EPFL

Master in Electrical and Electronics Engineering

EE-517: Bio-Nano-Chip Design

Lecture #3

Probe/Target interactions

Lecture Outline

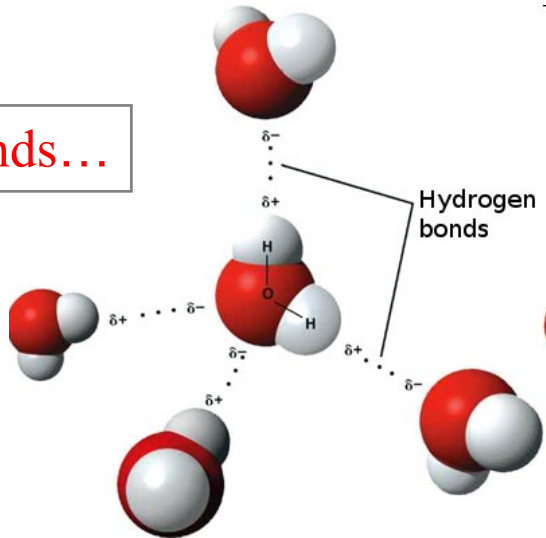
(Book Bio/CMOS, *second Edition*: Chapter 4)

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

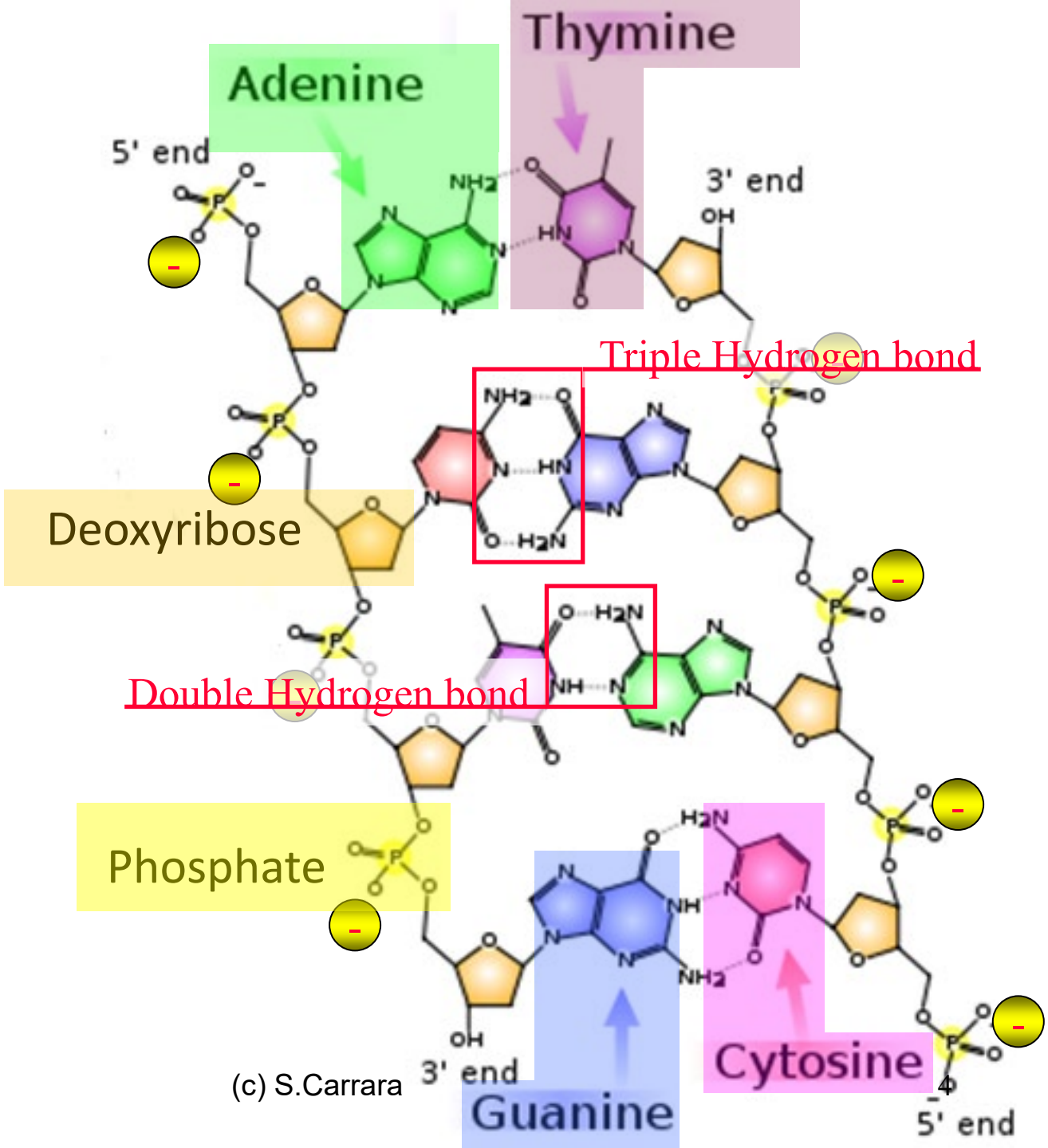
DNA Base pairing

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**.

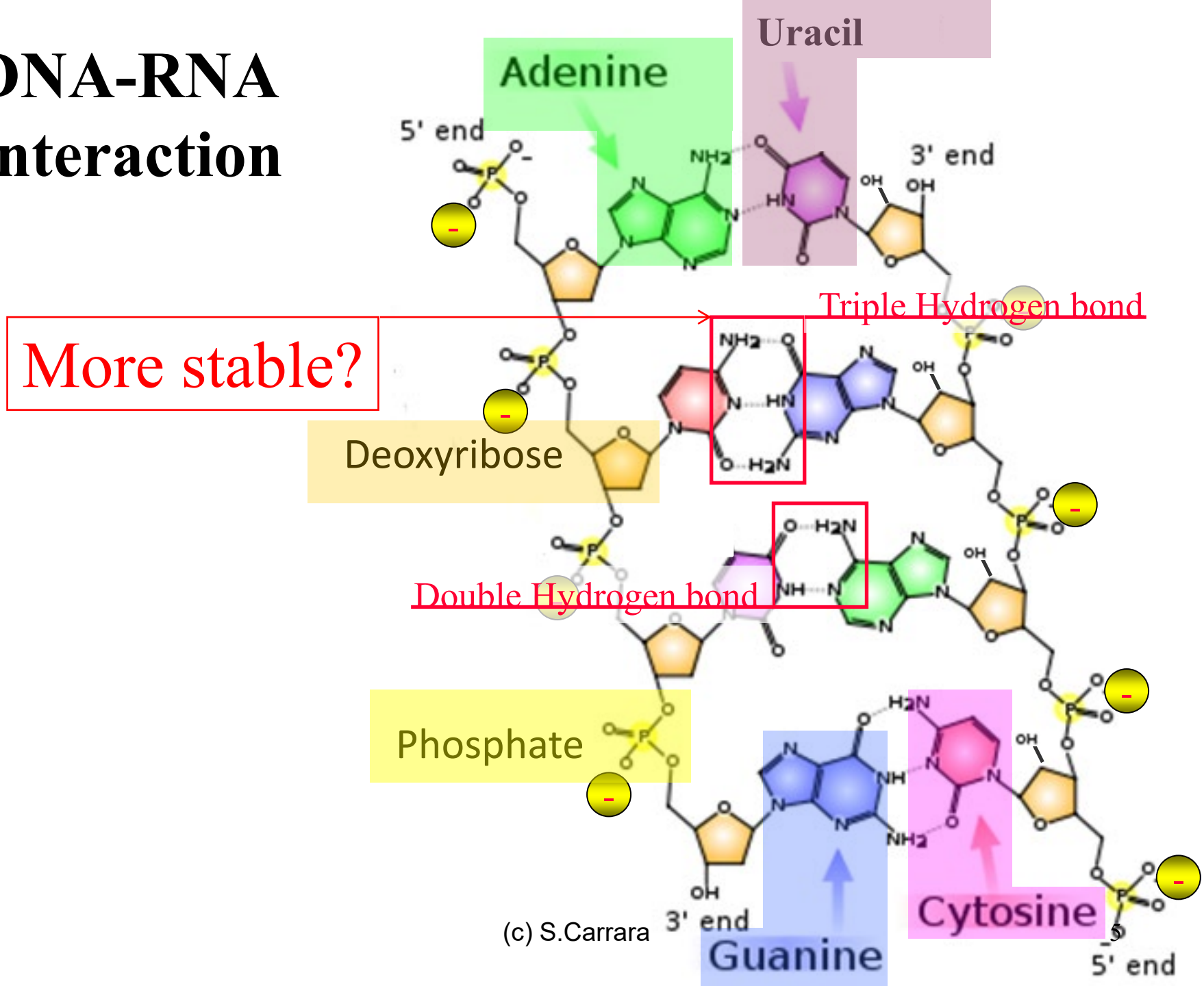
Thanks to Hydrogen Bonds…



DNA-DNA Interaction



DNA-RNA Interaction



Q1

Are more stable the pairs with three hydrogen bonds?

A. May be
B. Yes, since they have more bonds than two
C. Yes, because the pairing relies only on hydrogen bonds
D. Not necessarily, since other interactions take place
E. No, because the sugars in the backbone interact as well

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.

Q2

Do you think the structural conformation of DNA/RNA also contribute to pairing force?

A. No idea
B. Yes, since DNA/RNA may reorganize during the pairing
C. Yes, because the pairing occurs only with double helix structure
D. Not necessarily, since the pairing occurs only with the double helix structure
E. No, because the backbone has always the same conformation

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. Entropic forces also play a role

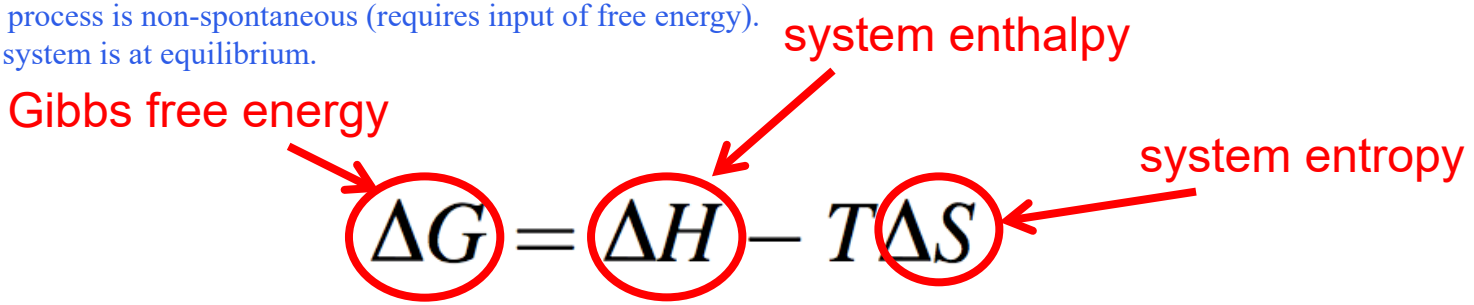
Q3

How to compute the total energy involved in DNA/RNA pairing?

A. Impossible to compute
B. By accounting the sticking energy due to any kind of bond
C. By accounting only the energy of the hydrogen of bonds
D. By computing the work done in creating the pair
E. By calculating the thermodynamic potential

Gibbs free energy

- **ΔG<0**: the process is spontaneous (releases free energy).
- **ΔG>0**: the process is non-spontaneous (requires input of free energy).
- **Δ*G*=0**: the system is at equilibrium.



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

Q4

Where it comes from the definition of the Gibb free energy?

A. I don't know
B. From the zeroth law of thermodynamics
C. From the first law of thermodynamics
D. From the second law of thermodynamics
E. From the definitions of Enthalpy and Entropy

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

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

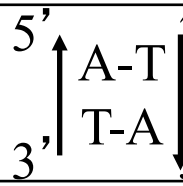
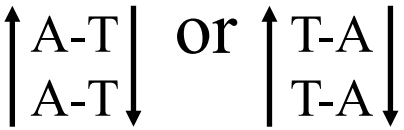
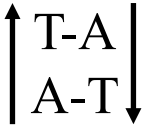
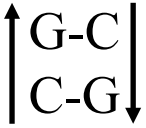
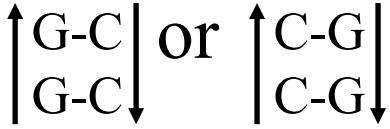
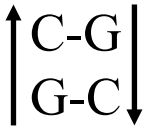
→ For any thermodynamic system, the change in internal energy (ΔU) equals the heat added to the system (Q) minus the work done by the system (W).

Q5

Pairing A-T returns always the same energy in different sequences?

A. Yes, of course!
B. Yes since they are the same nucleic acid
C. Yes, since the pairing of all nucleic acids has always the same energy
D. No since the require a different work to be inserted in different seq.
E. No since they may need of a different Enthalpy and Entropy in different sequences

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

number of occurrences of each nearest neighbor

account for the initial pair

$\Delta G=\sum(\Delta G_{\text{nearest-neighbor steps}})+\Delta G_{\text{initiation}}$

$$\Delta G = \sum_{\forall i} n_i \Delta G_i + \Delta G_i|_{init\ term\ A-T} + \Delta G_i|_{init\ term\ G-C} + \Delta G_{system}$$

Gibbs free energy change for the considered neighboring pairs

empirical parameter (+1.80 kJ/mol if the nucleic acid duplex is self-complementary, or equal to zero if not)

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

Q6

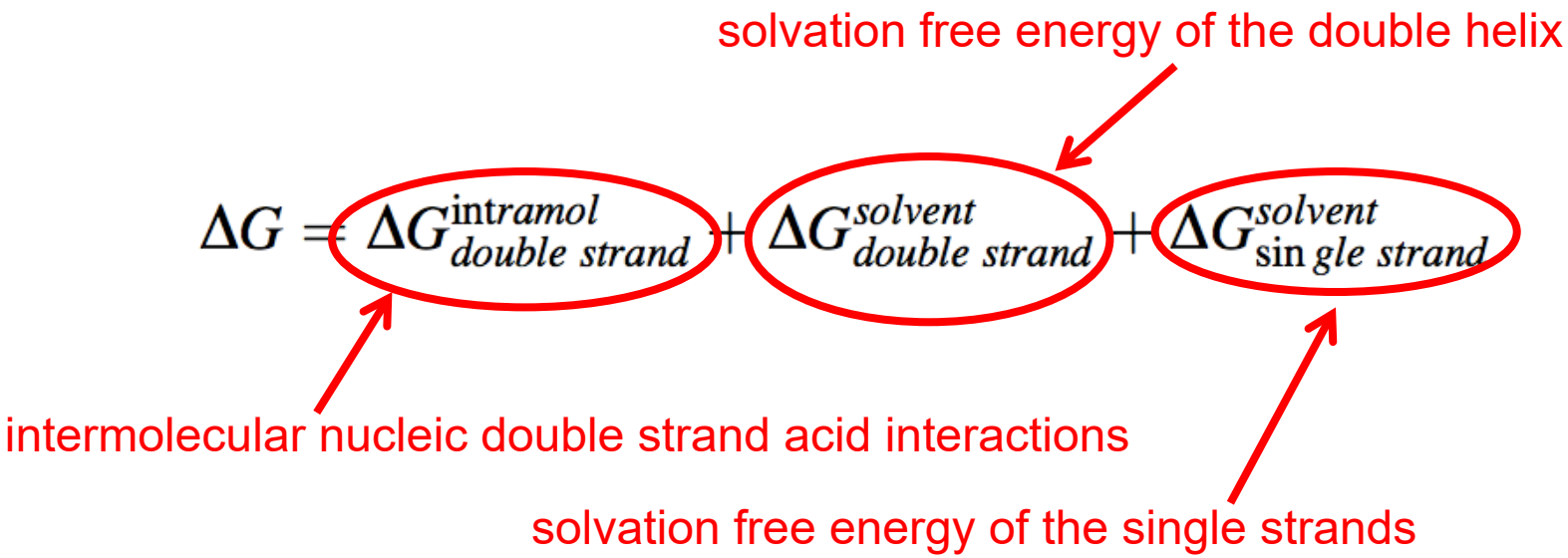
In a sequence of 9 bases, does a single-pair mismatch change enough the pairing energy?

A. No, of course!
B. Not really since it is one mismatch out of 8 matches
C. No since the right sequence requires a slightly different work in creating the full pairing
D. Yes since any pair counts
E. Yes since it changes enough both Enthalpy and Entropy

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:

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

→DNA is fully double-stranded = very ordered.
→It melts = entropy increases because single strands are more disordered.
→The entropy contribution also depends on the concentration of DNA molecules:

Thus, the melting temperature is:

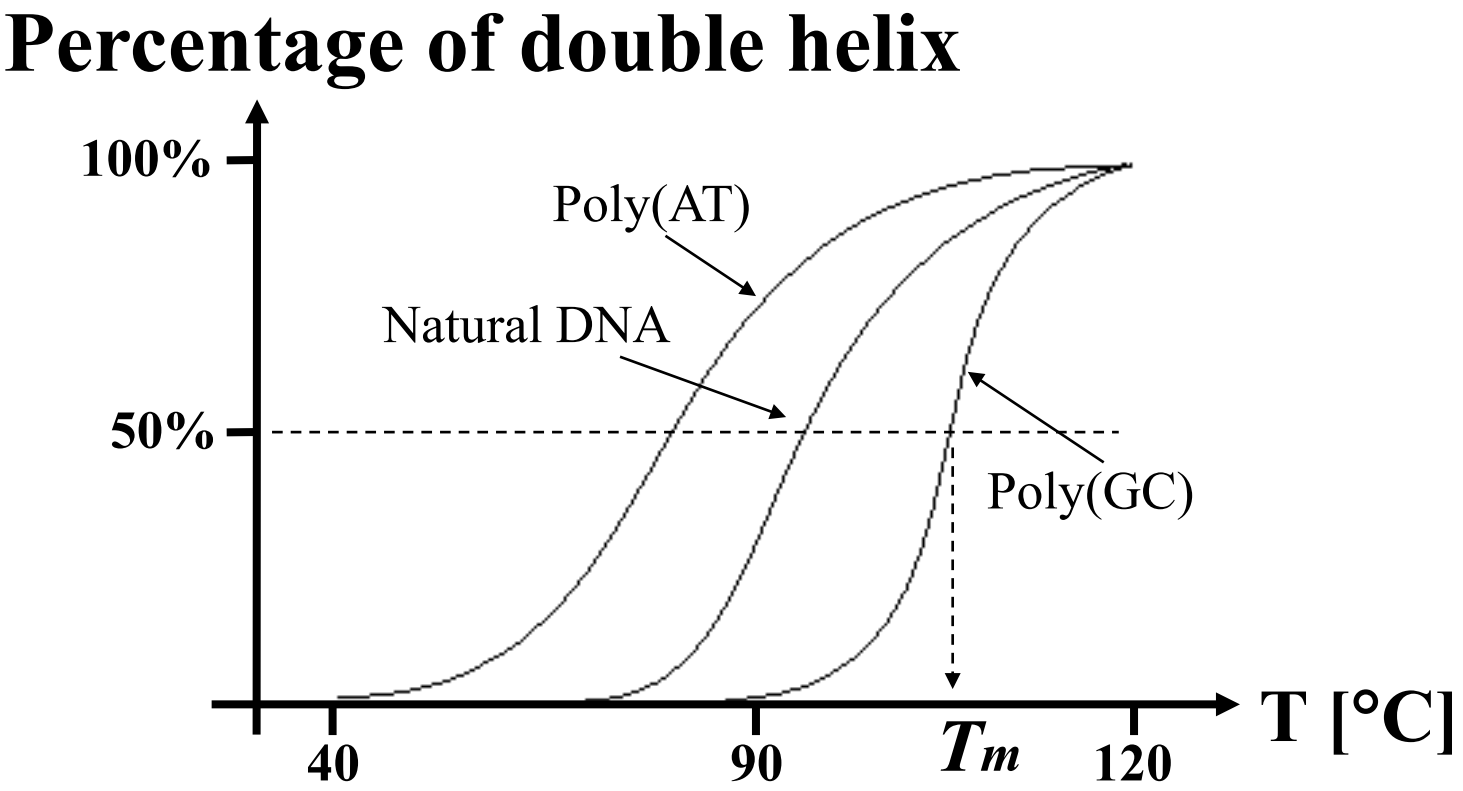
$$T_m = \frac{\Delta H}{\Delta S + R \ln C_{DNA}}$$

Q7

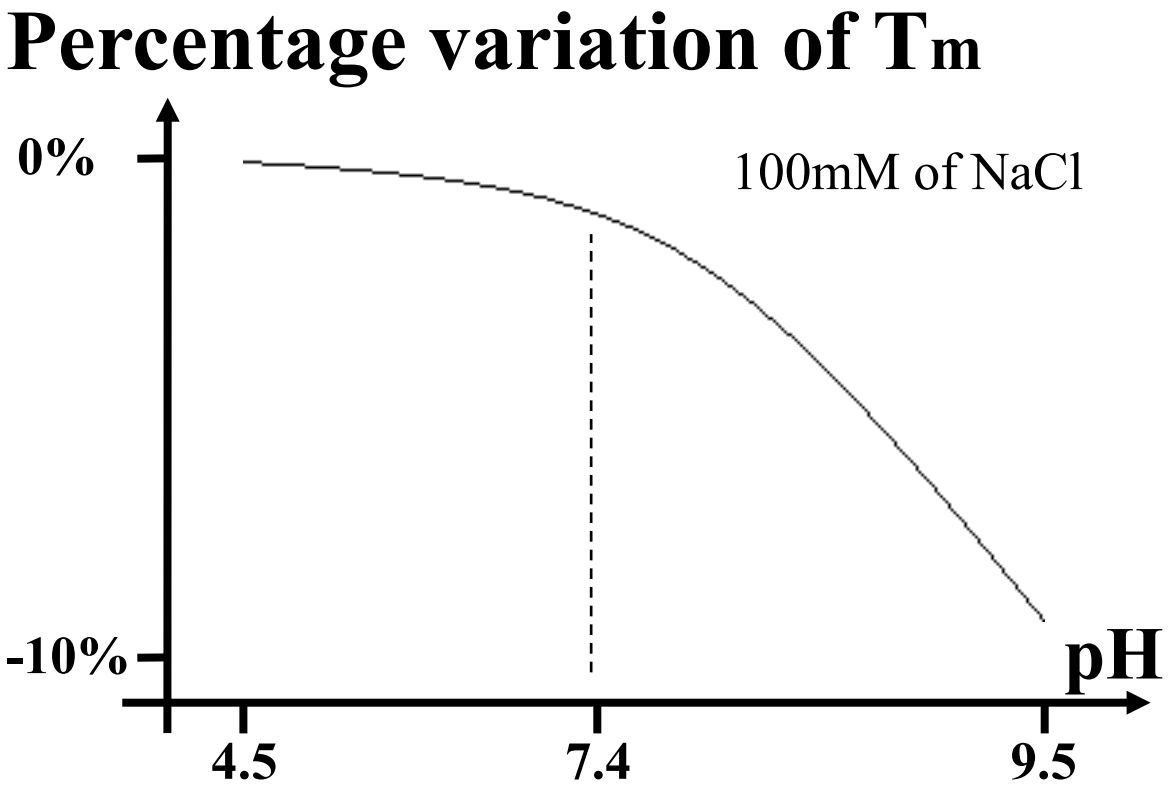
Have all DNA sequences a very similar melting temperature?

A. Not exactly, but very close
B. Yes since they are all same kind of nucleic acids
C. Yes, since the pairing of nucleic acids has always the same energy
D. No since pairing of nucleic acids has always a slightly different energy
E. No since they have a different Enthalpy and Entropy

Melting processes for different DNA sequences



Melting temperature of DNA versus pH



Q8

Why the melting temperature depends on pH?

A. Since pH changes the solution' temperature
B. Since pH affects directly the solvent
C. Since pH affects the enthalpy
D. Since pH affects the entropy
E. Since pH affects directly the hydrogen bonds

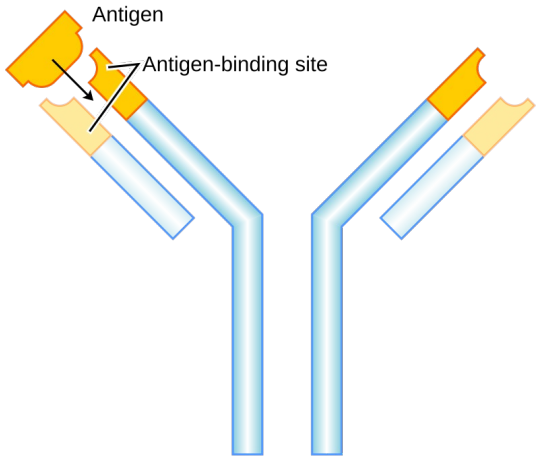
Antibody/Antigen interactions

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/Antigen interactions

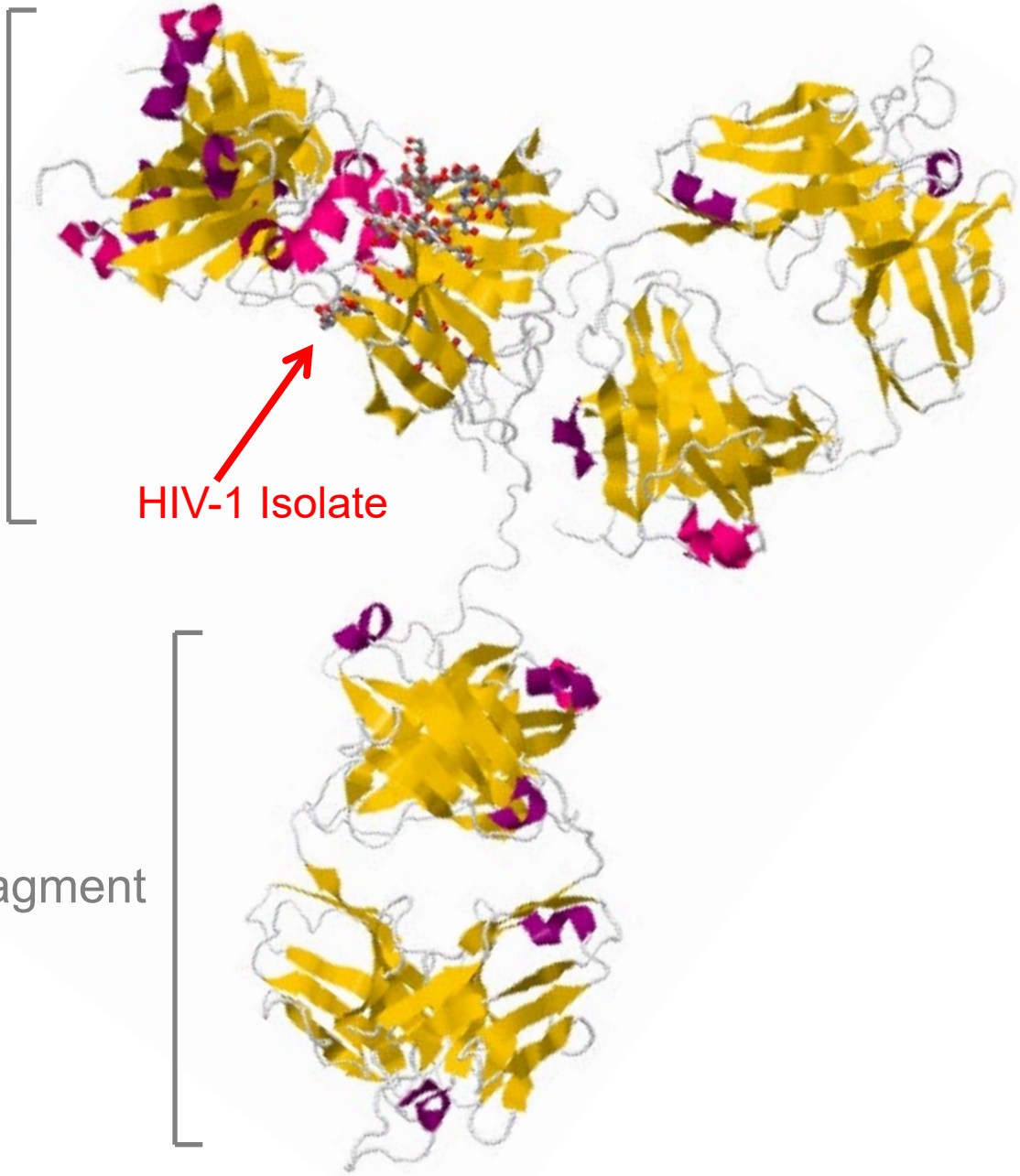
- An antibody (Ab), or immunoglobulin (Ig), is a large, Y-shaped protein belonging to the immunoglobulin superfamily.
- It is used by the immune system to identify and neutralize antigens (such as bacteria and viruses).
- Each individual antibody recognizes one or more specific antigens.



Antibody

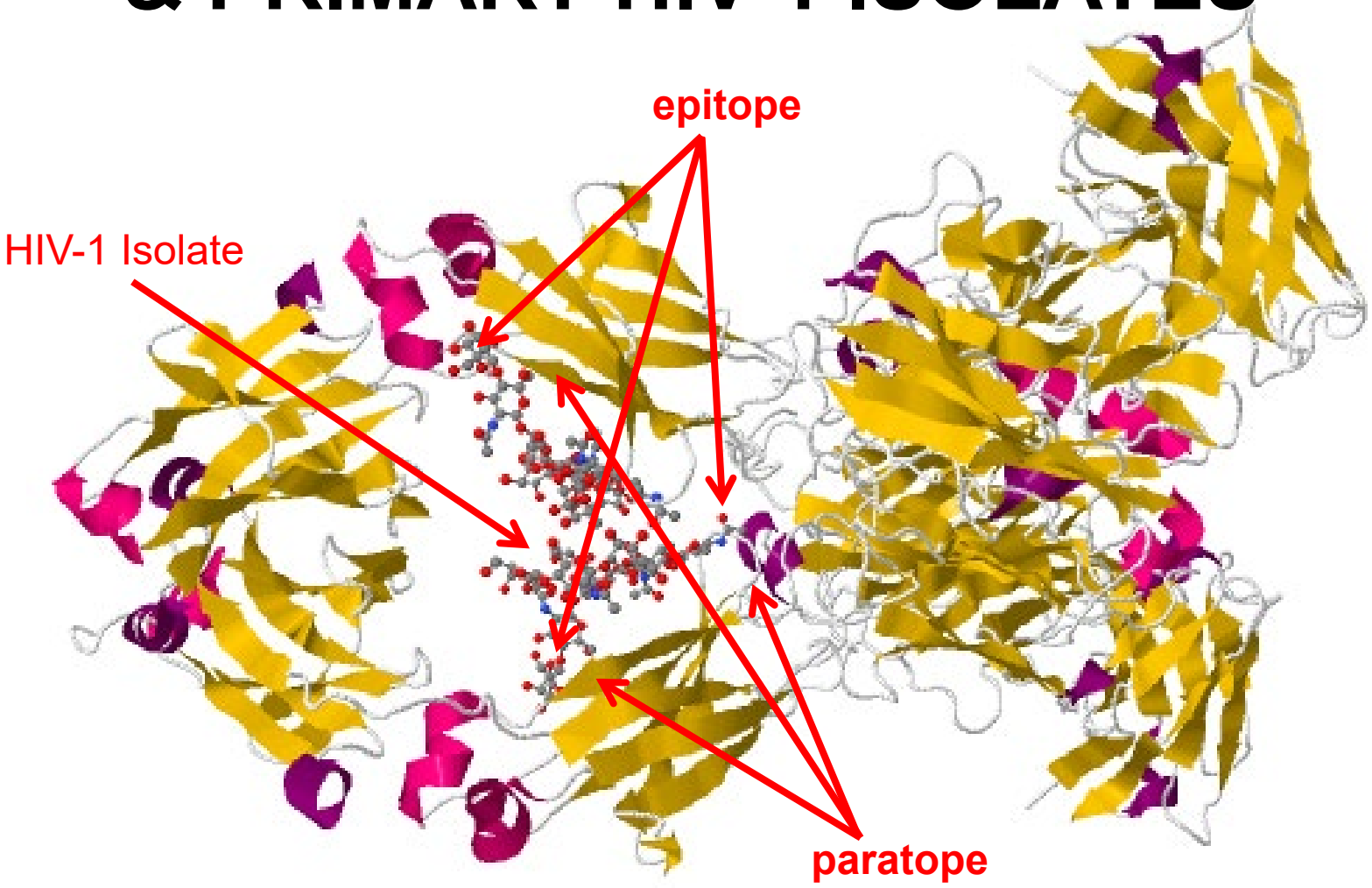
- Each Ab has the paratope that specifically binds to one particular epitope on an antigen, allowing the two molecules to bind together with precision

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



[Lateral View]

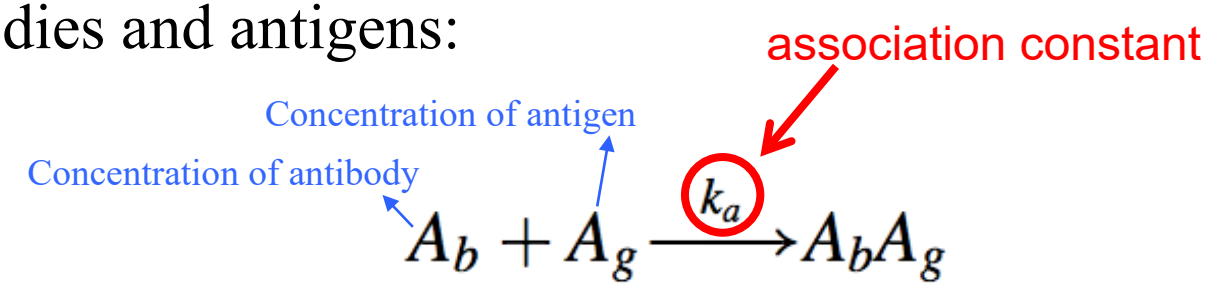
CRYSTAL HUMAN IGG B12 & PRIMARY HIV-1 ISOLATES



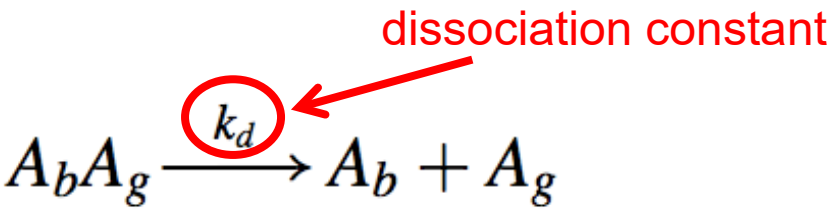
[Top View]

Uptake Process

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



That also has the reverse process of dissociation:



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

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

Uptake Process

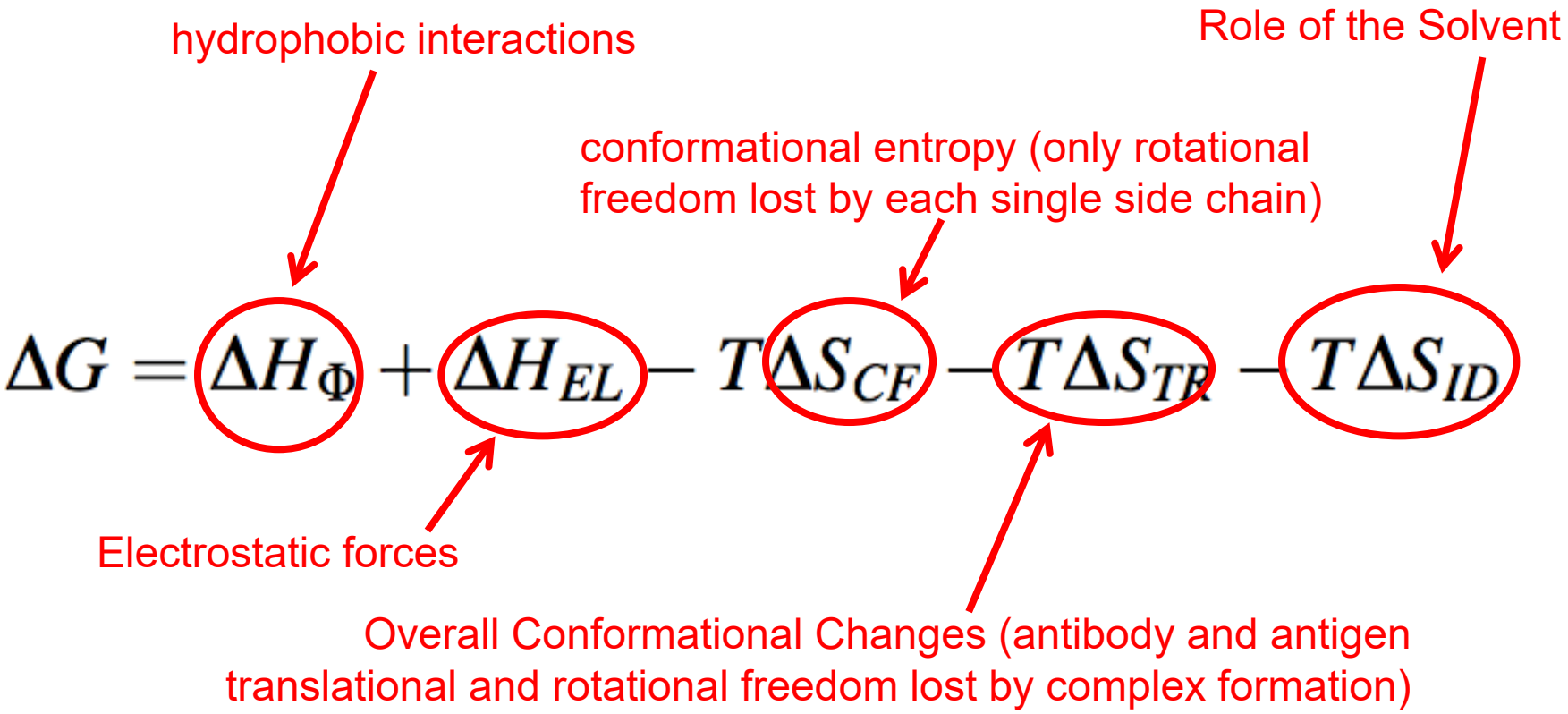
At equilibrium, we have: $$\frac{d[A_b A_g]}{dt} = 0$$

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

Which contributes to the Gibbs free energy:

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

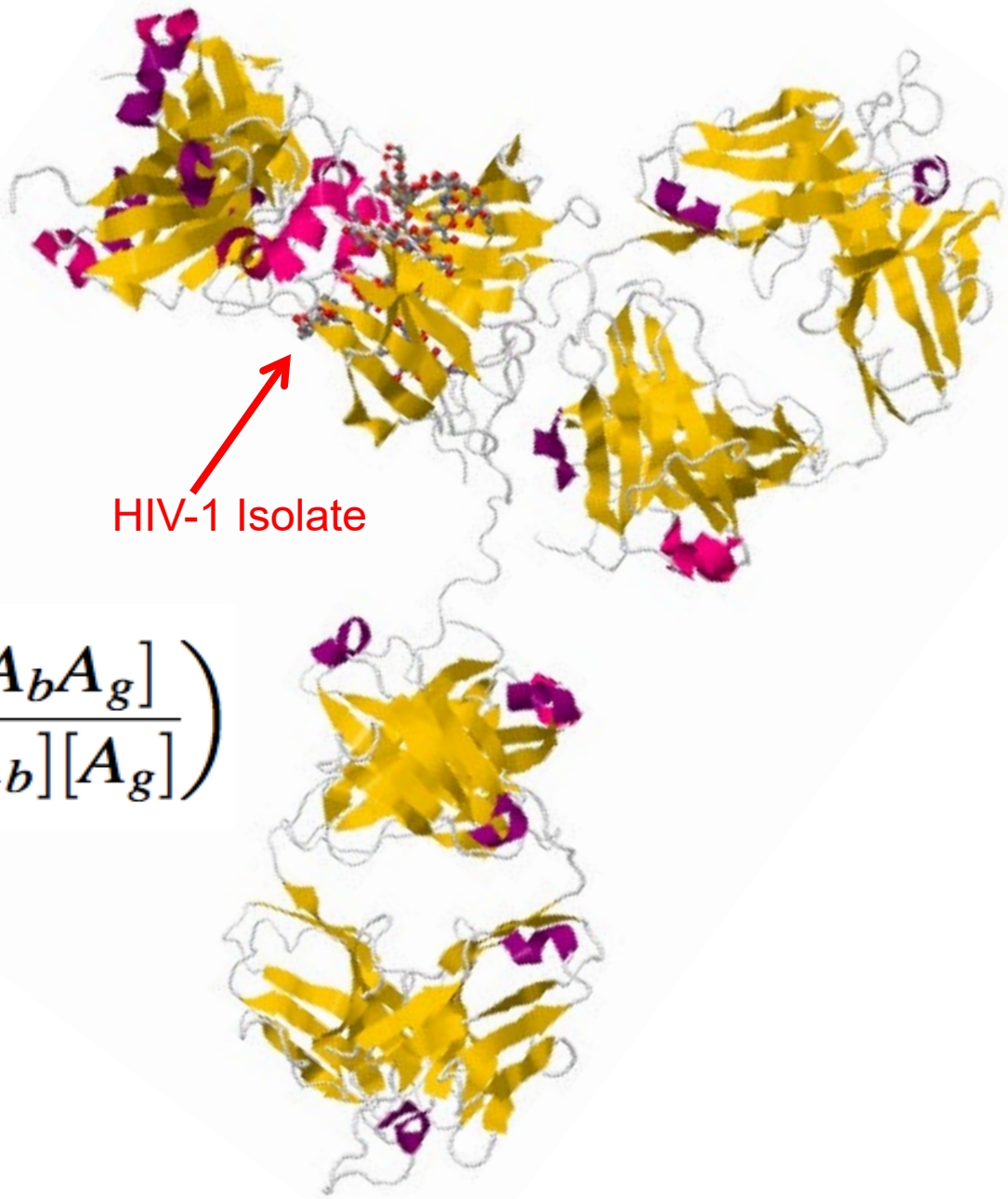
Gibbs free energy contributions



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

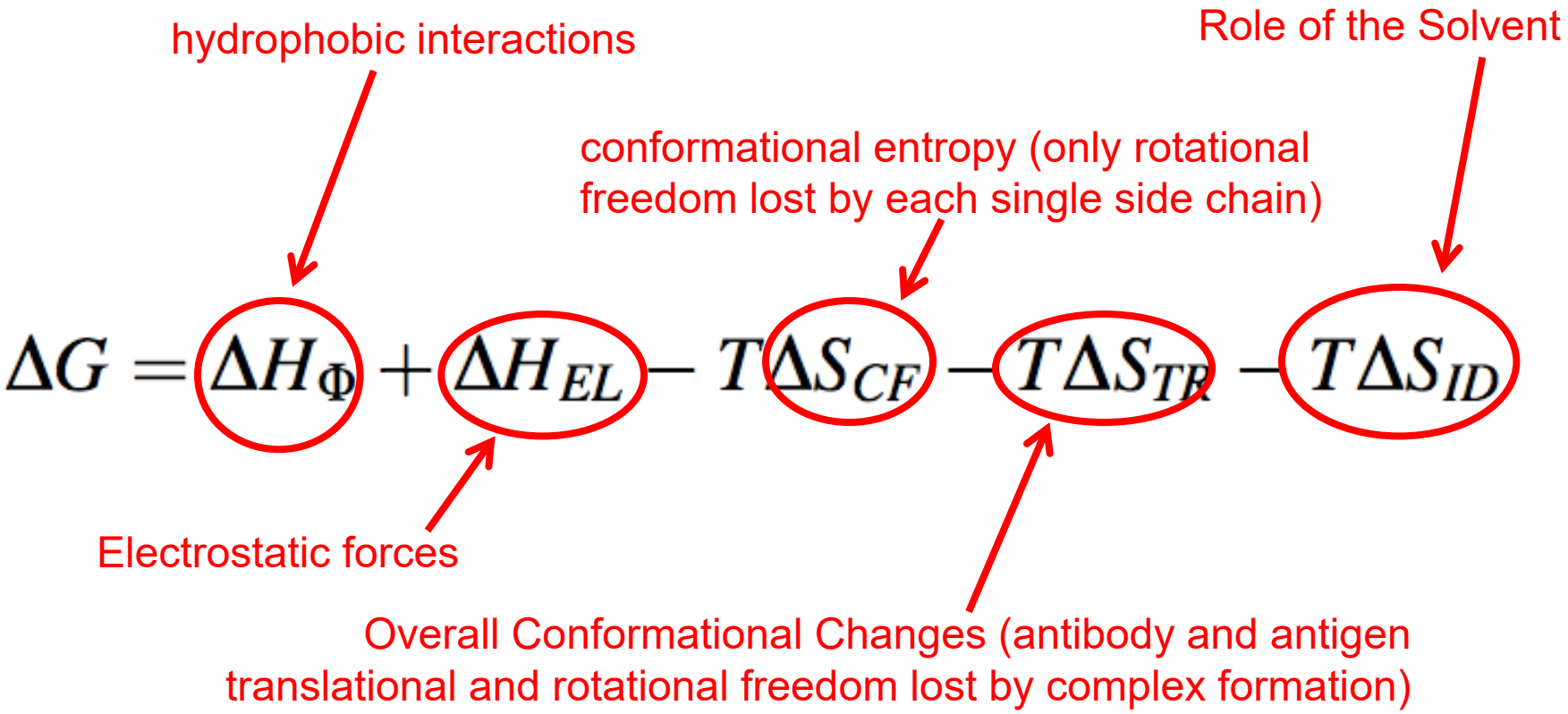
La pause

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



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

Gibbs free energy contributions



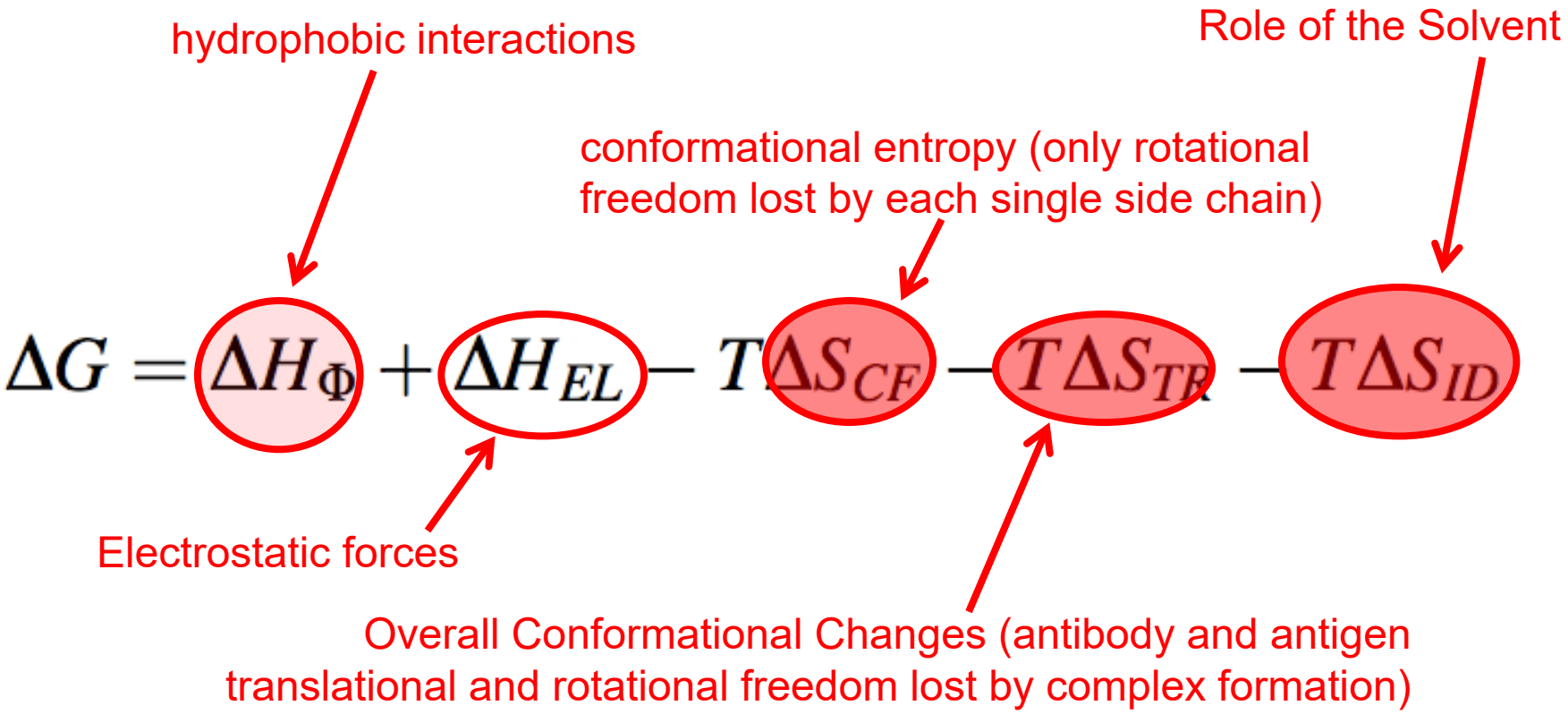
The total Gibbs free energy in antibody/antigen uptake is related to various sources of molecular interactions

Q9

Does the pH affect the antibody/antigen uptake?

A. No, never
B. Yes, since pH affects the enthalpy of the uptake
C. Yes, since pH affects the entropy of the uptake
D. Yes, since pH affects several components of the Gibb free energy
E. Yes, since pH affects only the solvent

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:

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

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:

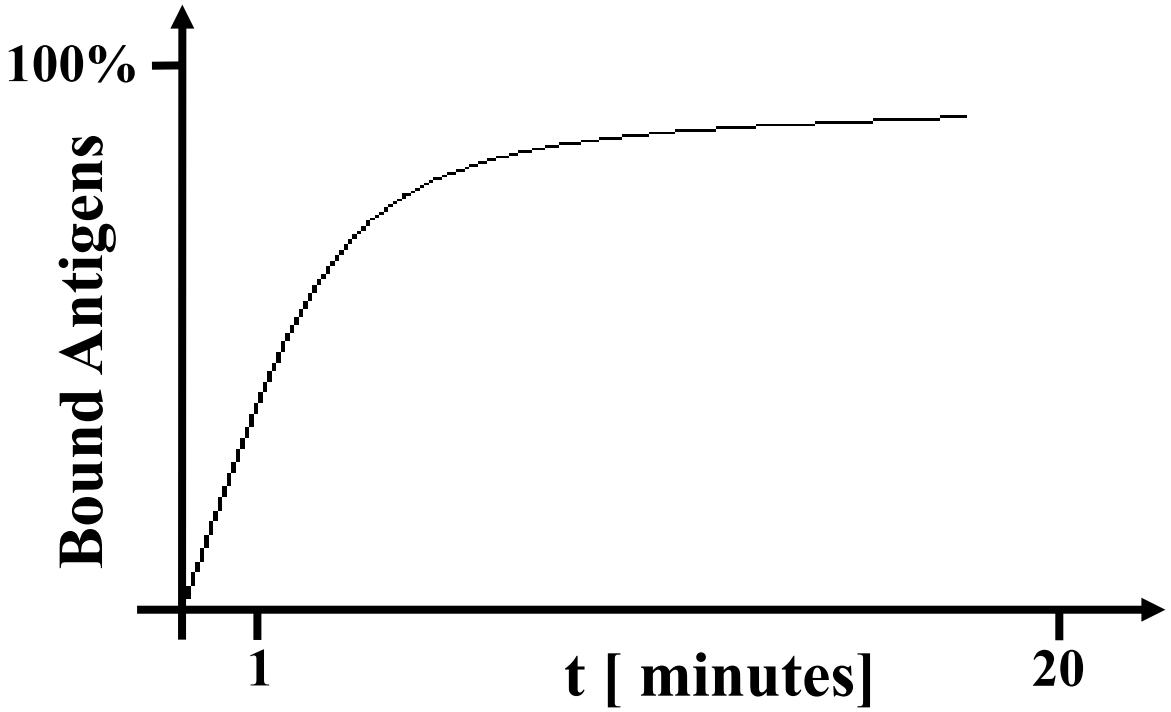
$$-T\Delta S_{TR}^{Empirical} = 29 \div 46\, kJ/mol$$

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.

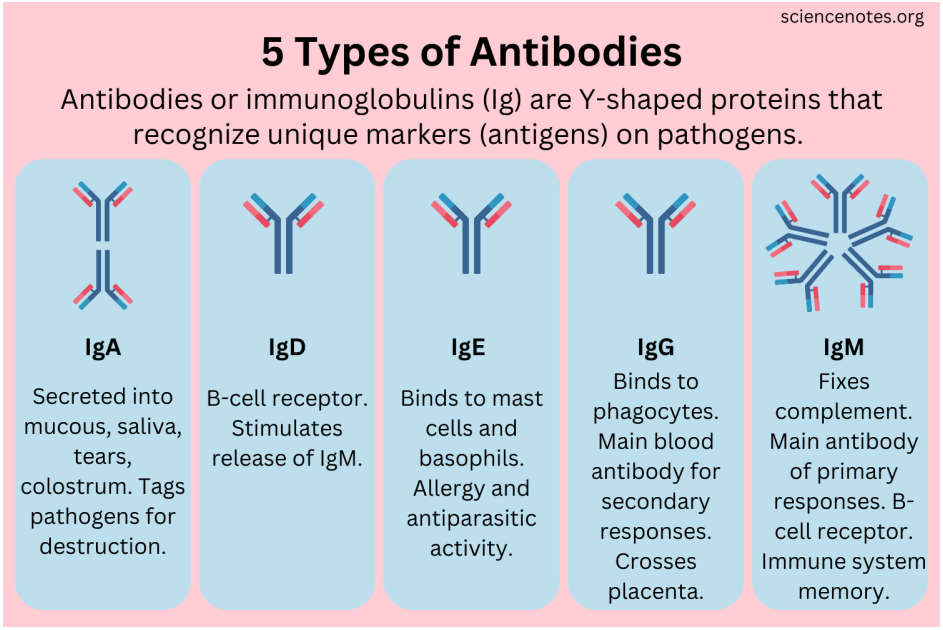
They recognize **only one epitope** on the antigen

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.

They recognize **multiple epitopes** on the antigen

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

Different Kinds of Antibody



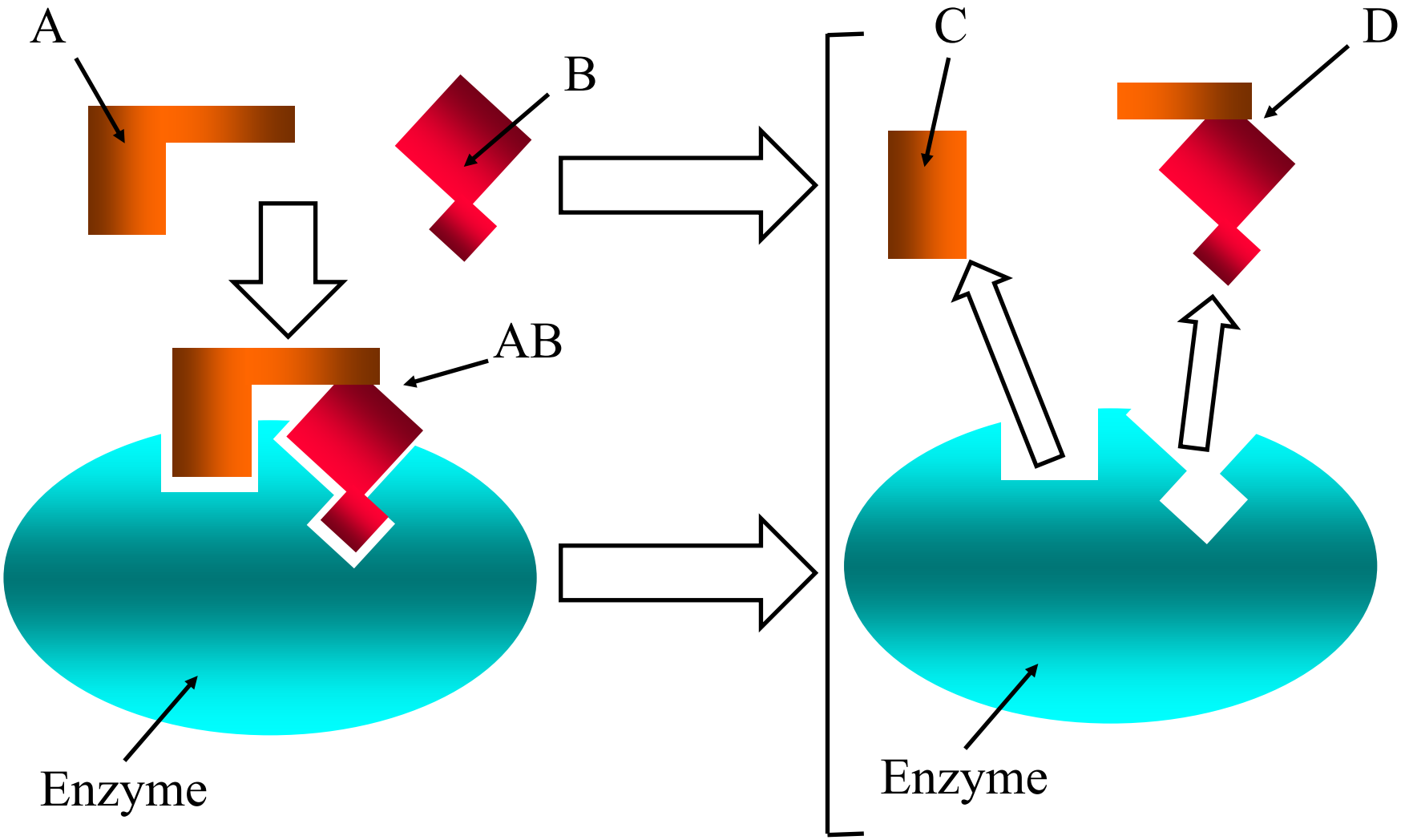
- **Immunoglobulin A (IgA)**: Primarily found in bodily secretions like saliva, tears, and mucus, IgA helps protect against pathogens on mucous membranes.
- **Immunoglobulin D (IgD)**: Found in the blood and tissue fluids, the primary role of IgD is less understood but it acts as a receptor on the surface of B cells.
- **Immunoglobulin E (IgE)**: IgE is involved in allergic responses and parasitic infections. It binds to allergens and triggers mast cells to release histamine.
- **Immunoglobulin G (IgG)**: The most abundant antibody in blood and tissue fluids, IgG is crucial for fighting bacterial and viral infections. It is the only antibody that can cross the placenta, providing passive immunity to a newborn.
- **Immunoglobulin M (IgM)**: Produced first during an immune response, IgM is the largest class of antibody and plays a key role in the initial defense against pathogens.

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



Q10

Why the enzyme is more efficient for the chemical reaction?

A. I don't know
B. Since it changes the equilibrium constant of the reaction
C. Since it changes the forward rate of the reaction
D. Since it diminishes the energy required to split the reagents
E. Since it diminishes the energy required by the reaction

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

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

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

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

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:

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

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:

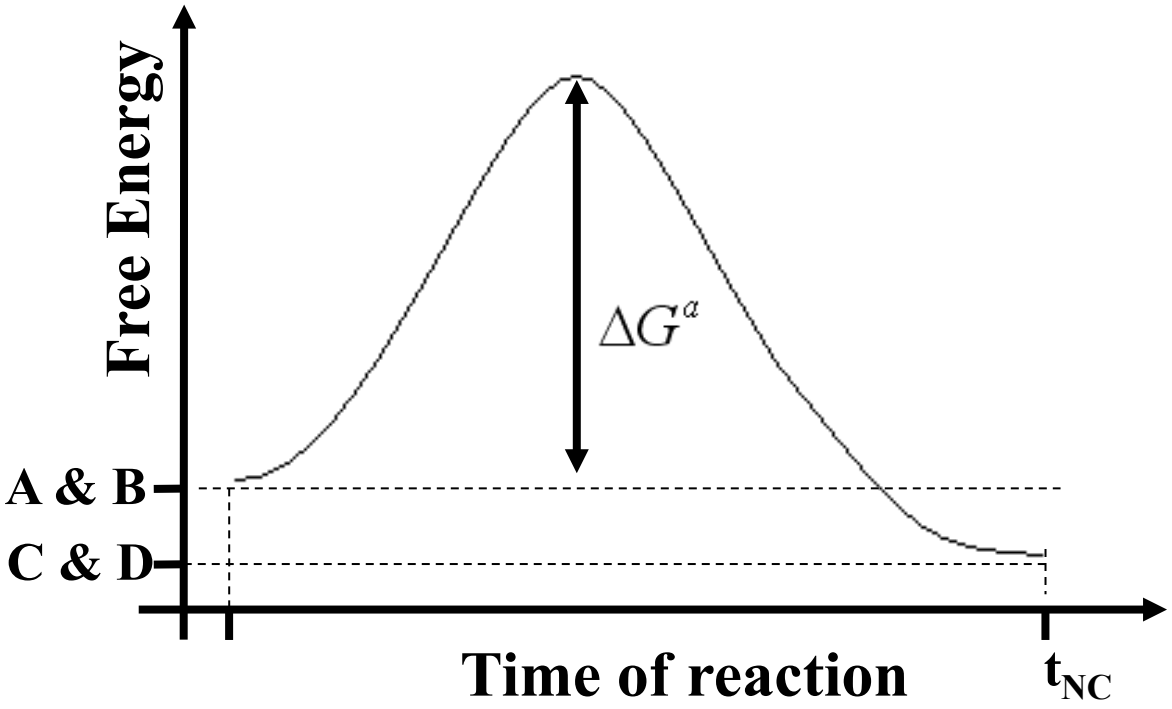
$$\Delta G^a = -RT \ln K_a$$

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:

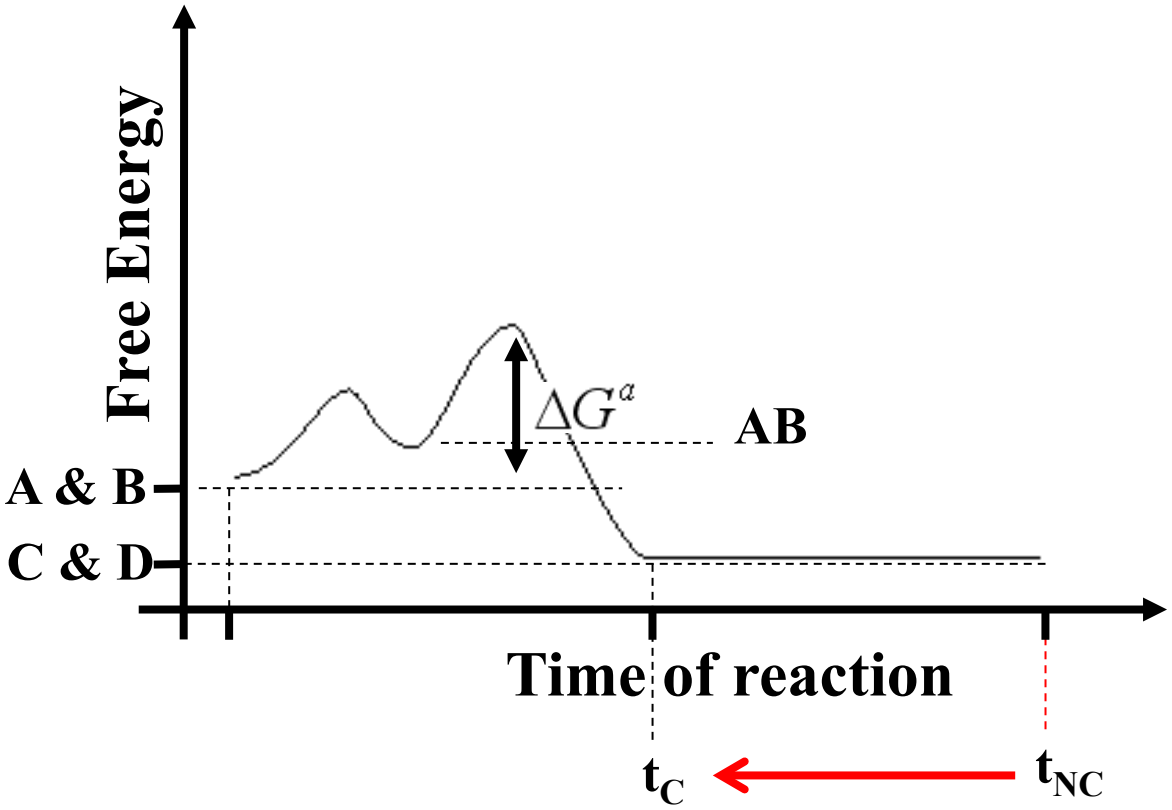
Gibbs free energy

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

Free energy of a reaction without enzymes

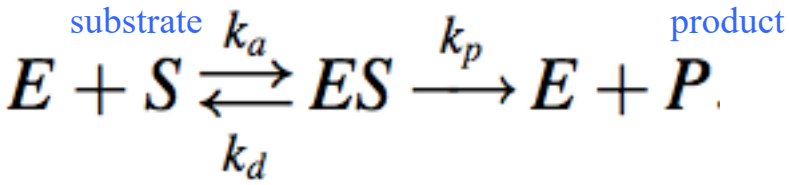


Free energy of a reaction with enzymes



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:



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:

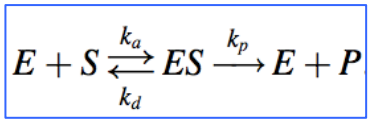
$$k_a[E][S] = (k_d + k_p)[ES]$$

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_{max}, respectively, as:

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

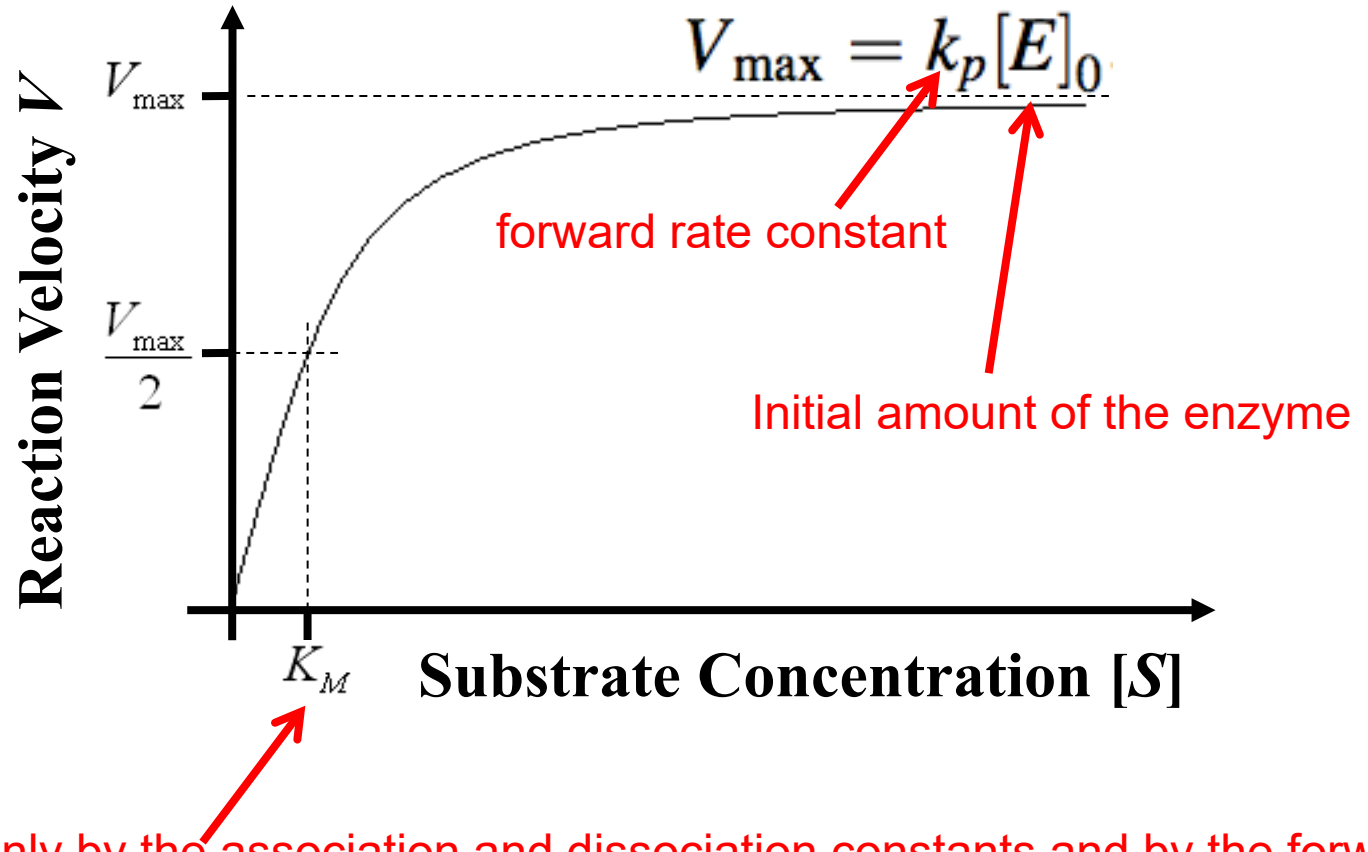
If we, then, go back to couple of previous equations:

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

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

→ *Question*: What does Km tell us about affinity?

→ *Answer*:

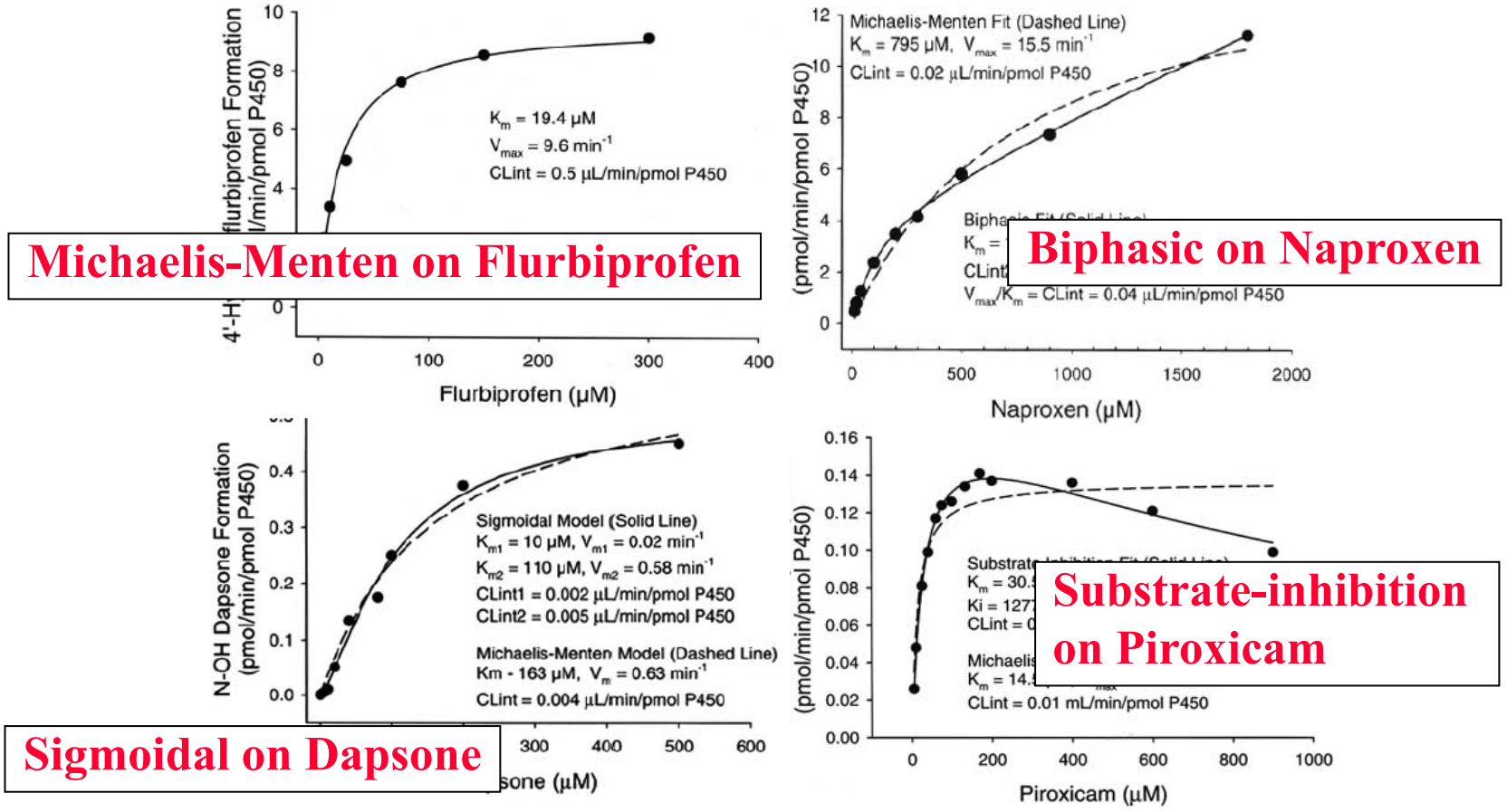
- *Low Km = high affinity (enzyme reaches half-max velocity at low substrate concentration).*
- *High Km = low affinity.*

Q11

Do all enzymes strictly follow the Michaelis-Menten kinetics?

A. Yes, all enzymes
B. Yes, not all but almost
C. No, only very few
D. No, it depends by the enzyme
E. No, it depends by the substrate

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!