Antibodies

Introduction

Antibodies (globulins) constitute a major component of serum

-- The **normal serum protein** level is **6 to 8 g/dl** (deciliter or 100 ml).

-- **Albumin** makes up **3.5 to 5.0 g/dl**, and the remainder is the total globulins.

-- **Serum globulin**: **2.0 to 3.5 g/dL**

IgM: 75 to 300 mg/dL

IgG: 650 to 1,850 mg/dL

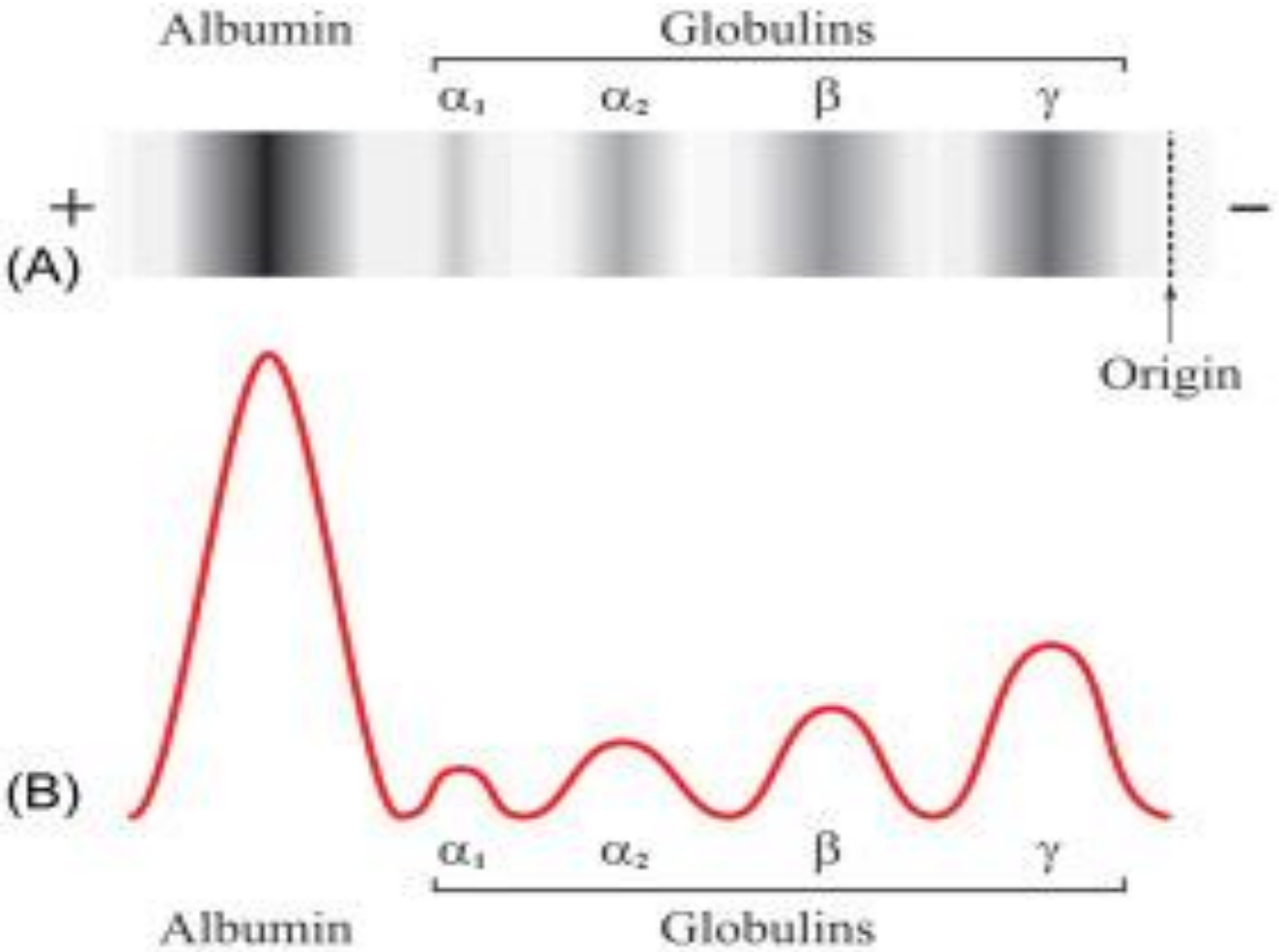
-- Globulins are **much larger** in size as compared to albumin.

-- There are 4 types of globulins:

α1, α2, β and **γ**

-- **Antibodies** (immunoglobulins) are **usually γ (gamma) globulins** which are **largest** in size among globulins**.**

-- But some immunoglobulins are not gamma globulins, and some gamma globulins are not immunoglobulins.


Albumin
Globulins
α1
α2
β
γ
+
(A)
−
Origin
(B)
α1
α2
β
γ
Albumin
Globulins

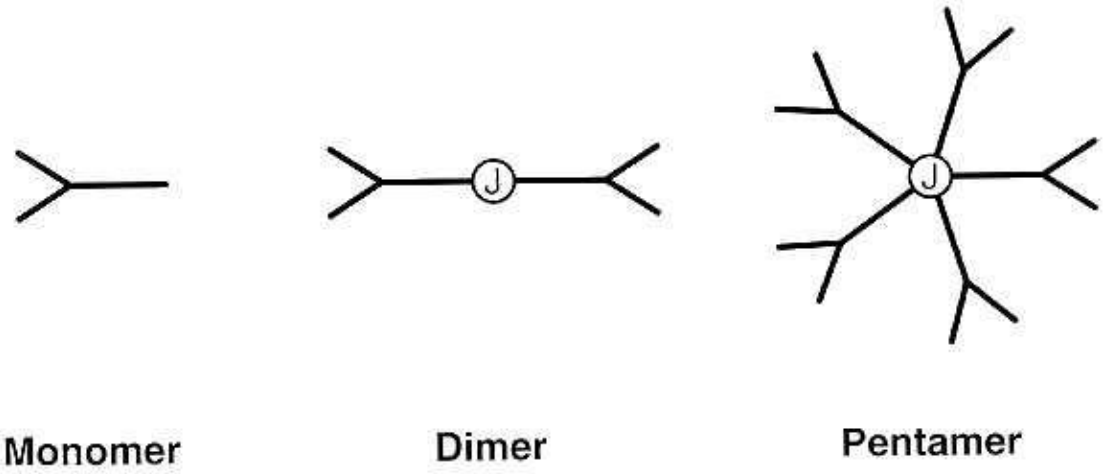
- Antibodies are found:

  (i) in the **plasma**

  (ii) **bound to specific receptors on immune cells** for the **invariant (Fc) region** of immunoglobulin.

- They are also found in **secretory fluids** such as mucus, milk and sweat.

- They may be present as a **single** unit or **multiple** units.

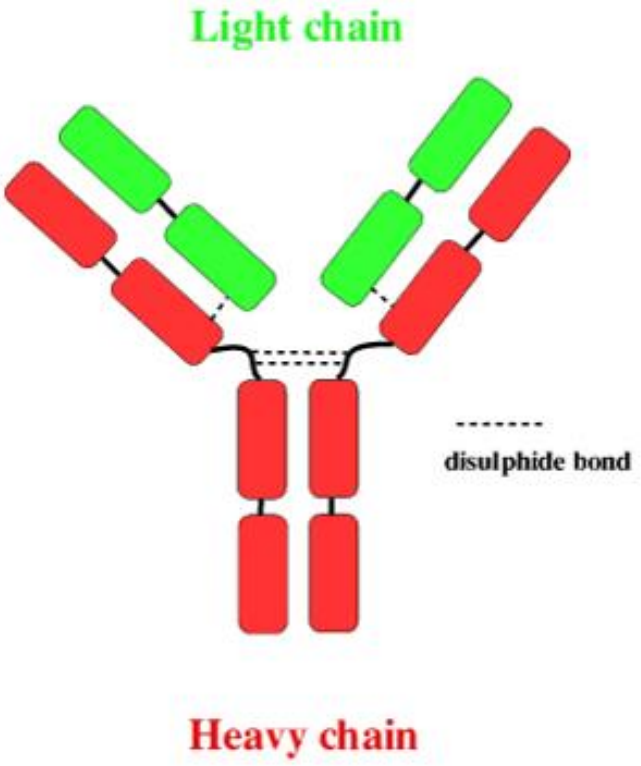


Antibody Structure

- Antibodies (with a few exceptions) are **Y shaped**. The lower part is called **Fc** and upper **two pronged** part is called **Fab.**

- All antibodies have a similar overall structure with **two light** and **two heavy** chains.

- These chains are linked by both **covalent (disulphide bridges)** and **non-covalent forces**.

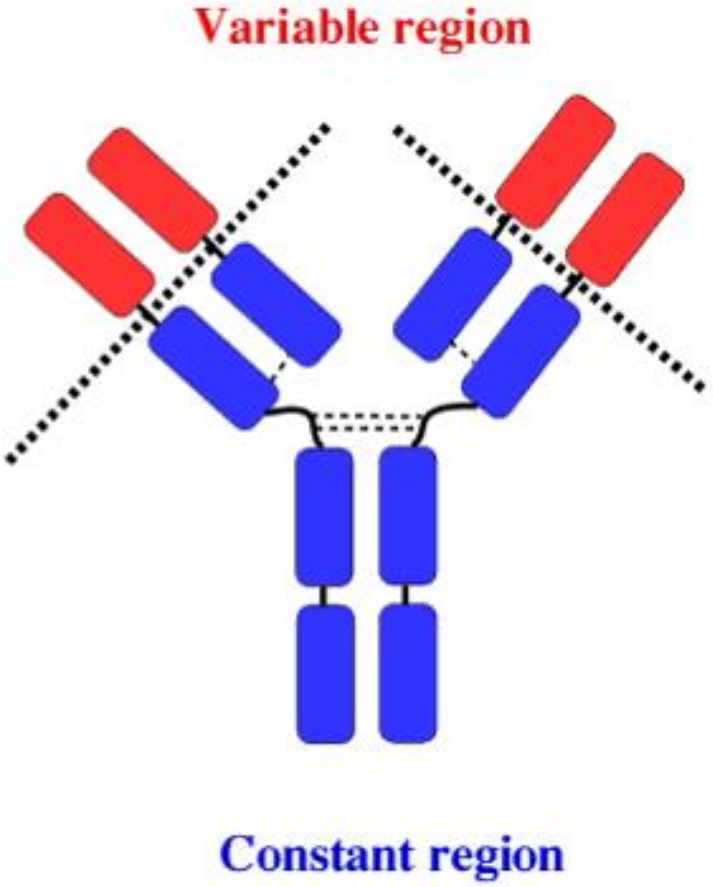


Immunological structure:

- Antibodies are made up of **V (for variable)** and **C (for constant)** regions

- The **antigen binding activity** is found in the **V region**

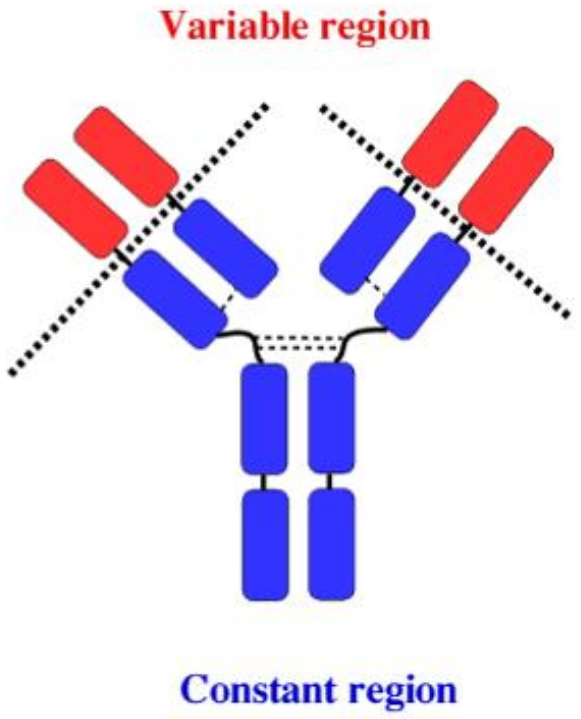
- The **complement fixing** and **Ig receptor** (on cellular surface) **binding activity** is found in the **C region**.



- The variable region of **Fab** is called **Fv**.

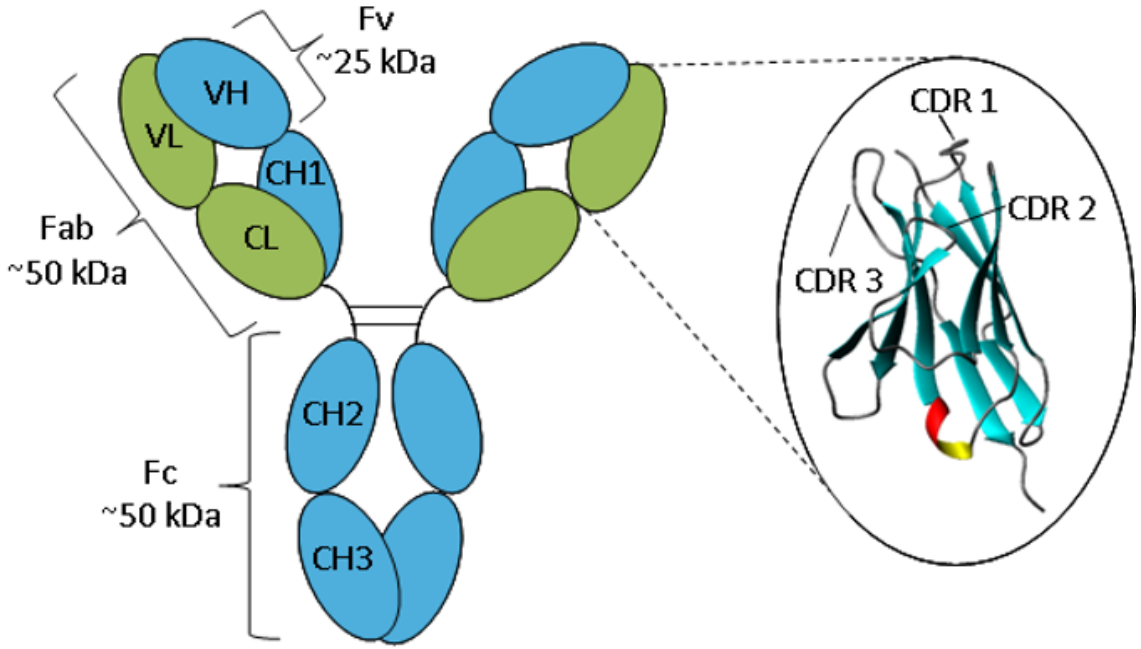
- The immunological variation is mostly **restricted to three regions** in **Fv** within the N-terminal domain of both the heavy (H) and light (L) chains.

- In the **3-dimensional structure** these regions form **loops** at the surface of the antibody molecule and these provide the **binding surface** between antibody and antigen.

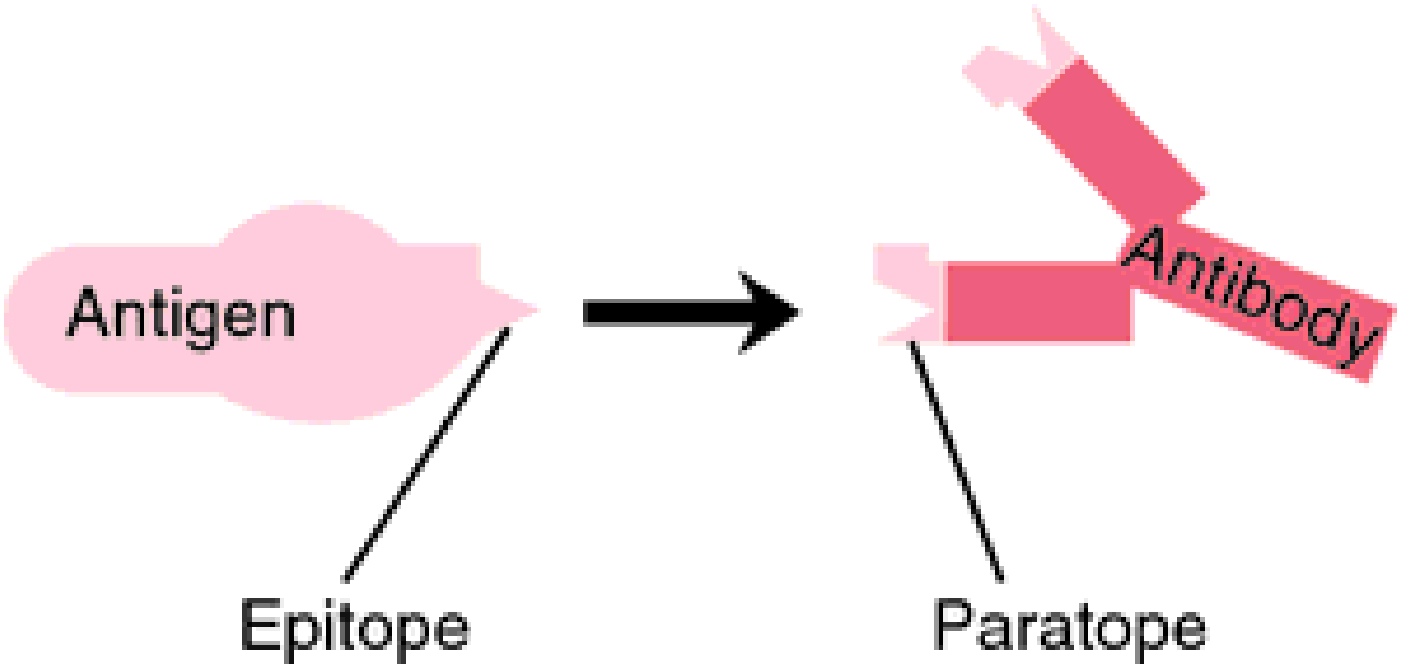


- Because these regions determine the '**fit**' between antibody and antigen they are referred to as the **complementarity determining regions** or **CDRs.**

- **CDR3** shows more variation that either **CDR1** or **2**.



- Collectively these CDRs form **paratope**.

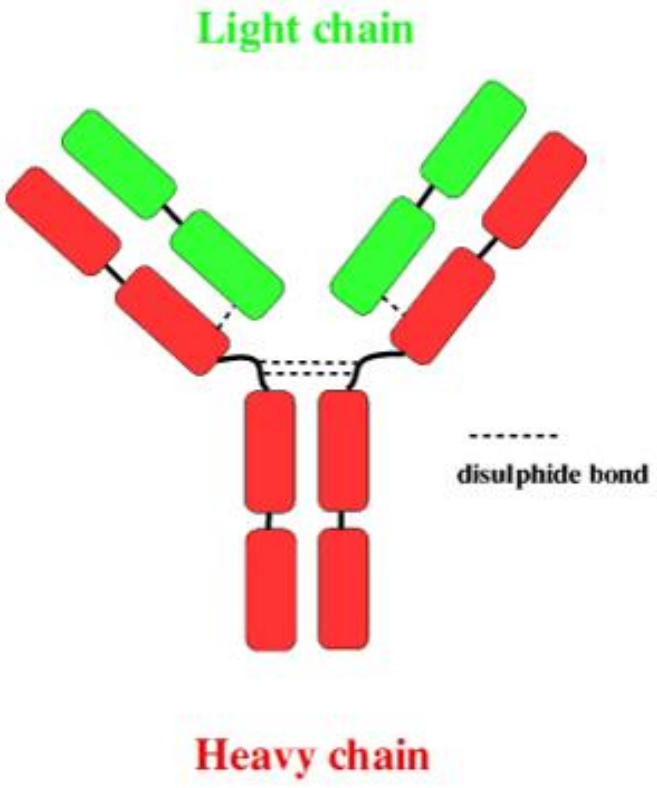


A set of CDRs constitute a paratope

Heavy and light chains:

2 heavy (H) and 2 light (L) chains:

- H: ~50 kD, five classes (m,d,g,a,e)
- L: ~25 kD, two classes (k, l)



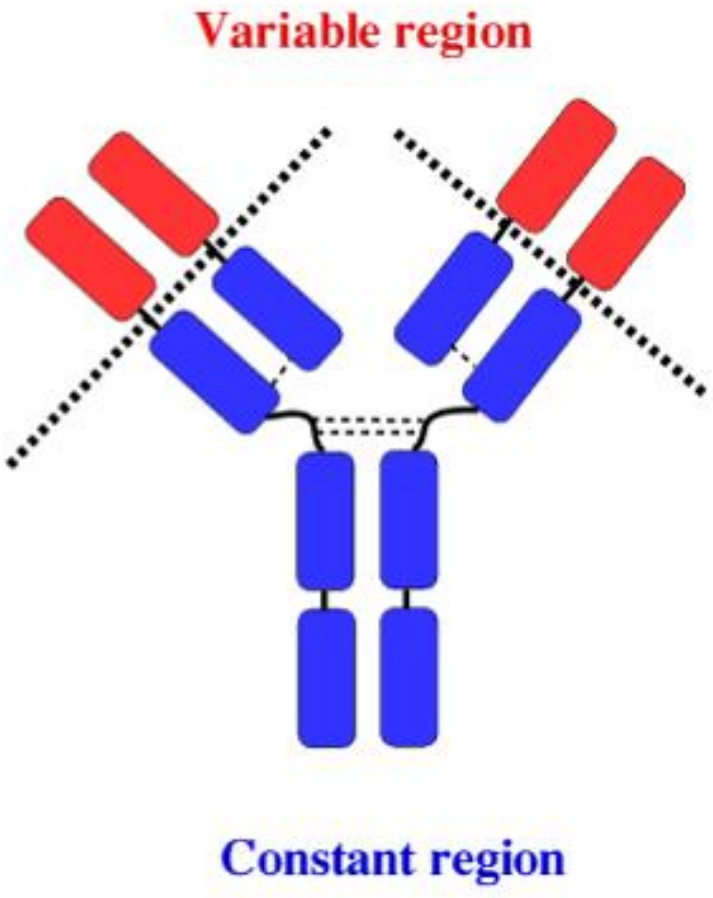
Two activities:

- Antigen binding (H + L)
- Biologic (effector) function (H only) it includes complement binding and attachment to cell surface

Each chain has one **Variable (V)** domain

There are one or more **Constant (C)** domains.

Flexibility is provided by **hinge** region.



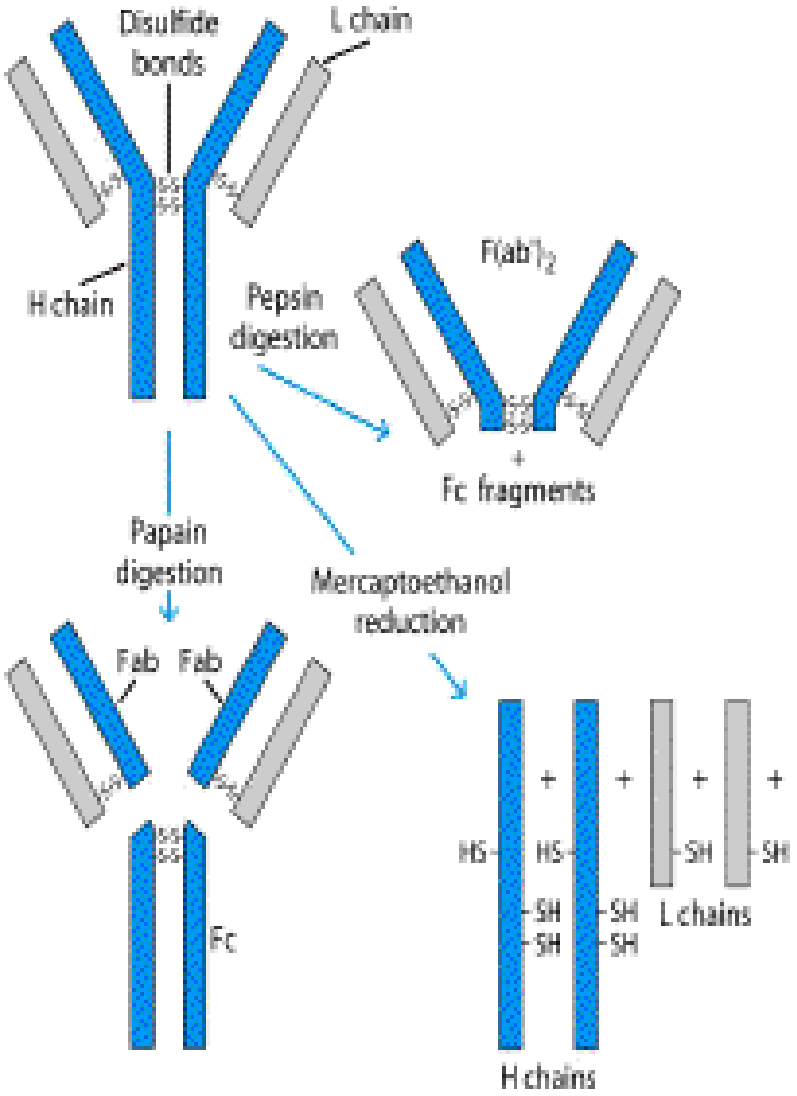
Enzymatic characterization of antibodies

Papain yields **F(ab)** and **Fc** fragments:

- **F(ab)** = “fragment, antigen binding”
- **Fc** = “fragment, crystallizable”

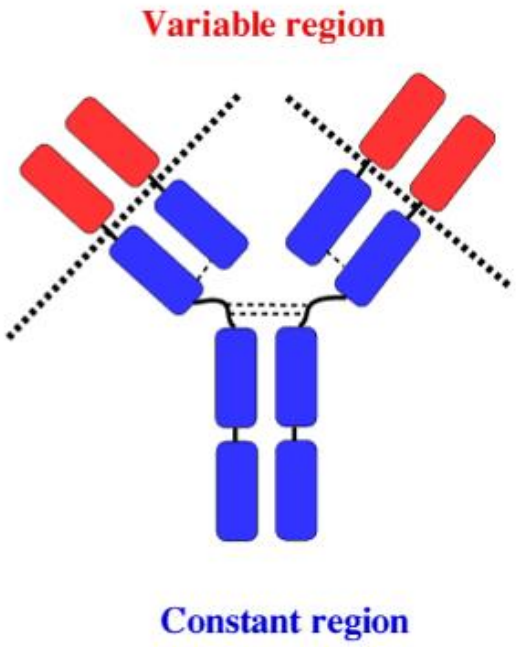
Pepsin yields F(ab’)2 + other small fragments

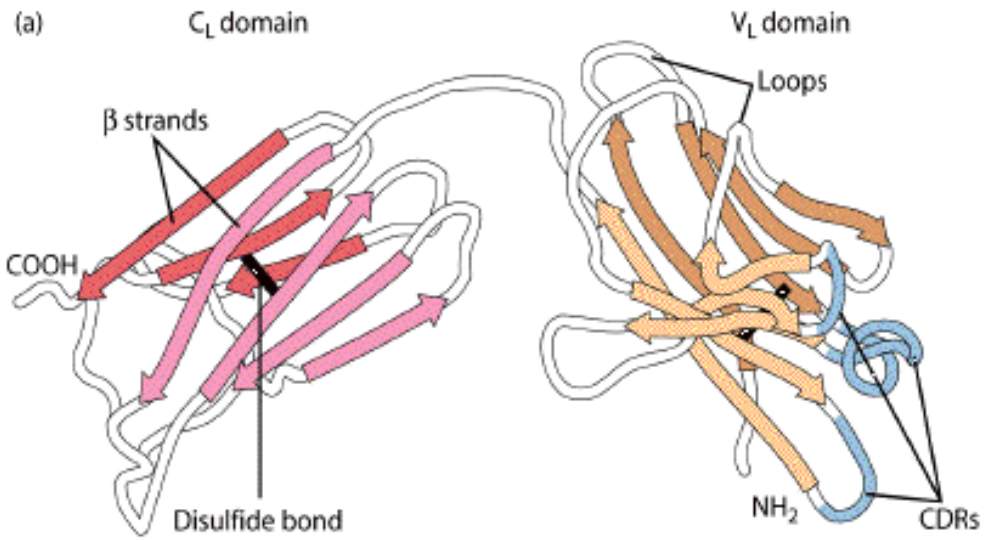
β-mercaptoethanol reduces disulfides and yields two polypeptide chains (H and L)


Disulfide bonds
L chain
H chain
Pepsin digestion
F(ab')2
+
Fc fragments
Papain digestion
Mercaptoethanol reduction
Fab
Fab
Fc
HS
HS
SH
SH
SH
SH
+
+
+
+
L chains
H chains

Crystallographic analysis of antibodies

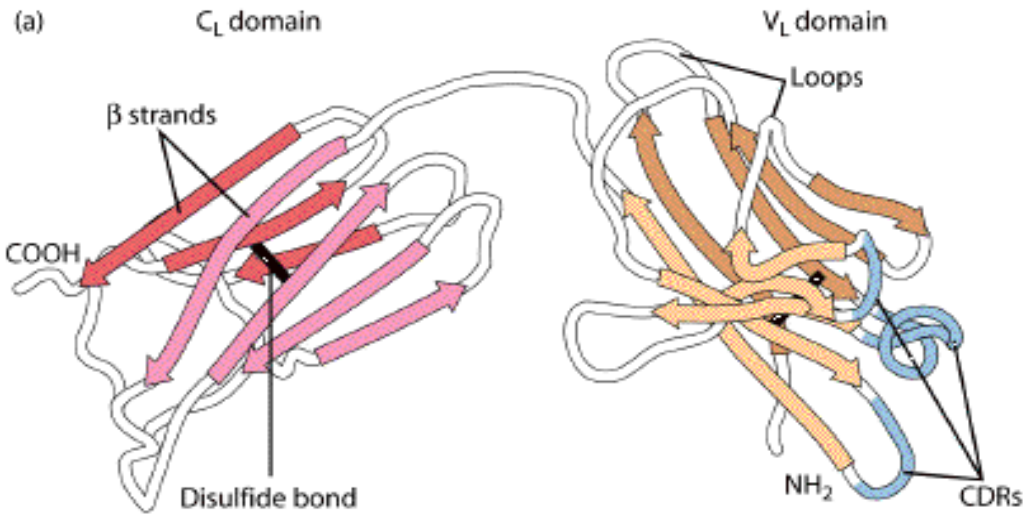
-- Each domain contains a characteristic **“immunoglobulin fold”**





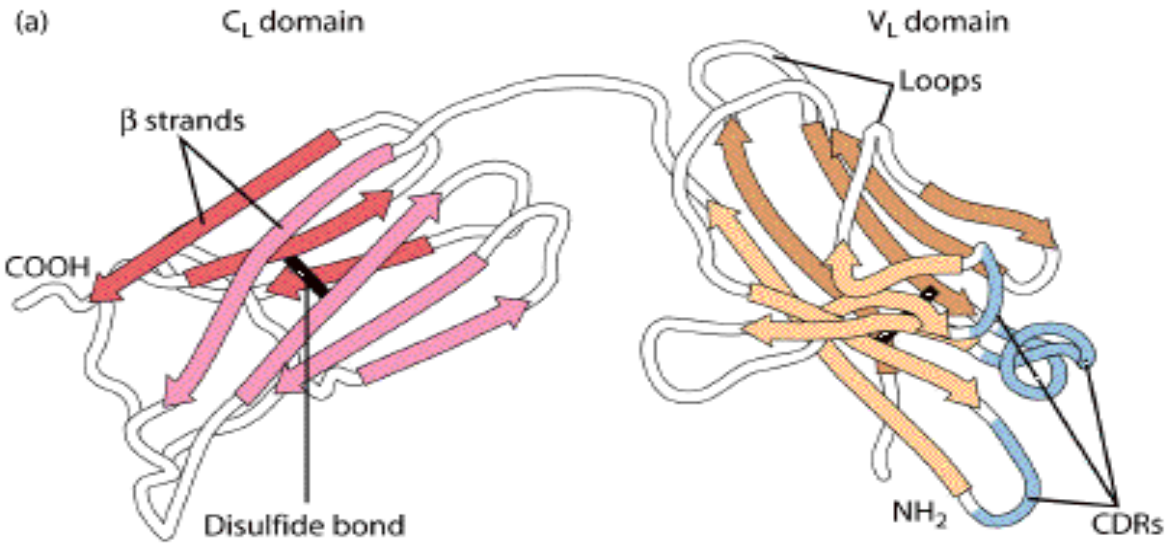
-- Each immunoglobulin fold has two layers of **β-pleated (twisted) sheets**

-- Each sheet contains **anti-parallel β-strands**.

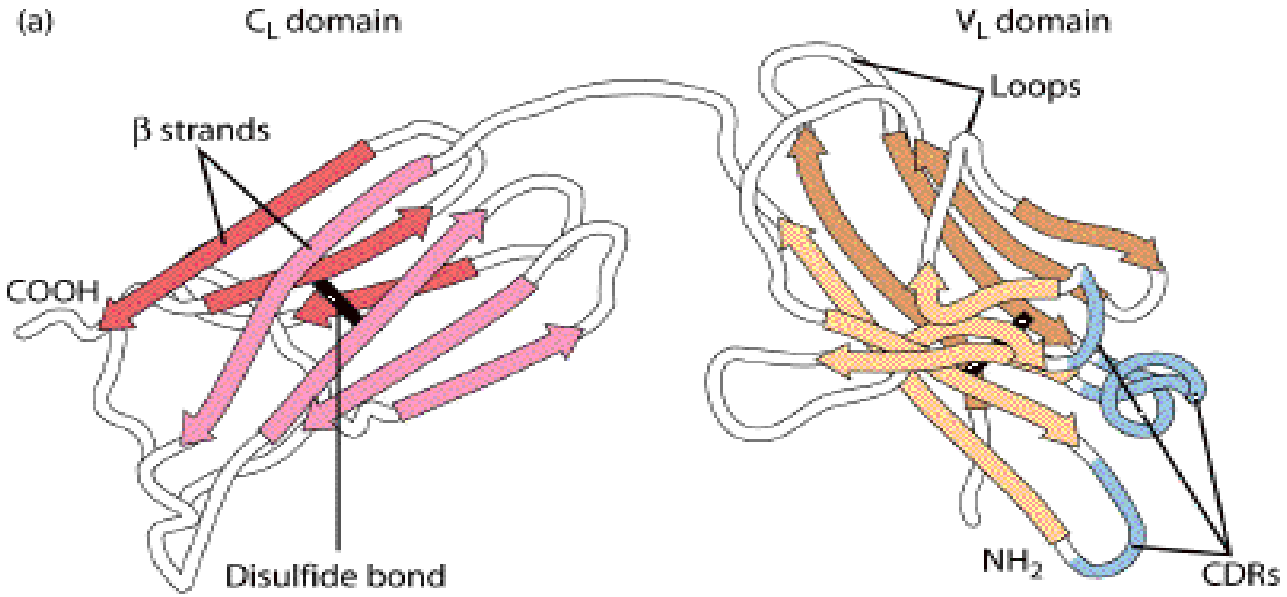


-- Each β strand is connected by **loops** of various length.

-- Three loops found in the **V_L domain** comprise the **CDRs.**



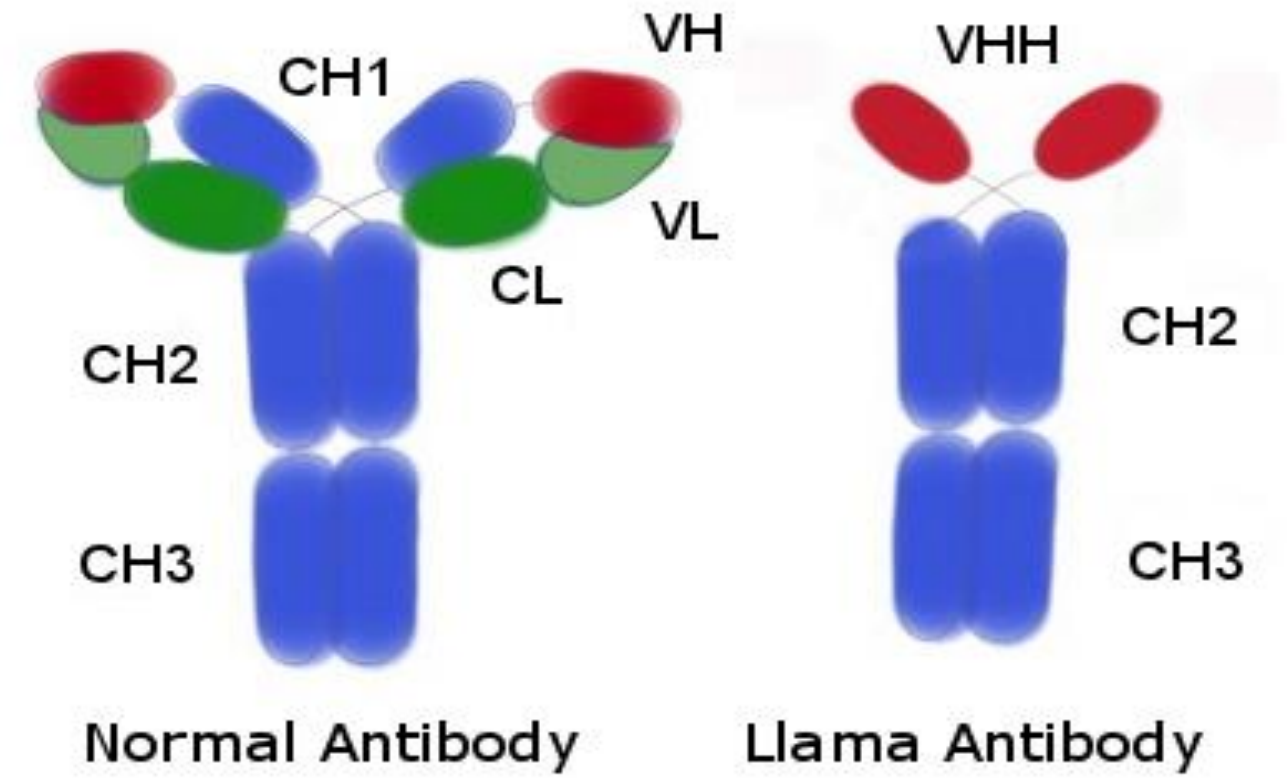
-- The two β-pleated sheets are **stabilized** by hydrophobic interactions and a disulfide bond acting as a bridge.



Mini-Antibodies

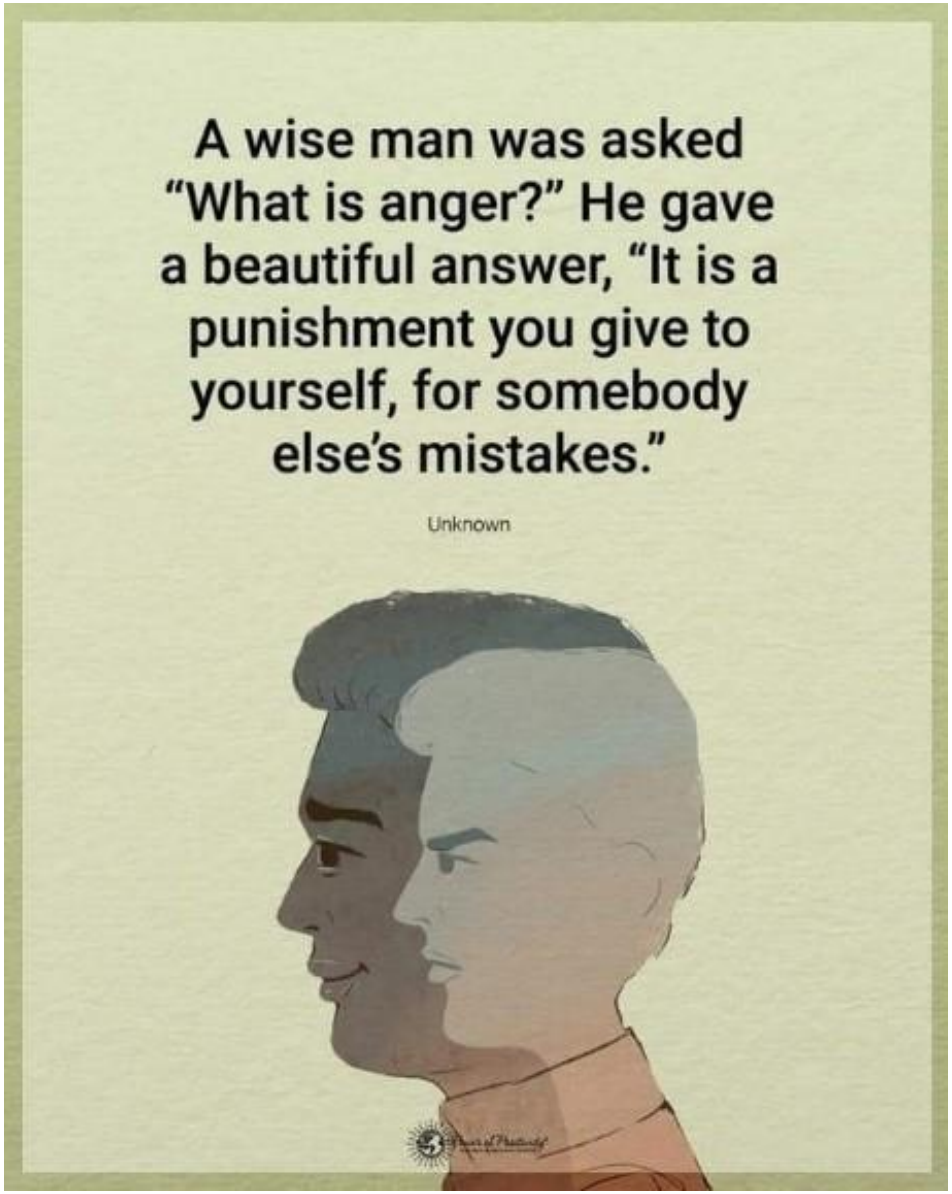
- **Sharks, llamas, camels**, and their relatives produce **unusual, diminutive antibodies** that are only **about half the size** of the conventional antibodies.

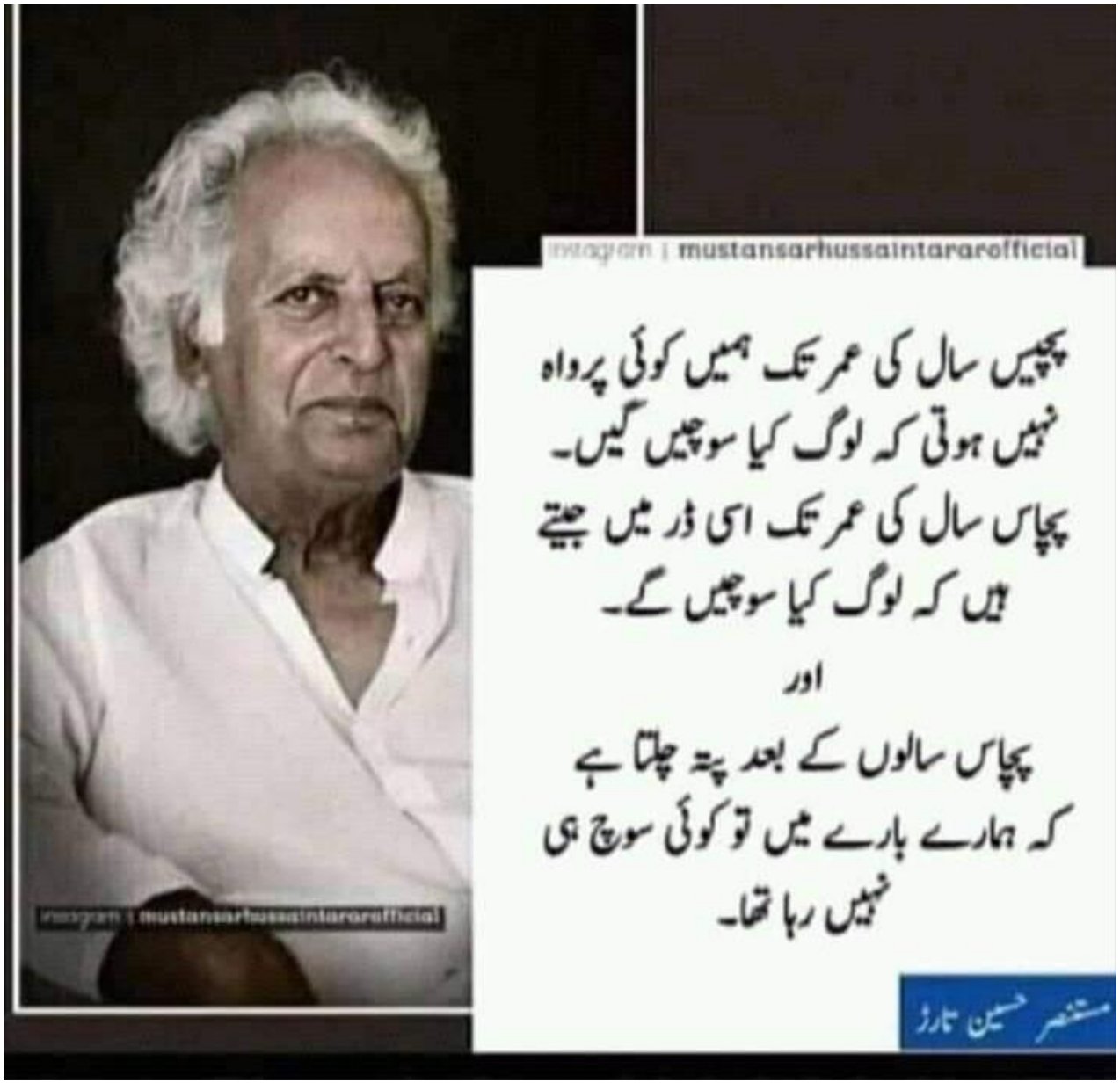
- These mini-antibodies have roughly the **same shape** as the conventional antibodies but are **missing the light chains**.



- These mini-antibodies can work **inside cells**, and their size allows them to **go deep into tissues**, which is difficult for conventional antibodies.

- It is hoped that as we know more about these mini-antibodies, their **real role in animal's life** and **potential use as a tool in laboratory** will become established.


A wise man was asked
“What is anger?” He gave
a beautiful answer, “It is a
punishment you give to
yourself, for somebody
else’s mistakes.”
Unknown


instagram | mustansarhussaintararofficial
پچیس سال کی عمر تک ہمیں کوئی پرواہ
نہیں ہوتی کہ لوگ کیا سوچیں گیں۔
پچاس سال کی عمر تک اسی ڈر میں جیتے
ہیں کہ لوگ کیا سوچیں گے۔
اور
پچاس سالوں کے بعد پتہ چلتا ہے
کہ ہمارے بارے میں تو کوئی سوچ ہی
نہیں رہا تھا۔
مستنصر حسین تارڑ
instagram | mustansarhussaintararofficial

At the residence of Mustansir Hussain Tarar Sb with Dr. Tabassum Kashmiri when he invited me after reading my book “Urun Khatola”

Dr. Abdul Haque


Thank You!