EPFL

Master in Electrical and Electronics Engineering

EE-517: Bio-Nano-Chip Design

Lecture #3

Probe/Target interactions

Lecture Outline

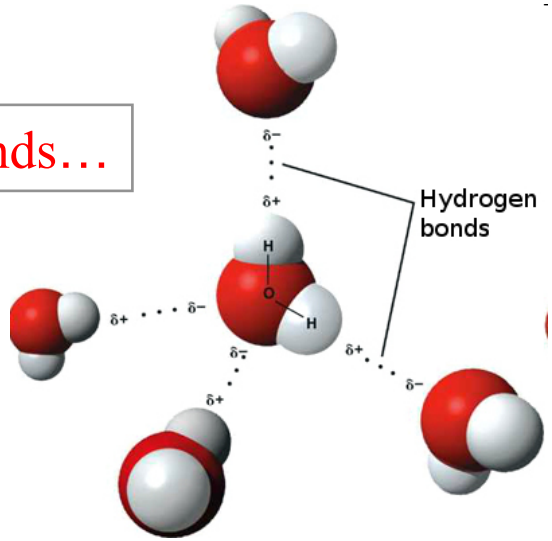
(Book Bio/CMOS: Chapter' paragraphs §4.4-16)

- DNA/RNA pairing & Bond energy
- Antibody/Antigen affinity & Bond energy
- Enzymes & Michaelis-Menten Kinetics

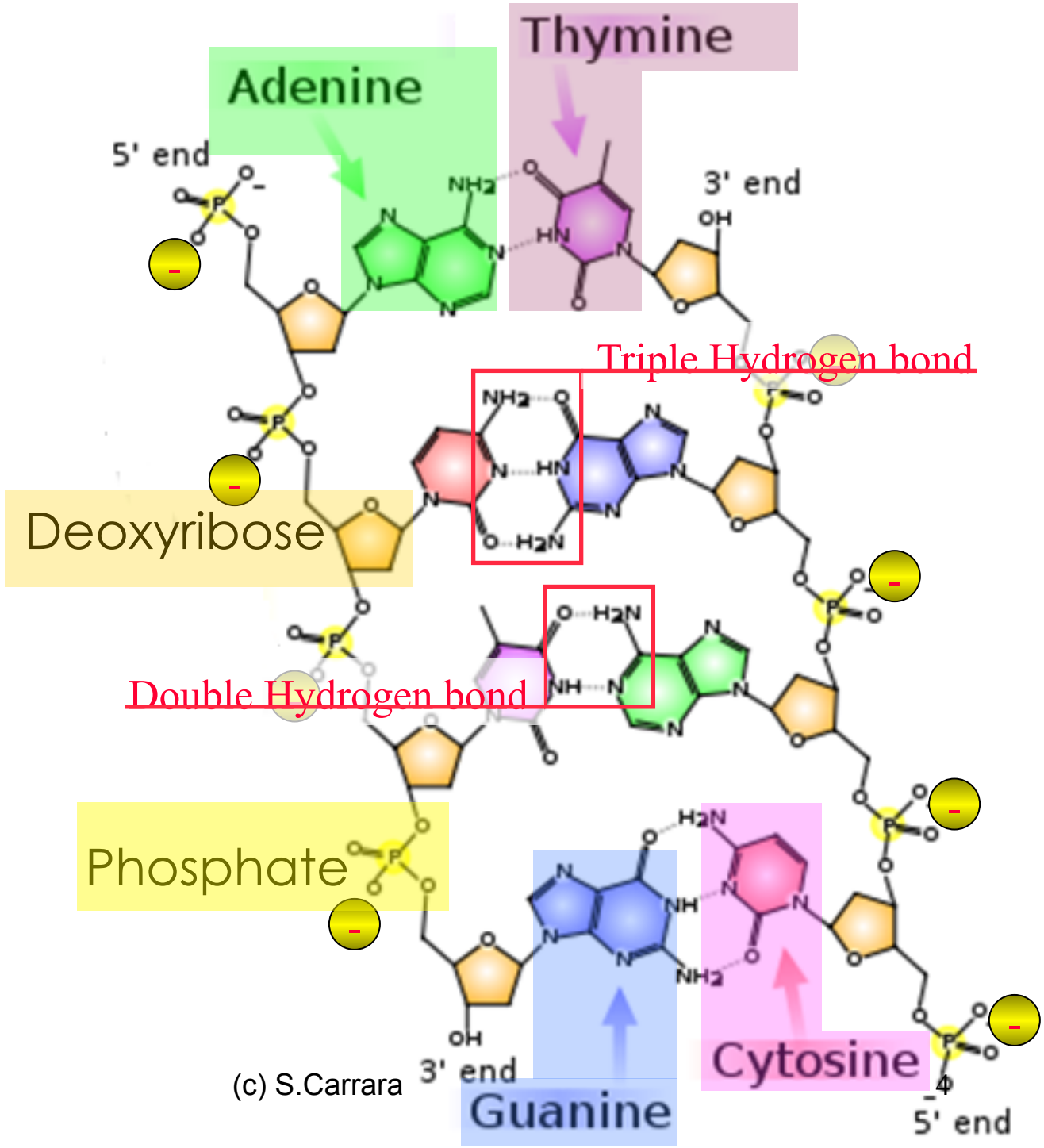
DNA Base pairing

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**.

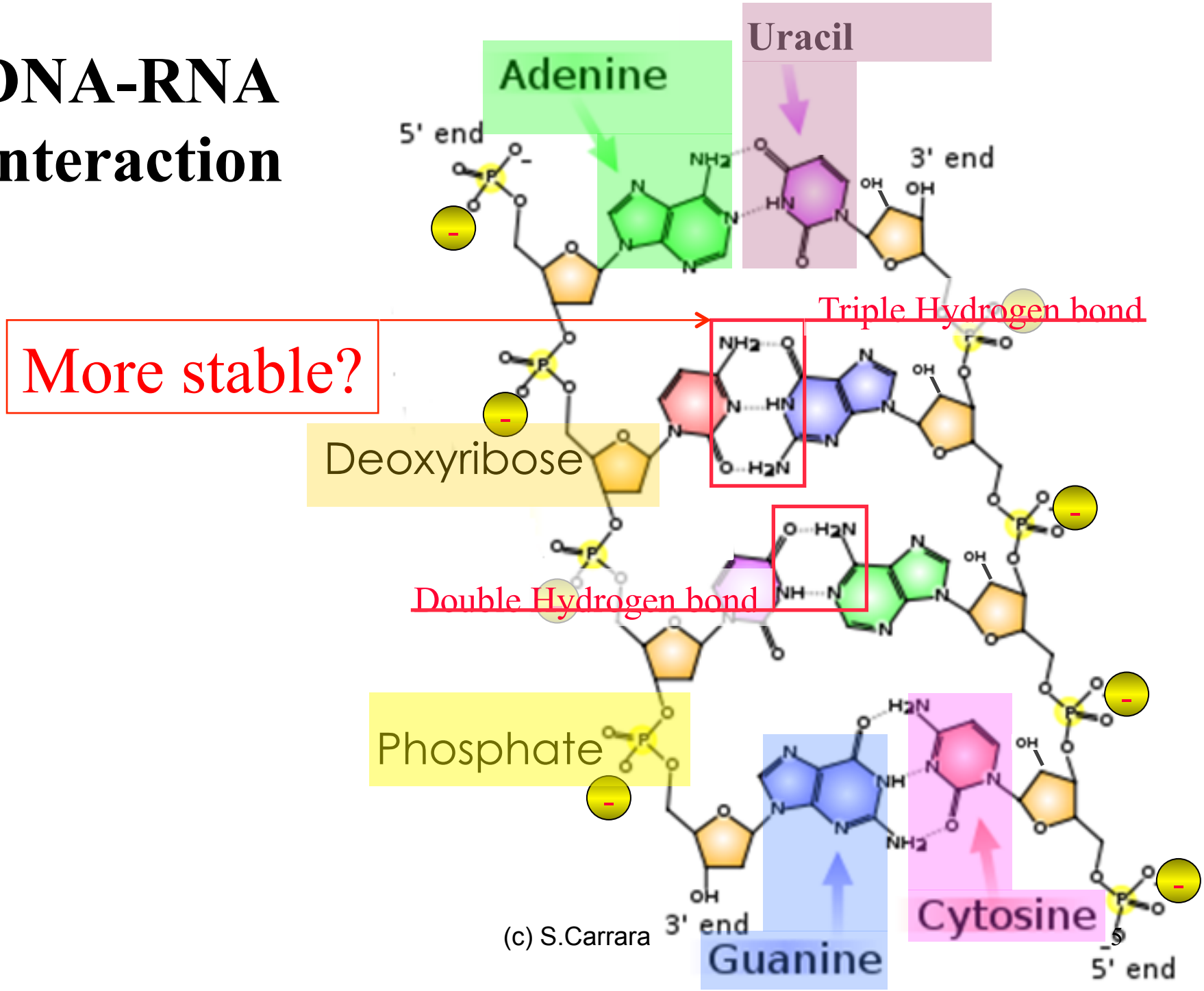
Thanks to Hydrogen Bonds…



DNA-DNA Interaction



DNA-RNA Interaction



DNA/RNA pairing

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.

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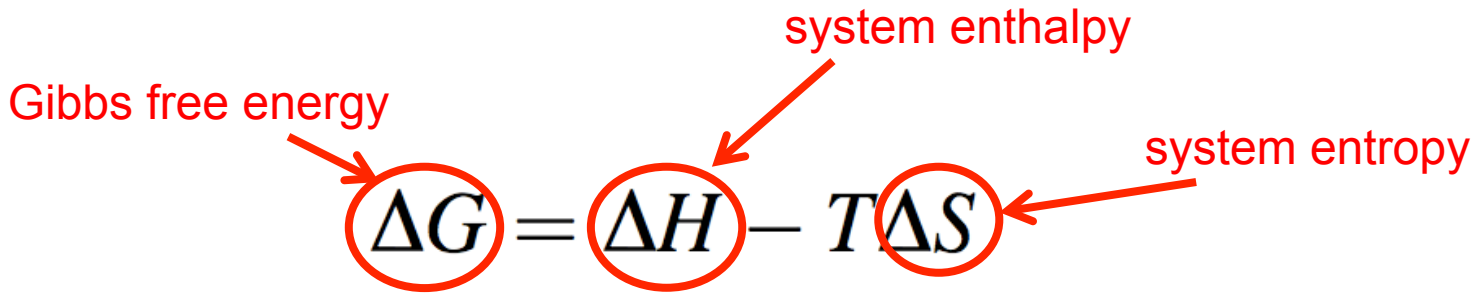
Gibbs free energy



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

Gibbs free energy

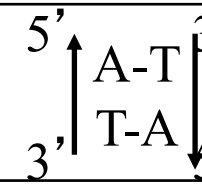
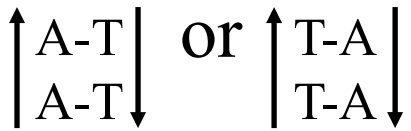
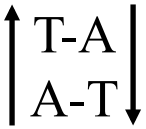
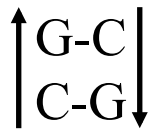
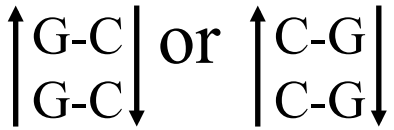
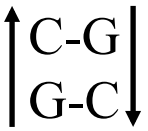
The **Gibb free energy** is the upper limit (some times called thermodynamic potential) of the total work obtainable by the system in processes at constant temperature and pressure

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

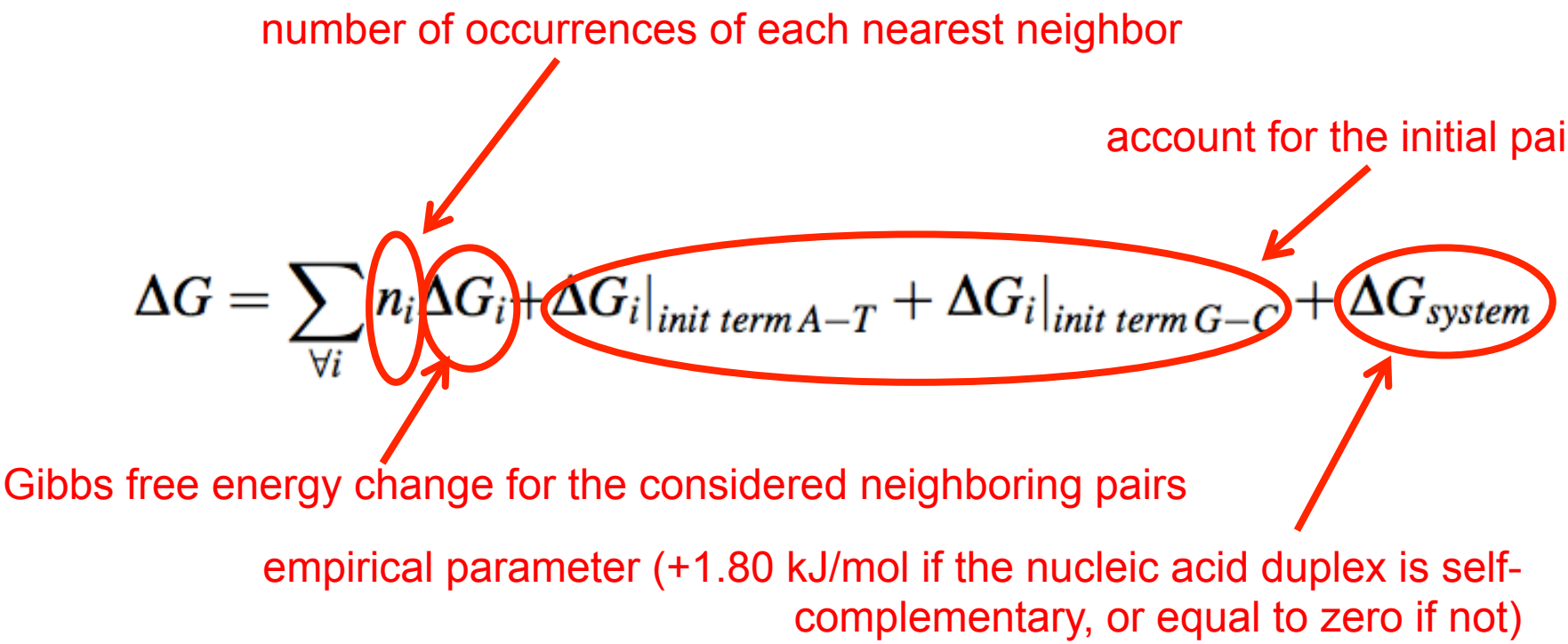
The **first law of thermodynamics** is the law of the energy conservation for the thermodynamic systems

$$\Delta U = Q - W.$$

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

The Nearest-Neighbor Model

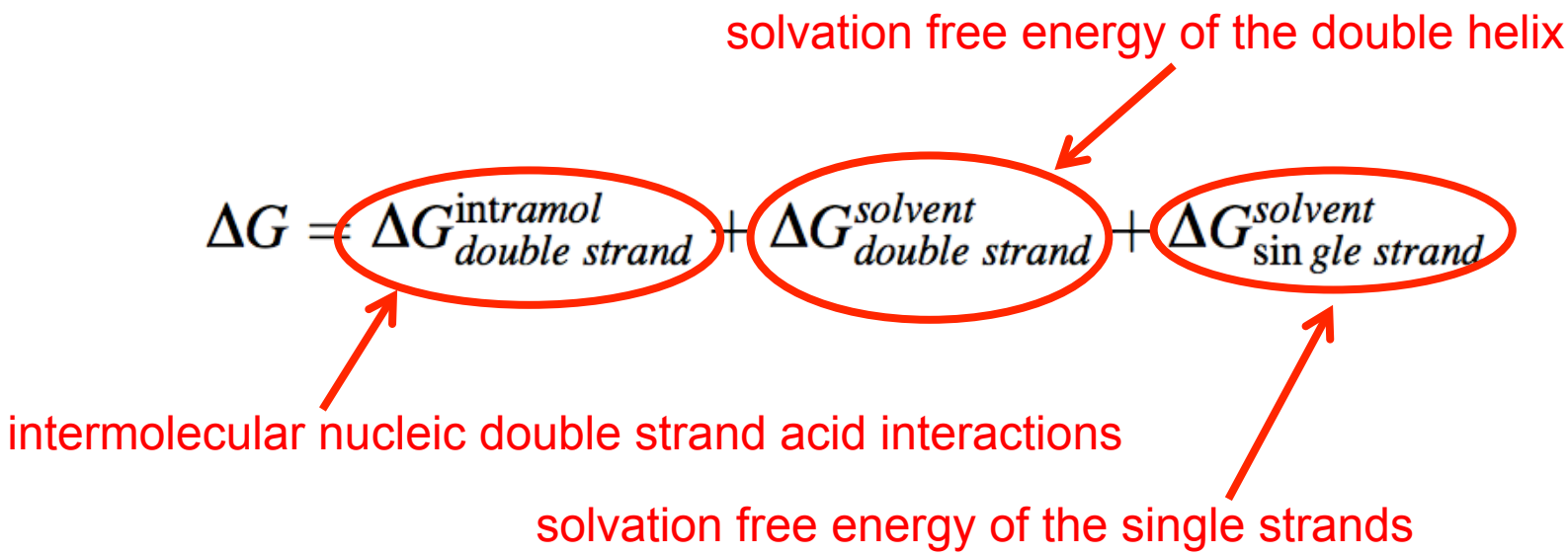


It introduces a computation for the total Gibbs free energy that takes into account the interactions from neighbours

Gibbs free energy

duplex	*Experimental* ΔG [kJ/mol]
GGTTATTGG CCAATAACC	-26.8
GGTTCTTGG CCAAGAACC	-31.4
GGTTTTTGG CCAAAAACC	-29.5
GGTTATTGG CCAAAAACC	-12.0
GGTTCTTGG CCAATAACC	-12.4
GGTTTTTGG CCAAGAACC	-17.5

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. 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:

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

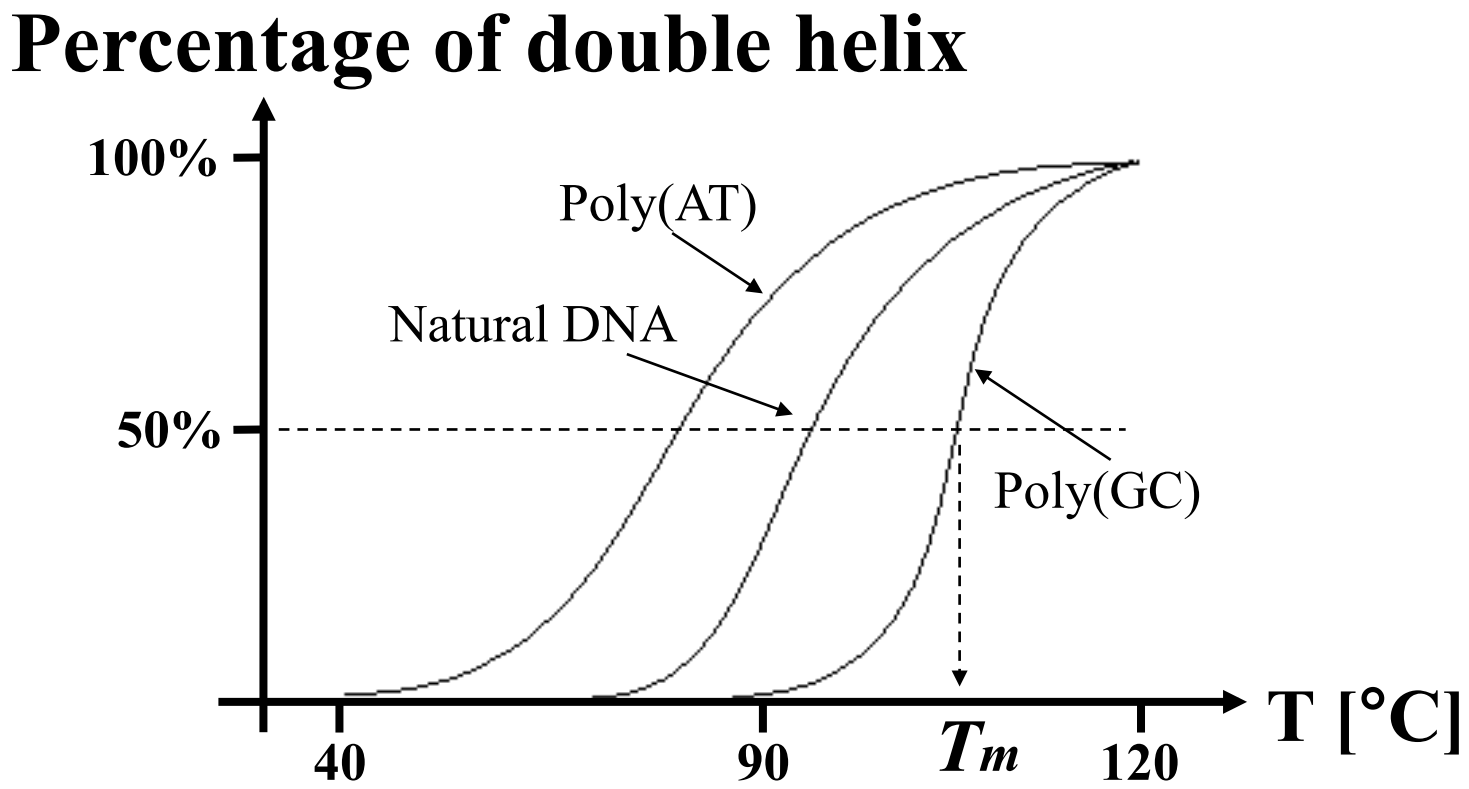
The variation in the entropy starts from the system status where all the nucleic acids are in double-helix conformation:

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

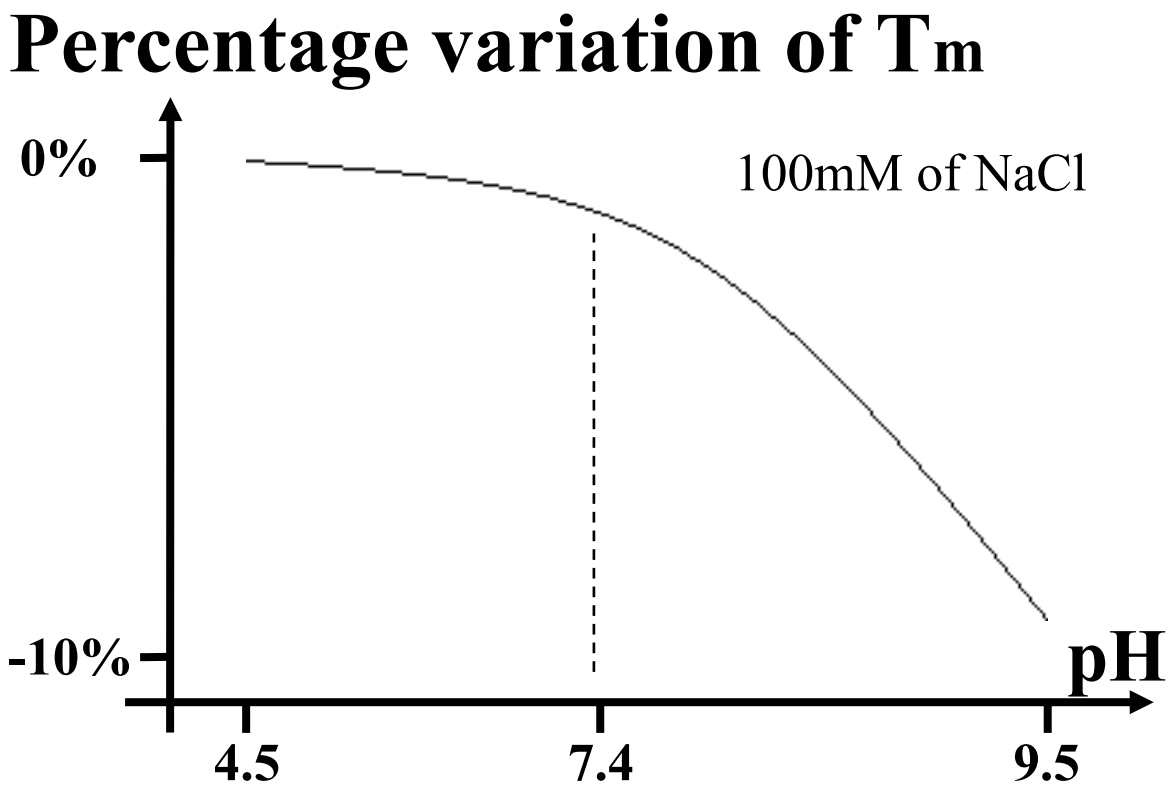
Thus, the melting temperature is:

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

Melting processes for different DNA sequences



Melting temperature of DNA versus pH

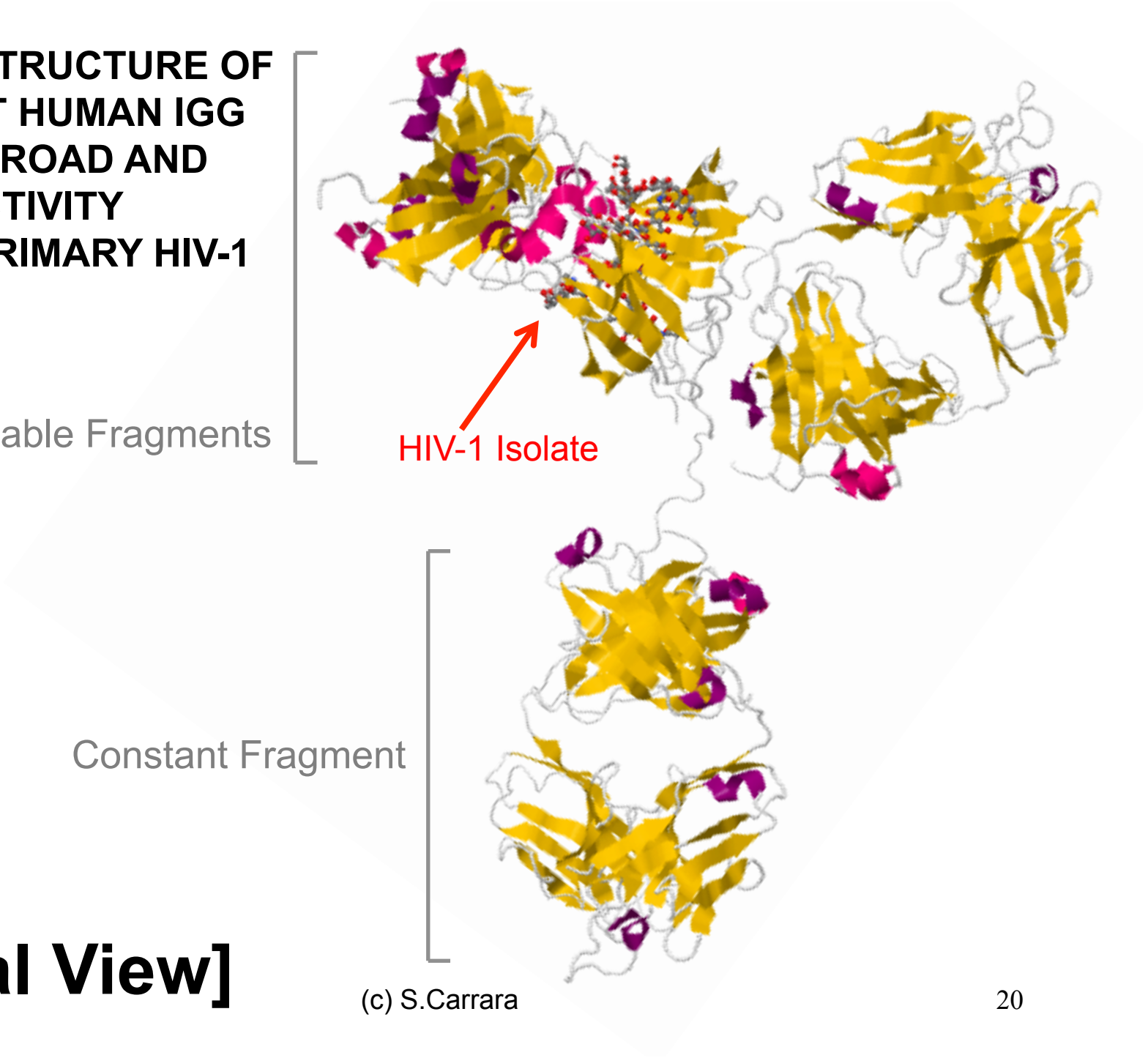


Antibody/Antigen interactions

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

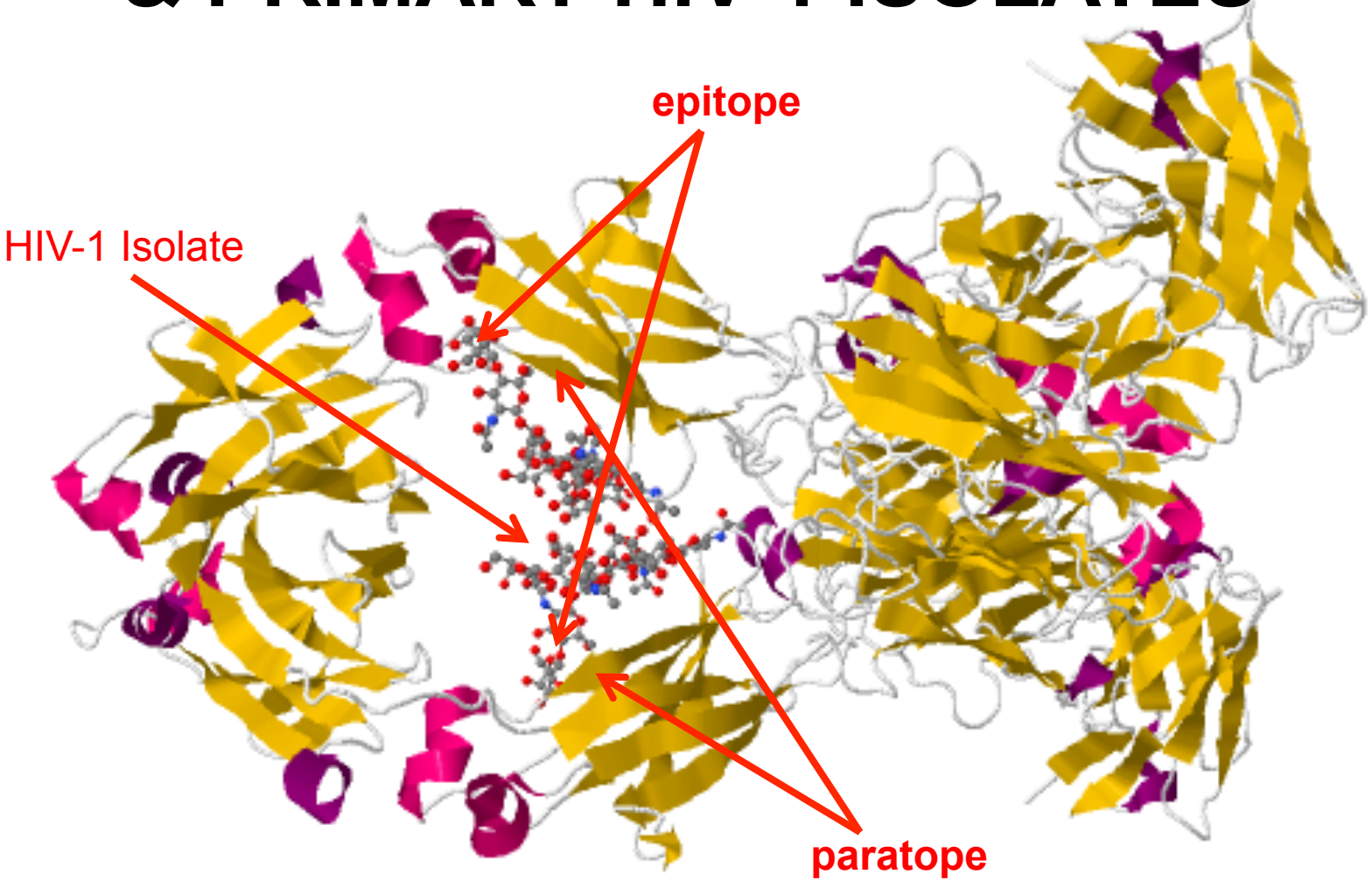
An efficient trapping is assured by the perfect match between the steric shape of the antigen and the 3D conformation of the antibody. The right part of the antigen in direct contact with the antibody is called the **epitope**, while the right part of the antibody in contact with the antigen is called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

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



[Lateral View]

CRYSTAL HUMAN IGG B12 & PRIMARY HIV-1 ISOLATES



[Top View]

Uptake Process

The uptake of antigens is a process of association among antibodies and antigens:

association constant

$$A_b + A_g \xrightarrow{k_a} A_b A_g$$

That also has the reverse process of dissociation:

dissociation constant

$$A_b A_g \xrightarrow{k_d} A_b + A_g$$

Both contributing to the net rate of formation of the complexes:

$$\frac{d[A_b A_g]}{dt} = k_a[A_b][A_g] - k_d[A_b A_g]$$

Uptake Process

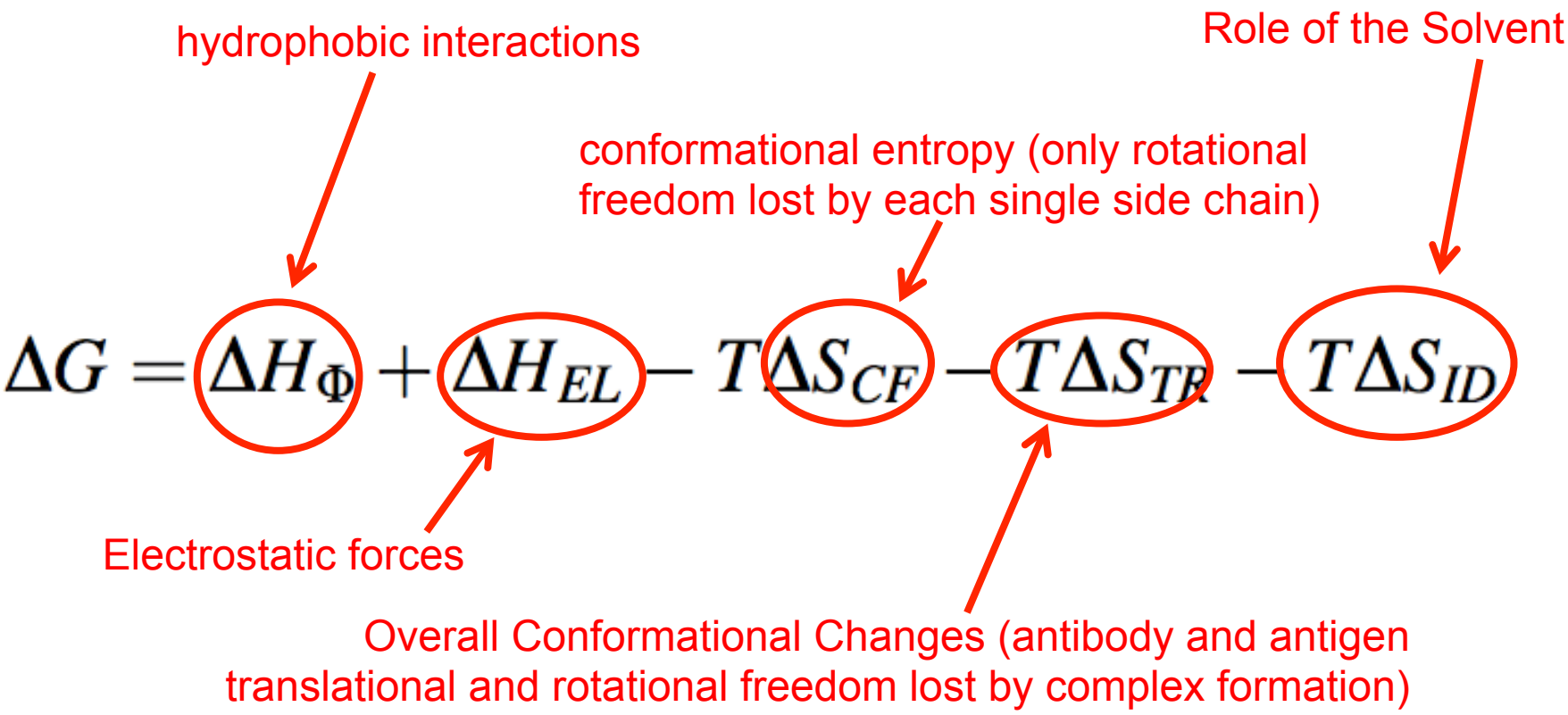
At equilibrium, we have: $$\frac{d[A_bA_g]}{dt} = 0$$

That defines the affinity constant: $$K = \frac{k_a}{k_d} = \frac{[A_bA_g]}{[A_b][A_g]}$$

Which contributes to the Gibbs free energy:

$$\Delta G = -RT \ln\left(\frac{[A_bA_g]}{[A_b][A_g]}\right)$$

Gibbs free energy contributions



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

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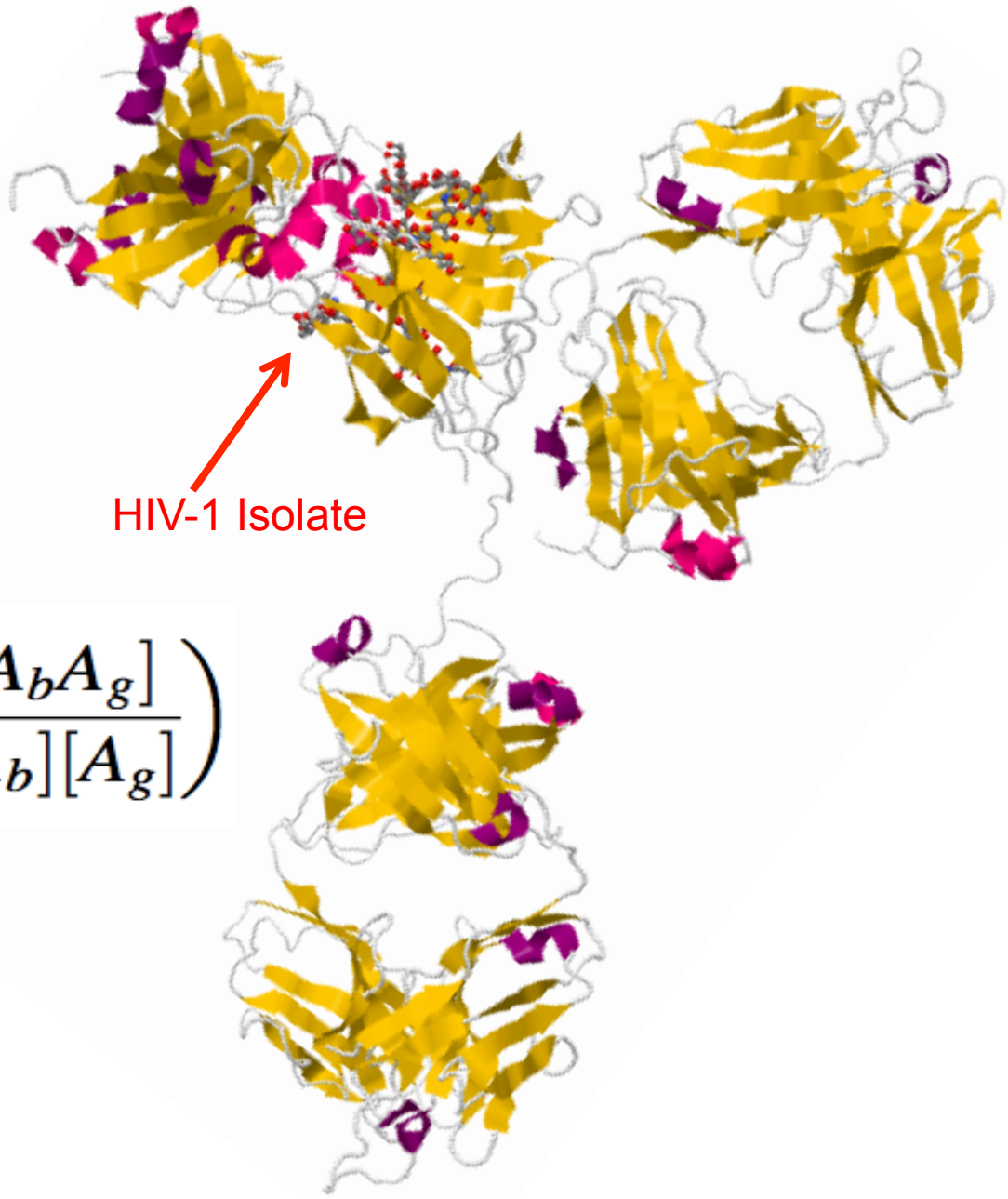
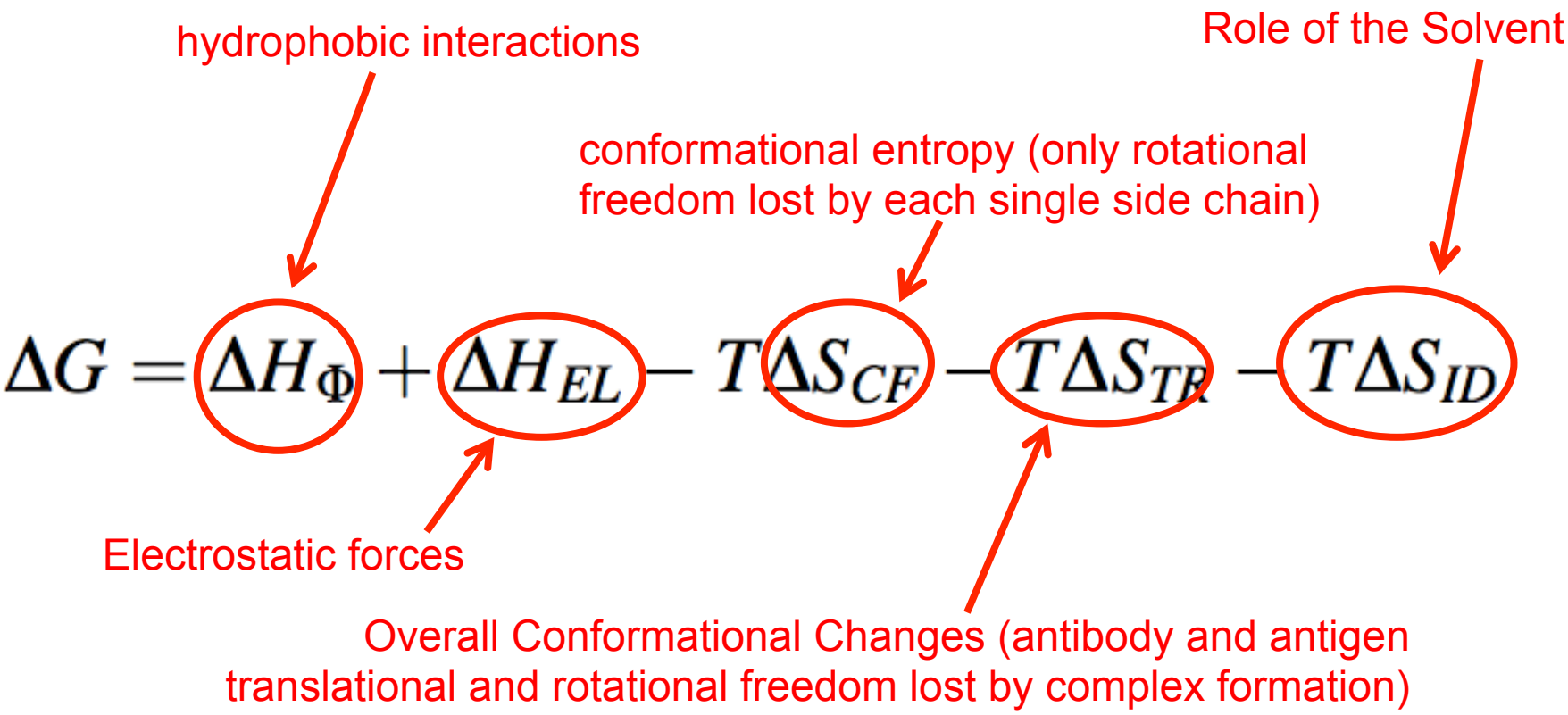


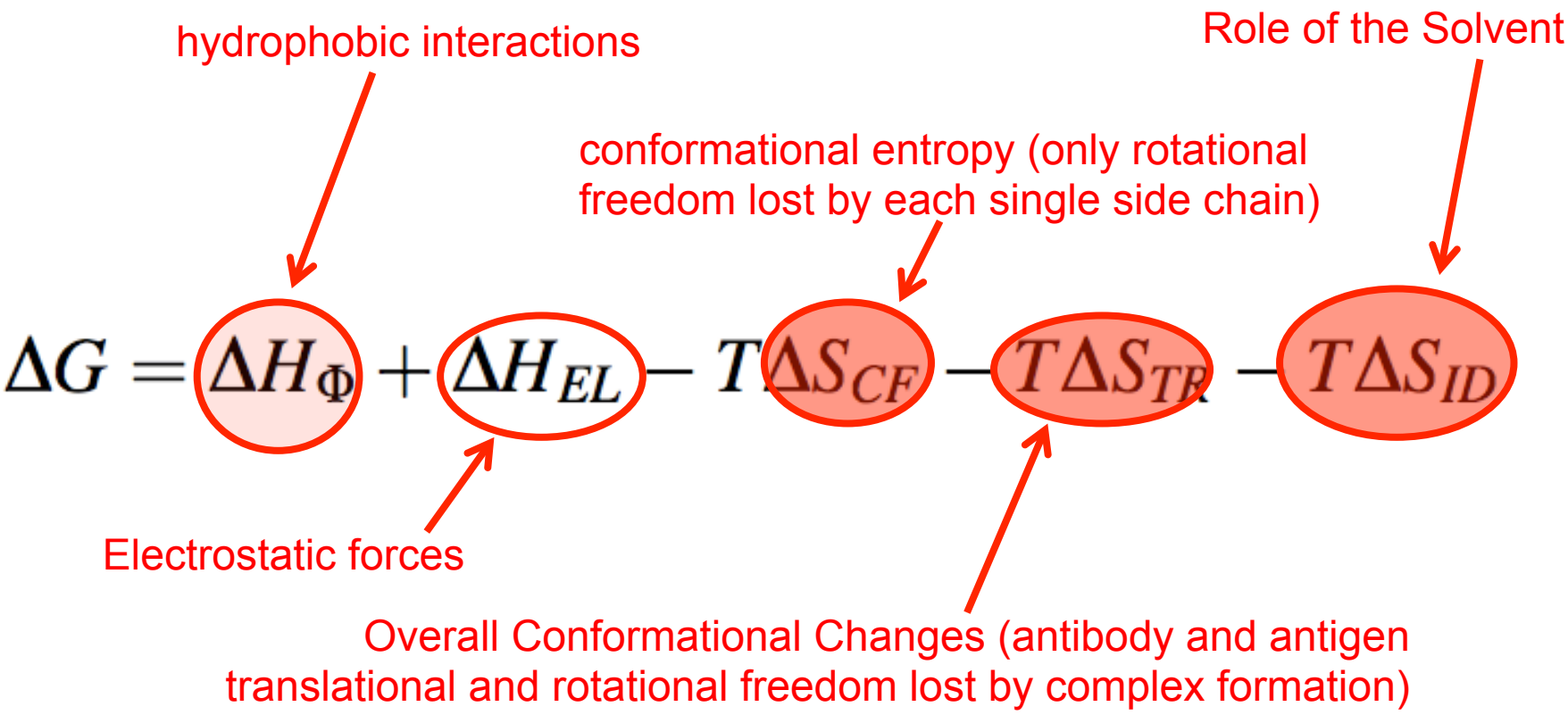
$$\Delta G = -RT \ln\left(\frac{[A_b A_g]}{[A_b][A_g]}\right)$$

Gibbs free energy contributions



The total Gibbs free energy in antibody/antigen uptake is related to various sources of molecular interactions

Gibbs free energy contributions



The total Gibbs free energy in antibody/antigen uptake is related to various sources of molecular interactions

Hydrophobic Interactions

First antibody/antigen interaction is based on hydrophobic interactions the epitope and the paratope:

$$\Delta H_{\Phi} = \alpha A_{contact}$$

The coefficient of proportionality in the previous equation is empirically determined in:

$$\alpha = -104.5 \frac{kJ}{mol\ \text{Å}^2}$$

The typical contact area of an antibody–antigen interaction ranges from 150 to 690 Å. Thus, the hydrophobic energy ranges from 15.7 kJ/mol to72.1 kJ/mol

Electrostatic Interactions

van der Waals forces and hydrogen bonds contribute to the electrostatic interactions:

$$\Delta H_{EL} = -\sum_{\forall i,j} \frac{q_i q_j}{16\pi r_{i,j}}$$

This electrostatic enthalpy is usually in the range of 88 to 163 kJ/mol for antibody/antigen interactions.

Conformational Changes of protein residues

Conformational changes due to rearrangements in the 3D organization of protein residues both in the epitope and in the paratope contribute to the entropy of the system. The torsional degrees of freedom are three in 3D space and, thus, we have for N side chains that lost their rotational freedom:

$$T\Delta S_{CF} = -RT\ln(3^N) = -NRT\ln(3)$$

This conformational entropy returns a value of 2.7 kJ/mol for each side chain.

Other contributions

The overall translational and rotational freedom that was partially blocked by the complex formation is usually empirically estimated in:

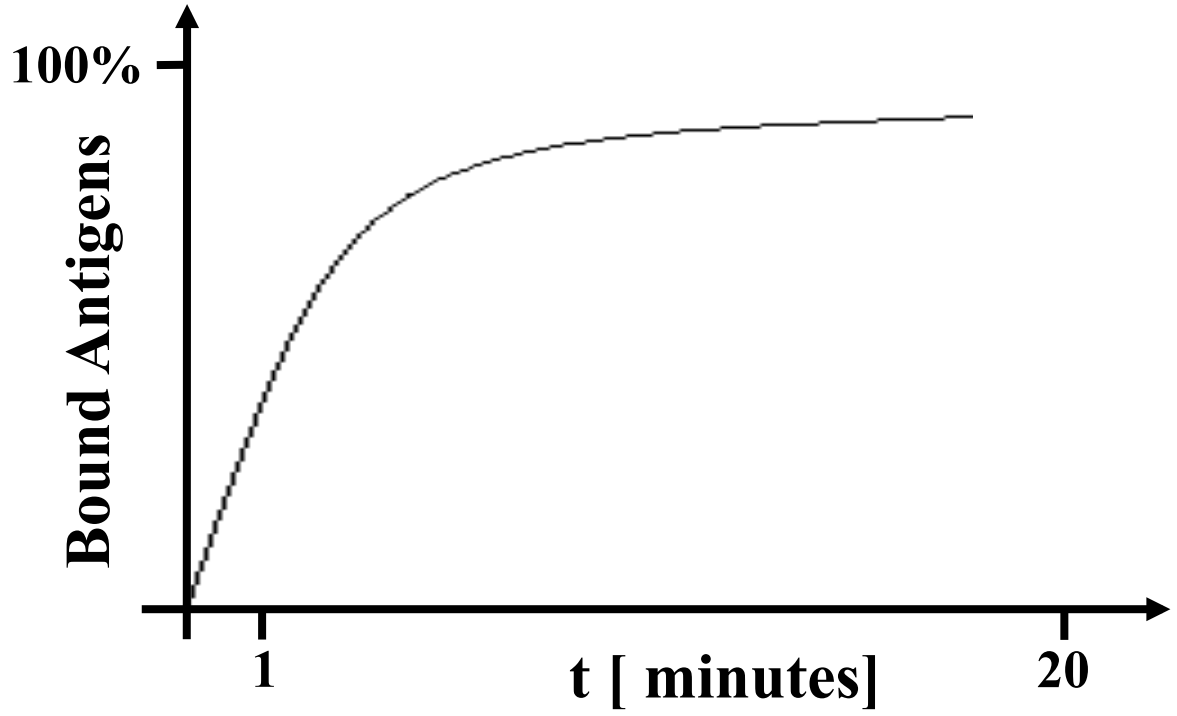
$$-T\Delta S_{TR}^{Empirical} = 29 \div 46\, kJ/mol$$

While the Role of the Solvent may be roughly estimated by considering an approximation to infinite dilutions:

$$T\Delta S_{ID} \approx -8.4\, kJ/mol.$$

So, the typical **total Gibbs free energy** of a complex antibody–antigen interaction is in a range of **27.6 to 60.3 kJ/mol**

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 kinetic on the same antigen.

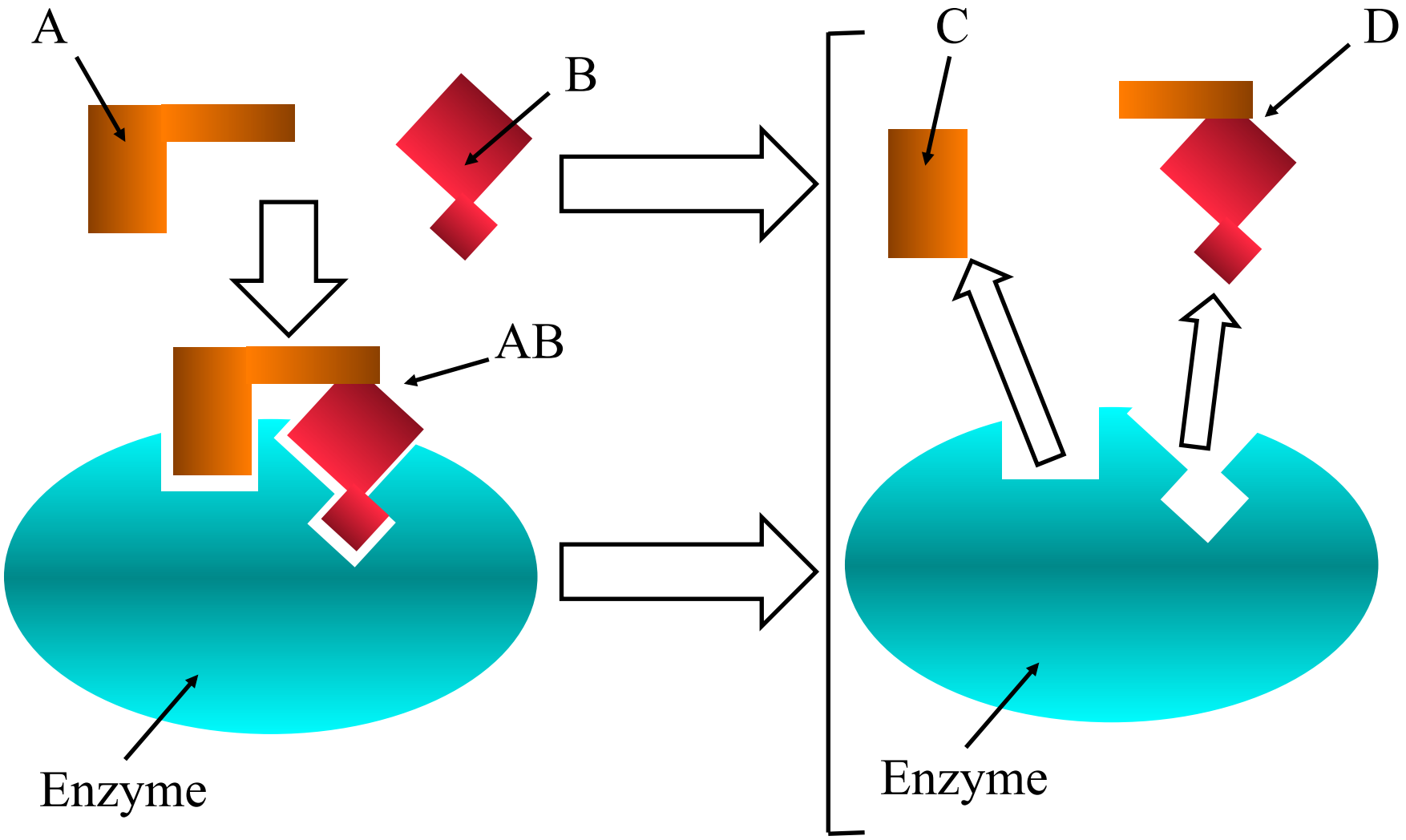
We then have different kinetics by involving polyclonal antibodies since they present different paratopes to address different epitopes of the same antigen

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**.

Mechanism of Enzymatic reactions



Equilibrium Constant

In any chemical reaction from two compounds to products, we have:

forward rate constant

$$A + B \xrightarrow{k_F} C + D$$

And we also have the reverse reaction as well:

backward rate constant

$$C + D \xrightarrow{k_B} A + B$$

Both defining the equilibrium constant of the overall reaction:

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

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

What the enzyme changes is the time within which the chemical reaction occurs. If the equilibrium is reached in, let say, 1 h in the absence of an enzyme, then it might be reached in few seconds in the presence of the suitable enzyme!

Enzymatic Process

In the presence of the enzyme, we have in the same chemical reaction :

forward rate constants

$$A + B \xrightarrow{k_a} AB \xrightarrow{k_p} C + D$$

And we also have the reverse reaction as well:

equilibrium constants

$$A + B \xleftrightarrow{K_a} AB \xleftrightarrow{K_p} C + D$$

Thus, the rate of products' formation now depends by the formation of the complex:

$$\frac{d([C][D])}{dt} = k_p[AB]$$

Enzymatic Process

AB is obtained by A and B with the equilibrium constant K_a:, and thus the Gibbs free energy of the reaction is now related to the equilibrium constant of the formation of the intermediate state:

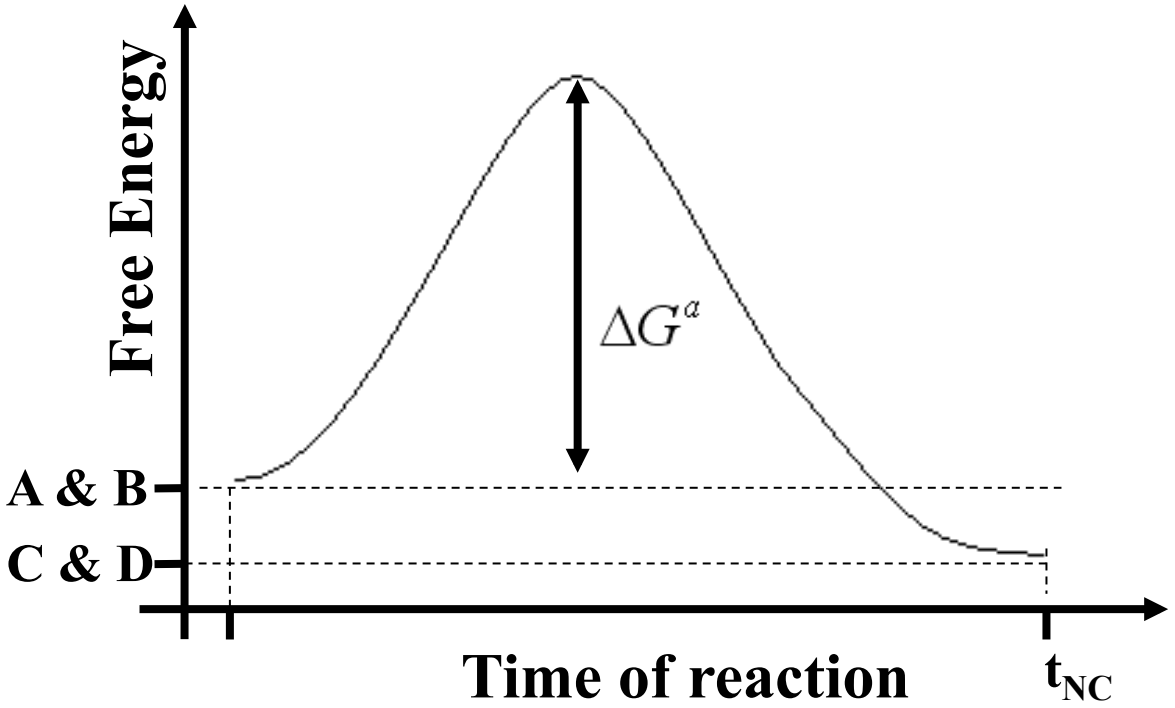
$$\Delta G^a = -RT \ln K_a$$

By neglecting the possibility for C and D to give back the intermediate state AB, we can now write the net rate of products formation as:

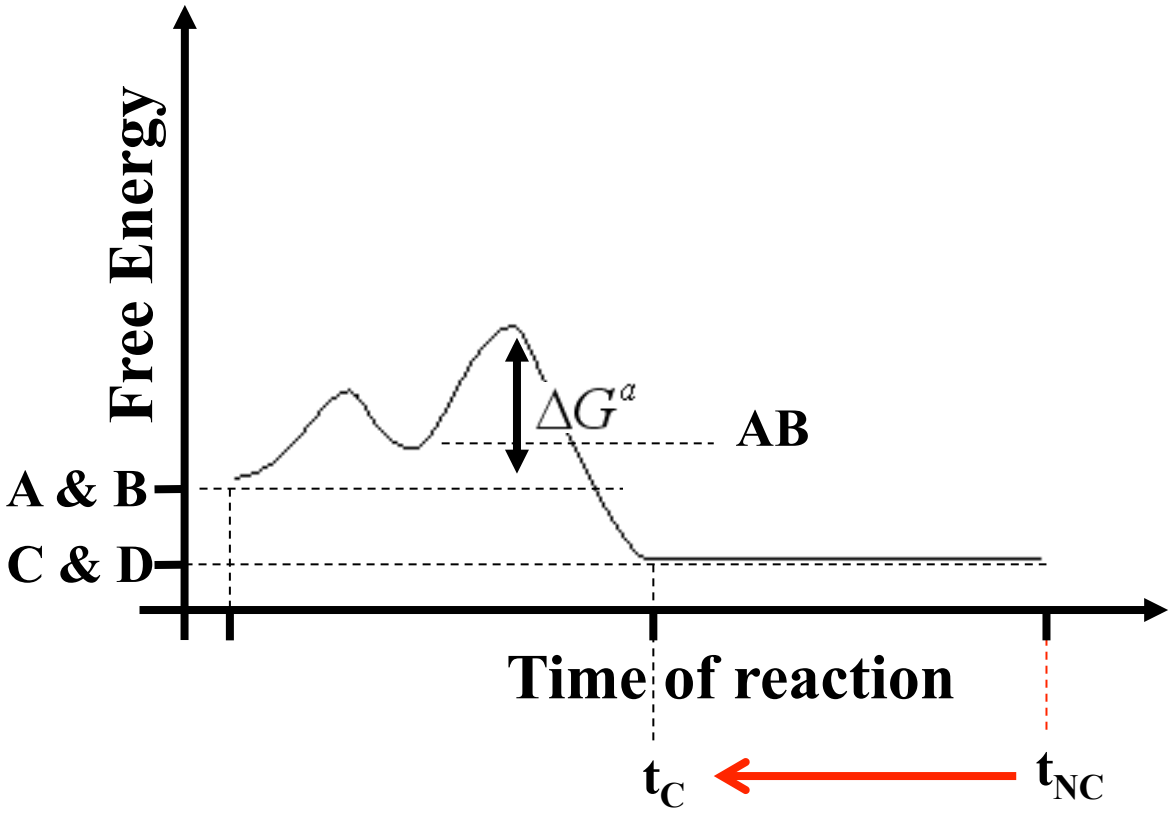
Gibbs free energy

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

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:

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

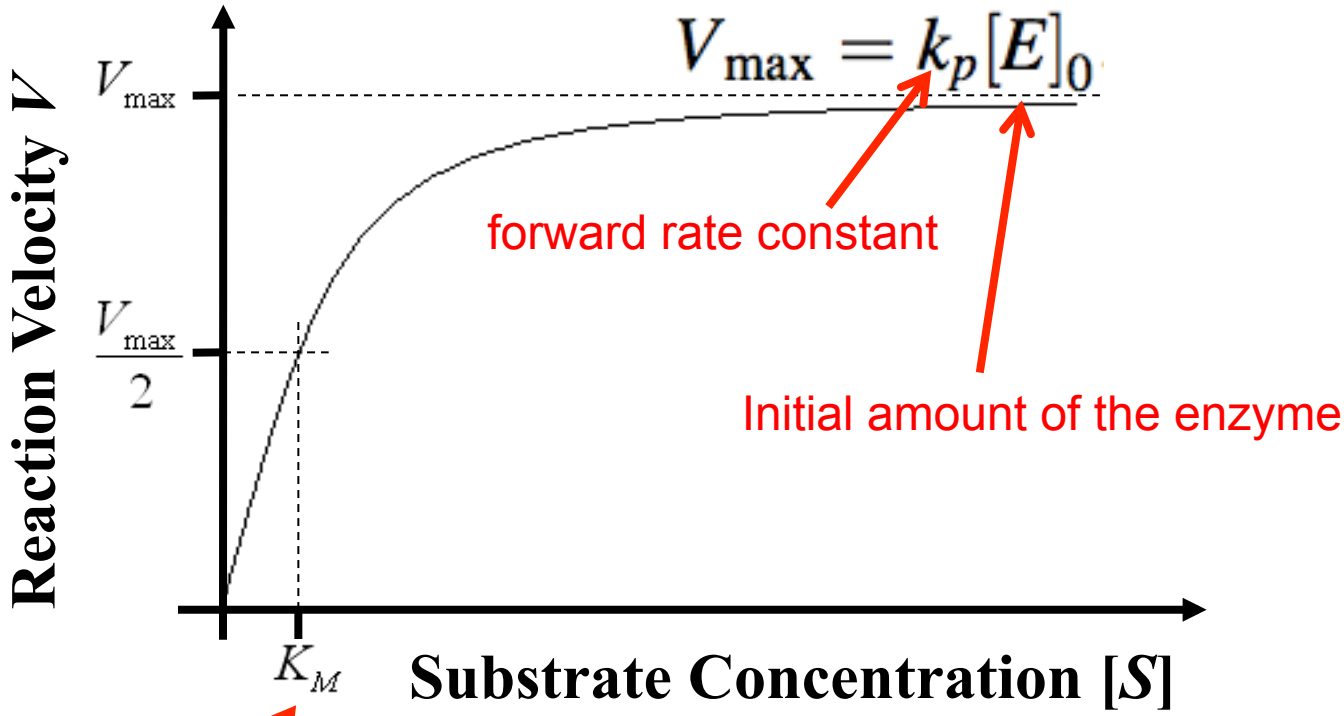
If we, then, go back to couple of previous equations:

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

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

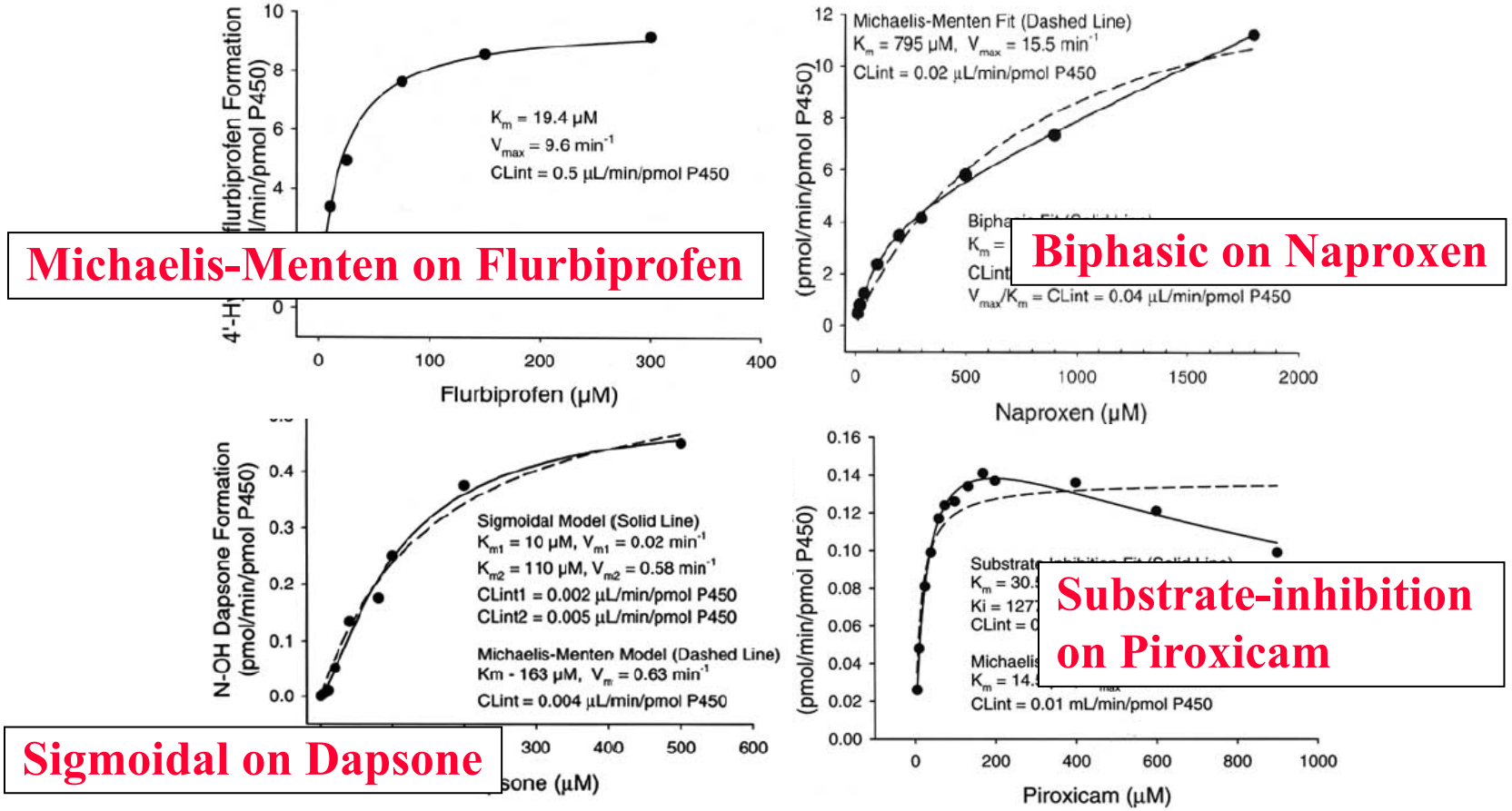
$$V = V_{\max} \frac{[S]}{[S] + K_M}$$

Michaelis-Menten Kinetics



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

Atypical Enzymatic Reactions



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

Some enzymes (e.g., the cytochrome P450 2C9) also present kinetics different than Michaelis-Menten on different substrates!