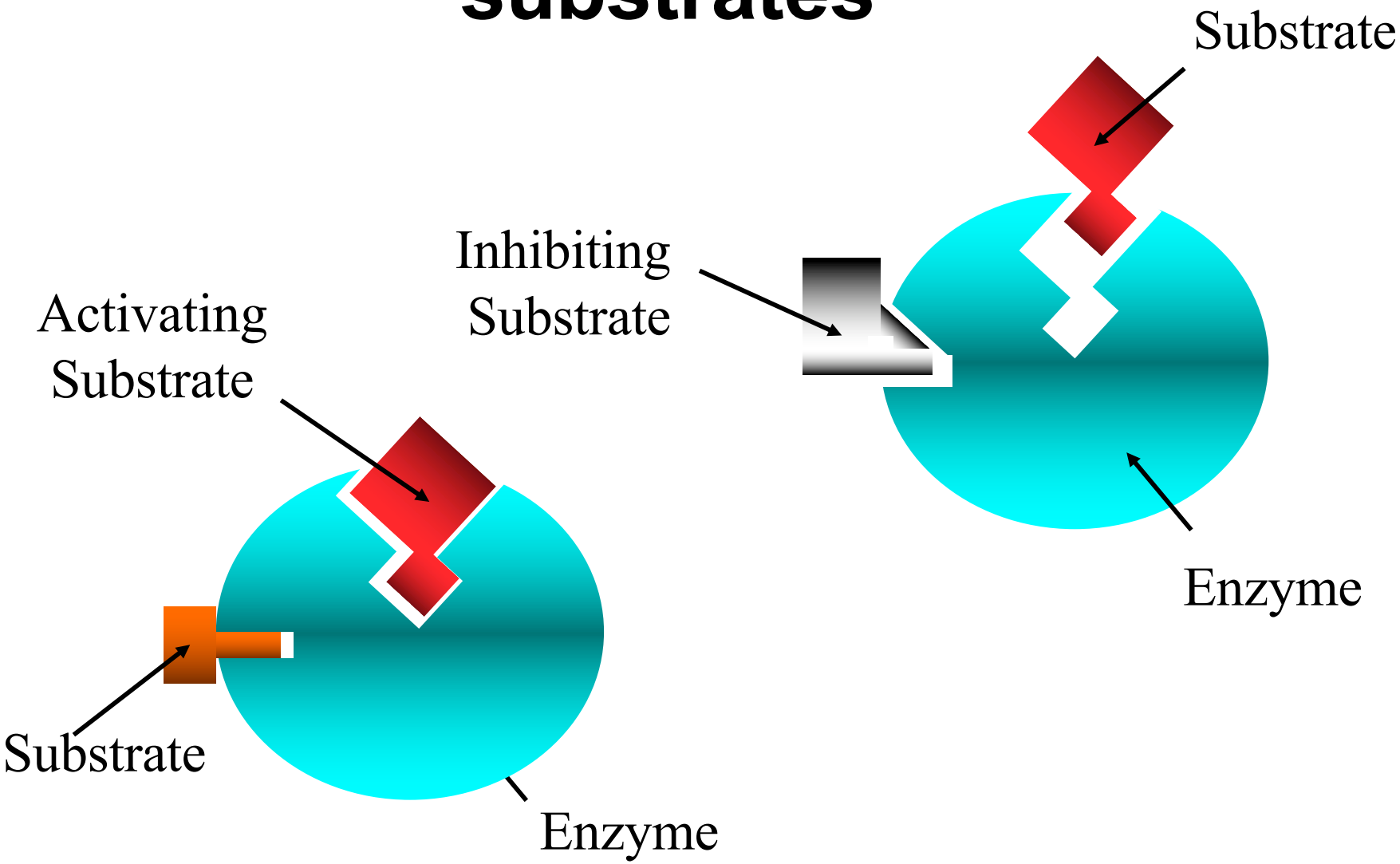


The presence of D2 inhibits
The catalysis on D1

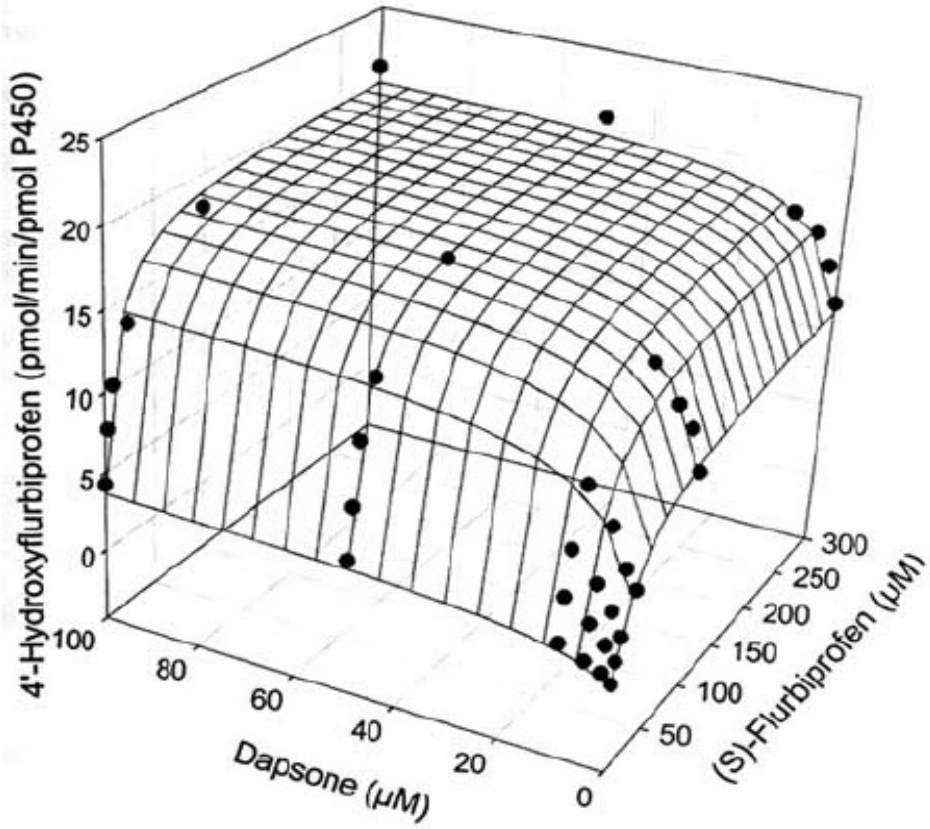
Competitive and noncompetitive enzyme inhibitors



Activating and inhibiting enzyme substrates



The case of Cytochrome P450 2C9



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$$v = \frac{V\max[S]}{km\left(\frac{1+\frac{[E]}{Ks}}{1+\frac{\varepsilon'[E]}{\varepsilon Ks}}\right)+[S]\left(\frac{1+\frac{[E]}{\varepsilon Ks}}{1+\frac{\varepsilon'[E]}{\varepsilon Ks}}\right)}$$

B= effector (activator compound)
Ks = [B] producing half maximal effect
ε = change in Km due to effector binding
ε' = change in Vmax due to effector binding

Heterotropic kinetics of flurbiprofen and dapsone when catalysed by the enzyme P450 2C9