Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 4

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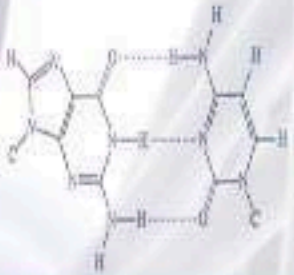
Biochemistry



Majida Al-Foqaraa'

Immunoglobulins

We started this semester talking about plasma proteins.

More than 500 plasma protein has been identified.

Plasma proteins → #Prealbumin #Albumin #Globulins #fibrinogens

globulins consist of 3 bands (alpha ,beta and gamma).

In gel electrophoresis of serum , the alpha band first appears then comes the beta band and at last the gamma band .

In this lecture we are focusing on the (gamma band) "**THE IMMUNOGLOBULINS**" as a part of the immune system , we will see their location and presence in big amounts.

-The immune system is divided into 2 subsystems :

1) Innate (non specific) : it is the native & natural system which comes with each individual from birth or even before.

- "it doesn't differ from a person to another".

-It is non-adaptive upon repeated infections , as every time it faces the foreign body it behaves the same way(doesn't have memory cells) .

-It acts through cells or products of cells and it only recognizes microbial agents .

-The innate system is composed of 2 lines of defense.

- If the foreign body crosses the two lines of defense in the innate immune system , it will come to the 3rd line of defense which is the specific immune system "the acquired one " .

Written by Asil Habash

2) Acquired (specific) : it differs between individuals ,it depends on things you are exposed to during your life , as it develops through life from facing foreign molecules .

non-specific(innate)		Specific (acquired)
First line	Second line	Third line
>Barriers: -physical: skin, hair, mucous membranes. -chemical: sweat, tears, saliva, stomach acid, urine.	-Phagocytic WBCs. -Antimicrobial proteins. -Inflammatory response.	-Lymphocytes. -Antibodies.

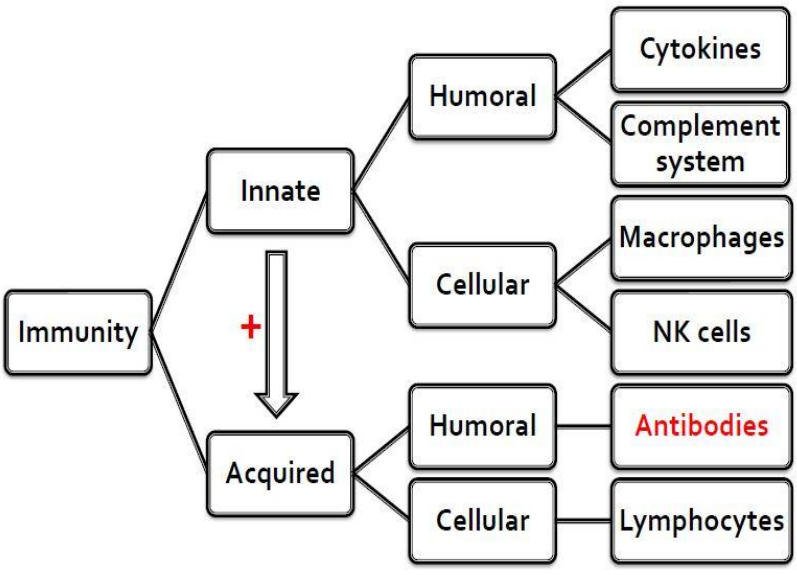
-It increases in magnitude every time you face the antigen.

-It is adaptable ; it takes it's adaptation through memory cells , which can identify the antigen when you face it again .

-It is widely specific and it recognizes microbial and non-microbial agents and even cell antigens .

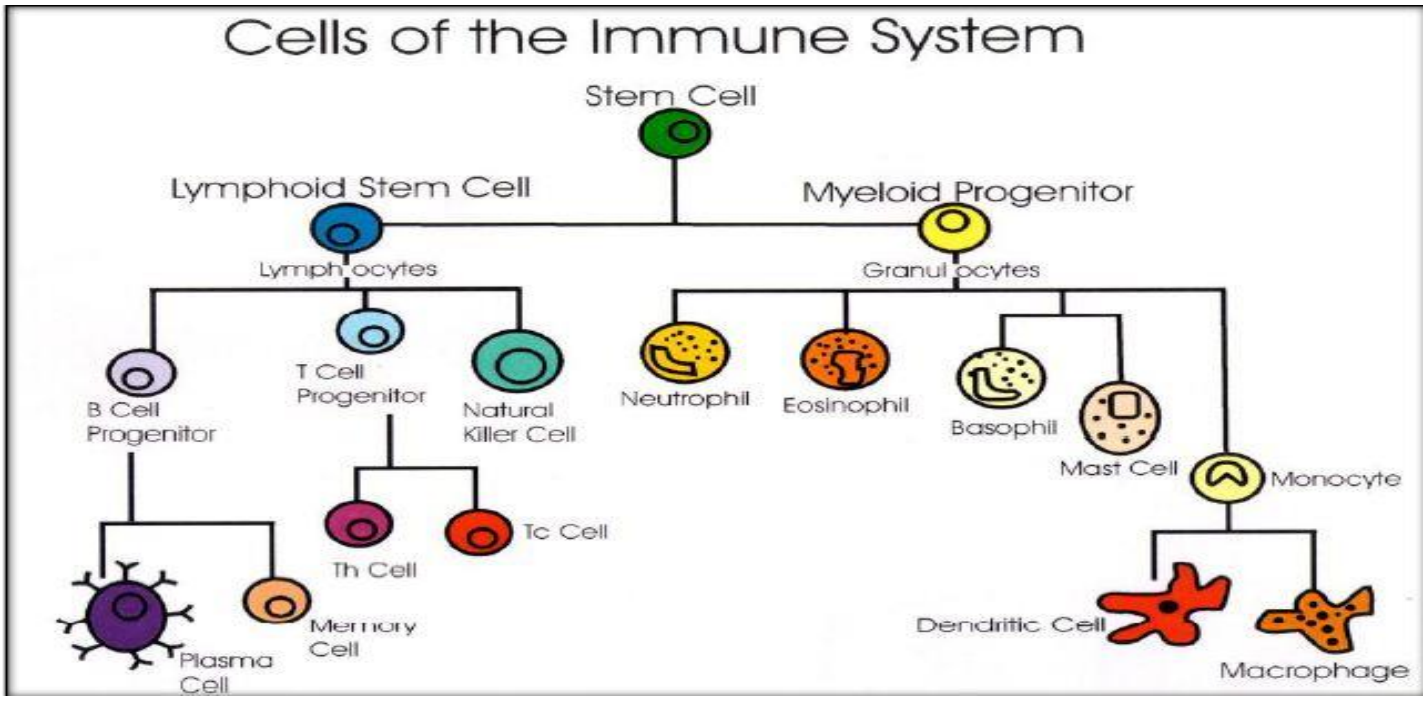
We fight foreign molecules in an acquired way either through cells or through products of cells which here are "Antibodies (as products of plasma cells)" .

In any subsystem either innate or acquired , you will face antigens through either cells or products of cells "products of cells could be either proteins or any other structure " .



So in the innate system there is cellular defense mechanism or products of cells defense mechanism "humoral immunity" .The same in the acquired immunity either humoral or cellular , the humoral is the one which acts through antibodies and the cellular acts through T-lymphocytes .

Antibodies are products of plasma cells , and this is where plasma cells are located in the big picture of the immune cells .



#Antibodies and antigens:

(immunoglobulins , Antibodies, gamma globulins) "synonyms"

-They are glycoproteins "as most plasma proteins ,except albumin" so there is a carbohydrate content connected to these antibodies .

-They are products of plasma cells (mature B-lymphocytes which are called plasma cells which produce immunoglobulins).

-They bind foreign molecules 'antigens' , this binding has specifications : the binding is highly specific and there is a high affinity between the antigen and immunoglobulin.

Q)) Why does the antigen have a high affinity to the immunoglobulin ??

A)Because it is highly specific ;

Every antigen has an antibody which has been made specially for it, so the affinity will be very high (due to high specificity).

Q)) How many kinds of antibodies do we have ?

-There are up to 100 million different kinds of antibodies in each individual.

-We do have 5 classes , but each class doesn't contain identical antibodies " for example not all IgG's are the same ".

- The number (quantity) not kinds of antibodies is infinite.

-The structure of an antibody is protein and proteins are products of genes , so if we have around 10^8 antibody , we should have 10^8 different genes , but We Only Have 40,000 gene (so this is a problem which we will discuss how they solved it next lecture).

-Antibody binds the antigen , this binding results in activation, degradation or lyses of the foreign molecule that you are dealing with (whatever the response was).

-Any material that can start an immune response is an IMMUNOGEN.

-ANTIGEN is the foreign body which binds the immunoglobulins.

*There is a certain molecular weight (MW) for the immunogen to start an immune response .If the MW is equal to or larger than this certain MW ,the immune response will be started.

-A small material(small MW) can't start an immune response even if it is foreign , unless :

1- If this material bound the albumin in your blood, it will become a big macromolecule (we consider the small material which is bound to the albumin, the antigenic determinant , now we have a whole complex which we will call a foreign molecules).

2- If it is attached to a micromolecule (like proteins or lipids or nucleic acids).

We call the small substance that can't start an immune response by itself ,**unless** it binds a micromolecule, ***HAPTEN***.

Usually when a bacterial cell or a virus cell or a foreign molecule enters your body , your body will not recognize it as whole as a foreign body , it will bind it first then know that its foreign , as when it binds it , it will bind at certain places (certain binding sites) we call each binding site between the antibody and the foreign molecule ***EPITOPE***.

So the EPITOPE is the antigenic determinant , it is what determines the foreign molecule to be antigenic or not .

-what determines any material to be antigenic or not is to have a certain sequence on its surface that can bind the antibody so on bacterial cells we expect to find 4 or 5 places where antibodies can bind .

Every epitope has a different antibody ; so if we have 5 different epitopes on the bacterial cell surface, we'll find 5 different antibodies .

-"*dinitrophenol* " can't elicit an immune response , although it is foreign , however it can when it binds the serum albumin , three of them are bound to the serum albumin . In the structure bound to albumin it can induce an immune response within .

The structure of immunoglobulins (antibodies):

It is a quaternary structure protein (consists of 4 subunits "polypeptide chain ") :-

-Two identical light chains (25 kda for each one).

-Two identical heavy chains (50 kda for each one).

We say heavy or light according to the molecular weight (high molecular weight →heavy low molecular weight→light)

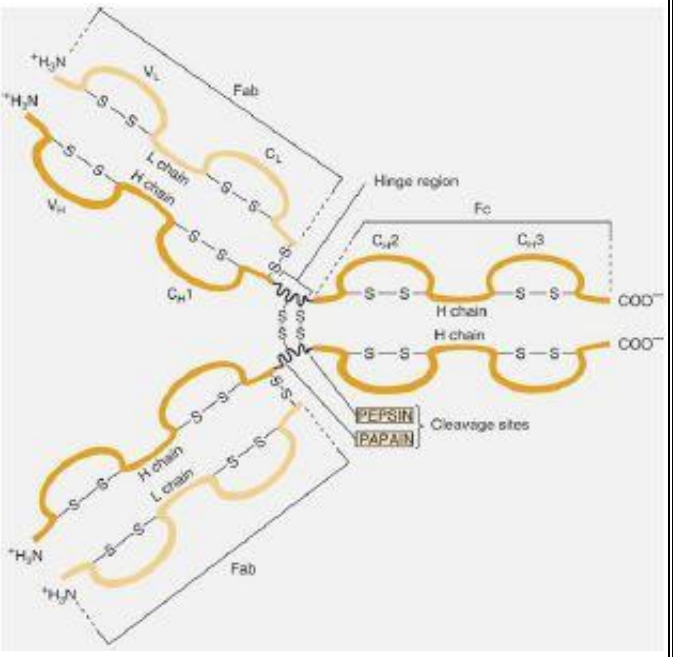
There are two bulges in the structure of the antibody.

We took in the summer that any sequence of amino acids that has a certain structure and applies a certain function is called a DOMAIN .

Immunoglobulins in general share the same function, so light chains and heavy chains are composed of domains (a supersecondary structure which has the same function in different proteins).

-The light chain has 2 domains.

-The heavy chain has at least 4 domains , sometimes it can be composed of 5 domains.



-The shape of the immunoglobulin 'antibody' is (Y-shaped) ; it has 2 tips and a stock . The antigen binds on the tips of the (Y-shaped) molecule , these tips are composed of the (N-terminal) of the light chain and heavy chain.

So both the light chain and the heavy chain are responsible for the binding of the antigen since it binds on the tips of the immunoglobulin which are composed of both kinds of chains (the dr. always asks about this in his exams)

- We want the binding of immunoglobulin with the antigen (which is a very specific binding) , and as the antigen has a different shape every time , we should make different immunoglobulins . The binding site of the antigen is on the tips of the (Y-shaped molecule) , so these tips should be changing all the time ,so as for each time to fit another antigen .

We have around 10^8 immunoglobulins which are all (Y-shaped molecules) , since they have the same shape, more or less they will have the same amino acid sequence (we can't obtain the same shape unless the amino acid sequence is more or less the same) .
"The final structure of a protein is preserved in its primary structure "
So if the amino acid sequence is different in each immunoglobulin they won't all have the same (Y-shape)
So all immunoglobulins have almost the same amino acid sequence , and all immunoglobulins bind with huge different kinds of antigens , so How could this happen ??
Here comes the purpose of domains ,the *light chains* consist of two domains (one is variable and the other is constant , 1/2 constant : 1/2 variable) whereas the *heavy chain* consists of 4 domains (3 constant and one variable , 3/4 constant : 1/4 variable)
-The antigen binds at the (N-terminal of each the light and heavy chains) " the variable domains which differ in their amino acid sequence " , the other domains (constant) don't change their amino acid sequence , so we maintain the same shape .
The other thing is that they have a high content of CYSTINE , so there is a lot of disulfide bridges .
The structure of Any protein which has a high content of disulfide bonds should be preserved so it's function doesn't get affected .

There are disulfide bridges in the variable domains (the light and heavy) , and within each domain there is a disulfide structure (between the heavy and light chains and between the heavy chains themselves).
-The high content of disulfide bridges is to maintain the structure of the protein and preserve its shape , so it can provide its function each time at the same way.
-Y-shaped molecules(consist of 2 tips and one stock) , the 2 tips bind the stock at the ***HINGE REGION*** which provides flexibility and free movement (its located between the first constant region of the heavy chains and the second one).

Since the hinge is free to move and it provides flexibility , we can observe that the structure of the hinge is a LOOP (an irregular sequence of amino acids with no defined secondary structures which can move easily).
So the loops and turns are supersecondary structures that connect two secondary structures together , if the number of amino acids is 4 or less it's a turn , if it's more than that it's a loop .
loops are connected together by disulfide bonds though they are still free to move
But why do we need free movement ? why do we need the two tips of the y-shaped molecule to be flexible ?

In the purpose of distance ; it allows free movement of the antibody , so if the epitopes on an antigen are close together the tips of the antibody will come closer . and if the epitopes on the surface of the antigen were far from each other the two tips of the antigen will get far from each other too, so they can bind the epitopes . This ability is due to the flexibility provided by the hinge region "which is a loop".

#since the tips of the Y-shaped molecule are responsible for binding the antigen , we call them Fab "antigen binding fragment" , the fragment which is responsible for the binding of the antigen .
So in each immunoglobulin we have two ANTIGEN BINDING FRAGMENTS (Fab) and (1)CRYSTALIZABLE FRAGMENT (Fc).(they called it "crystalizable" because during purification it appears as crystal)

Historically when they studied any protein , they used to make studies on it ; how to break it down , how to deal with it, it's amino acid sequence etc..

-HOW CAN WE BREAK DOWN A PROTEIN ??

By proteases

-Historically they have fragmented immunoglobulins by two kinds of proteases :

1). Pepain 2).pepsin

#all proteolitic enzymes will cut at the hinge region (which has the least disulfide bonds) .

When we try to break down the antibody at portions other than the hinge region we can't fragmentate the protein ,as its parts will keep holding themselves by disulfide bridges between them , eventually we are not breaking down the molecule . We can break it into separate fragments by the Hinge region.

The hinge region contains disulfide bridges , proteases either cut after the disulfide bridges toward the (N-terminal domain) or after the disulfide bridges toward the (carboxylic side) , SO WHAT IS THE DIFFERENCE BETWEEN THE TWO PROTEOLITIC FRAGMENTATION PROCESSES IN THE RESULT OF FRAGMENTATION ?

#If we break the molecule after the disulfide bridges (at the hinge region) toward the amino side of the domain (as PAPAIN does) we will get : -two antigen binding fragments "FAB" and –one crystallizable fragment "FC".

#If we break the molecule after the disulfide bridges (at the hinge region)toward carboxylic side of the domain (as PEPSIN does) we will get :-- one antigen binding fragment "FAB"(as the two antigen binding fragments come out of the fragmentation as one unit , because the disulfide bridges are still there). And – one crystallizable fragment "FC".

(((the difference between papain and pepsin fragmentation is a common question in exams ^^)))

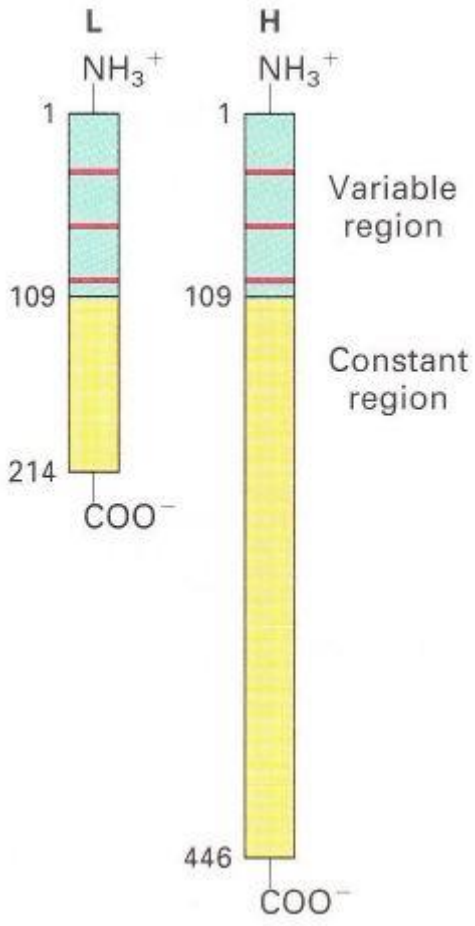
***linear (primary) structure of each light and heavy chains :

-the light chain consists of two domains (one is constant and the other is variable)

-the heavy chain (1/4 is variable and 3/4 are constant)

-Although the variable domains are responsible for the antigen binding process ,still not the whole variable domain binds the antigen , only a specific part of it binds the antigen . so we don't need the amino acid sequence in the whole variable domain to keep changing all the time .

There are certain places in the amino acid sequence of the variable domains that keep changing all the time(3 sequences of amino acids in the variable domain of the light chain and 4 amino acid sequences in the variable domain of the heavy chain),so they can change the region they bind to the antigen and they can keep binding to new antigens . The rest of amino acids in the variable domain are constant , more or less .



See the three stretches (sequences) of amino acids in the heavy or light chain , the length of each one is 7-12 amino acids , these are which keep changing all the time. The others are more or less constant , so they can finally form the same shape (domain).
So in the light chain one half of the domains is constant and the other half is variable
In the heavy chain 1/4 is variable an 3/4 are constant .

-Within the variable domains (amino acid sequences) there are THREE sequences (stretches) that keep changing all the time we call them HYPERVARIABLE REGIONS the others are more or less constant .

Antibodies don't only bind antigens , as the main purpose of this binding is to let the body know that there is a foreign body, so it can start a response and produce new antibodies . So logic wise antibodies should bind cells, or proteins, so it can transmit the message to the body.
-How is this message transmitted ?
The antigen binds on the tips of the Y-shaped molecule then this molecule binds through the FC portion to cells (immune cell) or products of cells (proteins which have the ability to start an immune response "COMPLEMENT PROTEINS ") so the body will understand that there is something foreign in it and it will start its immune response .

*The binding between the antibody and the antigen could be anything (electrostatic , hydrogen , van der waal's, hydrophobic) except covalent ; because if it was covalent it will act as a toxin to the antibody and it will block the antibody's work . The binding is temporary so it can detach from it again .

-We have an infinite number of antigens (foreign bodies) that bind with an infinite number of antibodies , as each antigen has its new antibody . So the binding between the antibody and the antigen is infinite (WHY ?) ; because the amino acids of the antibody (that part of the variable domain) are changing all the time .
-Whereas the binding between the antibody and the cells or proteins is finite , as every antibody is going to bind with a certain cell or one of the complement proteins (WHY IS THIS BINDING FINNITE ?); because the amino acid content of the crystallizable fragment (FC) is constant "it slightly changes",so its binding will be finite .

IMMUNOGLOBULINS – INTERACTIONS

After the antigen binding fragment (Fab) binds , it can :

1- Detect and bind the antigen .

2-Block the active sites of toxins .

3-Block interactions between host and pathogen .

After (Fab) binds the antigen , now the immunoglobulins needs to transmit the message either to cells or to proteins , so (FC) can induce an inflammatory function associated with cells or complement proteins and then it induces the cell signaling mechanism.

-After it binds cells or proteins, at the end it will induce within the cells a second messenger system .

Structure of the immunoglobulin :

When we look at the heavy chain of the immunoglobulins we find that it consists at least of 4 domains(3 constant and 1 variable, and between the first and the second constant domains is the HINGE) , we say "at least 4 domains " because there could be a fifth domain in the hinge region. This fifth domain is found in two immunoglobulins (igE and igM)

-The presence of a domain at the hinge region decreases the free movement of the tips of the antibody .

The heavy chain is a polypeptide chain which is a product of gene .we have 5 different genes producing 5 different heavy chains .

As the name implies

Gene	Antibody
α	igA
ε	igE
γ	igG
μ	igM
∫	igD

So every gene gives a different heavy chain , but this gene is not responsible for the whole amino acid sequence in the heavy chain , it just determines the constant region , because the variable domain keeps changing all the time so the sequence of the gene should change each time .

So the gene determines the class of antibody by determining the constant region of the *heavy chain*.

-The light chain consists of two domains (one variable and one constant), the constant domain is what determines the kind of the light chain , because the variable domain keeps changing according to antigen it is facing .

The constant domain is a product of two genes :1)kappa 2)lambda (never a mixture in the same antibody)

Be careful : it is the constant region of the heavy chain which determines the antibody class . The constant region of the light class only adds to diversity to the antibodies.

Constant regions of the light chain are either kappa or lambda(two different genes) .within the same immunoglobulin (for example igG) if one arm of the Y-shaped molecule had a kappa type , the other arm should have a kappa type "you'll never find a mixture in the same immunoglobulin of one light chain to be of a kappa type and the other of the lambda type"

(never a mixture)

The full shape of the immunoglobulin molecule (the Y-shaped molecule) : two FAB 'S and one FC with disulfide bridges connecting them together.

-The result of pepsin cleavage : - (two fragments) one crystallizable fragment and one $(Fab)^2$ {which consists of two antigen binding fragments which are connected to each other by disulfide bridges} .

-The result of papain cleavage : (three fragments)one crystallizable fragment and two separate antigen binding fragments .

-Each the light and the heavy chains are synthesized separately from each other, there are even cancers affecting one chain (you may find a high increase in the light chain without the heavy chain) , because they are synthesized separately

Remember that:

- all immunoglobulins are glycoproteins (the carbohydrate content binds the crystallizable fragment (FC) "the stock , within the two heavy chains , it has nothing to do with the light chains "

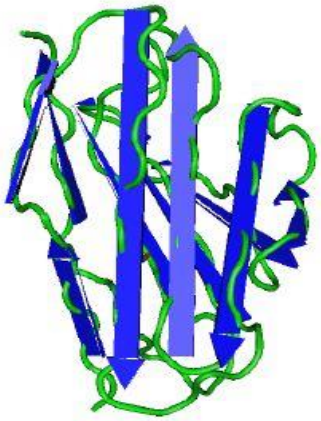
- the structure of the hinge region is a loop structure , it provides free movement .

The immunoglobulin fold :

A barrel is a supersecondary structure which is formed by folding of beta-sheets in the shape of a cylinder , and there are disulfide bridges at the middle (center) of the cylinder that maintain the cylinder shape and its 3-d structure without changing .

In each domain in the immunoglobulins "light or heavy " is a beta-barrel .

All immunoglobulin domains are barrels "immunoglobulin fold ".


Single V_L domain

If we cut this cylinder shape from one side and open it (as a paper) we will find beta sheets connected to each other and disulfide bridges at the middle.

In the light chain at the constant domain we have 7 beta sheets and 8 beta sheets at the variable domain .

I recommend passing through the slides quickly , Best of luck :D
و تذكروا : من لم يتحمل ذل العلم ساعة , بقي في ذل الجهل أبدااا

Done by : Asil Habash .

Corrected by : Mohammed Nawaiseh .