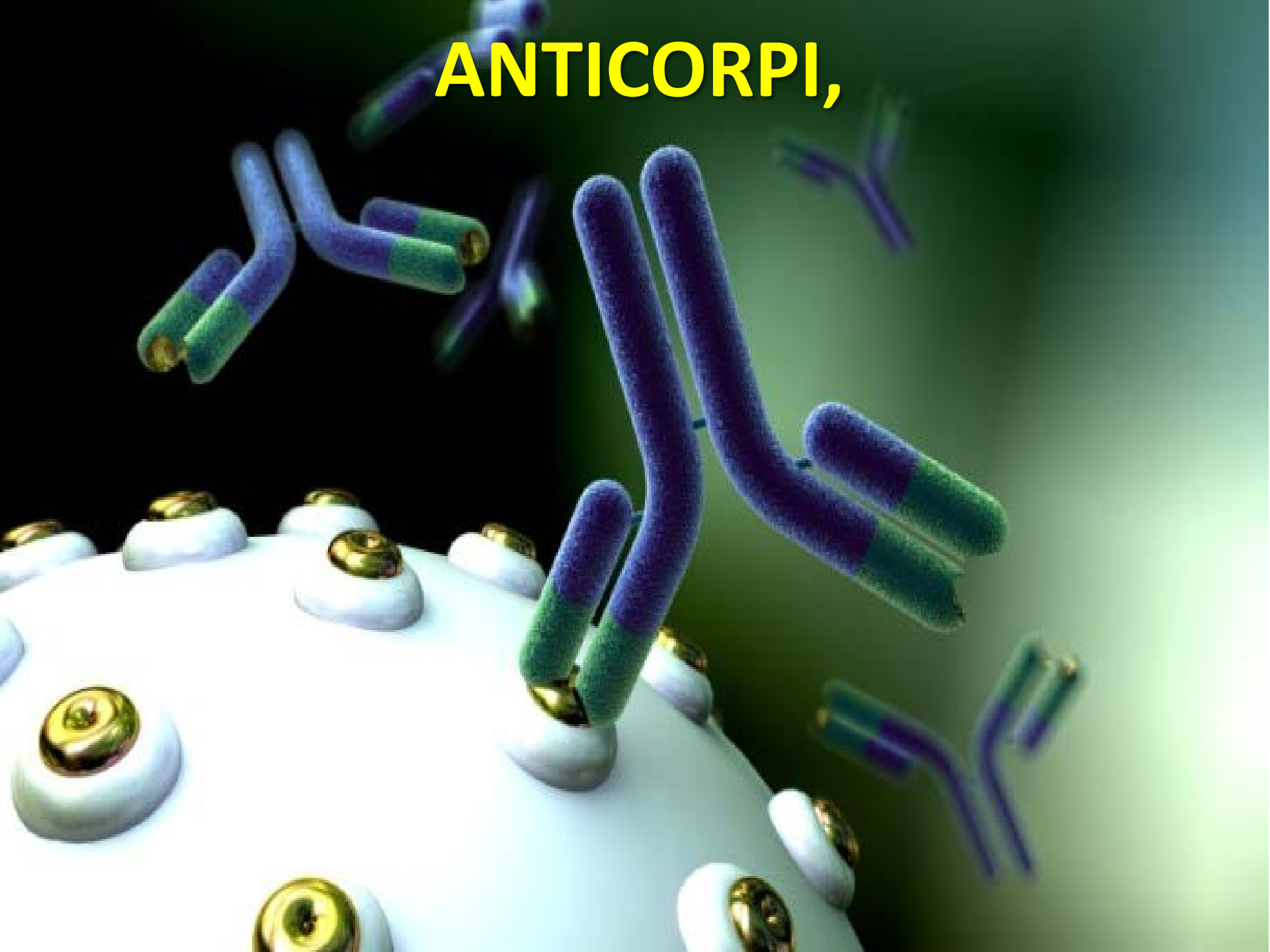


# ANTICORPI,



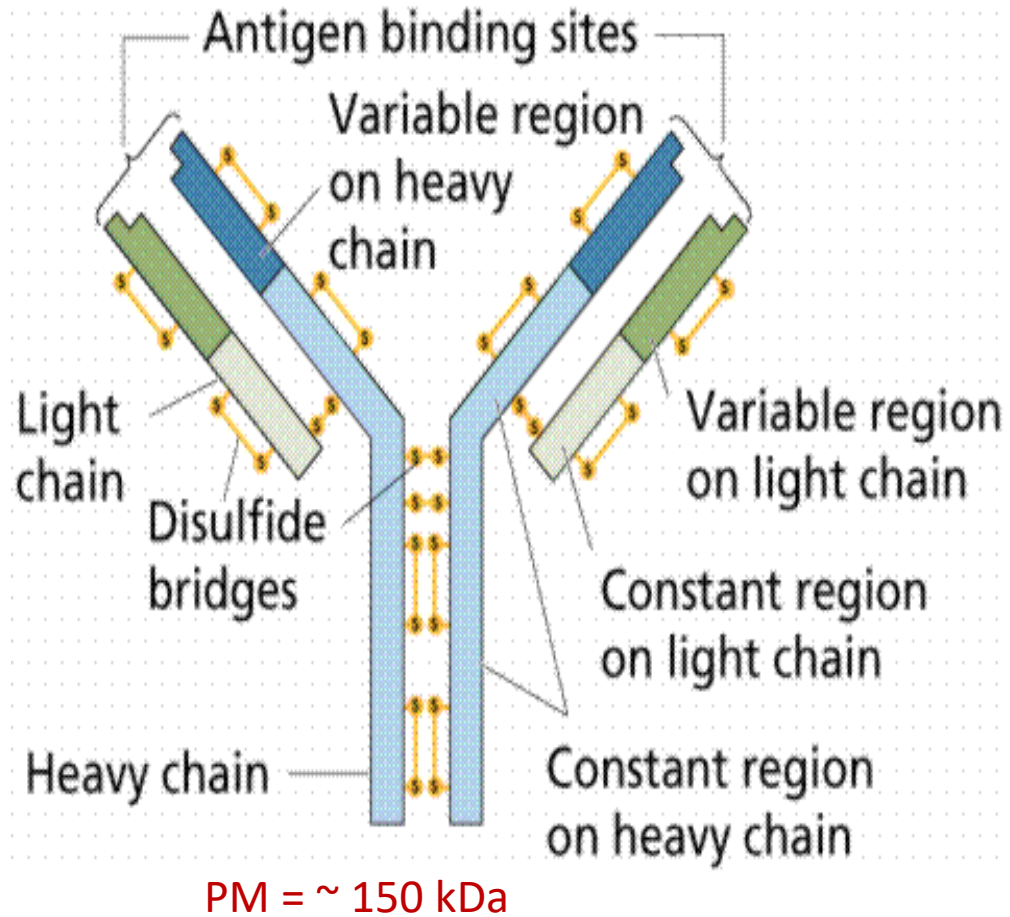
# GLI ANTICORPI (IMMUNOGLOBULINE)

## Anticorpo (Ab):

glicoproteine **solubili**, della classe delle immunoglobuline (Ig), prodotte e **secrete** dai linfociti B.

## Antigene (Ag):

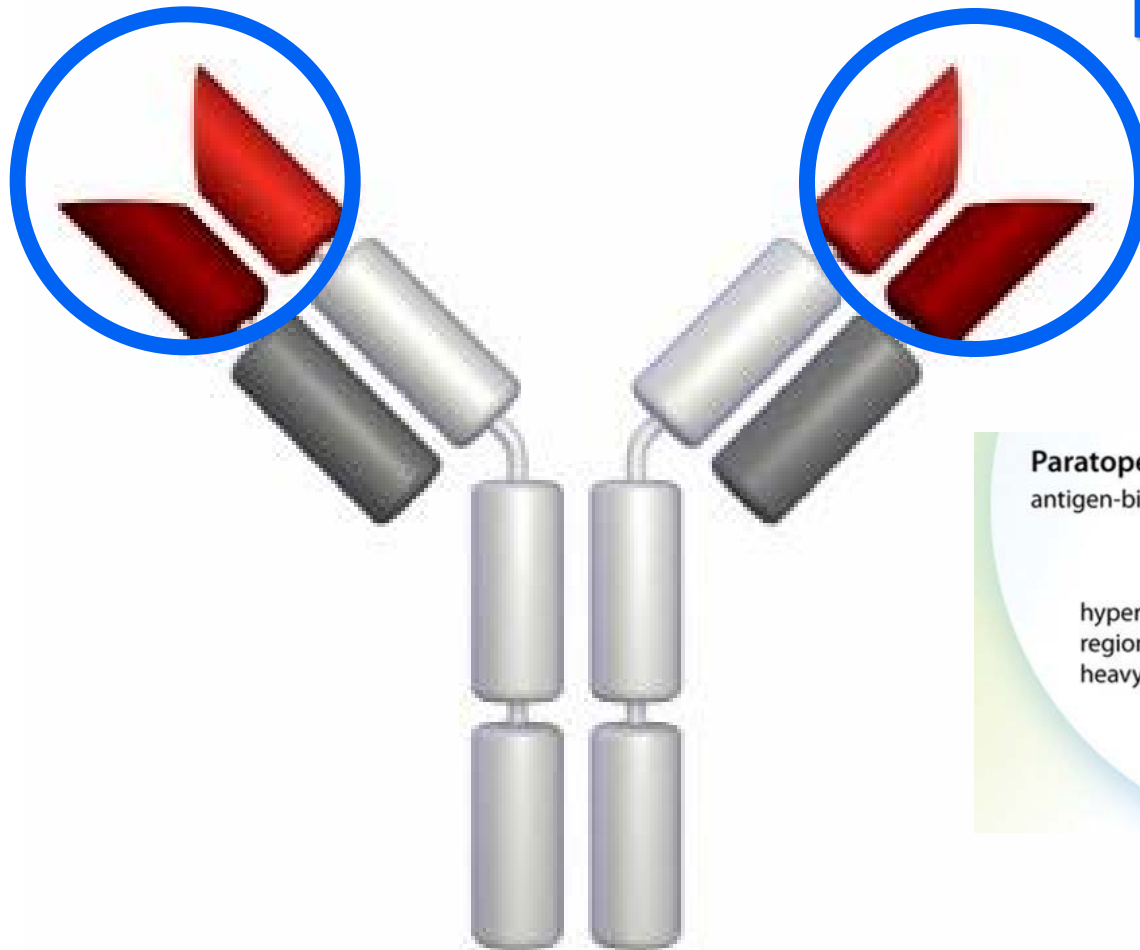
Qualsiasi sostanza estranea che induca risposta immunitaria (es. **polisaccaridi, proteine**). Sostanza riconosciuta e **legata** da un anticorpo.



## Epitopo (o determinante antigenico):

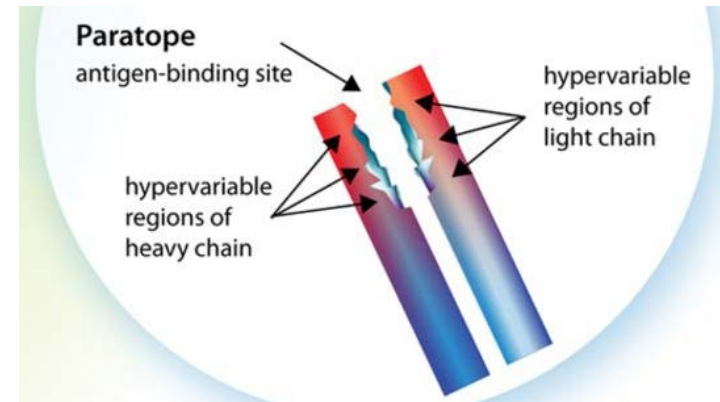
**Parte** dell'antigene **riconosciuta** dall'anticorpo

# SCHEMATIZZAZIONE DI UN IgG

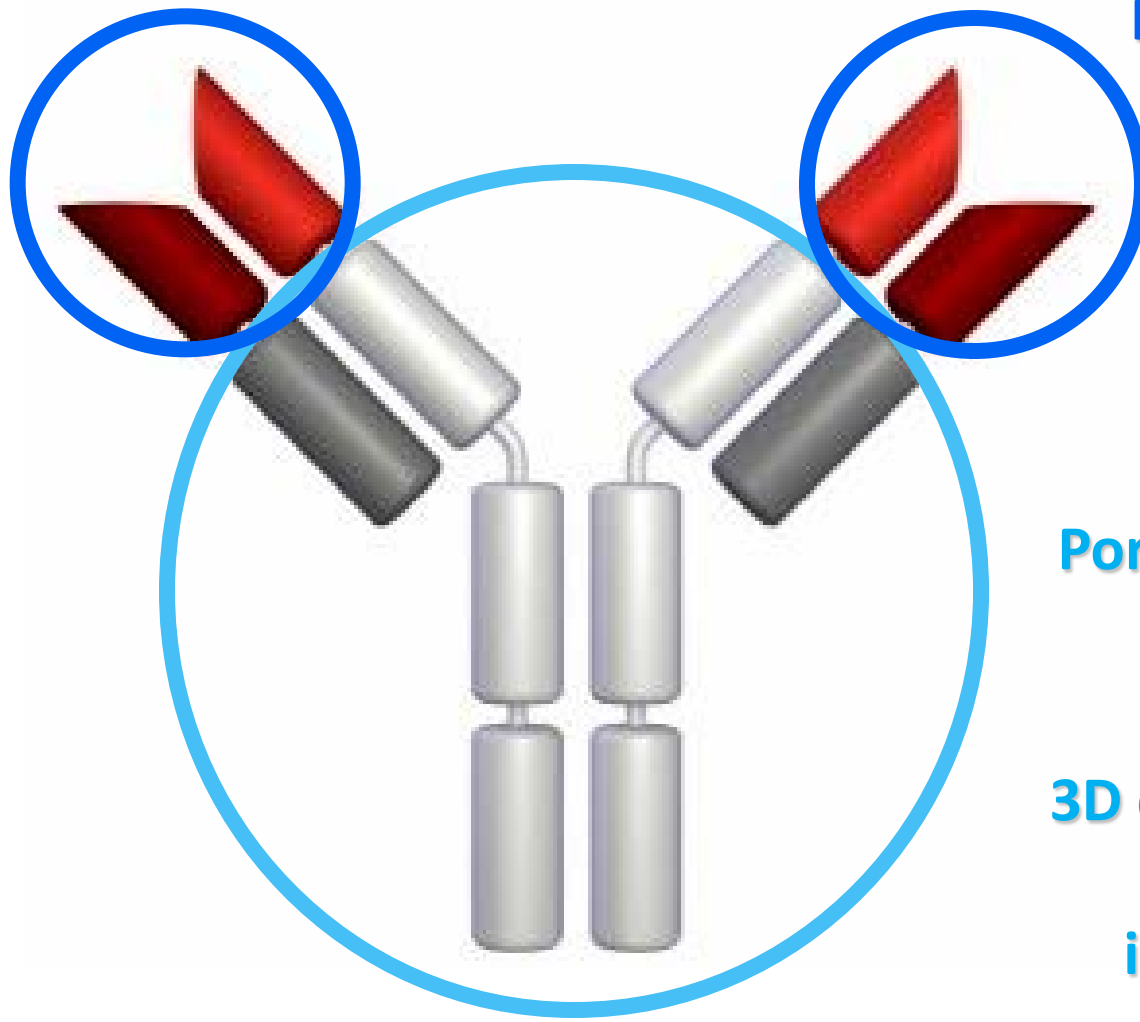


Porzioni variabili

Legame con  
l'antigene



# SCHEMATIZZAZIONE DI UN IgG



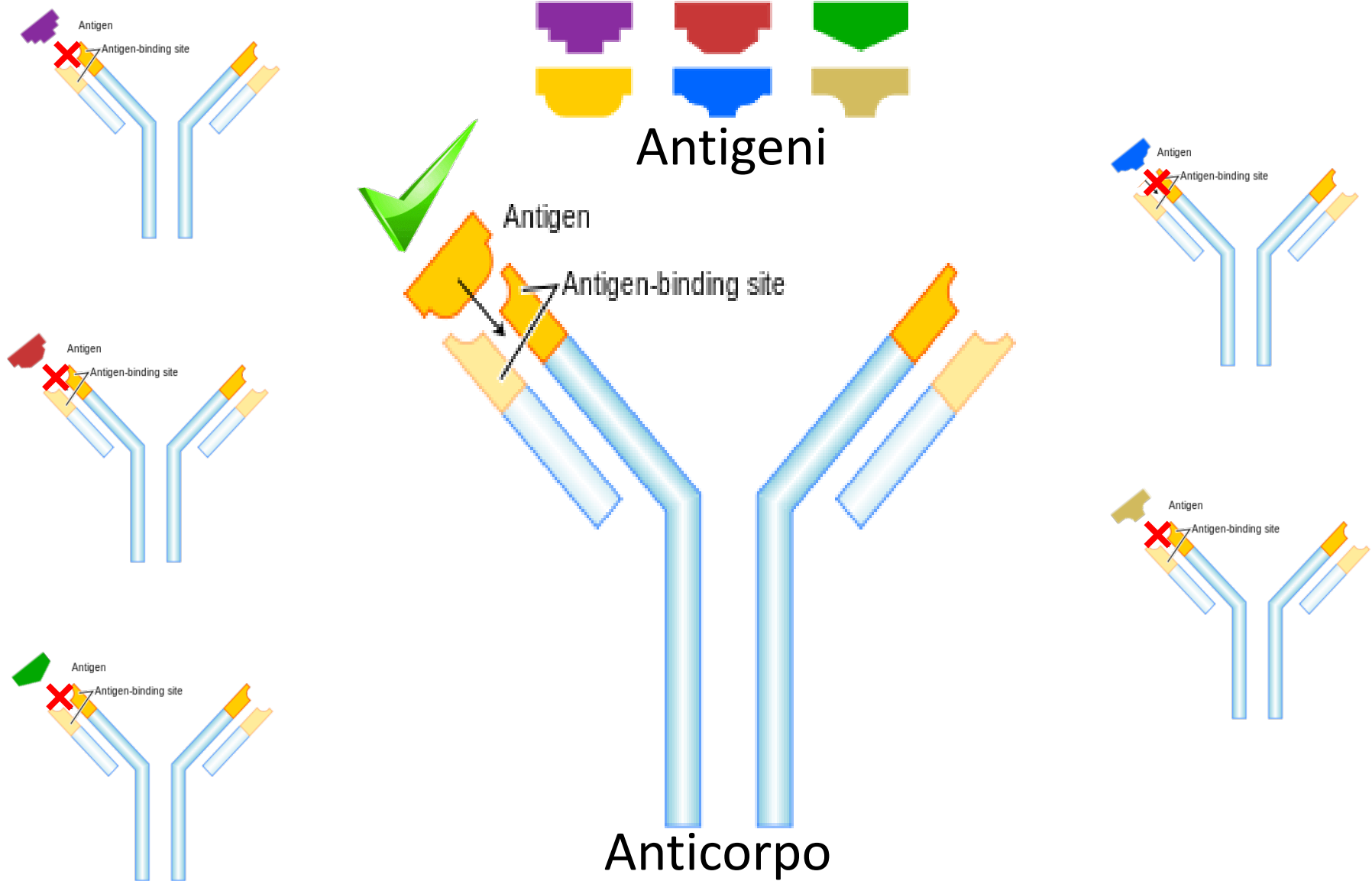
**Porzioni variabili**

**Legame con  
l'antigene**

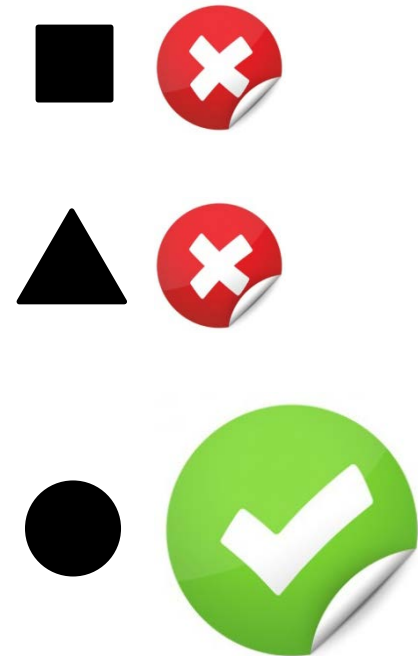
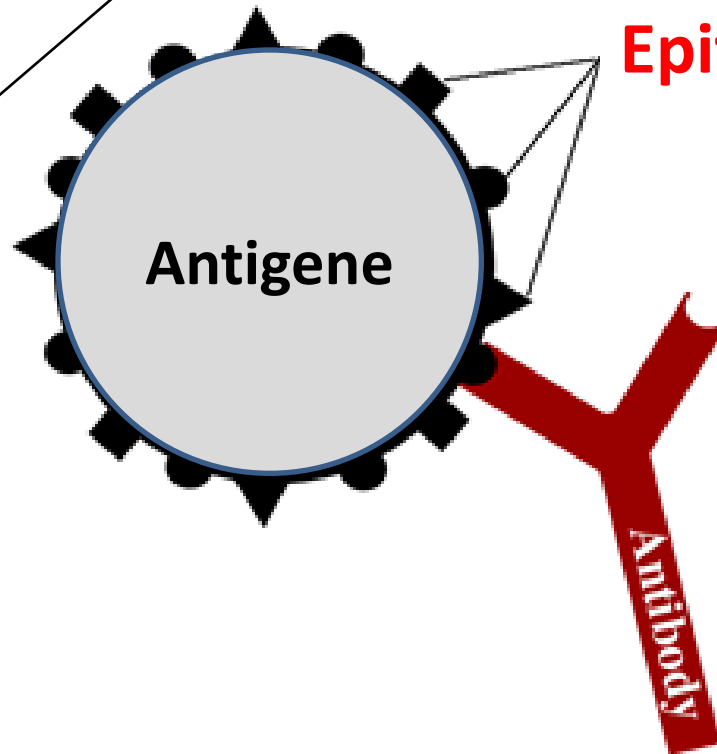
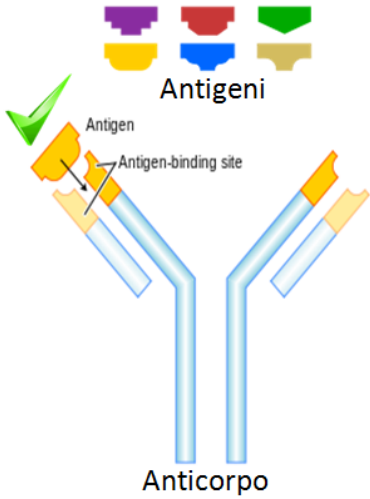
**Porzione costante**

**Mantiene la struttura  
3D dell'Ab, attiva il sistema  
del complemento,  
interagisce con recettori  
cellulari...**

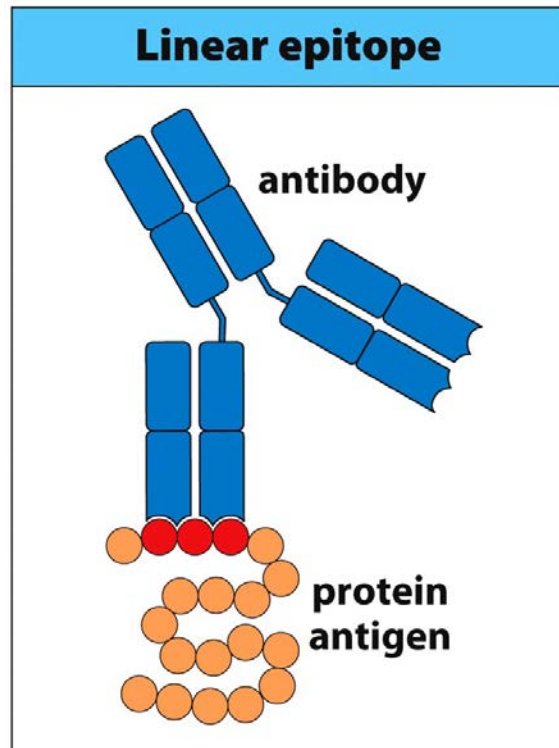
# Un **ANTICORPO** riconosce in modo specifico un **ANTIGENE**



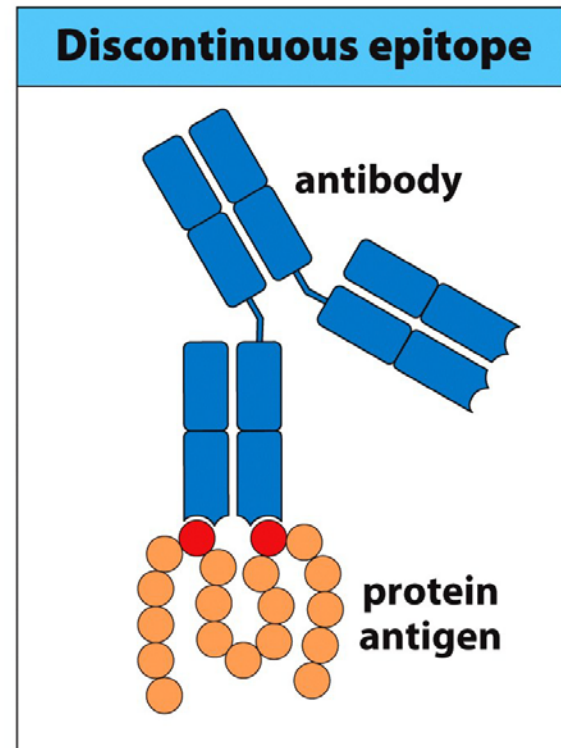
# Un **ANTICORPO** riconosce in modo specifico un **EPITOPO** presente sull'**ANTIGENE**



# EPITOPI



**Linear epitope**  
Amino acid residues  
are adjacent in the  
polypeptide chain



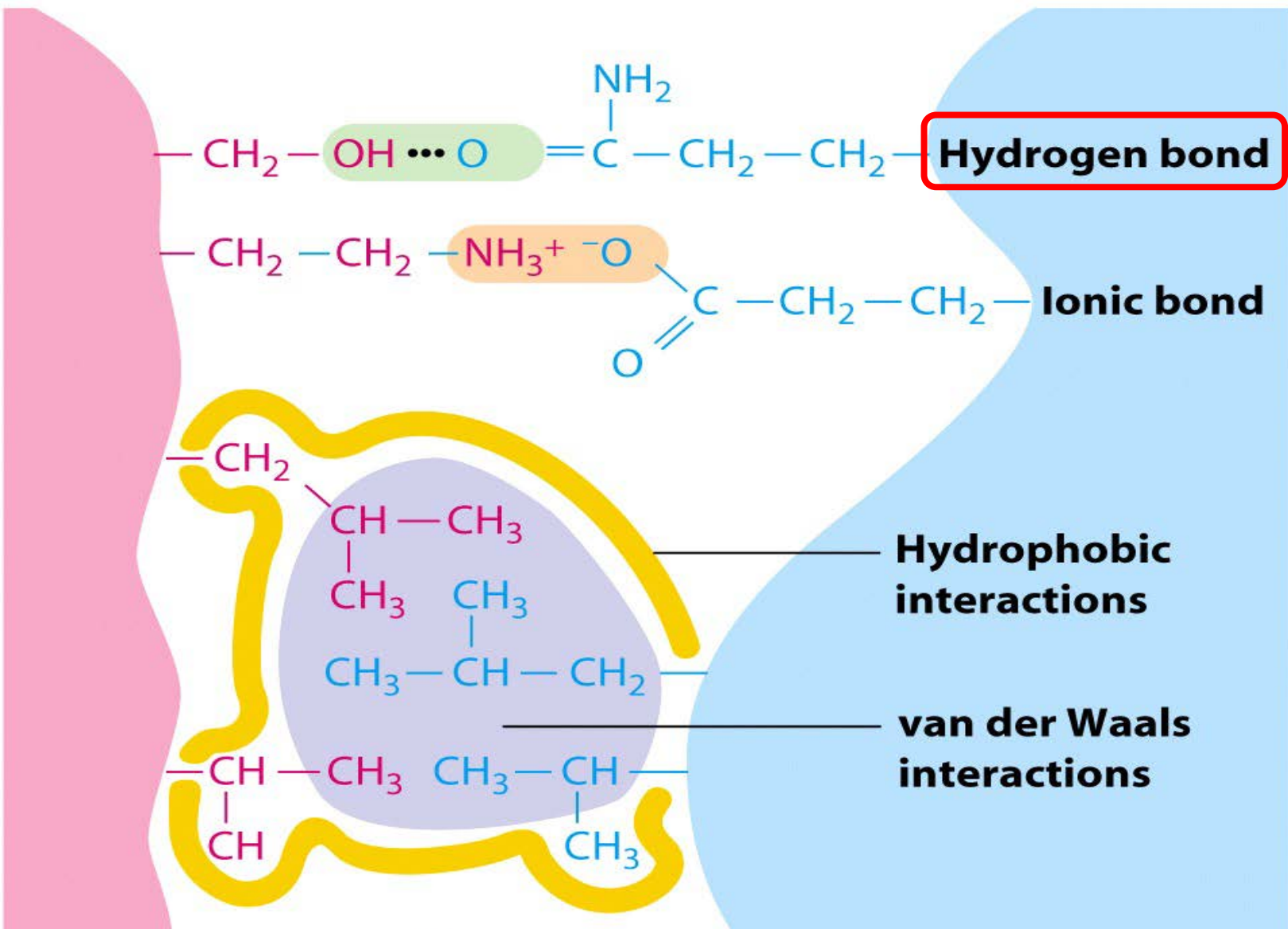
**Discontinuous epitope**  
Created from amino acid  
residues located in different  
parts of the polypeptide chain

Epitopo **conformazionale**

# BASI MOLECOLARI DELL'IMMUNOCOMPLESSO

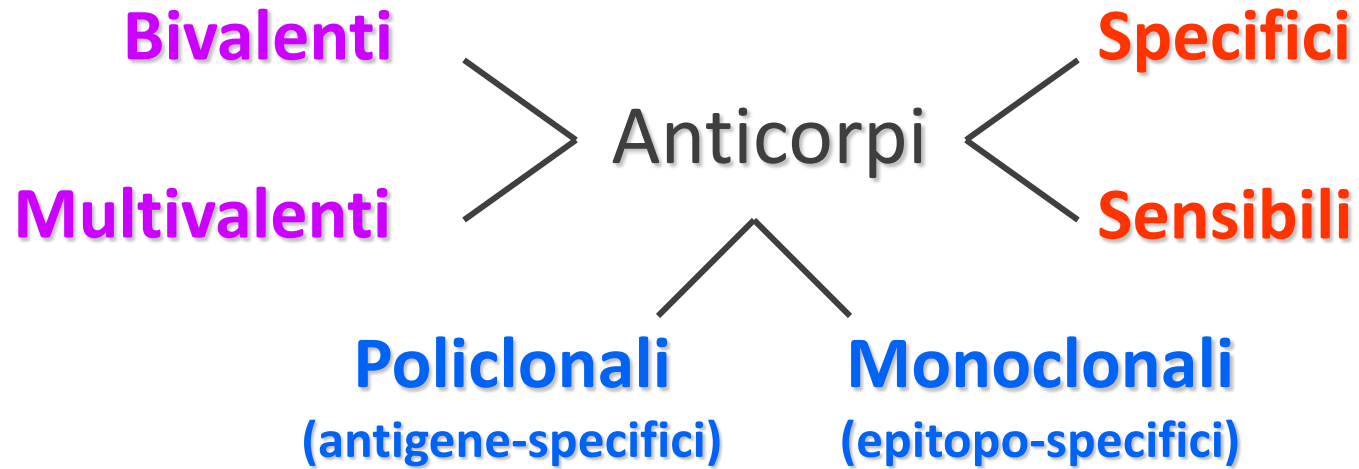
ANTIGEN

ANTIBODY

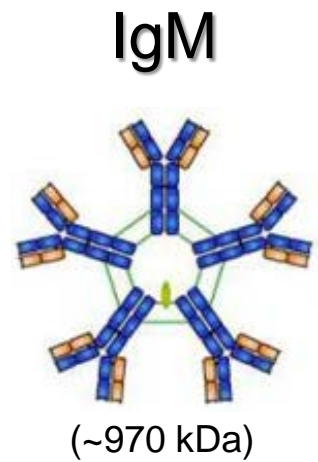
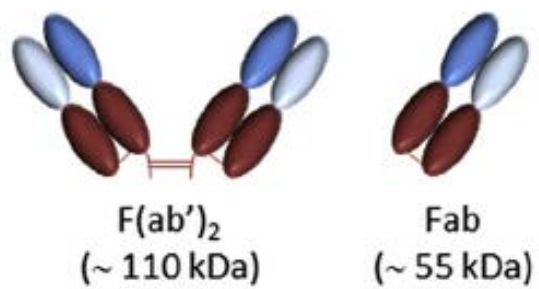
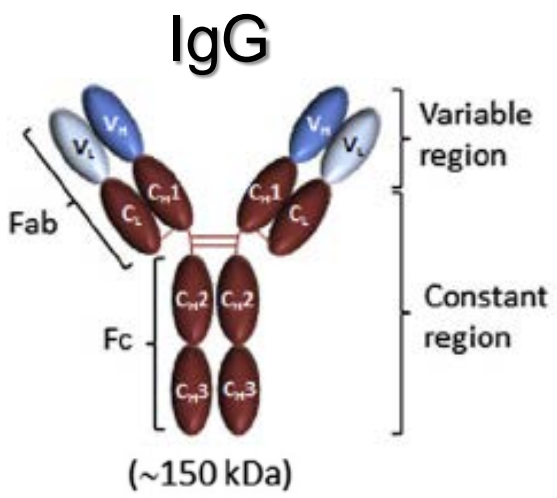
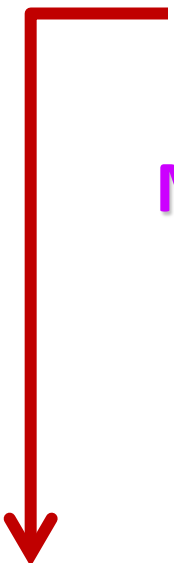
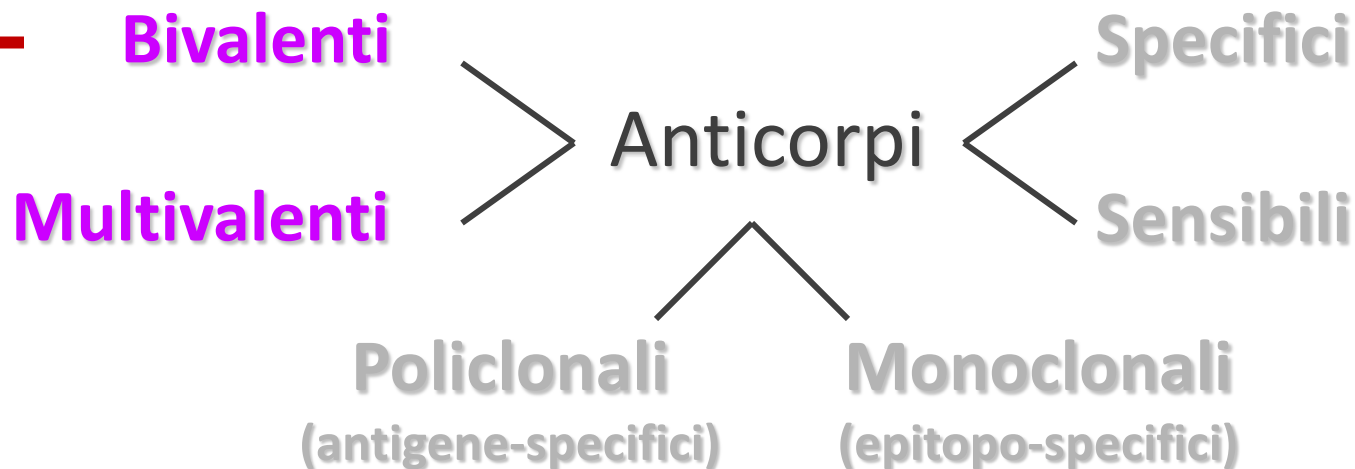




# CARATTERISTICHE DI UN ANTICORPO

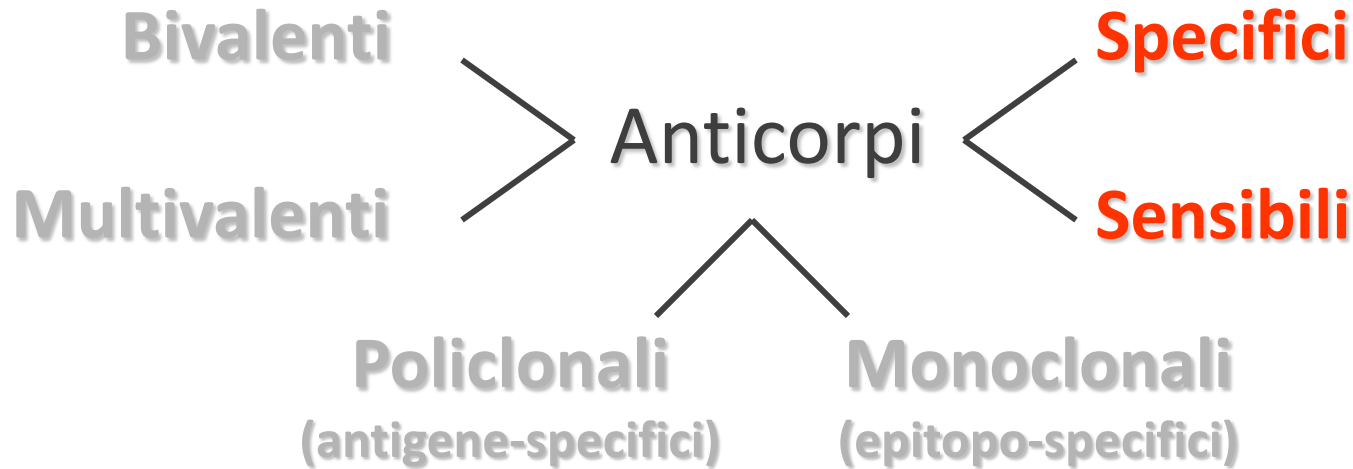


# CARATTERISTICHE DI UN ANTICORPO



Valenza                      2                      2                      1                      10

# CARATTERISTICHE DI UN ANTICORPO



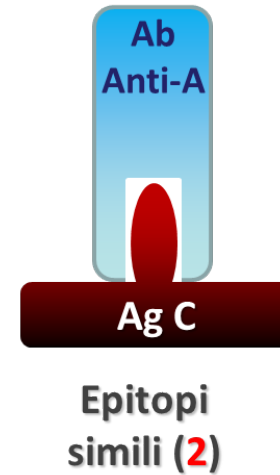
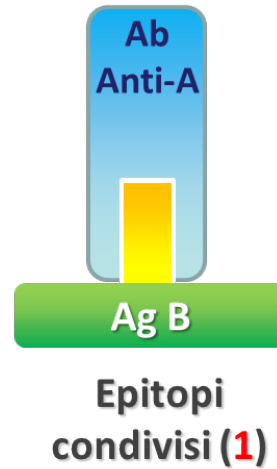
## Cross reattività

Capacità di un singolo Ab di legarsi a **più epitopi** o di una popolazione di Ab di reagire con **più antigeni**.

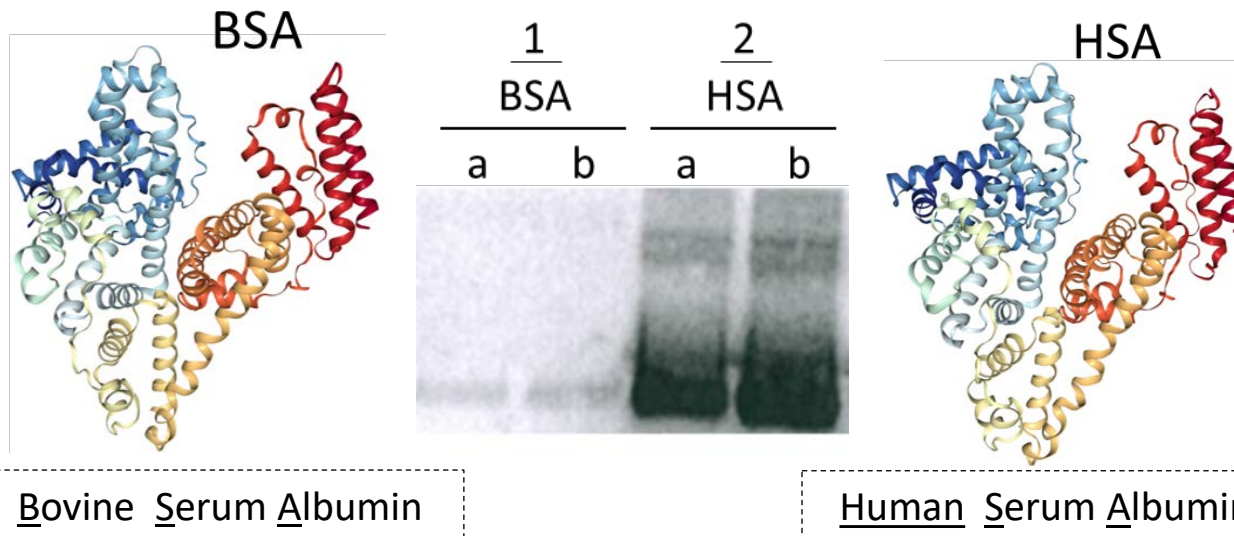
### Cause:

- Epitopi condivisi (**1**)
- Epitopi strutturalmente simili (**2**)

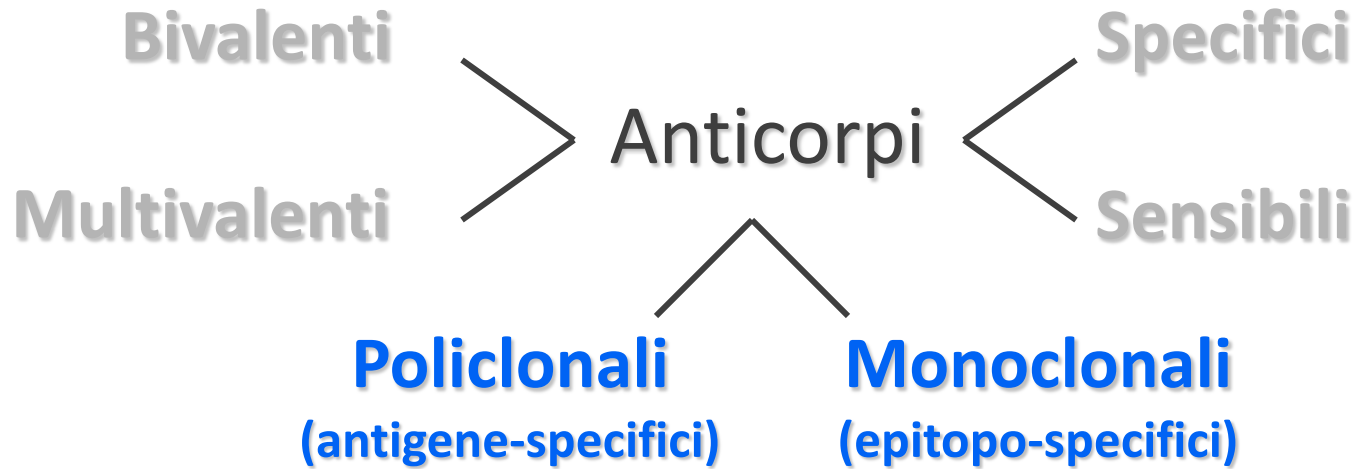
# CROSS REATTIVITÀ



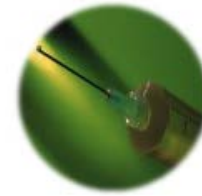
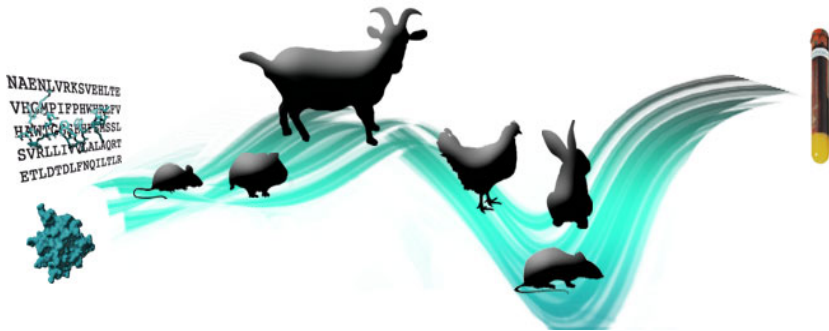
## Esempio reale - anticorpo contro albumina UMANA



# CARATTERISTICHE DI UN ANTICORPO



# PRODUZIONE DI ANTICORPI POLICLONALI



1. Immunization with antigen



2. Antibody production



3. Serum retrieval

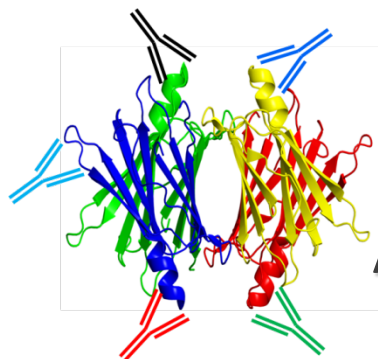
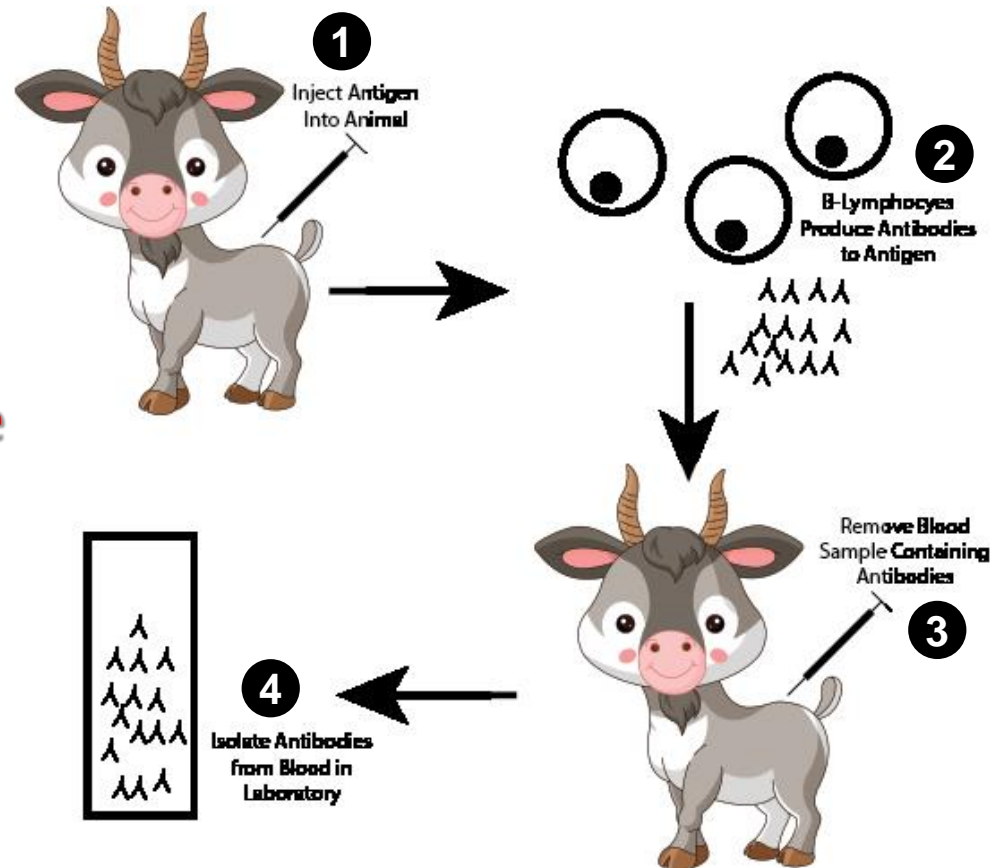


4. Serum purification

Anticorpi policonali:

-prodotti da **più tipi (cloni)** di cellule

-**antigene-specifici**: riconoscono **più epitopi** di uno **stesso antigene**



Proteina  
+  
Ab policlonali

# PRODUZIONE DI ANTICORPI MONOCLONALI

*Nature Vol. 256 August 7 1975*

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**Continuous cultures of fused cells  
secreting antibody of predefined specificity**

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*Köhler and Milstein. Nature. 1975;256(5517):495-7.*

1984



Fisiologia o  
Medicina



Niels K. Jerne



Georges J.F. Köhler



César Milstein

Basato sulla tecnologia degli **ibridomi**

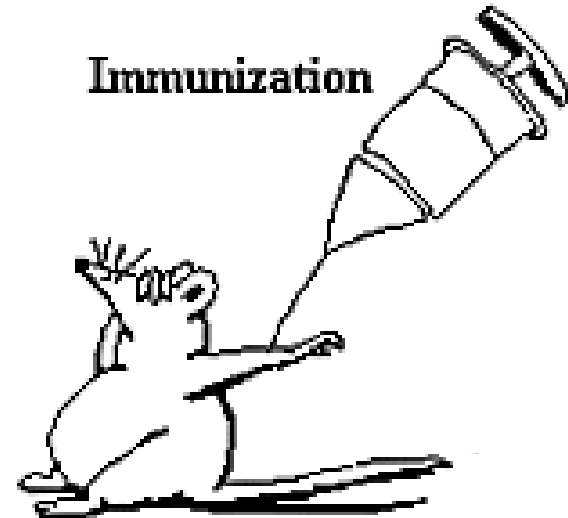
-Cellule normali di topo vengono fuse con cellule tumorali (es. mieloma)

-La nuova cellula ibrida ha proprietà di entrambi i tipi cellulari:

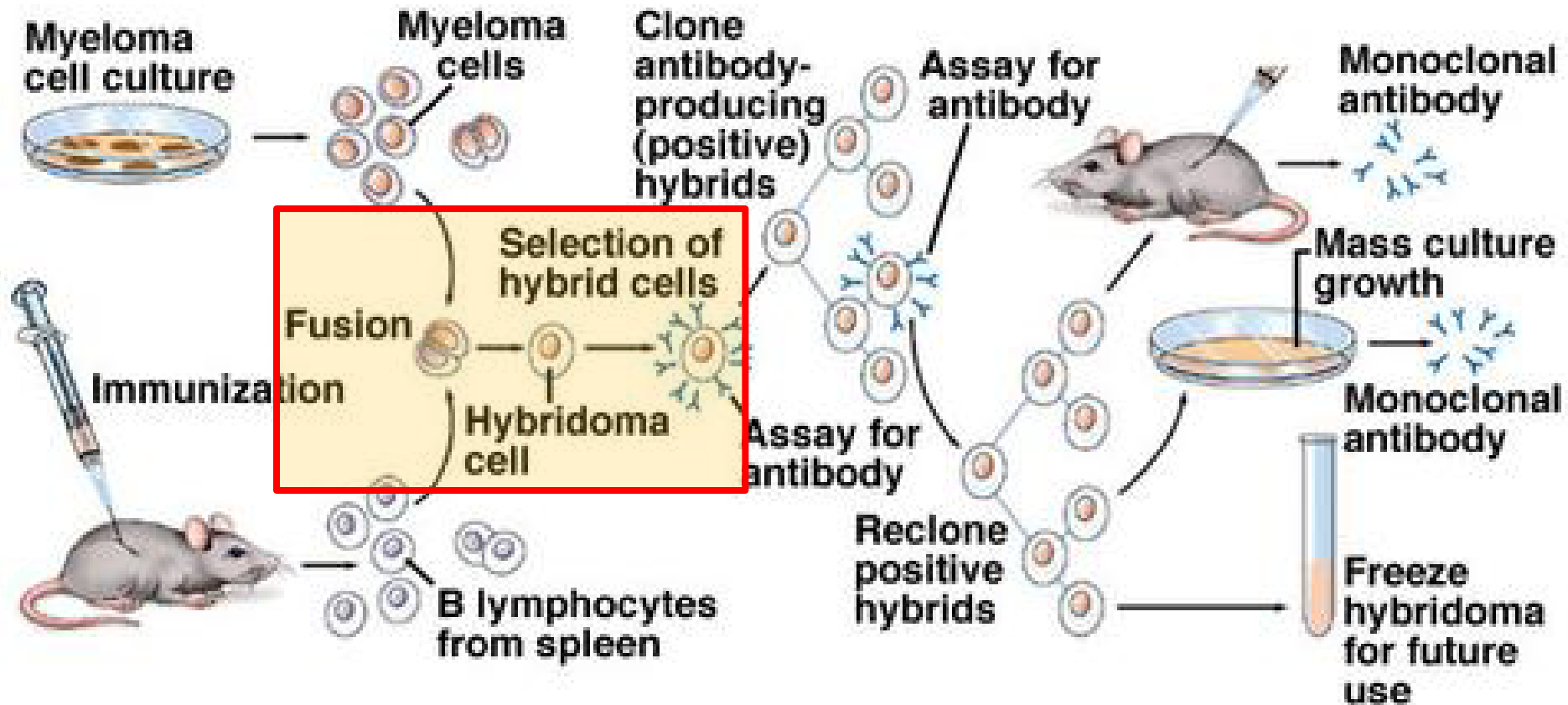
-Crescita illimitata;

-Secrezione di **anticorpi monoclonali**

Immunization

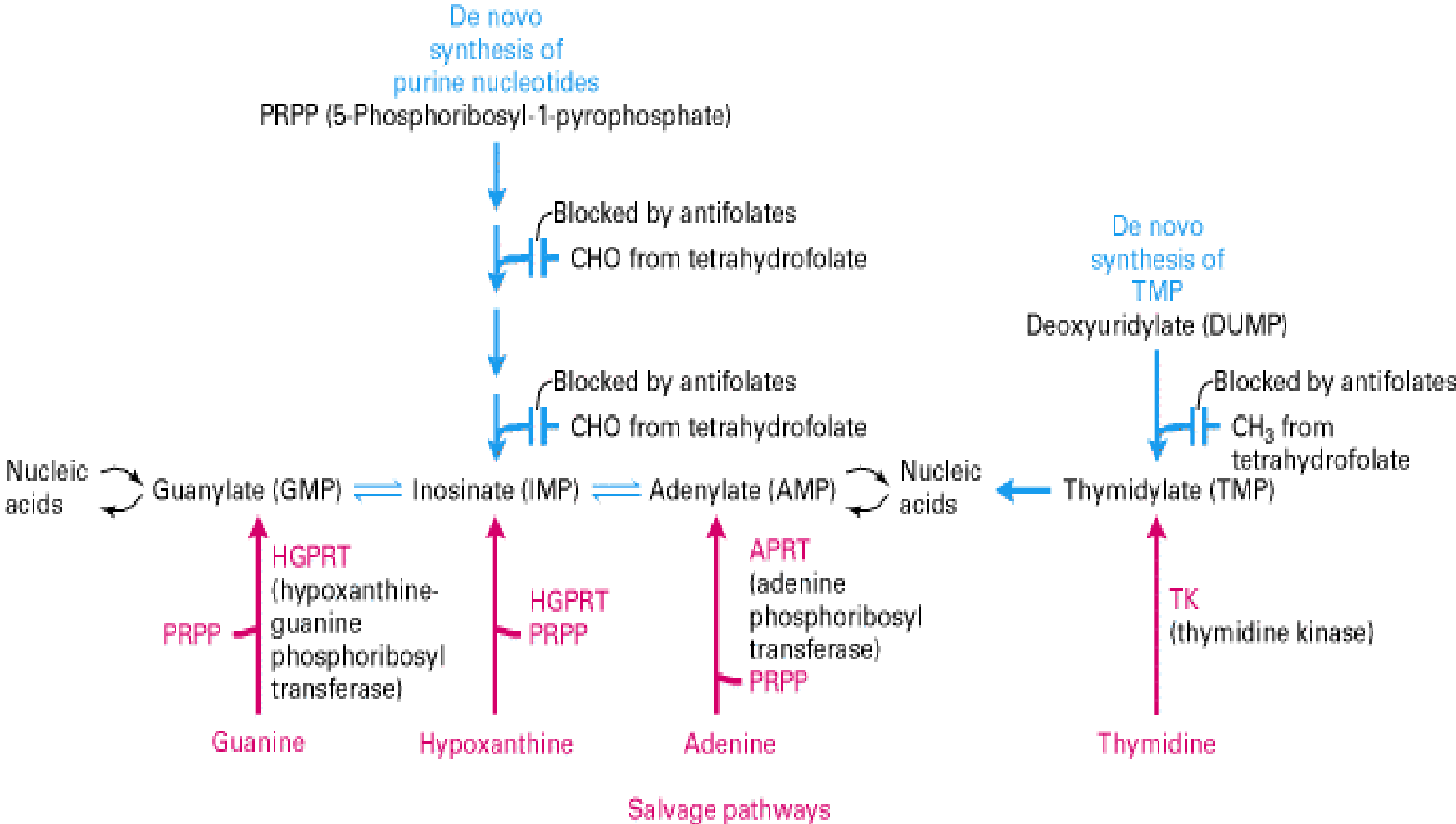


# PRODUZIONE DI ANTICORPI MONOCLONALI



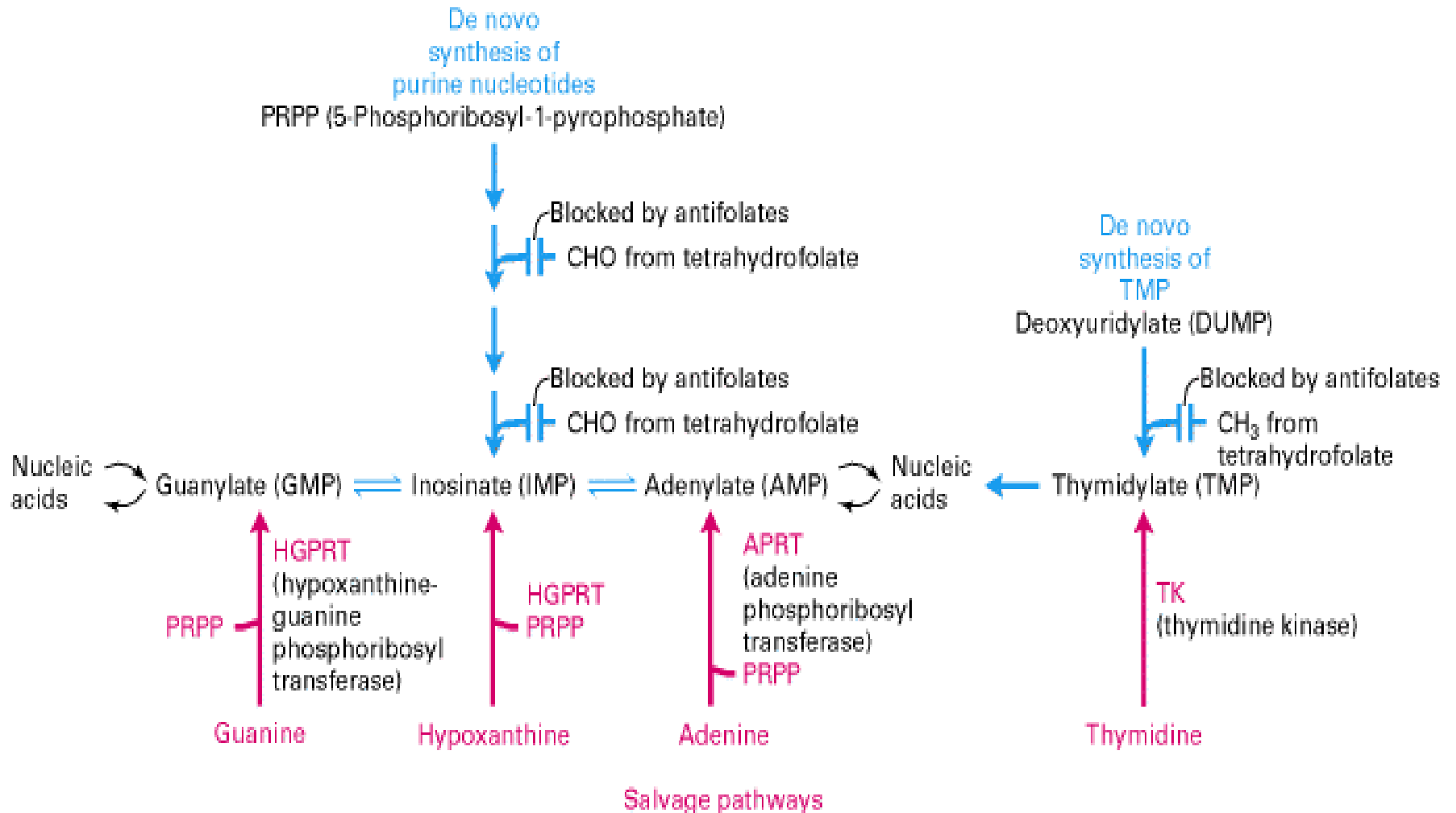


# PRODUZIONE DI ANTICORPI MONOCLONALI - *HAT selection*



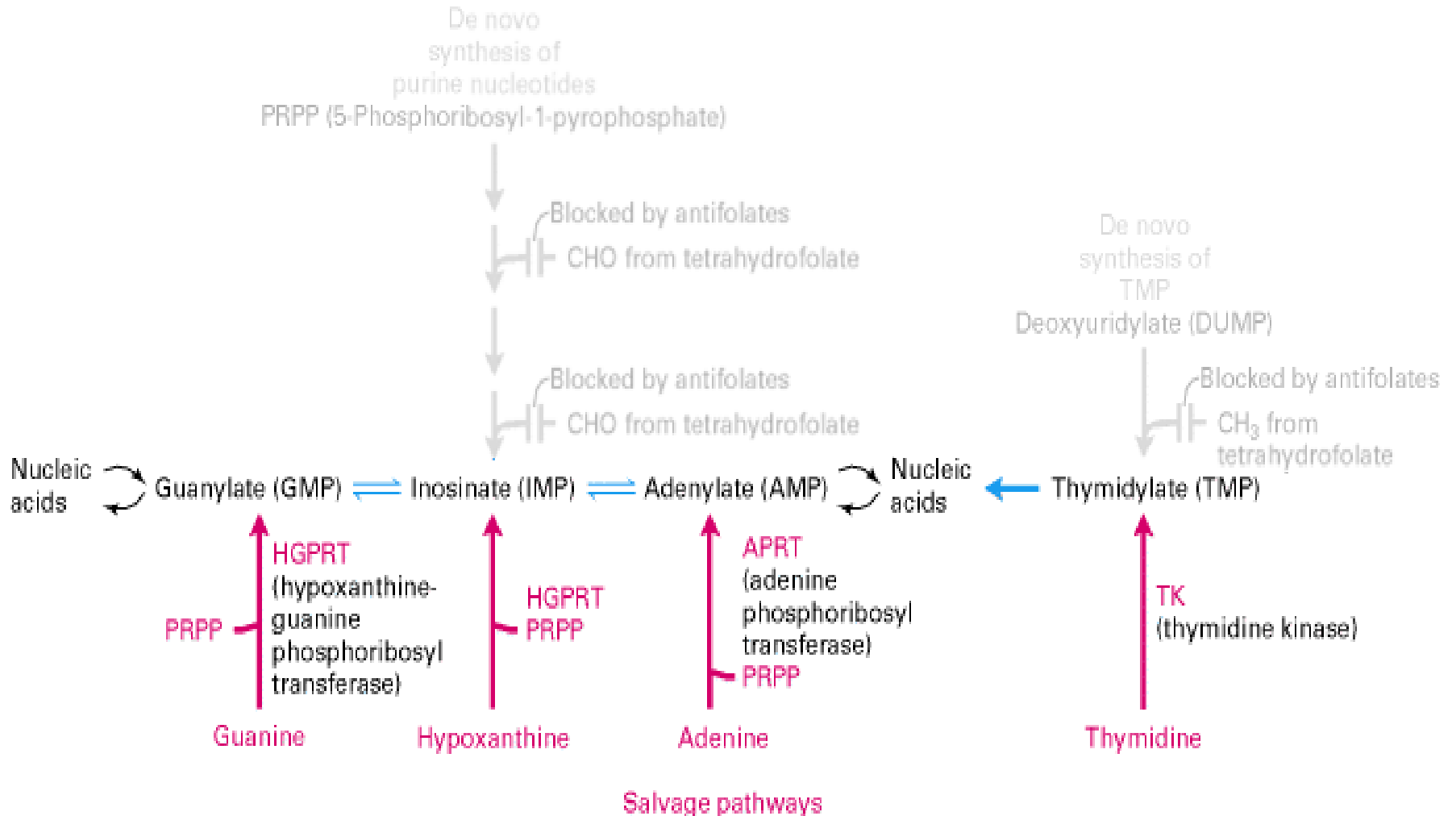
# PRODUZIONE DI ANTICORPI MONOCLONALI - *HAT selection*

- HAT:** **Hypoxanthine** → indispensabile per la sintesi di recupero delle basi puriniche  
**Aminopterin** → blocca la sintesi *de novo* delle basi puriniche e pirimidiniche  
**Thymidine** → indispensabile per la sintesi di recupero delle basi pirimidiniche



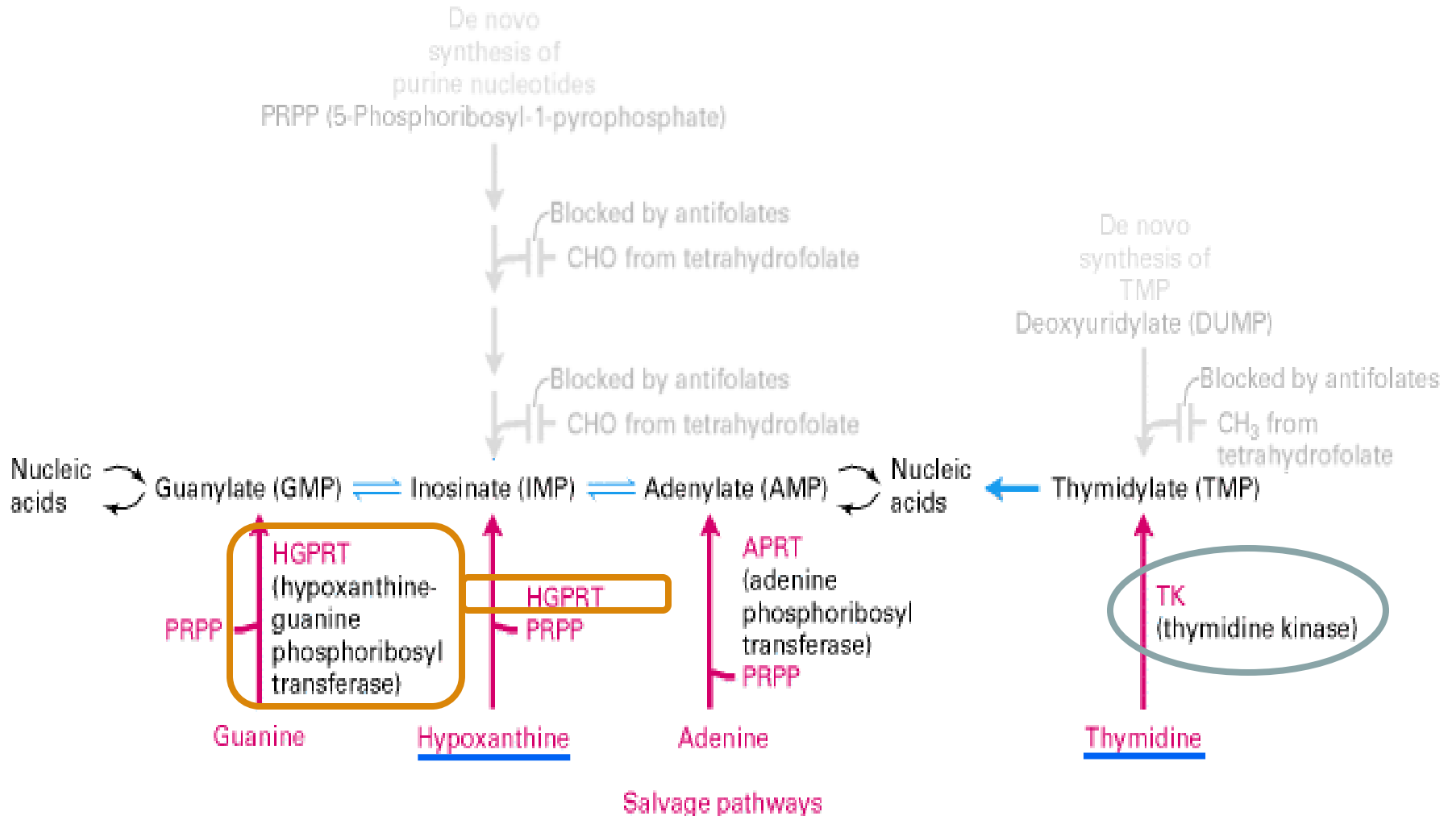
# PRODUZIONE DI ANTICORPI MONOCLONALI - *HAT selection*

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# PRODUZIONE DI ANTICORPI MONOCLONALI - *HAT selection*

- HAT:** **Hypoxanthine** → indispensabile per la sintesi di recupero delle basi puriniche  
**Aminopterin** → blocca la sintesi *de novo* delle basi puriniche e pirimidiniche  
**Thymidine** → indispensabile per la sintesi di recupero delle basi pirimidiniche



# PRODUZIONE DI ANTICORPI MONOCLONALI - *HAT selection*

**HAT:** **Hypoxanthine** → indispensabile per la sintesi di recupero delle basi puriniche  
**Aminopterin** → blocca la sintesi *de novo* delle basi puriniche e pirimidiniche  
**Thymidine** → indispensabile per la sintesi di recupero delle basi pirimidiniche

**Genotipo:**

**Tipo di cellula:**

**Selezione HAT:**

**Motivo:**

HGPRT -



HGPRT +



HGPRT +



**NO sintesi DNA:**  
- carenza di HGPRT nella "salvage pathway"  
- l'aggiunta di aminopterin blocca la sintesi *de novo*

**Linea continua e sintesi DNA:**  
1) Caratteristiche di cellula **immortalizzata** (**mieloma**)  
2) Capacità di **sintesi DNA** (enzima **HGPRT**; **cellule B** di milza)

**Mortalità:**  
HGPRT normale e sintesi DNA *ma* limitato numero di cicli di replicazione in coltura.

*HGPRT = hypoxanthine guanine phosphoribosyl transferase (oppure anche TK-, TK = thymidine kinase)*

# PRODUZIONE DI ANTICORPI MONOCLONALI - *HAT selection*

## Hybridoma selection using HAT medium

Hybridoma selection after fusion of myelomas and spleen cells is a critical step in monoclonal antibody production.

Often scientists use the **HAT (hypoxanthine-aminopterin-thymidine) method**.

During the fusion process, three types of cells are present: (1) **unfused myeloma cells** that are **deficient in the HGPRT enzyme**, (2) **unfused spleen cells**, and (3) **fused hybridoma cells**.

**Unfused spleen cells** are easily selected against since they **do not replicate in culture**.

**Unfused myelomas** can be **selected** against **using** media containing **HAT**. The **aminopterin** found in the medium **blocks** the **de novo DNA** nucleotide **synthesis** pathway. Typically when the *de novo* pathway is blocked, cells will then utilize the **salvage pathway** as an alternative means to replicate (only if hypoxanthine and thymidine are present). However, these myelomas are unable to do so since they are **deficient in the HGPRT enzyme**, which is **required for the salvage pathway**. Hence, myelomas are unable to replicate in culture.

**Only hybridomas survive**. Hybridomas inherit a functioning **HGPRT enzyme from** the **spleen cells**, so even though the *de novo* pathway is blocked, they can still use the salvage pathway to replicate, and are "**immortal**" due to features conferred by **myeloma cells**.

# PRODUZIONE DI ANTICORPI MONOCLONALI

*Nature Vol. 256 August 7 1975*

**Continuous cultures of fused cells  
secreting antibody of predefined specificity**

*Köhler and Milstein. Nature. 1975;256(5517):495-7.*

1984



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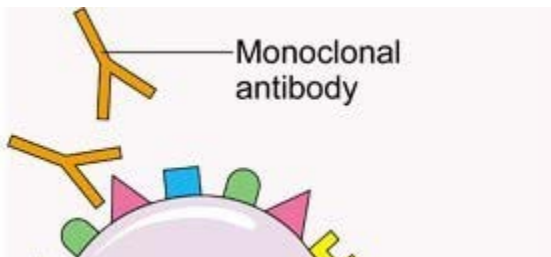
Georges J.F. Köhler



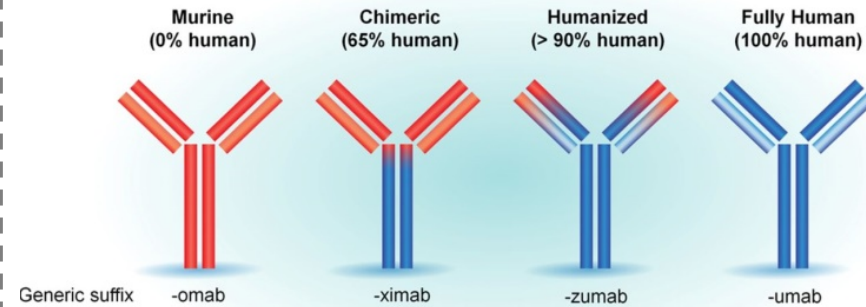
César Milstein

Anticorpi **monoclonali**:

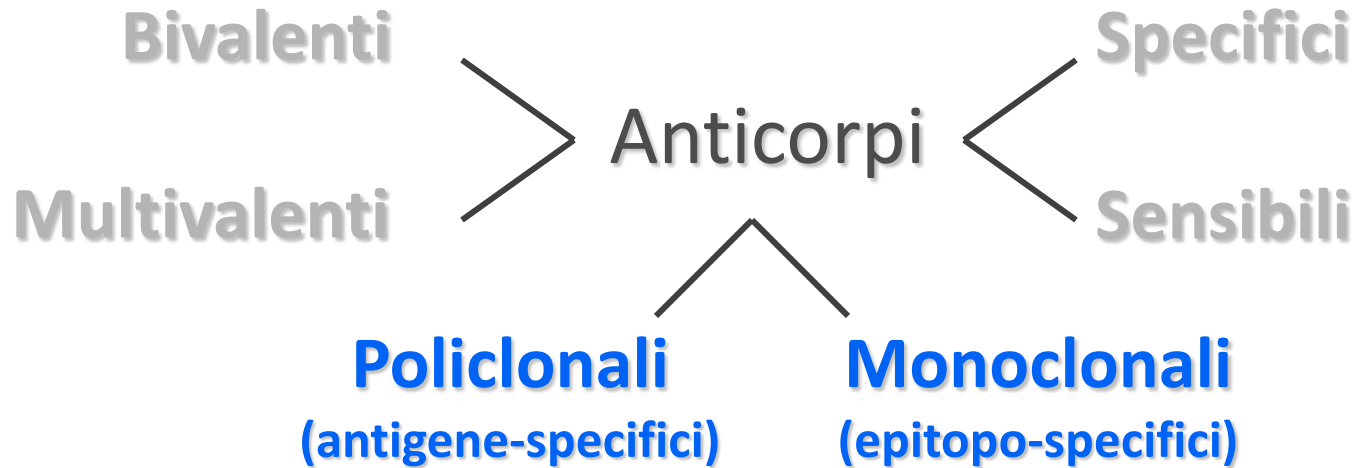
- anticorpi identici** prodotti da **un solo tipo (clone)** di cellule
- epitopo-specifici**: riconoscono **uno specifico epitopo** sull'antigene



Anticorpi monoclonali  
ad uso terapeutico:



# CARATTERISTICHE DI UN ANTICORPO



	<u>Polyclonal antibodies</u>	<u>Monoclonal Antibodies</u>
<i>Produced by:</i>	Many B cell clones	A single B cell clone
<i>Bind to:</i>	Multiple epitopes of all antigens used in the immunization	A single epitope of a single antigen
<i>Antibody class:</i>	A mixture of different Ab classes (isotypes)	All of a single Ab class
<i>Ag-binding sites:</i>	A mixture of Abs with different antigen-binding sites	All Abs have the same antigen binding site
<i>Potential for cross-reactivity:</i>	High	Low



# Alcune applicazioni degli anticorpi in ricerca (tecniche immunochimiche)

## Scopo sperimentale:

- ❑ **IDENTIFICARE** un determinato antigene (tecnica **QUALITATIVA**)
- ❑ **QUANTIFICARE** un determinato antigene (tecnica **QUANTITATIVA**)

## **Approccio** sperimentale:

- ❑ **Western Blotting** (tecnica **QUALITATIVA**)
- ❑ **ELISA** (saggio **QUANTITATIVO**)