EPFL

EDMI Microsystems and Microelectronics

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

Lecture #3

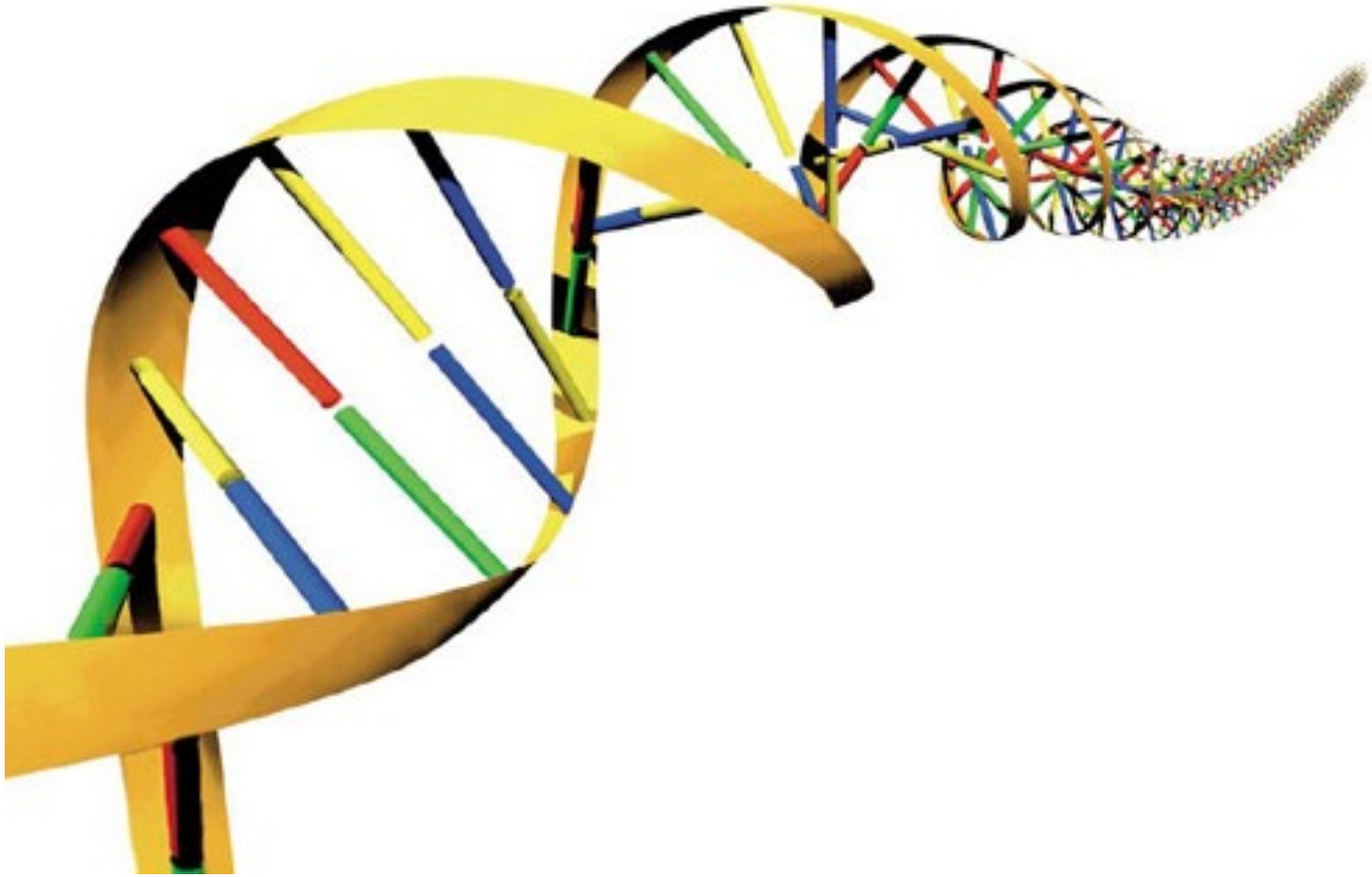
Probe/Target interactions (DNA/Antibodies/Enzymes)

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



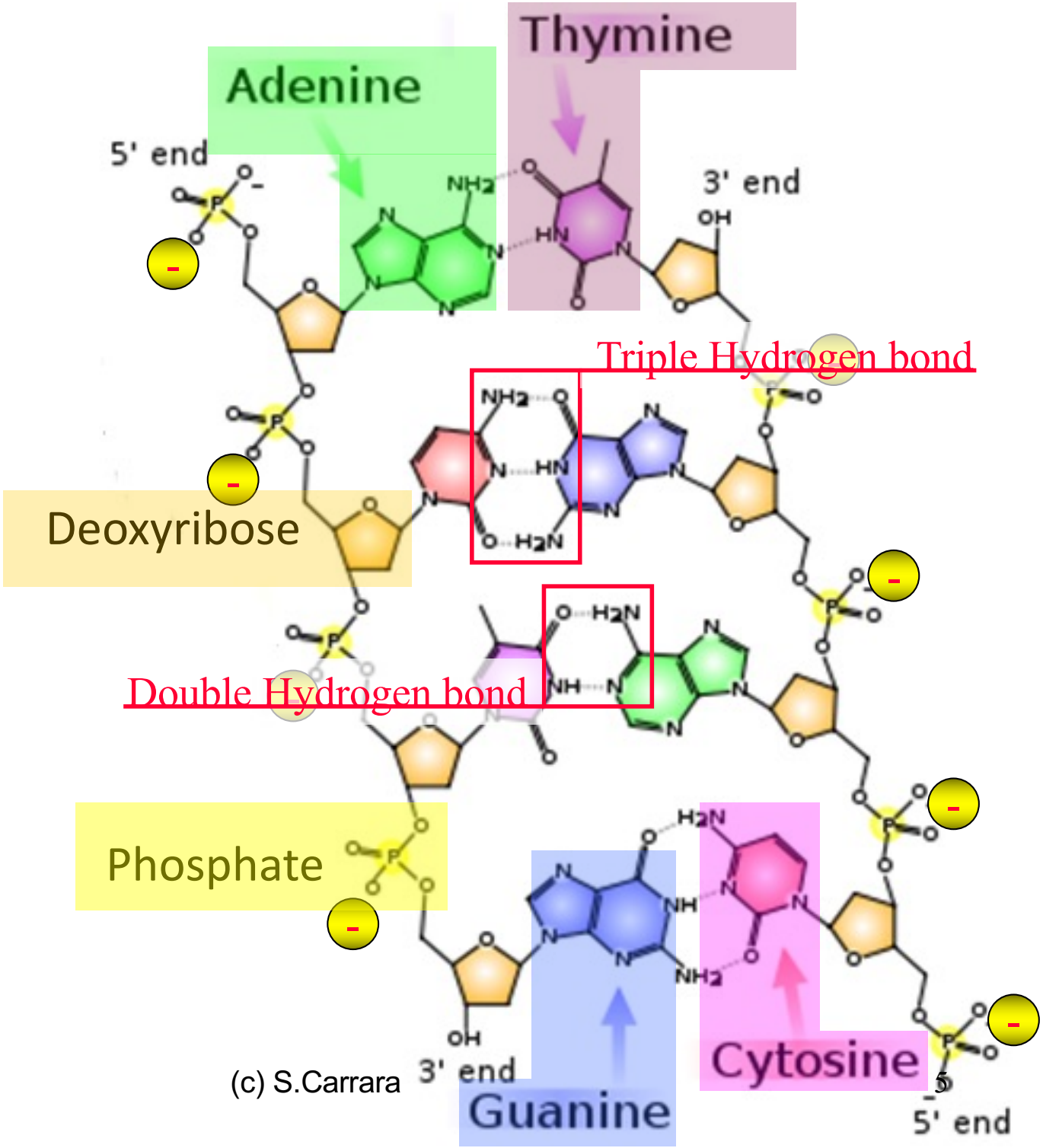
Another fundamental molecules in Biochemistry is the DNA

DNA

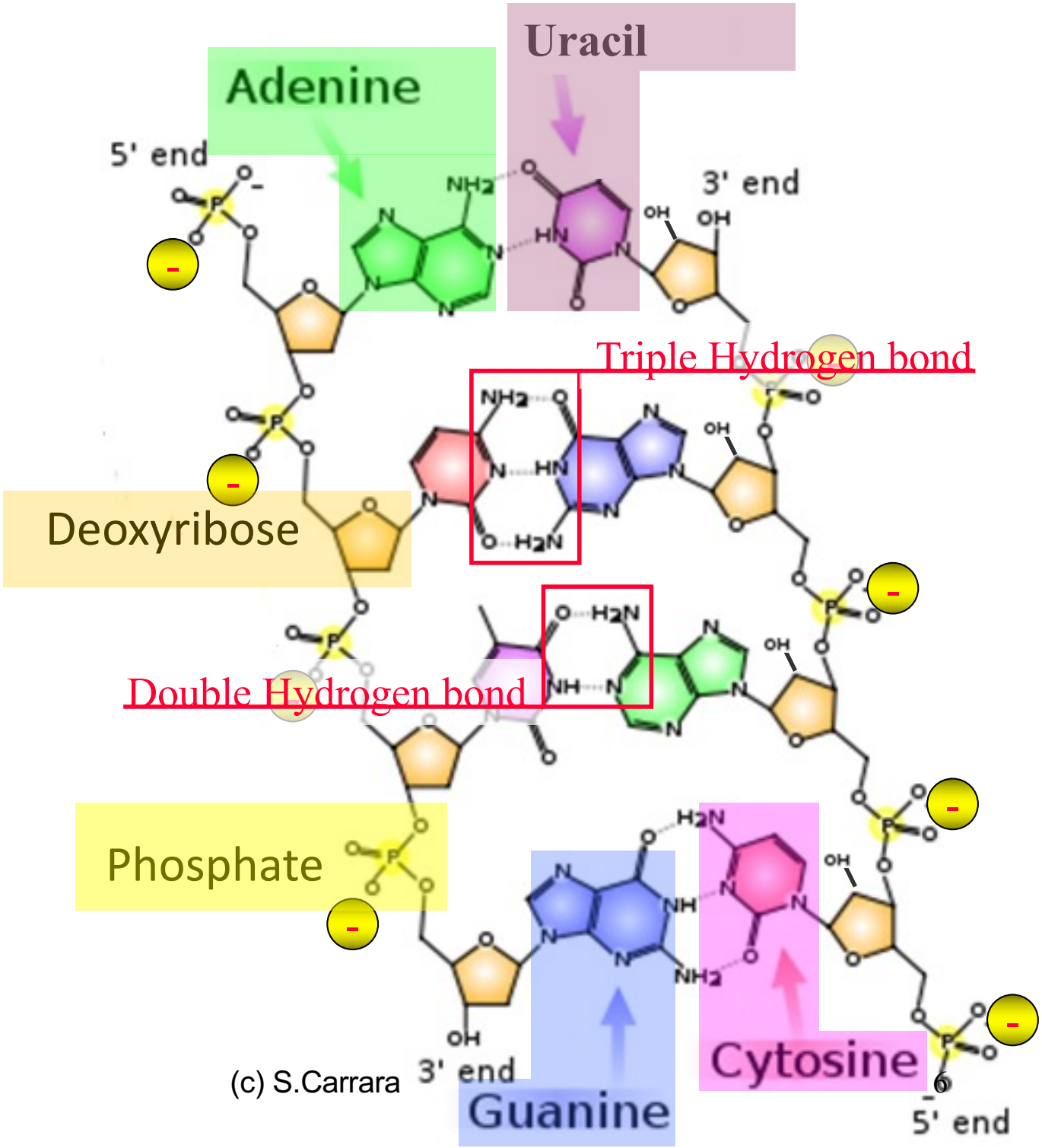
Definition

is a **nucleic acid** that contains the genetic instructions used in the development and functioning of all known living organisms and some viruses.

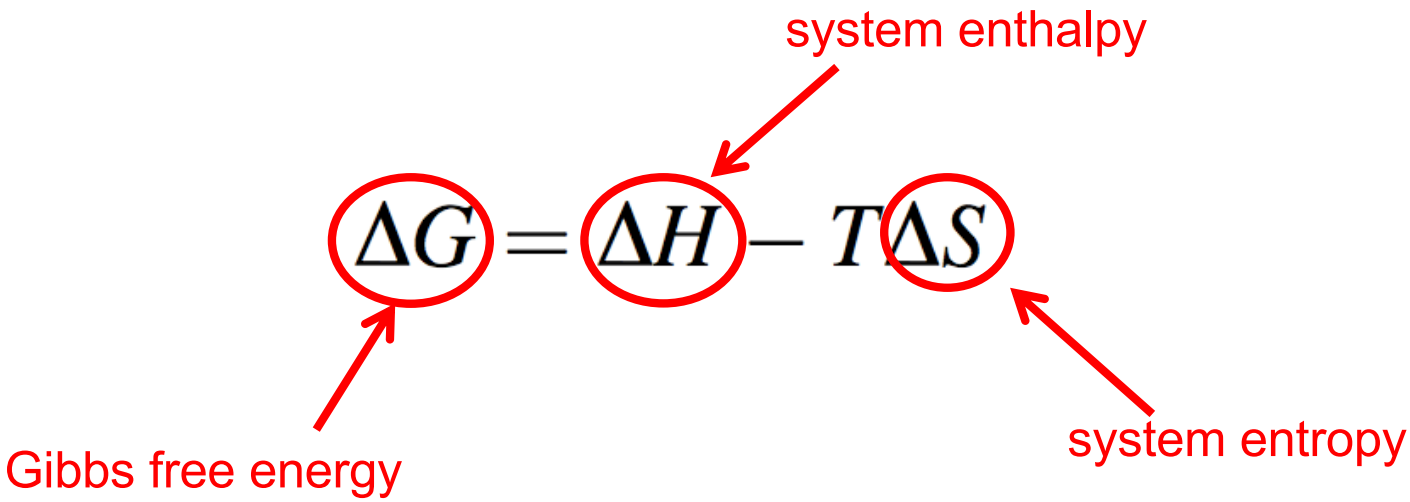
DNA-DNA Interaction



DNA-RNA Interaction

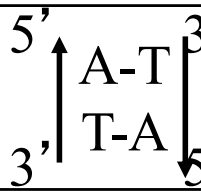
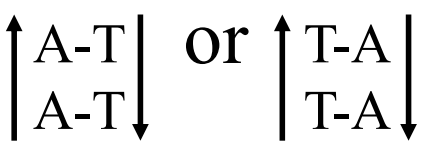
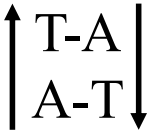
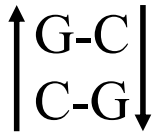
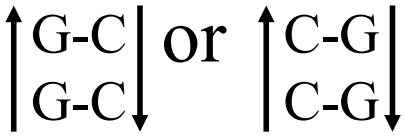
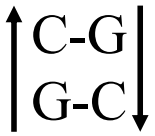


Gibbs free energy



Several interactions play a role in DNA-DNA and in DNA-RNA interactions. They include those between adjacent bases, those between electrons on the upper and lower parts of the base rings, and those between the phosphate groups in the nucleic acid backbone and the polar water molecules and other ions in the solution that interact directly with the paired molecule. Entropic forces also play a role

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

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

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. The word **denaturation** is more often used when the unwinding and opening of the double helix are performed by means of a pH change or by means of a change in ion content in the solution.

Melting Temperature

By definition, the maximum of double-helix unfolding and interaction interruption happens at the melting temperature. Therefore, it is the moment of transformation where the enthalpy supplied by heating contributes to nucleic acid melting. Therefore, the Gibbs free energy is null at this temperature:

$$\Delta G = \Delta H - T\Delta S = 0.$$

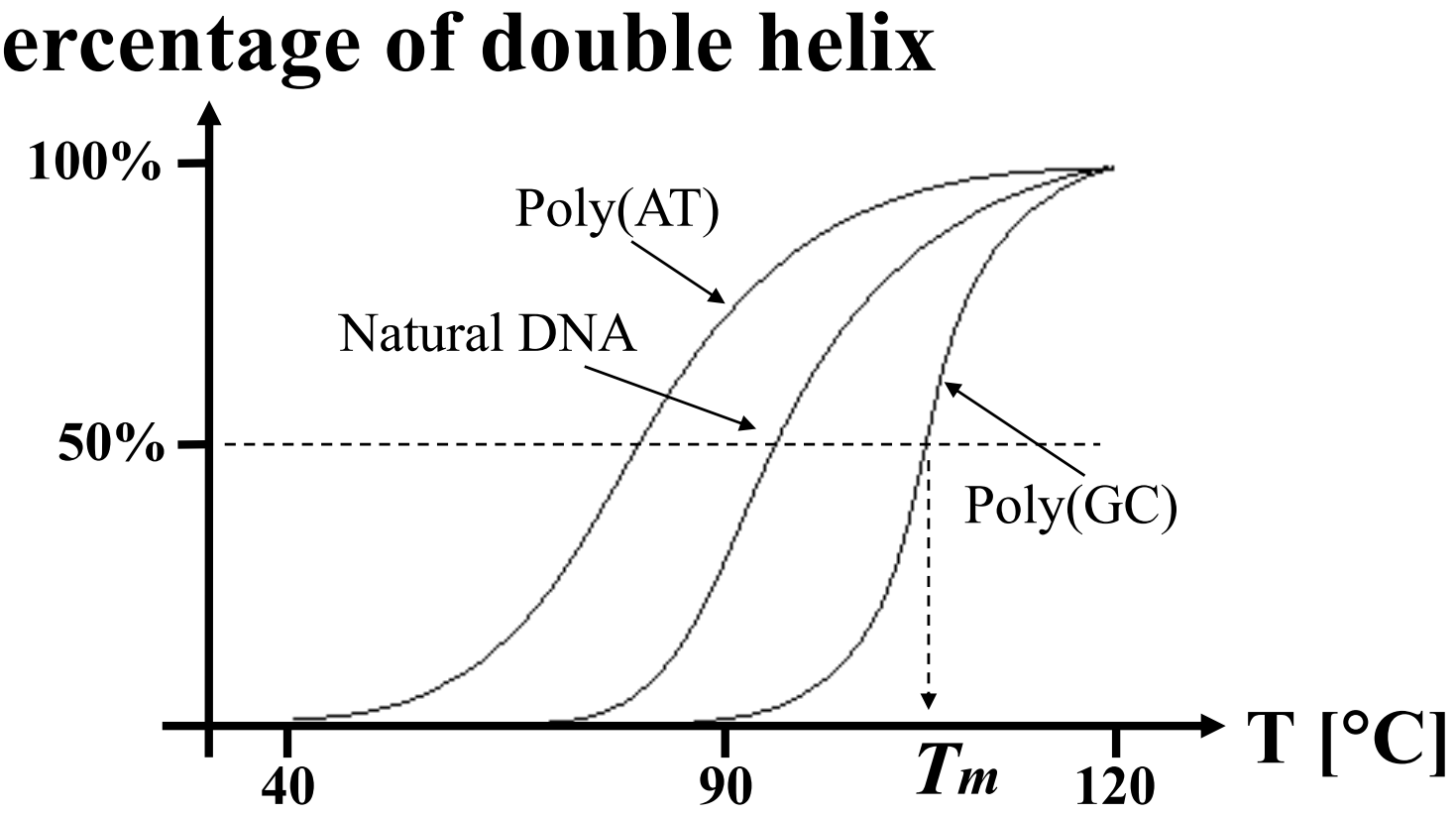
The variation in the entropy starts from the system status where all the nucleic acids are in double-helix conformation:

$$S_0 = R \ln(C_{DNA})$$

Thus, the melting temperature is:

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

Melting processes for different DNA sequences



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

Dependence by solution ions

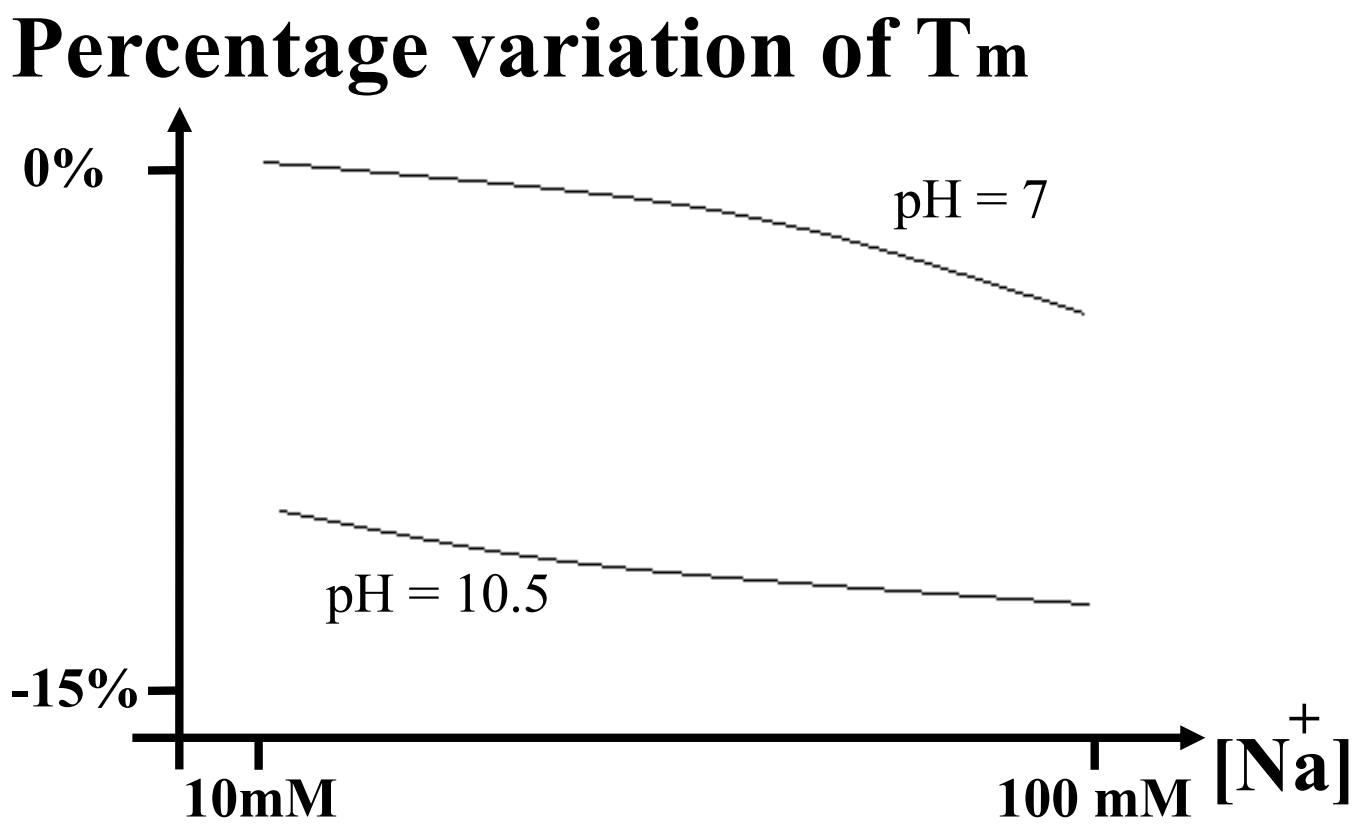
$$T_m = \frac{\Delta H}{\Delta S - R \ln C_{DNA}}$$

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

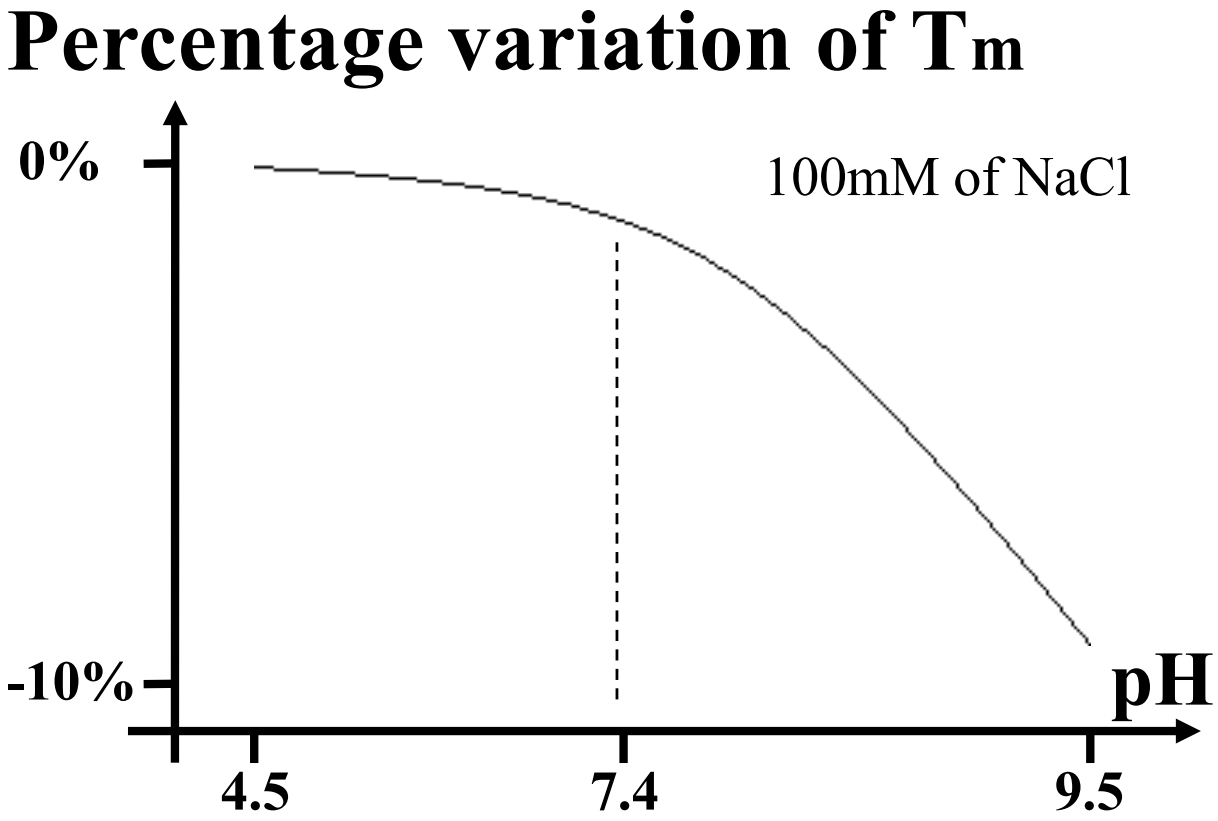
$$\begin{cases} \Delta G_{37^\circ}^{[Na^+]} = \Delta G_{37^\circ}^{[1\,Mole\ \ of\,Na^+]} + \alpha N \ln[Na^+] \\ \Delta S_{37^\circ}^{[Na^+]} = \Delta S_{37^\circ}^{[1\,Mole\ \ of\,Na^+]} + \beta N \ln[Na^+] \end{cases} \qquad \begin{cases} \alpha = -0.114 \\ \beta = +0.368 \end{cases}$$

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



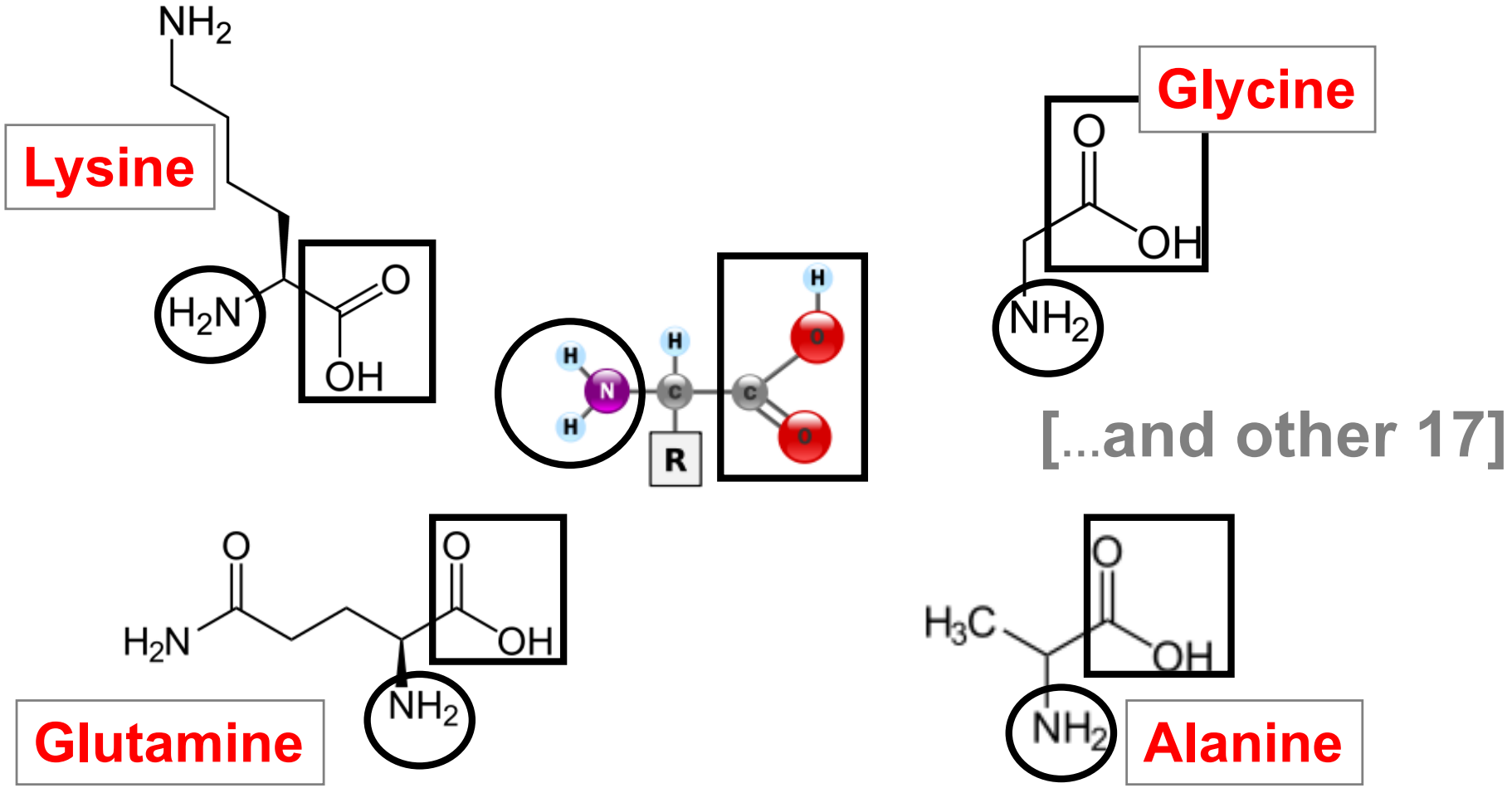
Melting temperature of DNA versus pH



Proteins

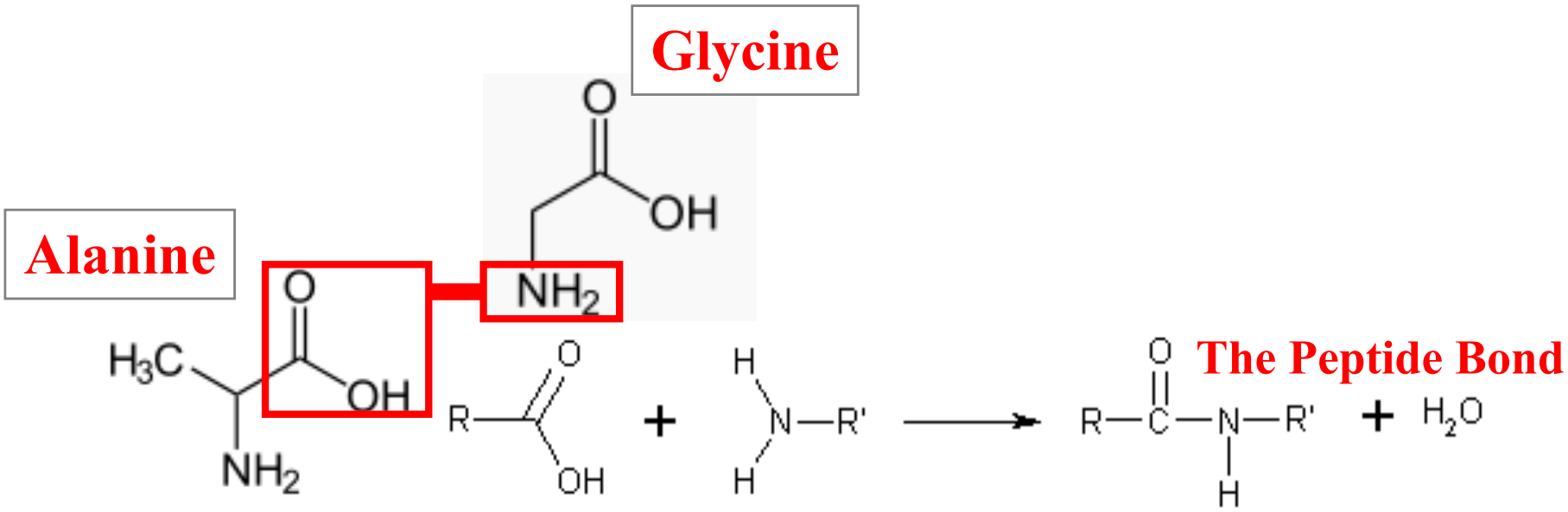
More complex bio-molecules are called Proteins, which are polypeptides, organic compounds made of **amino acids** arranged in a linear long chain and folded into a 3D usually complex form organized in beta-sheets, alpha-helices, and random-coils conformations

Amino Acids



Amino acids are molecules containing an amine group (NH2), a carboxylic acid group (COOH) and a side chain that varies between different amino acids

The Peptides



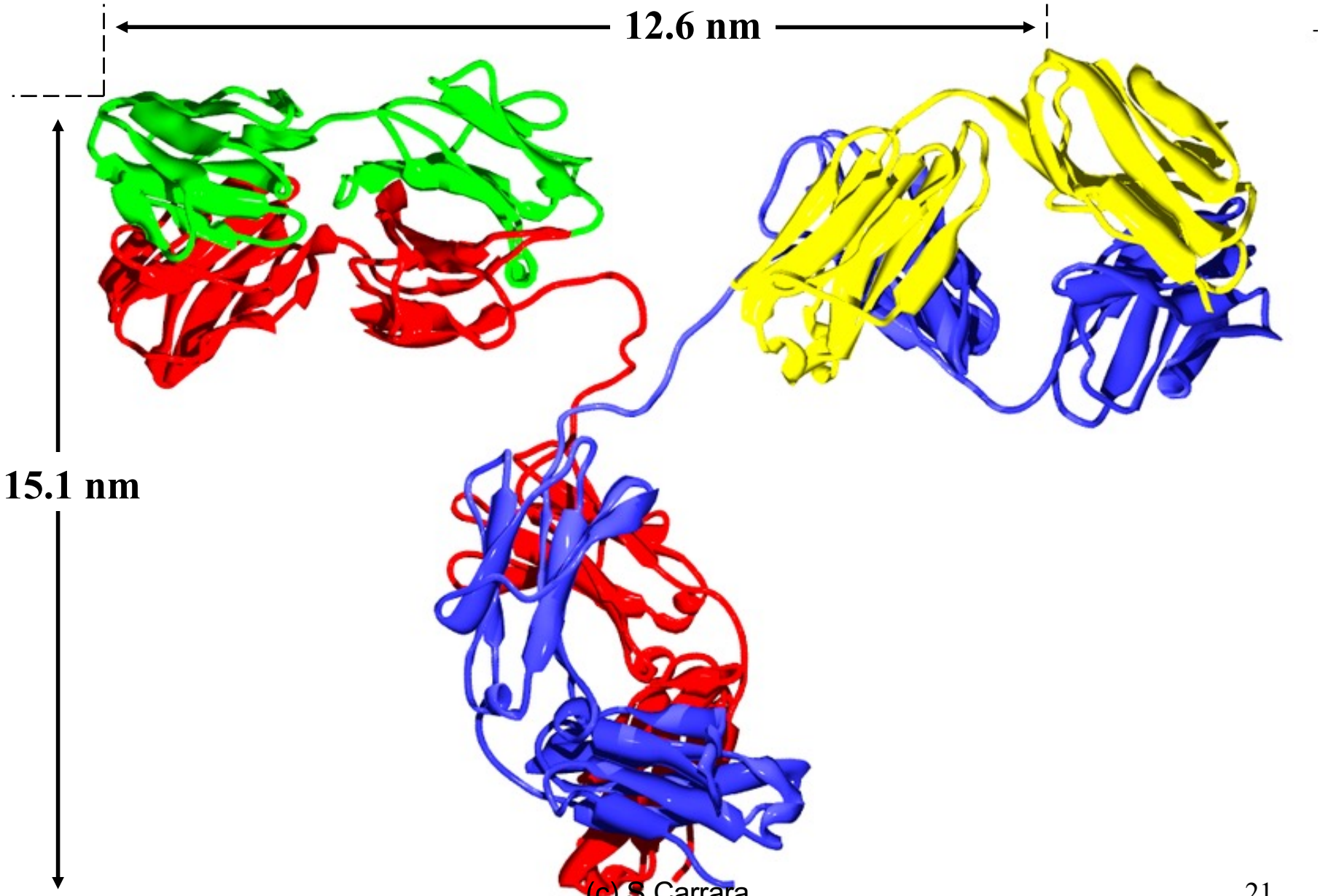
Peptides are short polymers formed by linking amino acids in a defined order

Antibody/Antigen interactions

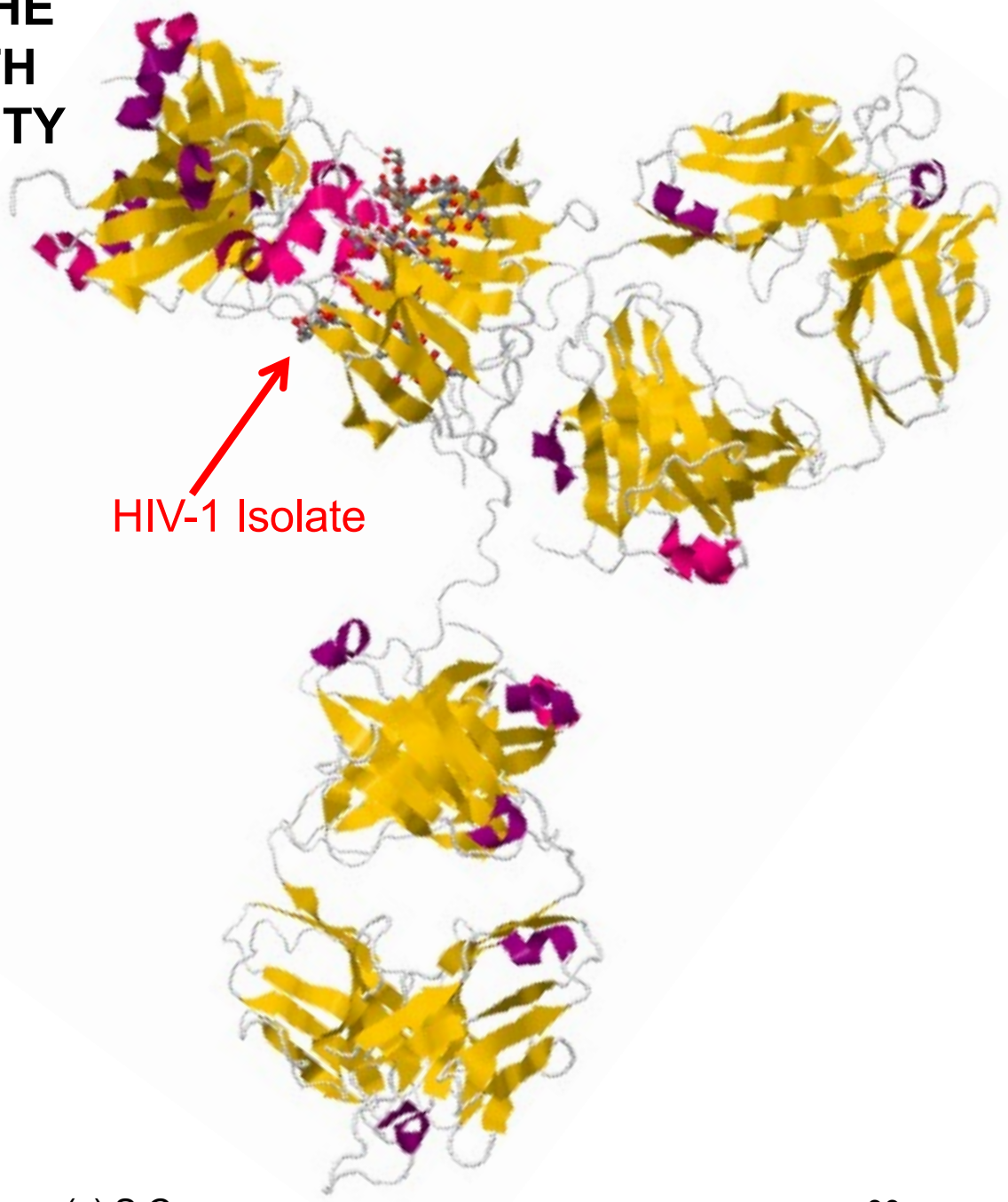
Antibodies are a large class of y-shaped **proteins** called immunoglobulins. An antibody shows a Y-structure with three main protein regions: a constant region and two variable regions. The variable regions are suitable for blocking the antigens. The protein blocks the antigen by closing the b-sheets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

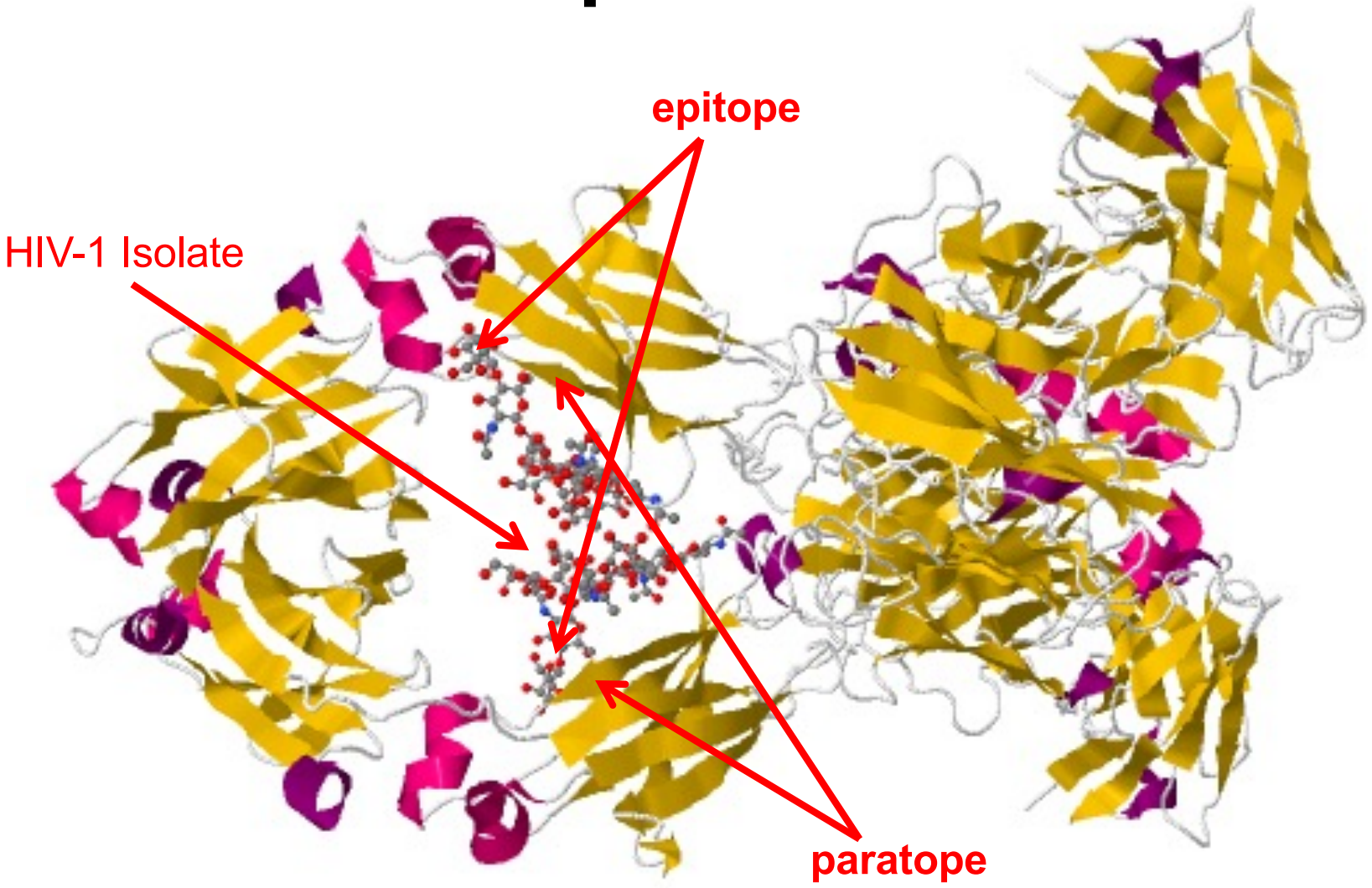
Antibody



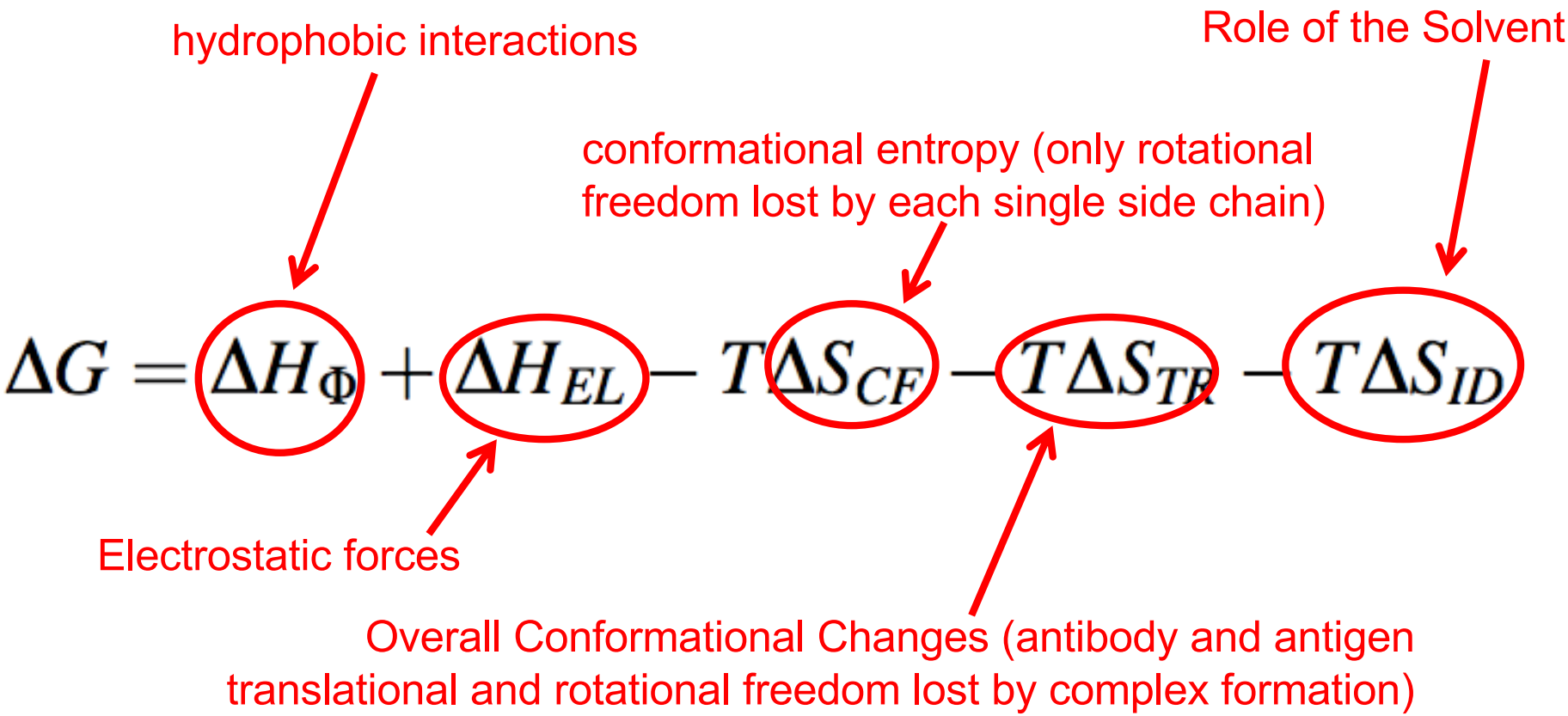
CRYSTAL STRUCTURE OF THE INTACT HUMAN IGG B12 WITH BROAD AND POTENT ACTIVITY AGAINST PRIMARY HIV-1 ISOLATES



Top View

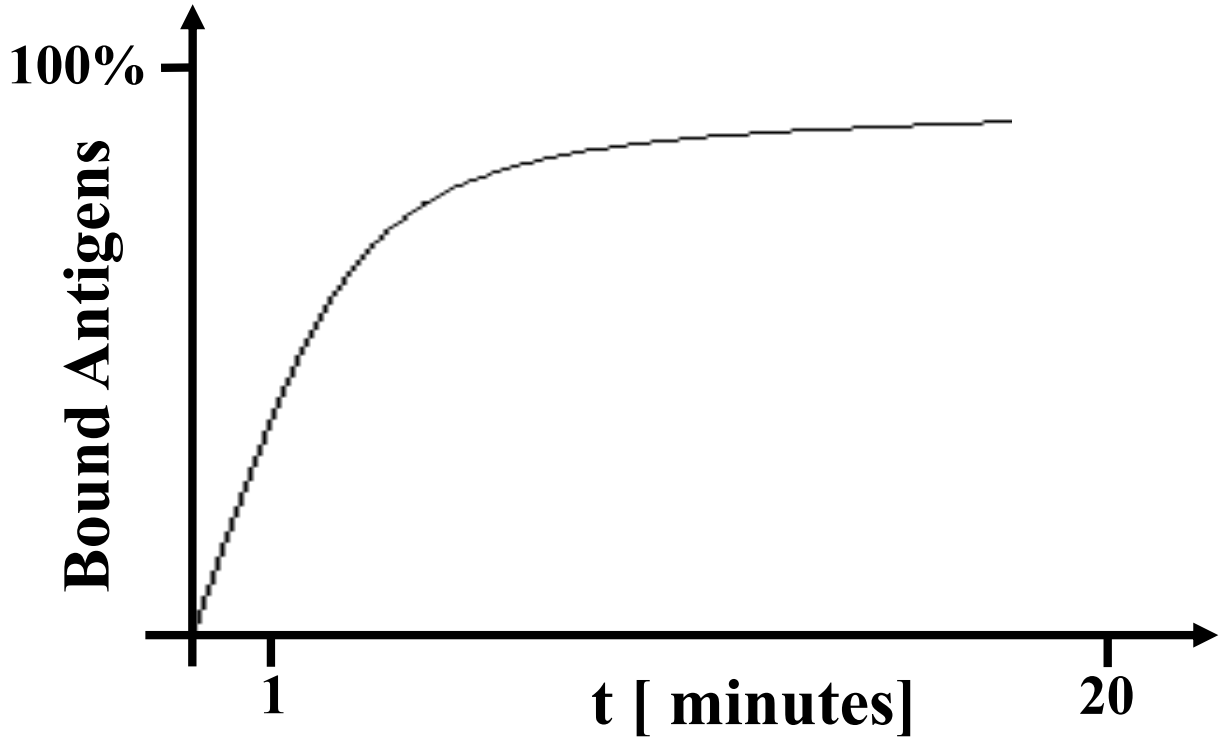


Gibbs free energy contributions



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

Typical trend of antigen binding versus time

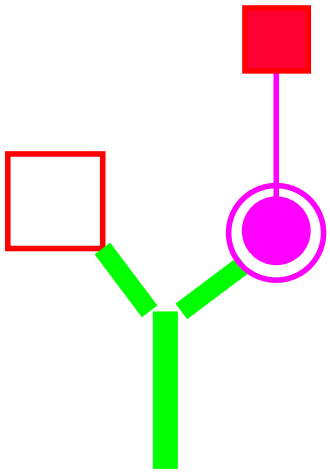


Different Kinds of Antibody

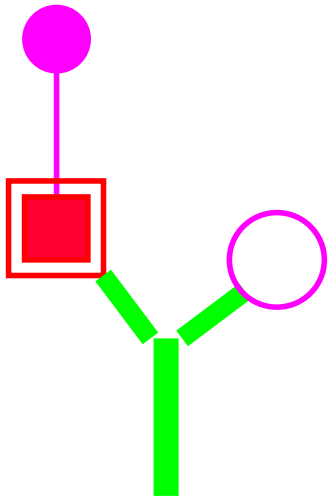
Dealing with real cases results in a bit more complex situation than just adding antigens to antibodies with a unique perfect match. Monoclonal antibodies are, then, all antibodies that have exactly the same specificity because they are from the same cloned single cell. However, antibodies are in general 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

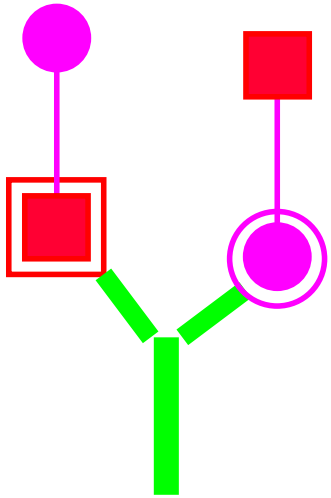
Different monomers with same antibody



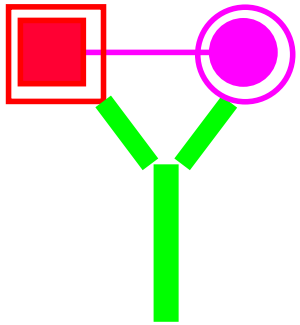
Simple Monomer



Simple Monomer

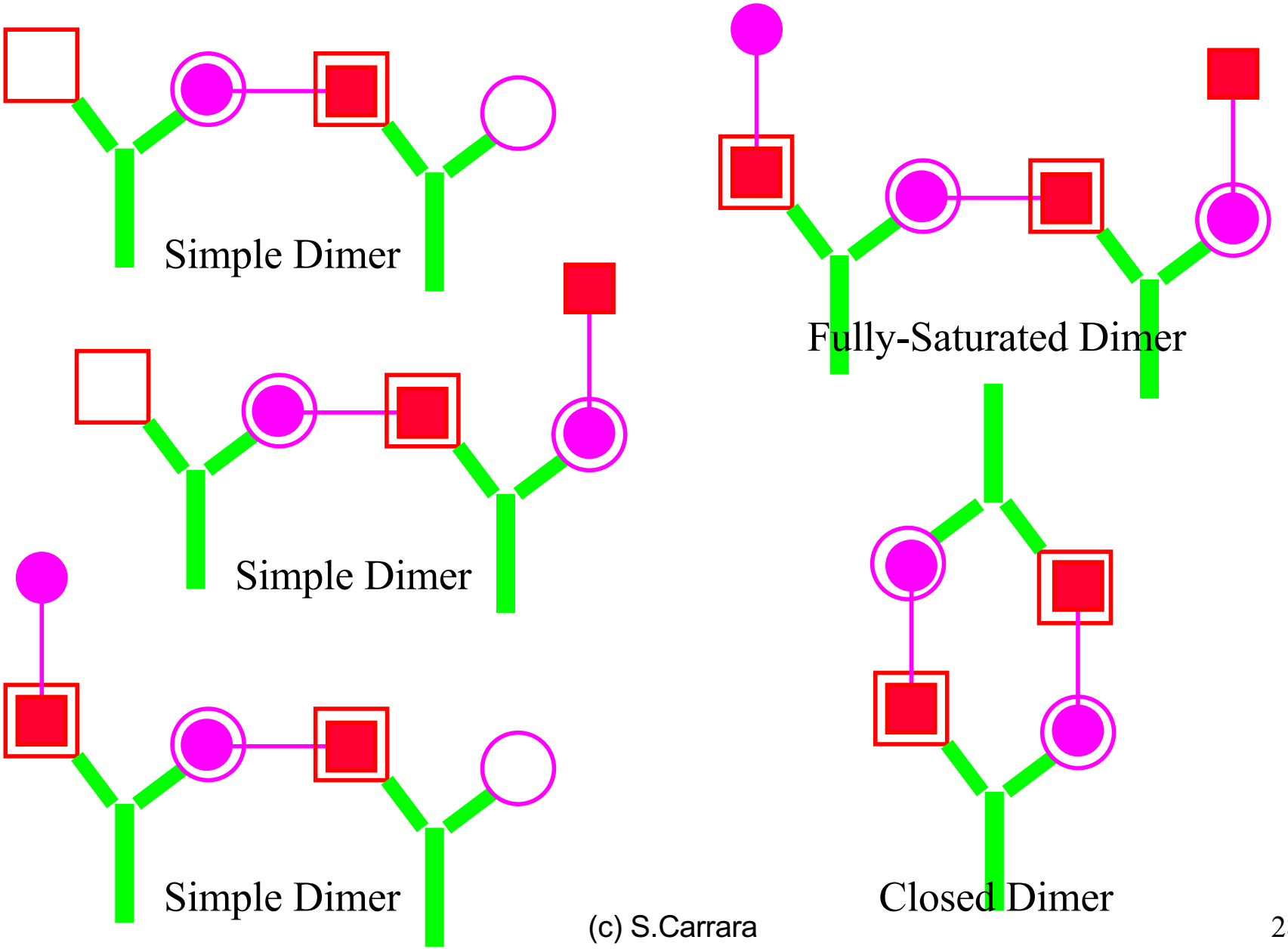


Fully-Saturated Monomer

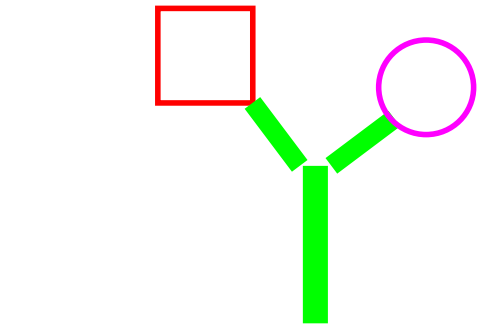


Closed Monomer

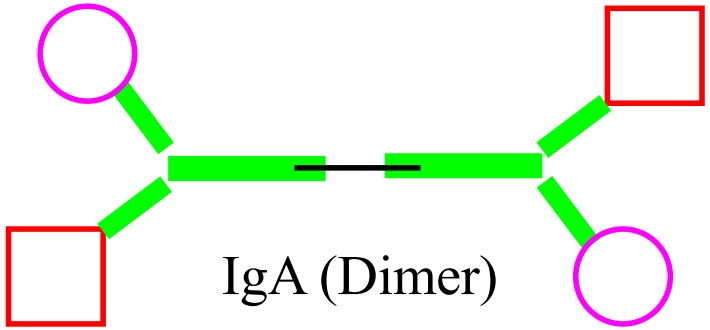
Different dimers with two antibodies



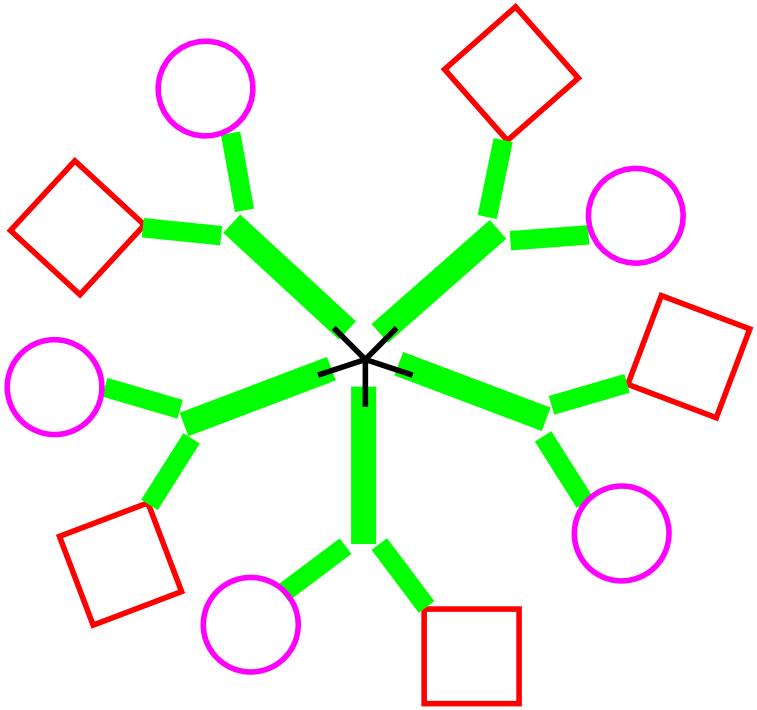
All possible classes of immunoglobulins



IgG, IgE, and IgD (Monomer)



IgA (Dimer)



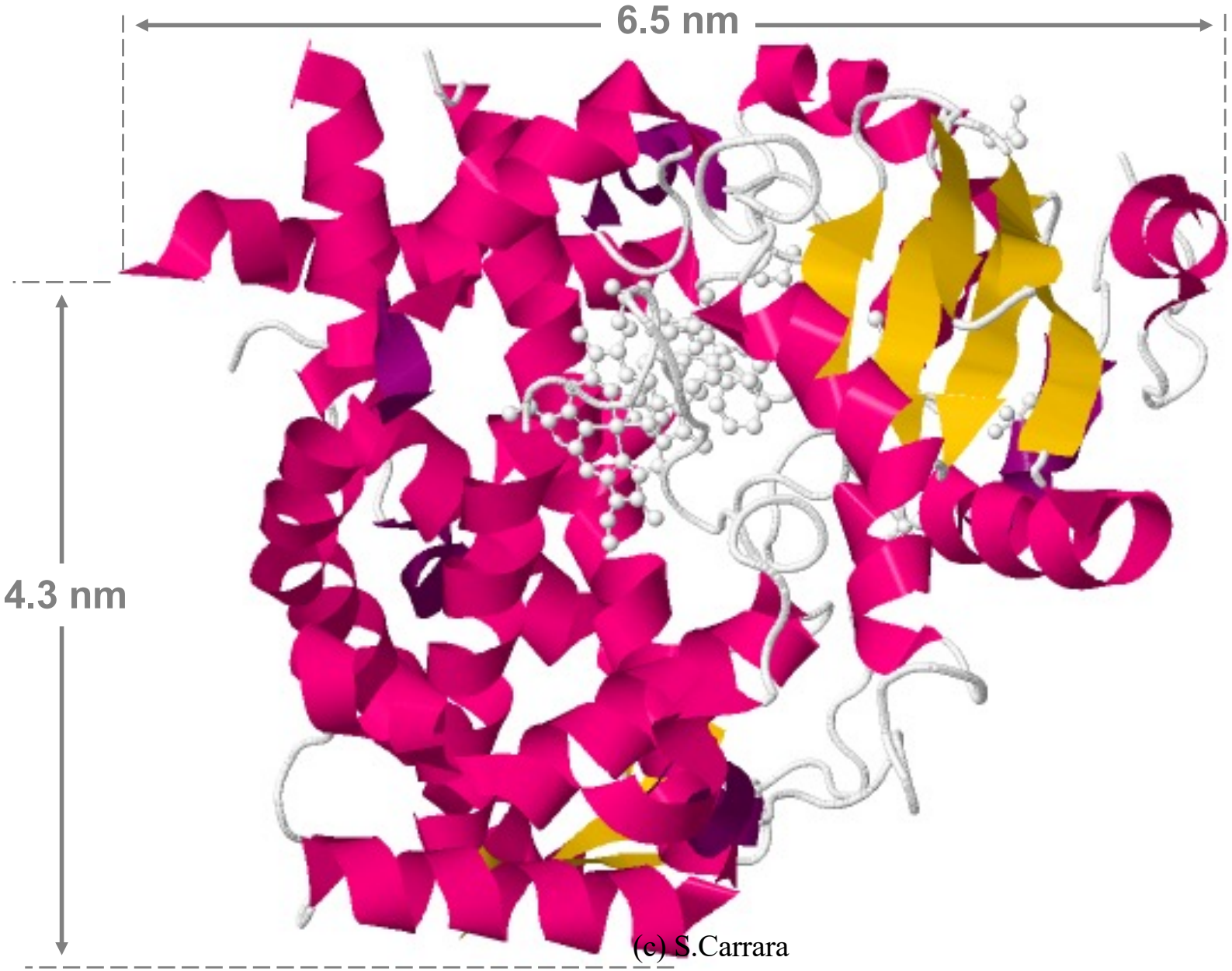
IgM (Pentamer)

Enzyme/Substrate interactions

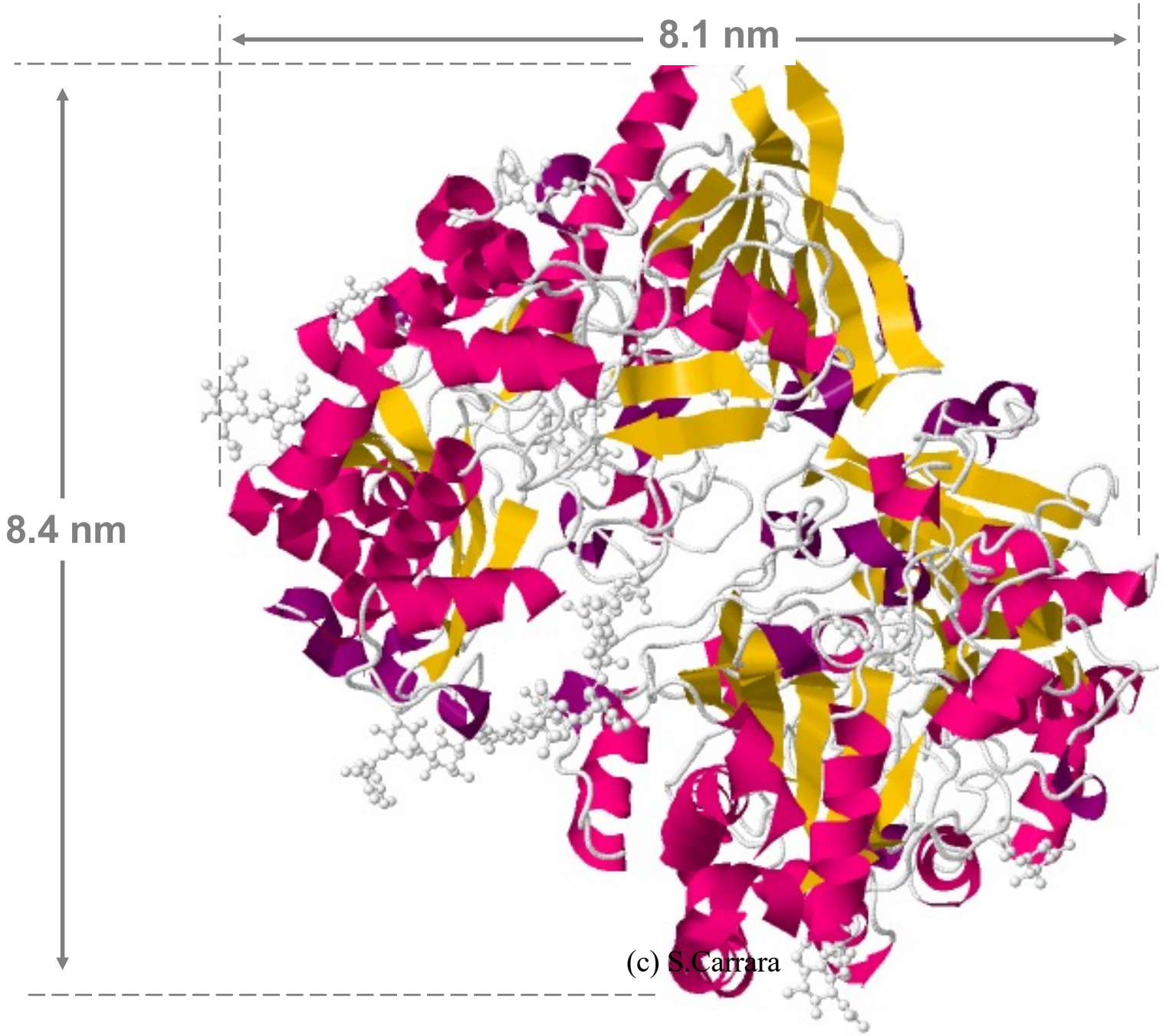
Enzymes are highly selective catalysts, accelerating both the rate and specificity of metabolic reactions. Almost all chemical reactions in a biological cell need enzymes in order to occur at a sufficient rates compatible with life. Most enzymes are proteins, although some catalytic RNA molecules have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

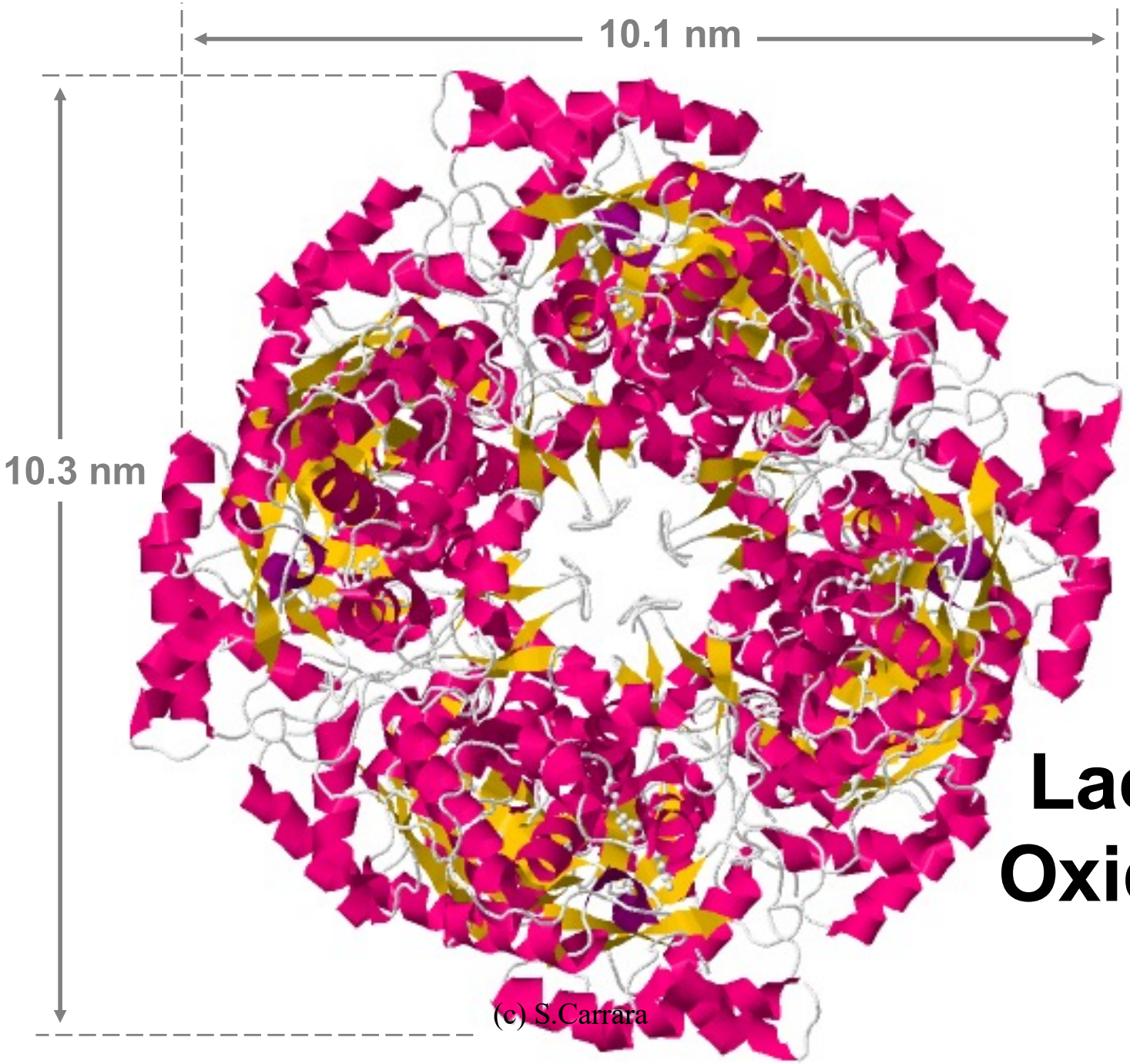
At the beginning of an enzymatic reactions, is called **substrates** the molecules which transformation is catalysed. This molecules are converted into different ones, called **products**.

Cytochromes P450



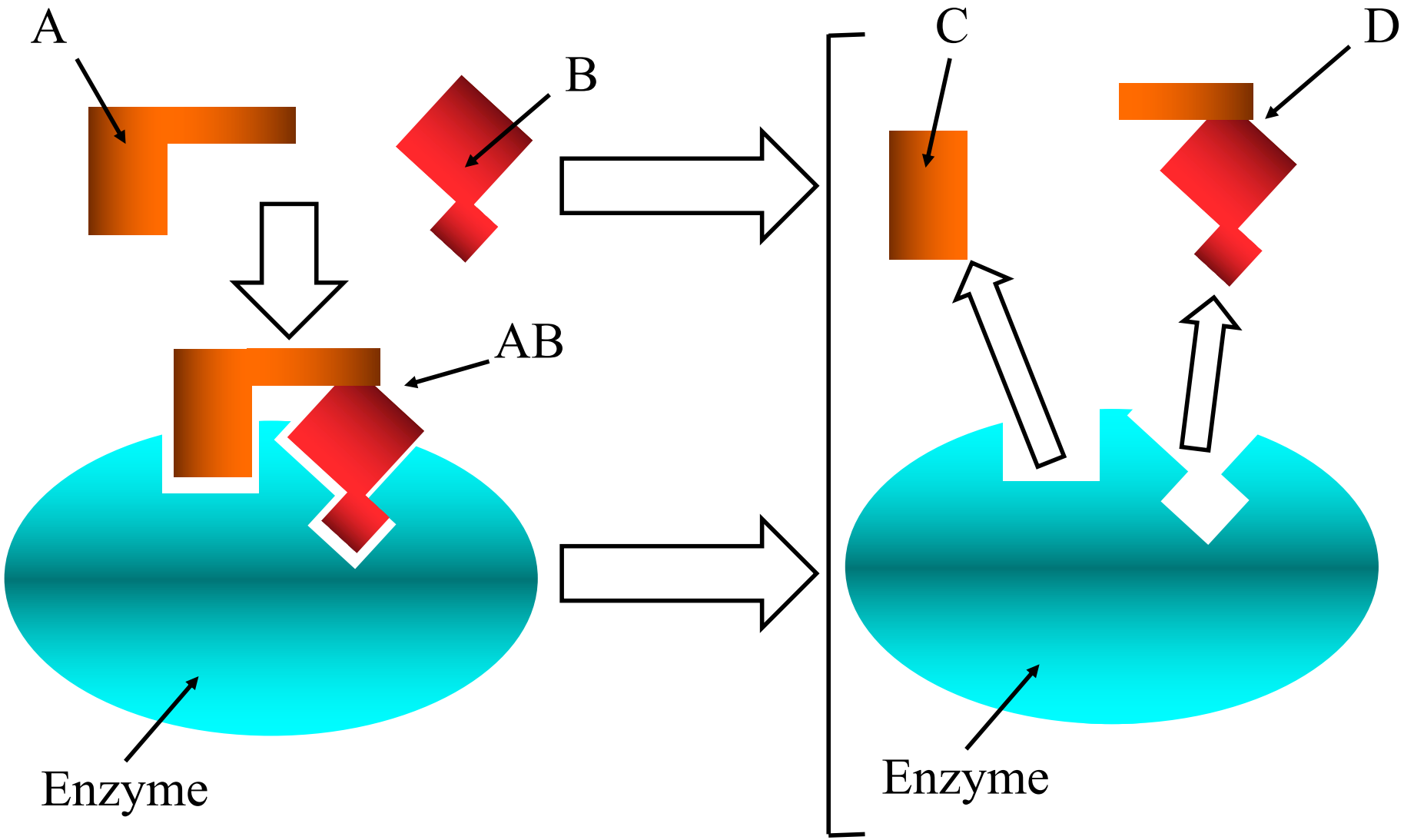
Glucose Oxidase





Lactate Oxidase

Mechanism of Enzymatic reactions



Enzymatic Process

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

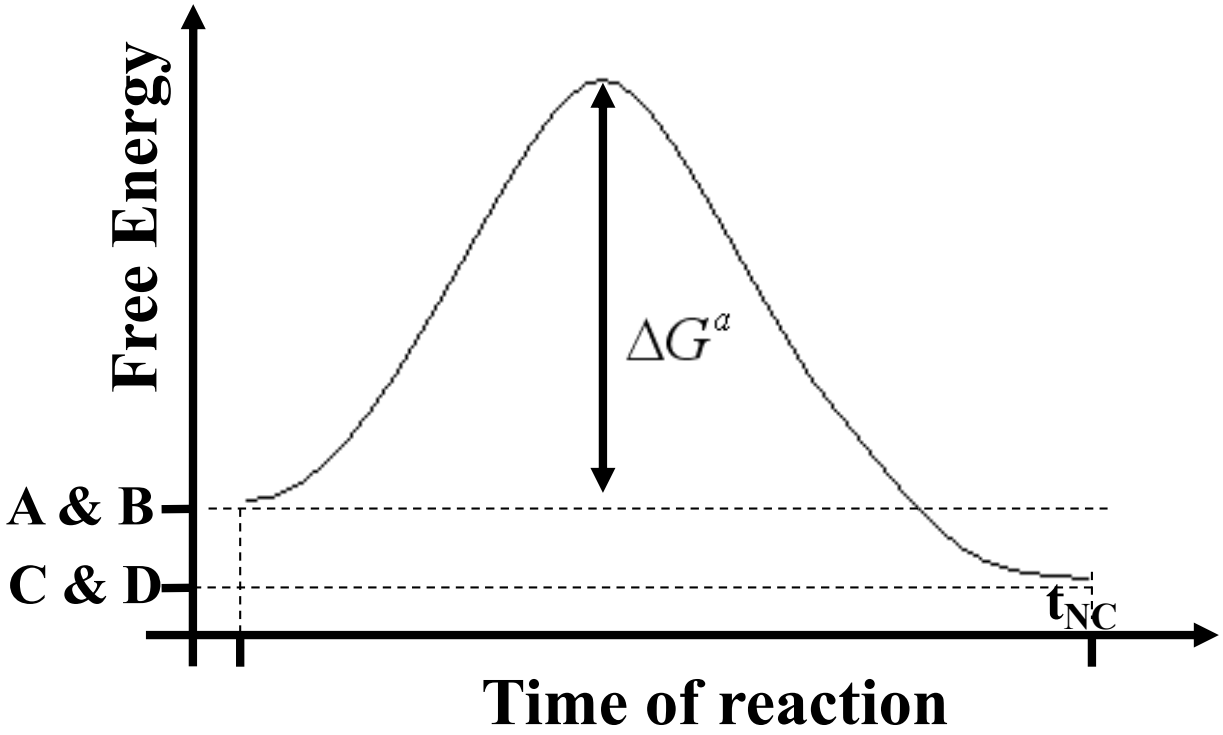
It is worth noting that the presence of an enzyme changes neither the equilibrium constant nor the two forward and backward rates

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!

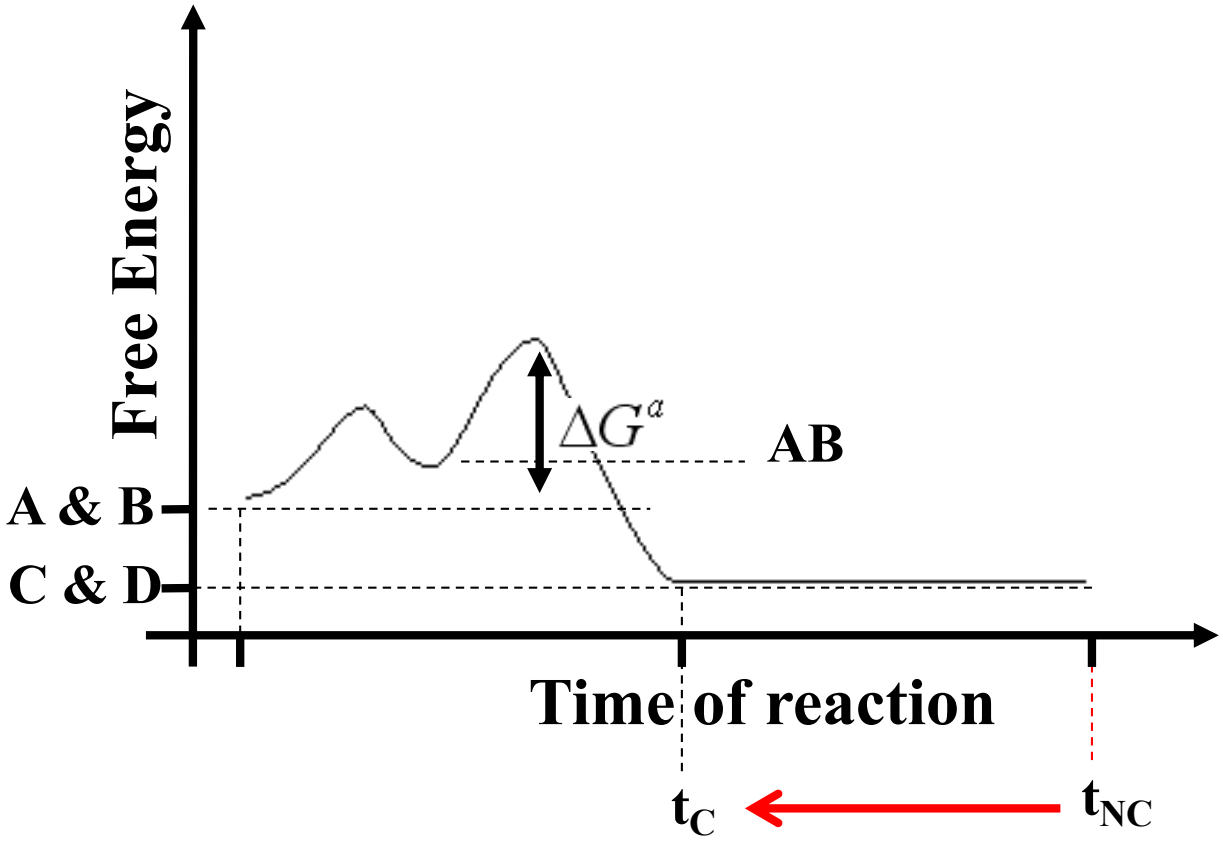
Gibbs free energy

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

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 (e.g., oxygen), and then the enzymatic process is re-written in a form that explicitly introduce the presence of the enzyme:

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

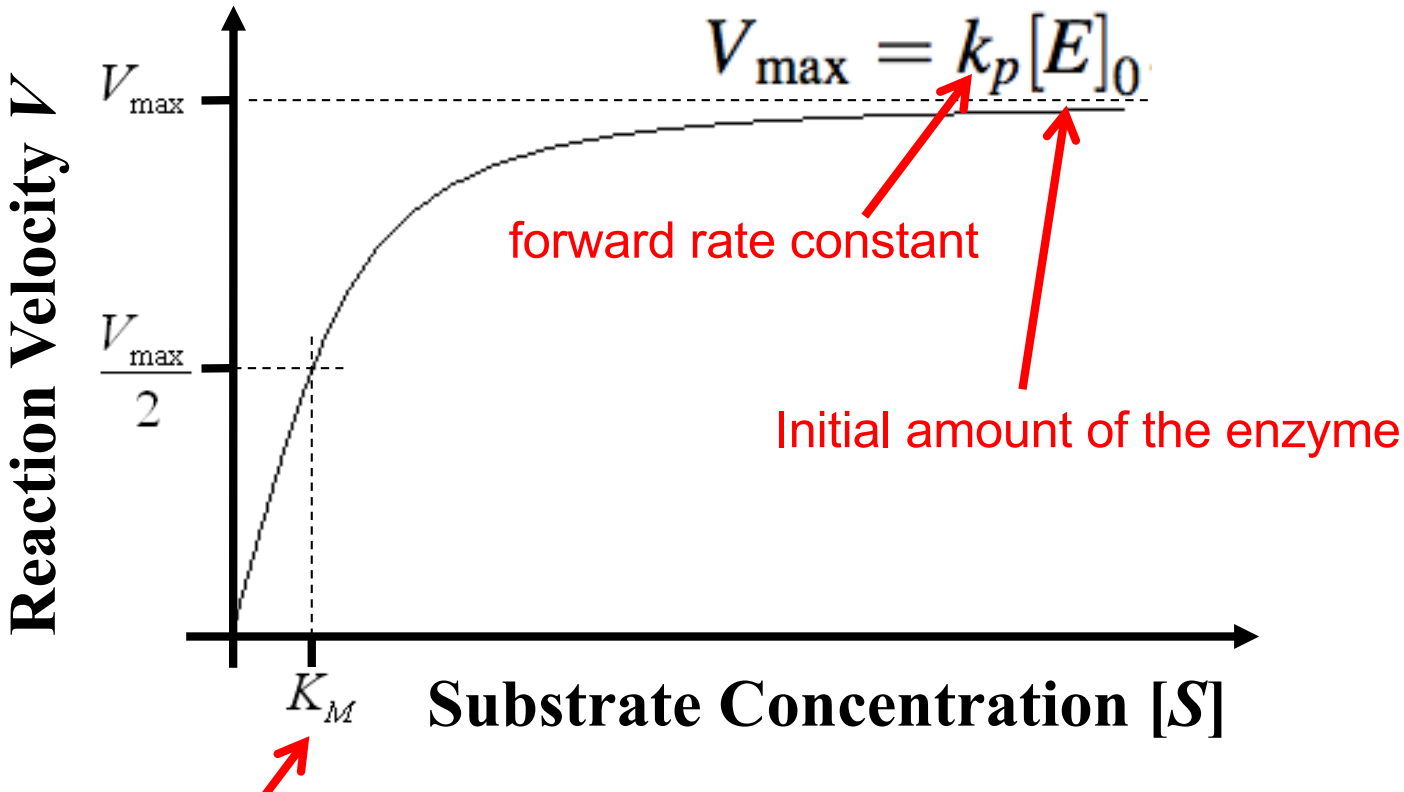
Now, by defining the reaction velocity V and the maximum reaction velocity V_m, respectively, as:

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

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

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

Michaelis-Menten Kinetics

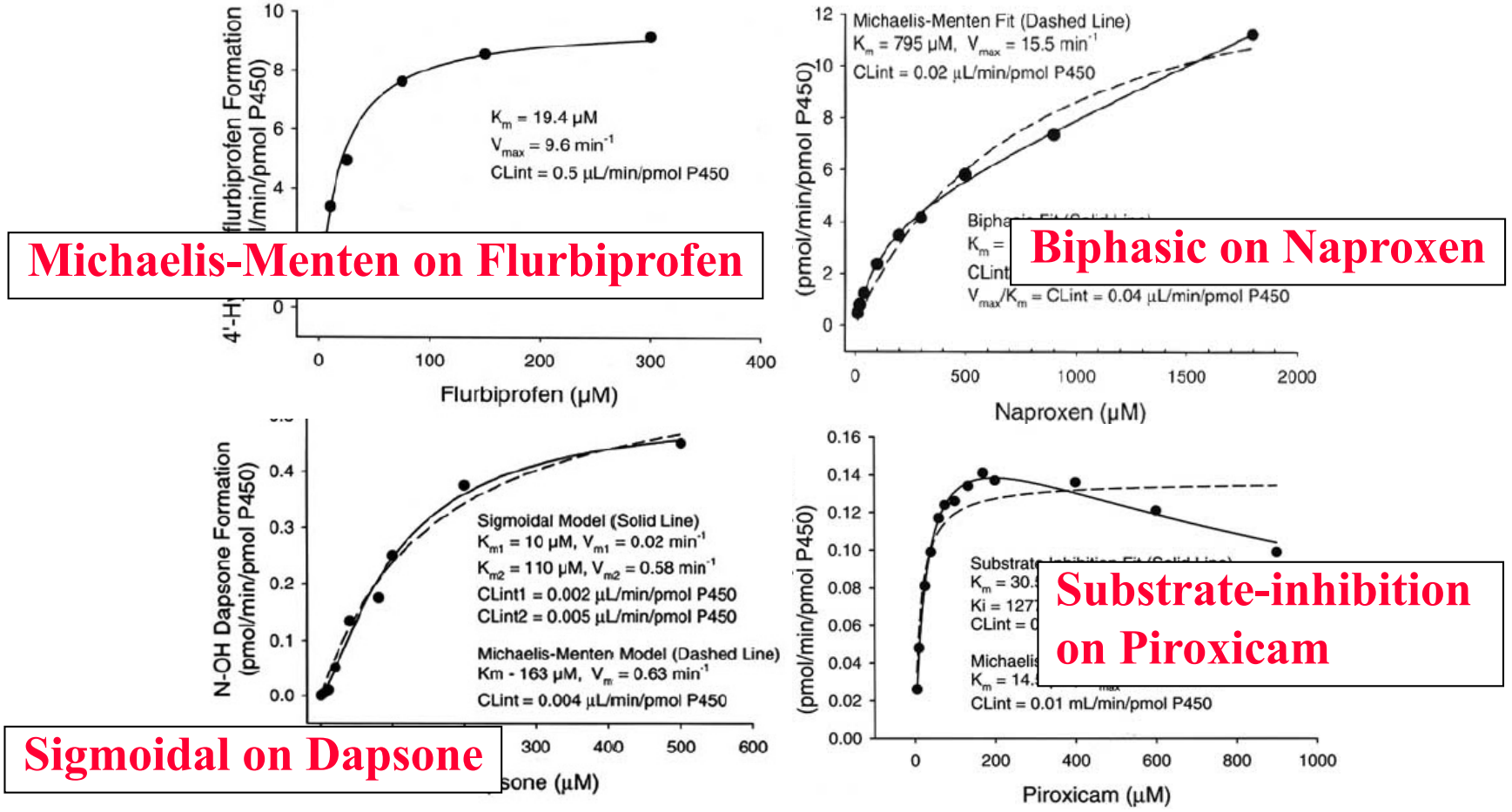


Depends only by the association and dissociation constants and by the forward rate

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

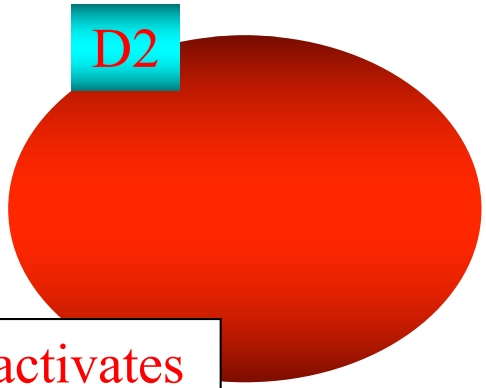


Timothy S. Tracy, Current Drug Metabolism, 2003, Vol. 4, No. 5

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

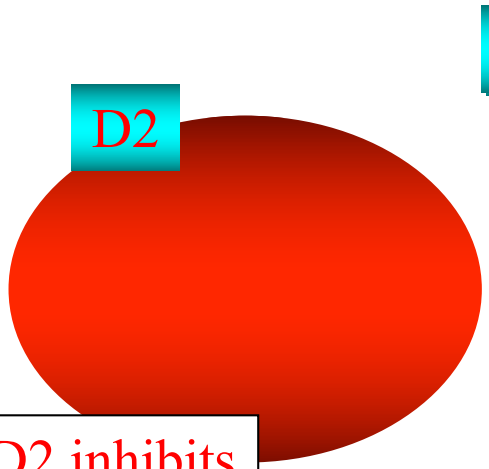
Phenomena of Heterotropic Kinetics

- **HETERO ACTIVATION**



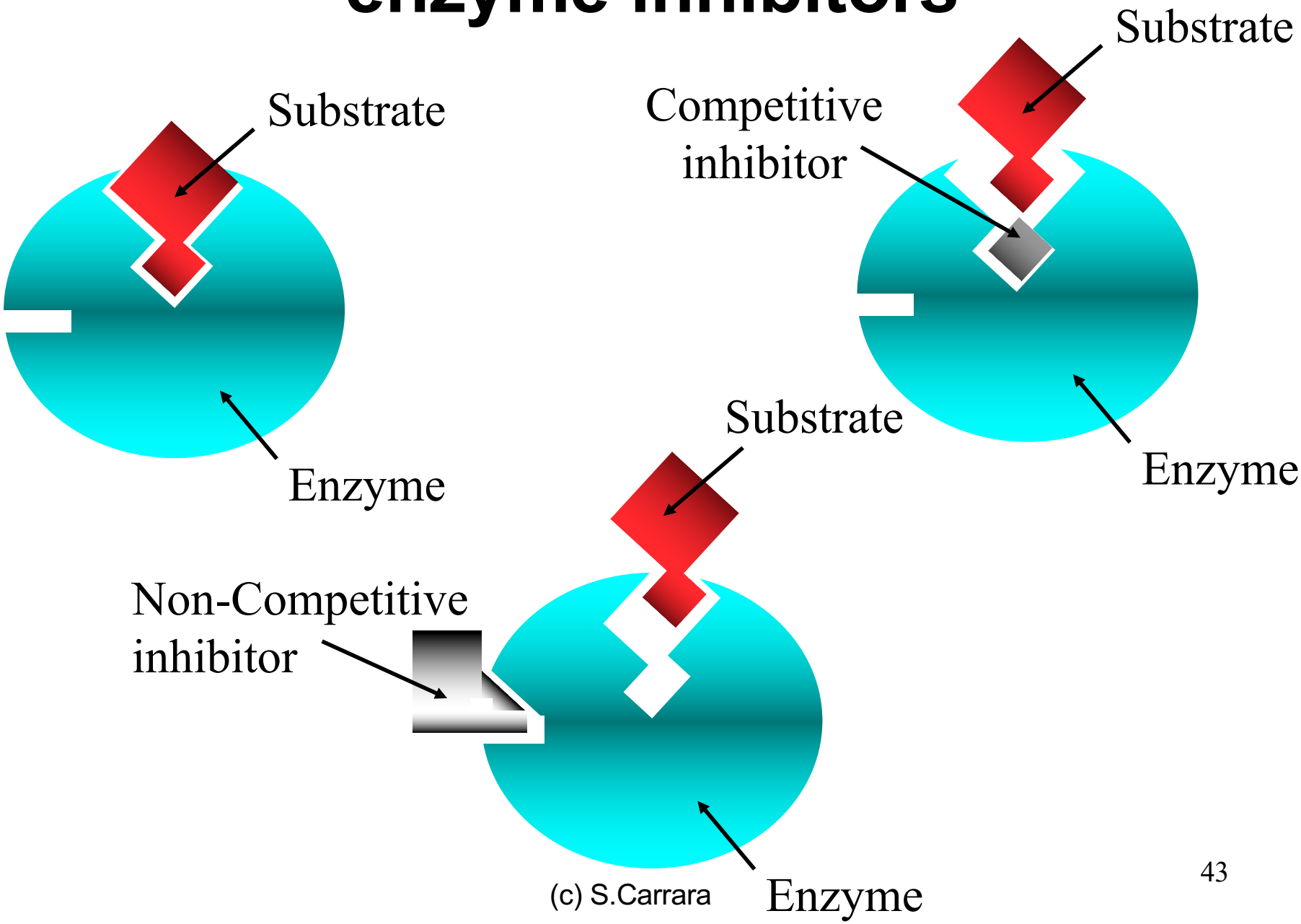
The presence of D2 activates
The catalysis on D1

- **PARTIAL INHIBITION**

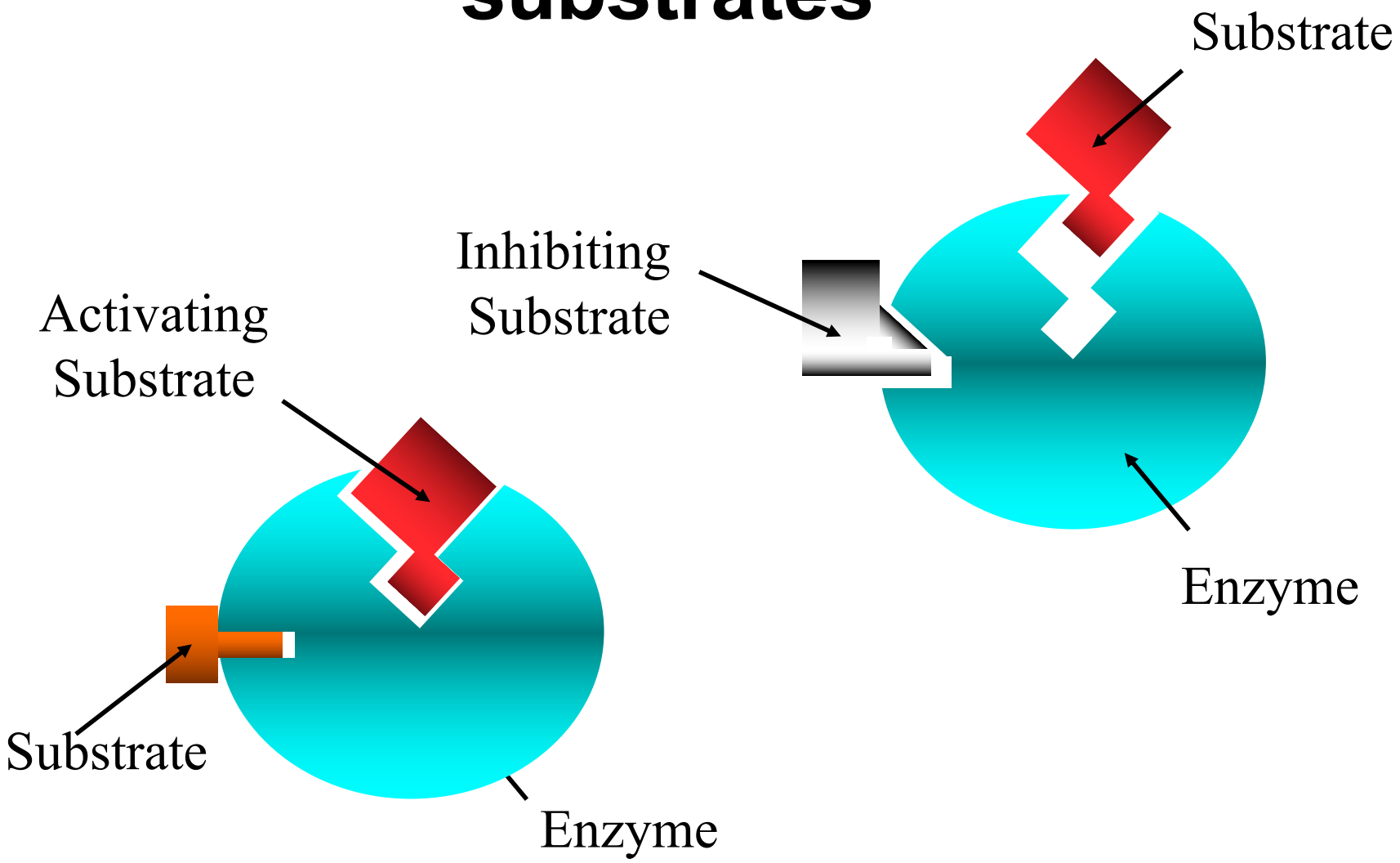


The presence of D2 inhibits
The catalysis on D1

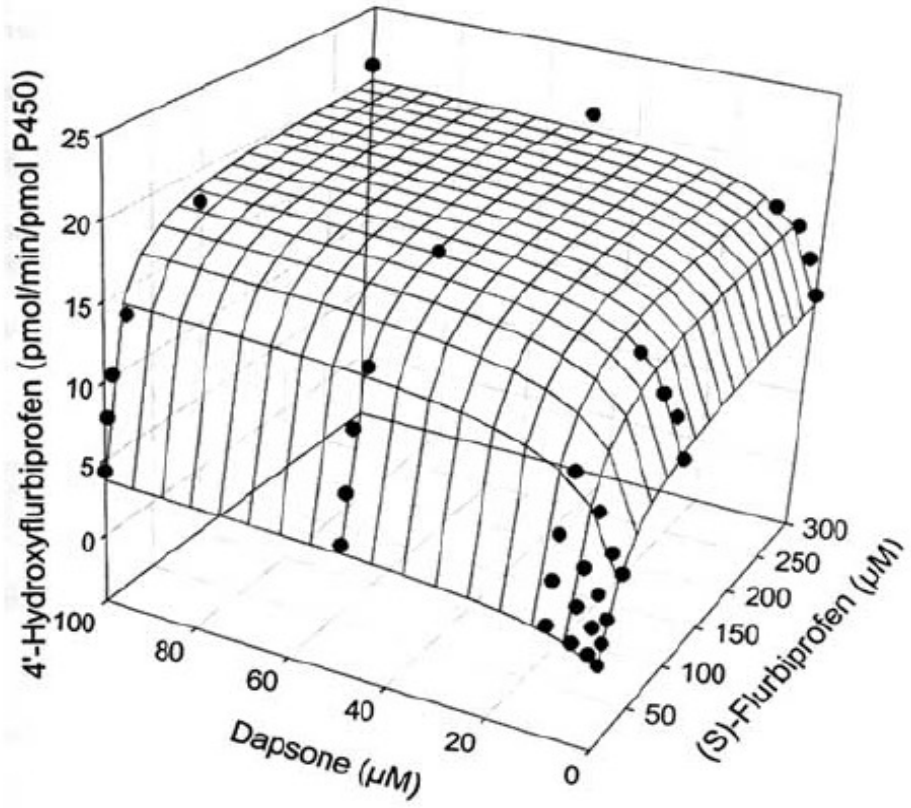
Competitive and noncompetitive enzyme inhibitors



Activating and inhibiting enzyme substrates



The case of Cytochrome P450 2C9



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

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

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