20.109 MOD1
Measuring Genomic Instability

Fall 2023
Lecture 4

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Professor of Biological Engineering

A look at BER Chemistry

More on γH2AX

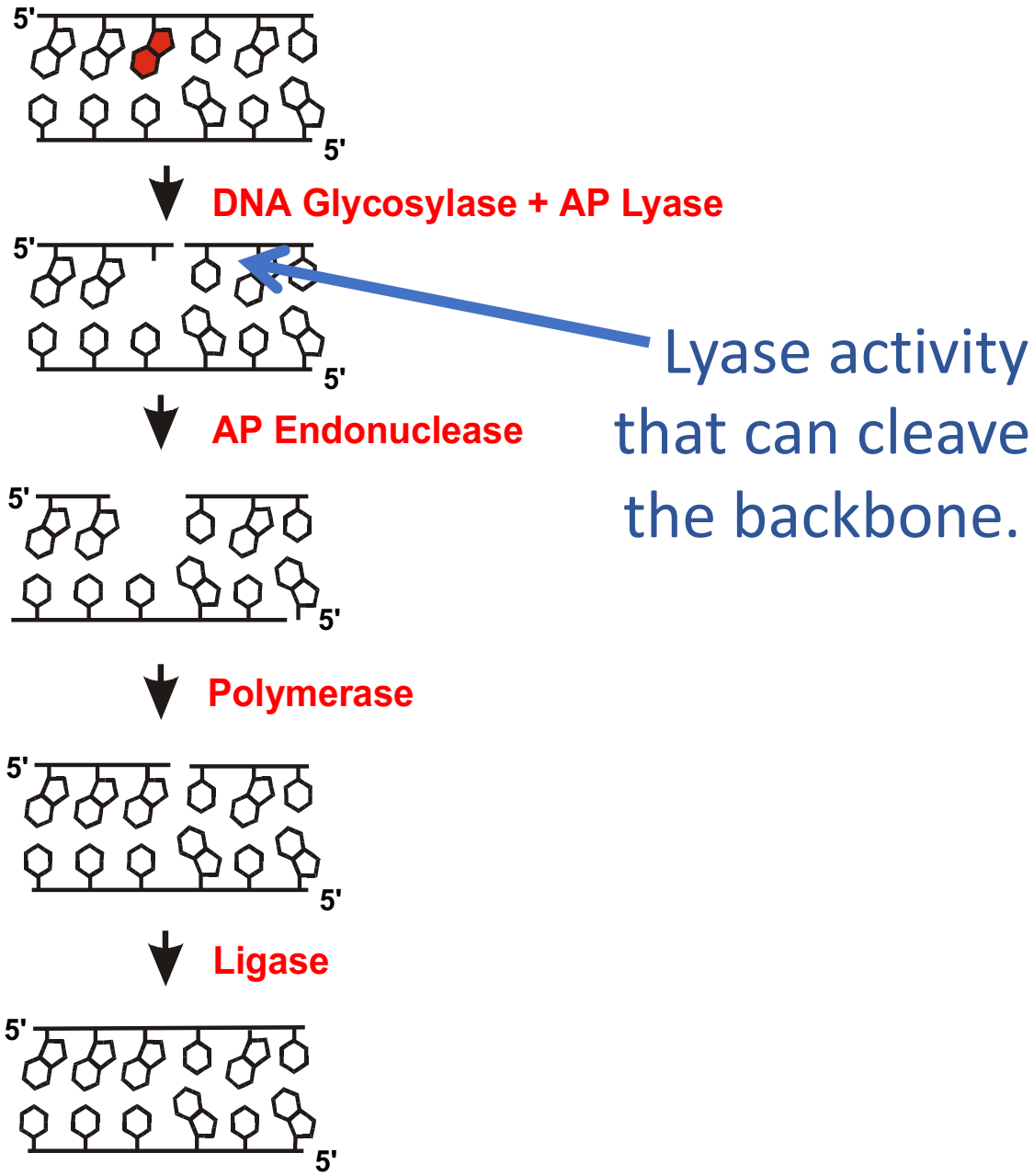
Base Pair Drawing Competition

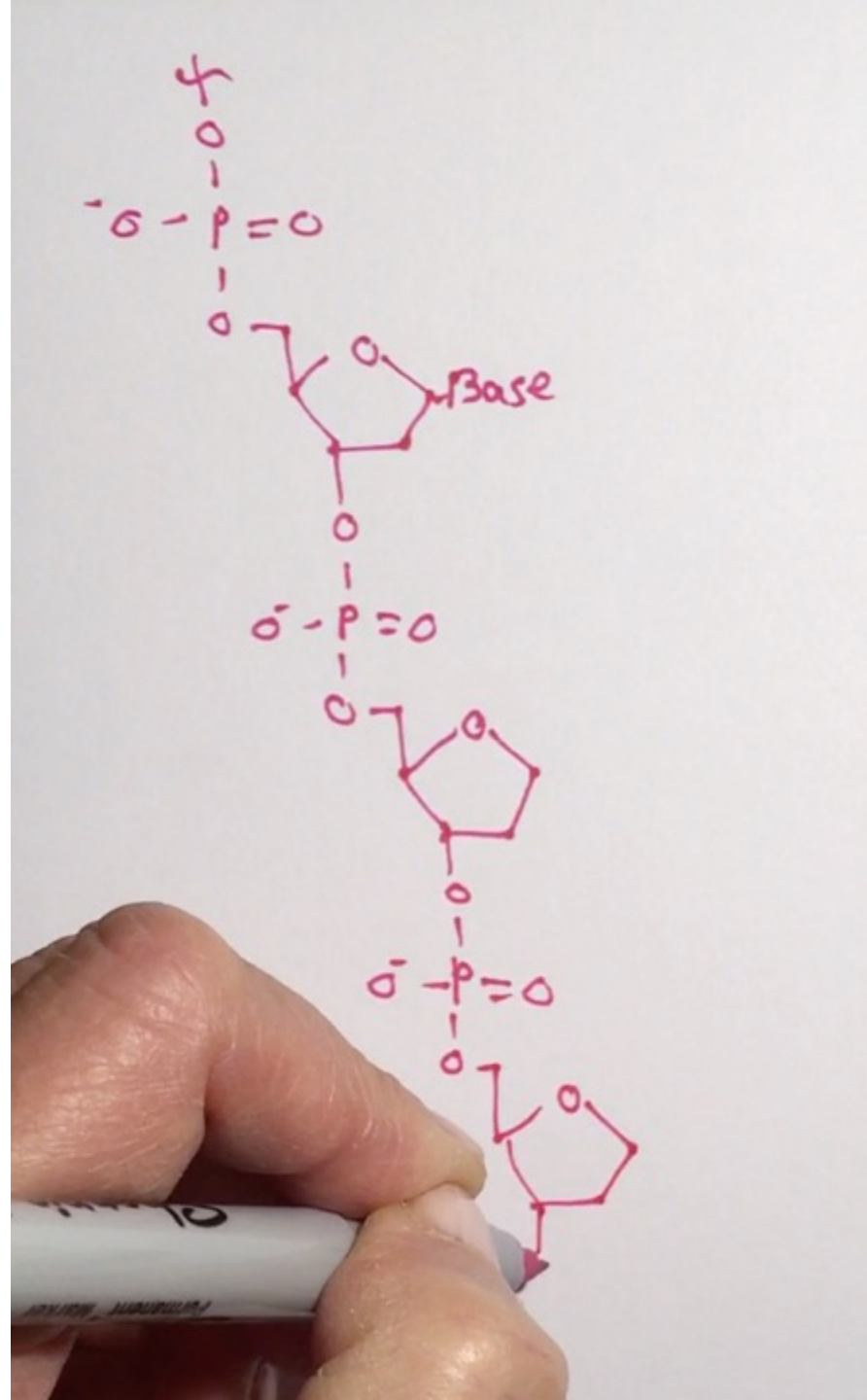
Antibodies and Affinity Maturation

Base Excision Repair

8-oxoguanine DNA Glycosylase (OGG1)

Removes the damaged base by cleaving the glycosylic bond.




Base

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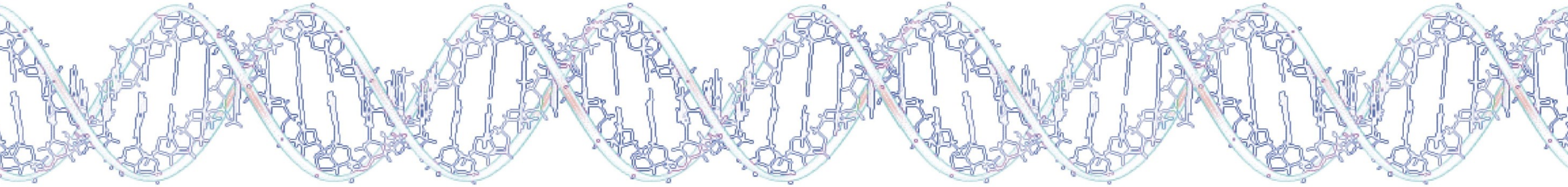
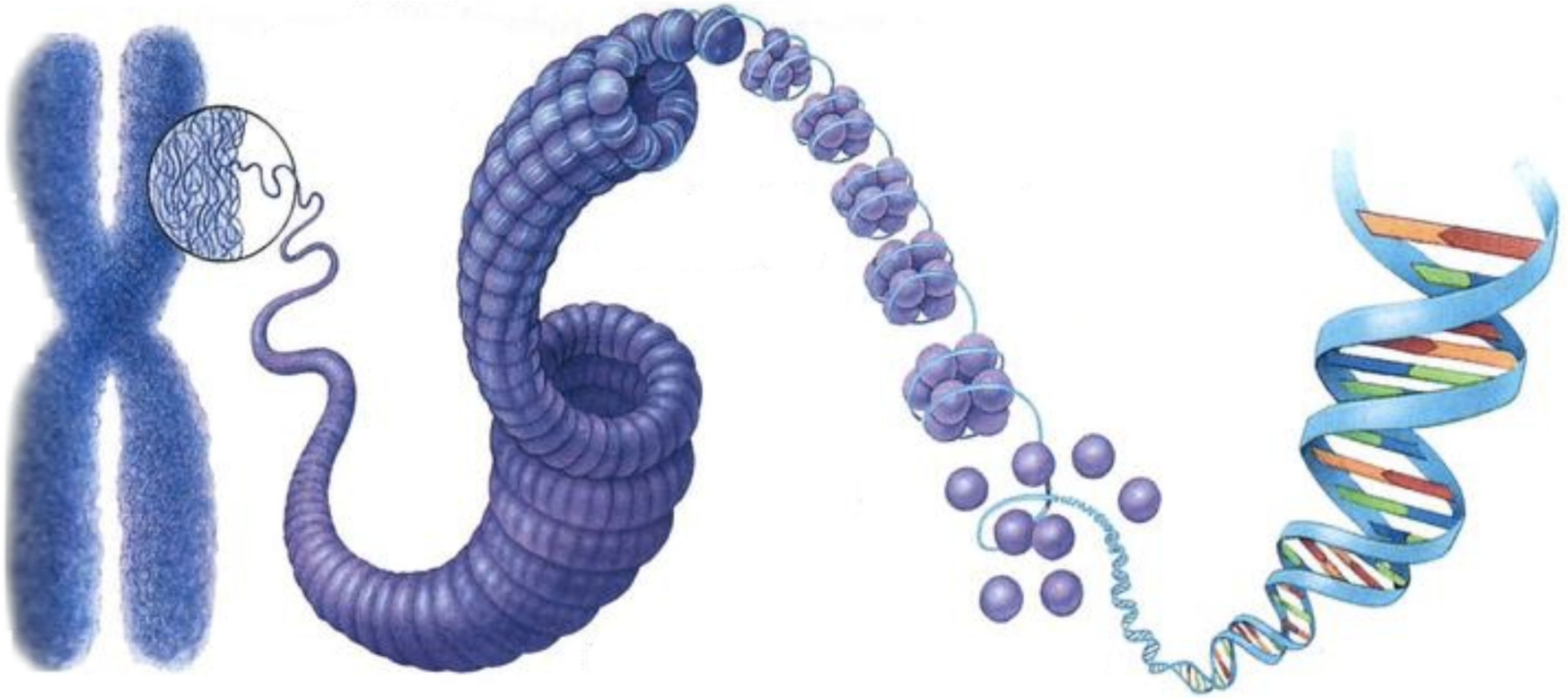
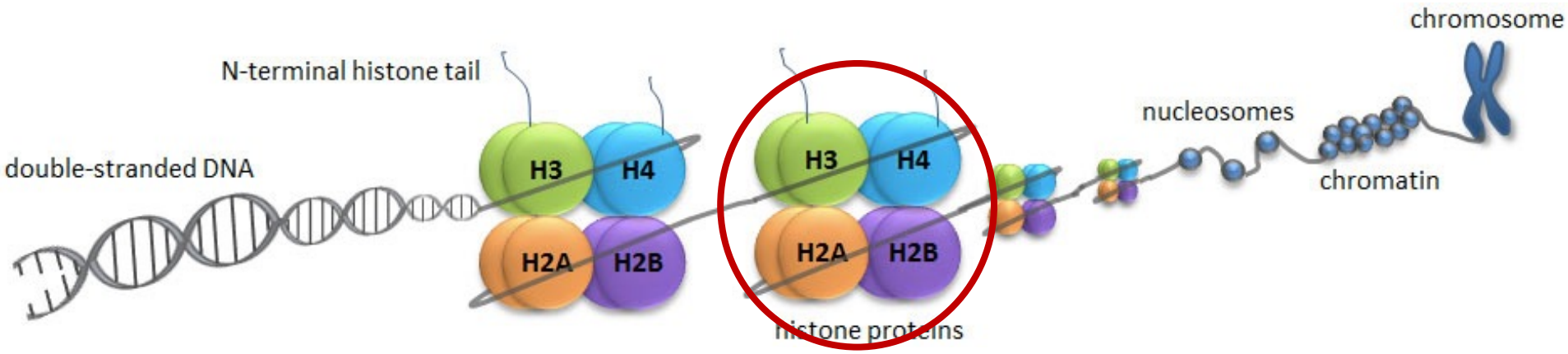
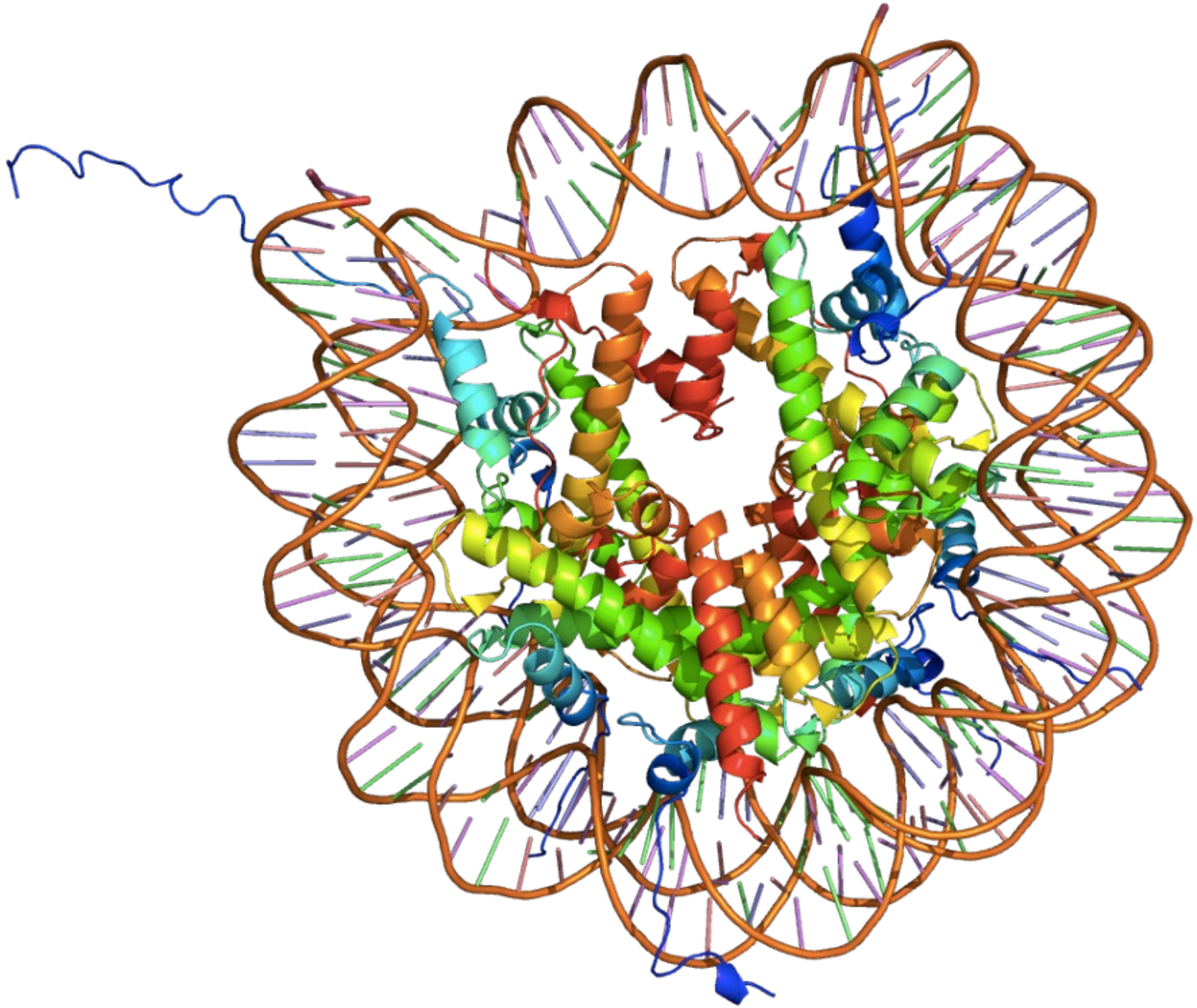
The Genome is Immense – Need to Fit DNA into a Nucleus



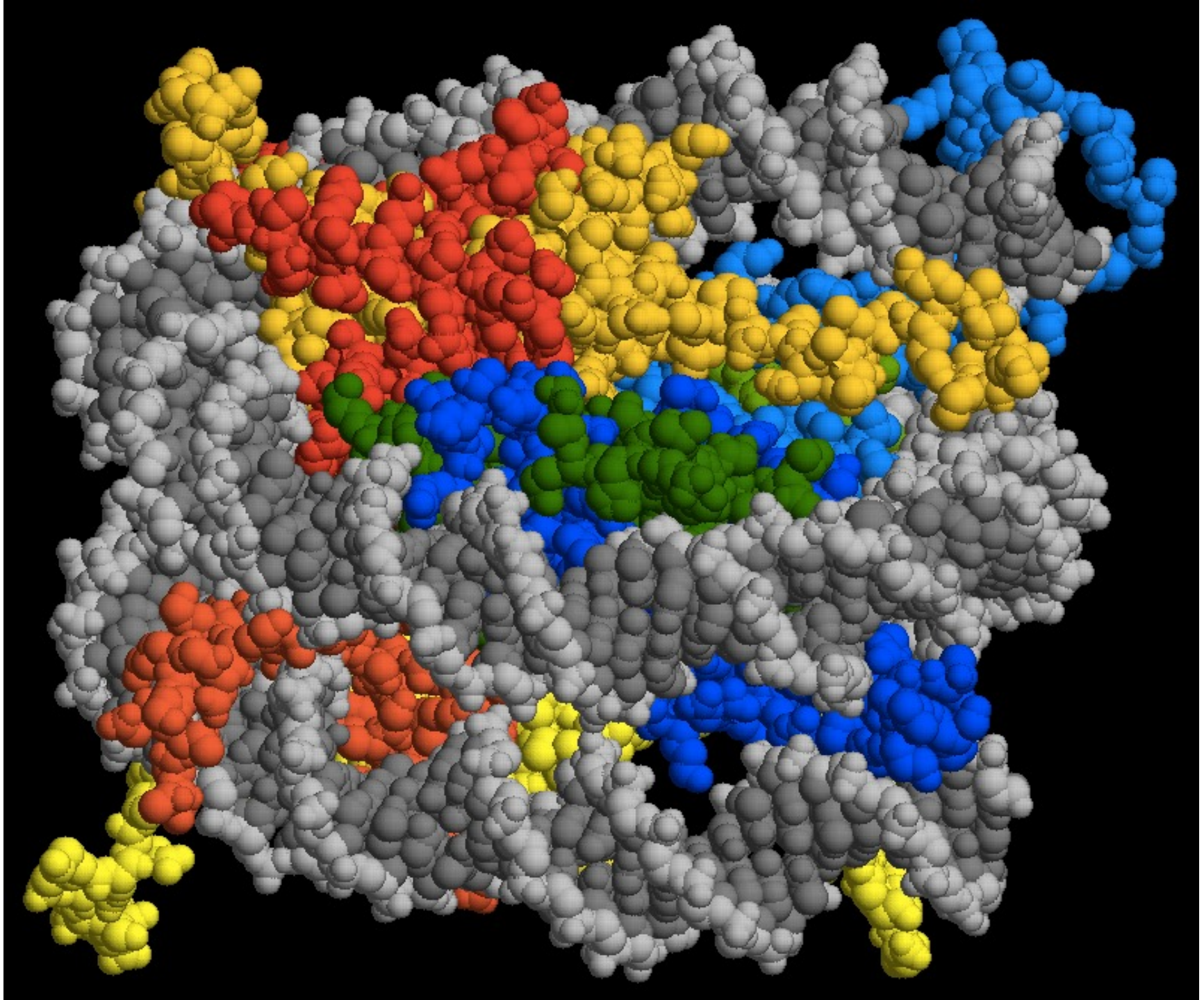


Histone Octamer or Nucleosome

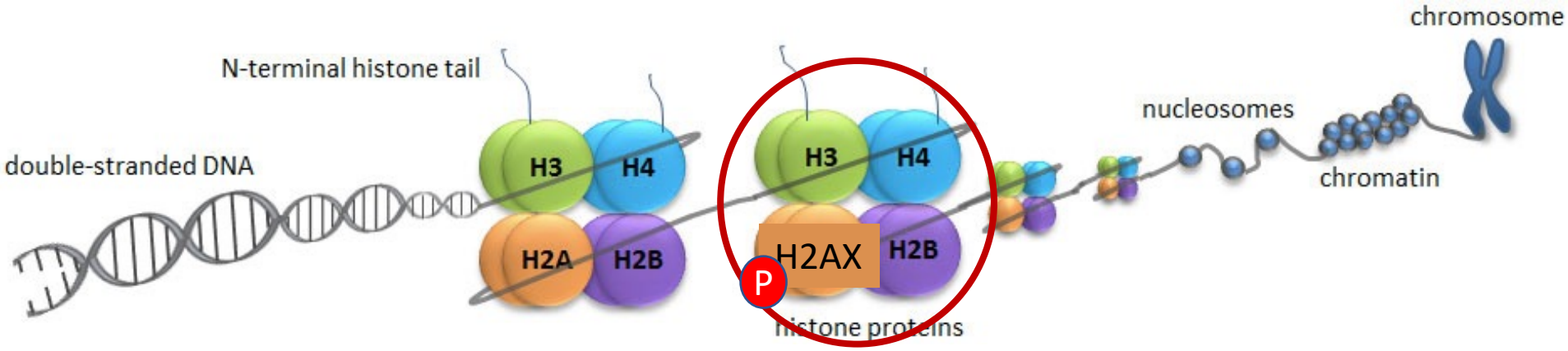




Histone tails
are
sites for
modification



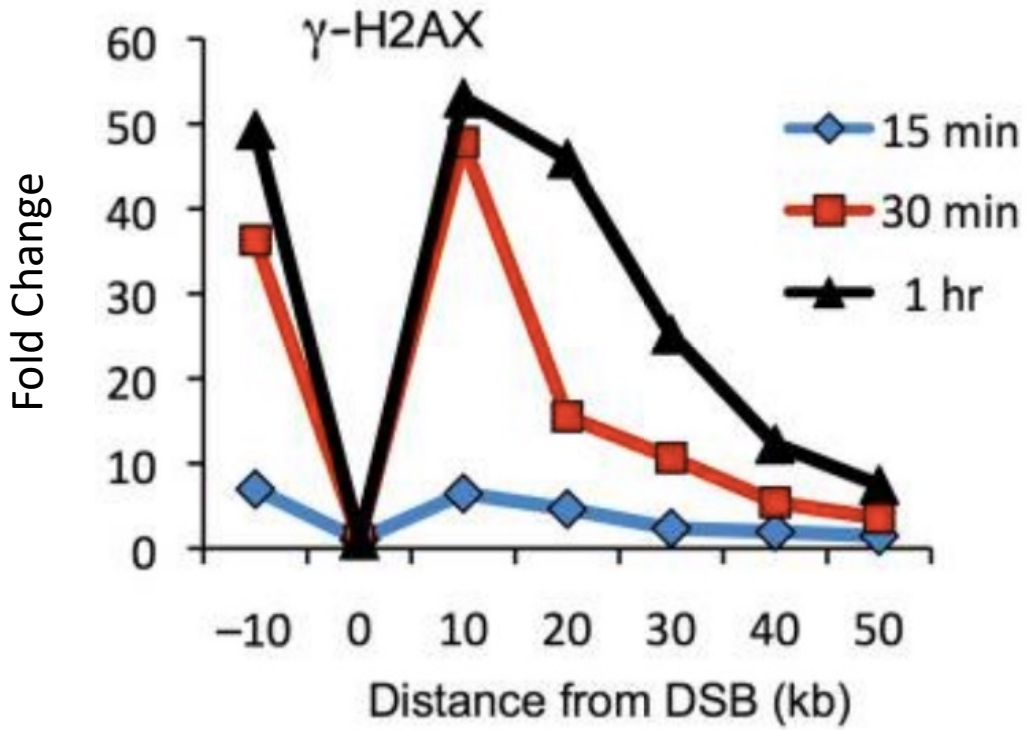
Histone Octamer or Nucleosome



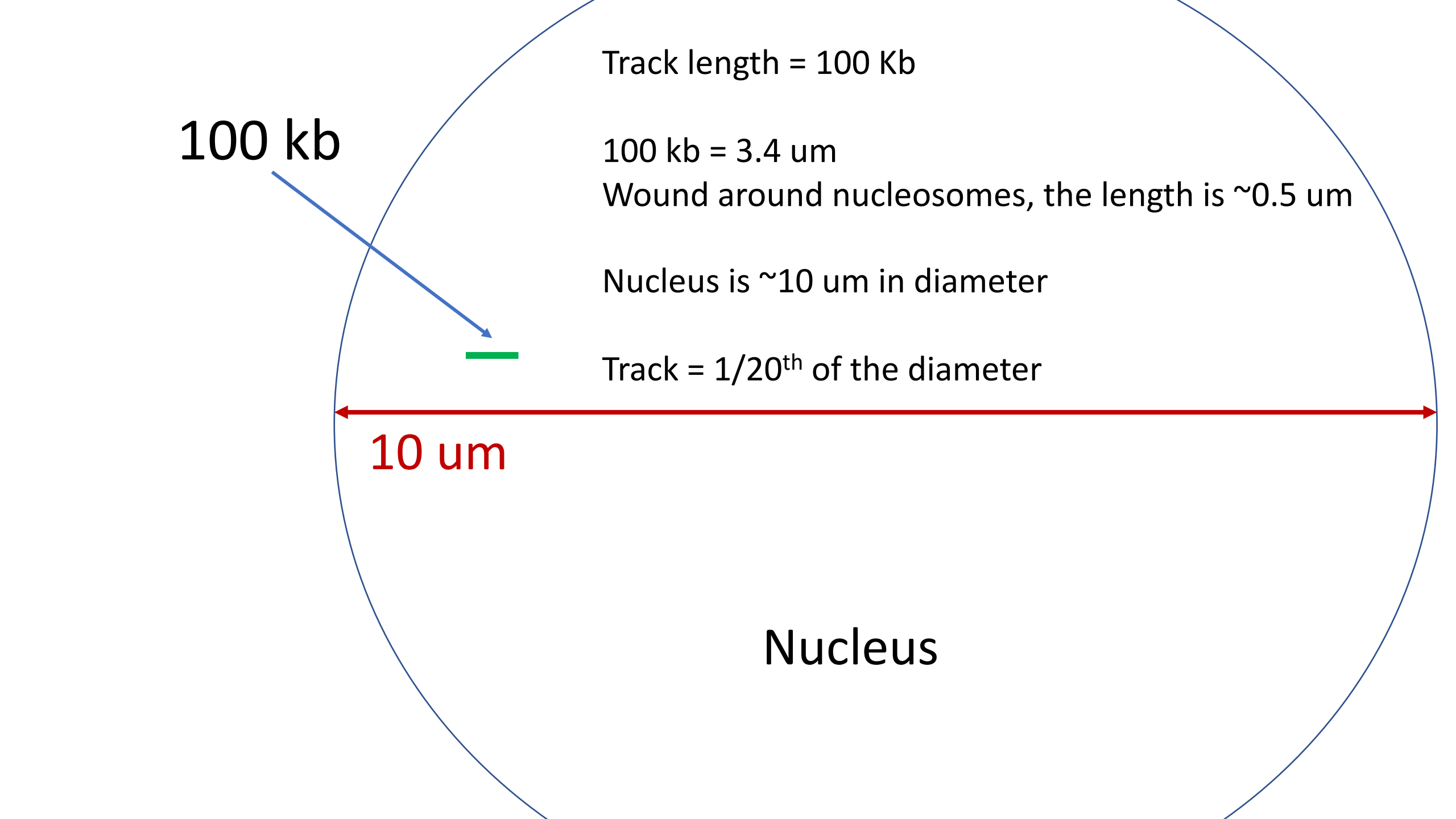
Occasionally H2A is replaced by H2AX

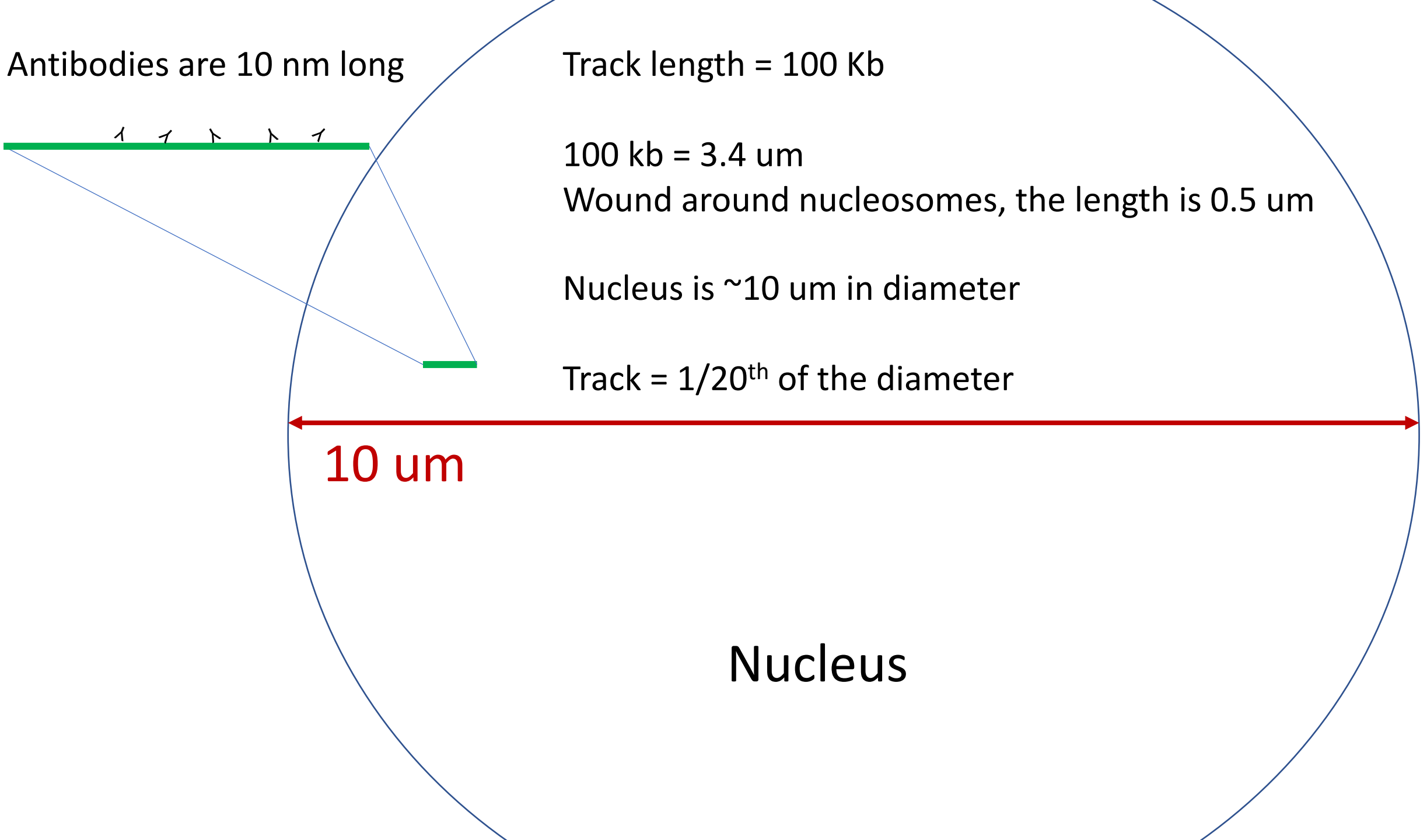
(Not all histones are the same; their structure can vary.)

Phosphorylated H2AX = γH2AX



Study performed in *S. cerevisiae* by Lee, Legube and Haber


100 kb
Track length = 100 Kb
100 kb = 3.4 um
Wound around nucleosomes, the length is ~0.5 um
Nucleus is ~10 um in diameter
Track = 1/20th of the diameter
10 um
Nucleus


Antibodies are 10 nm long
Track length = 100 Kb
100 kb = 3.4 um
Wound around nucleosomes, the length is 0.5 um
Nucleus is ~10 um in diameter
Track = 1/20th of the diameter
10 um
Nucleus

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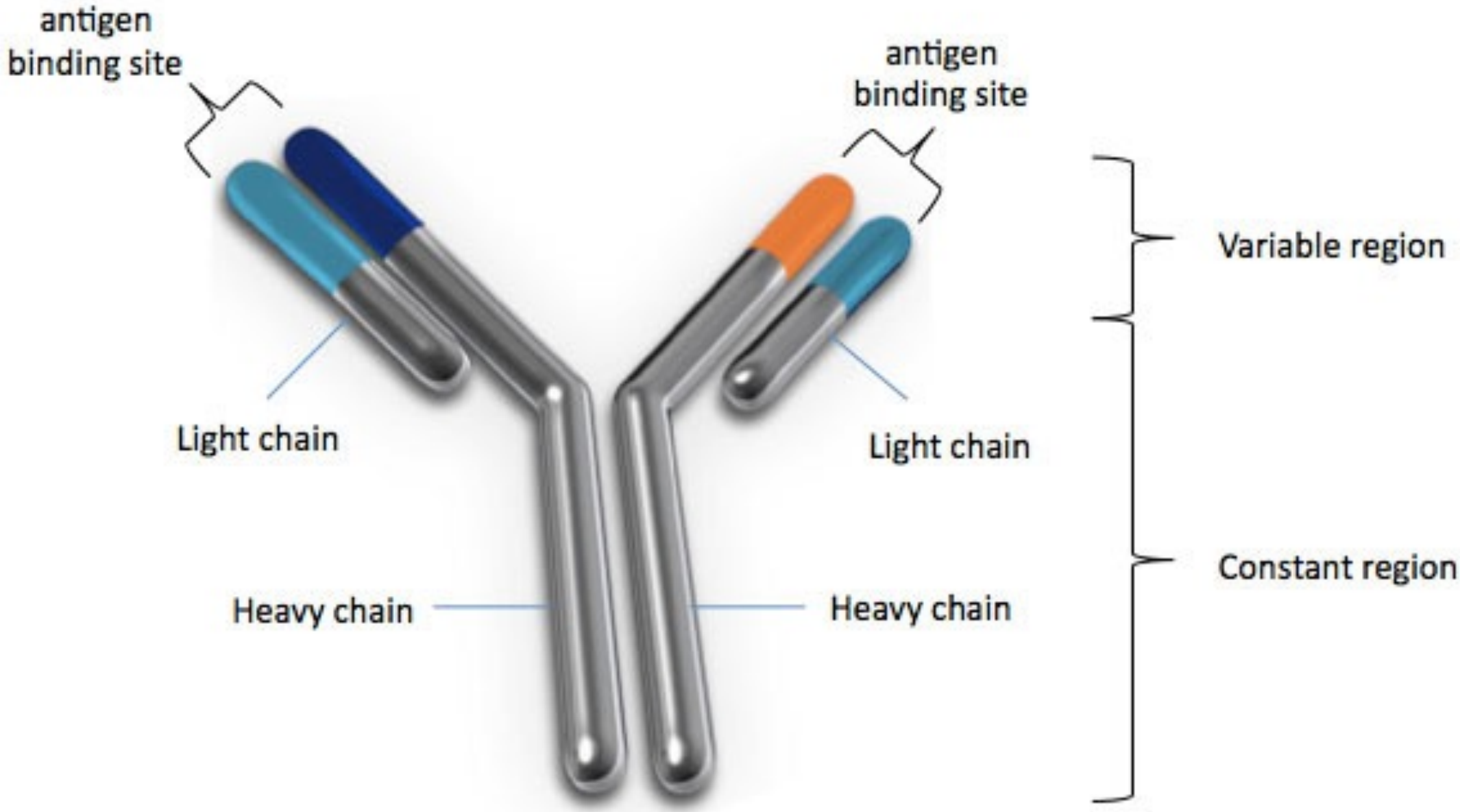
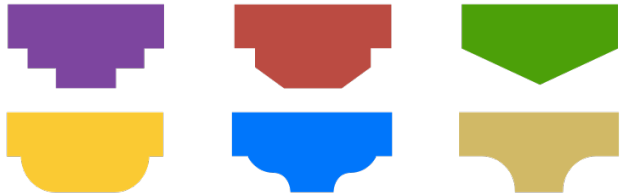
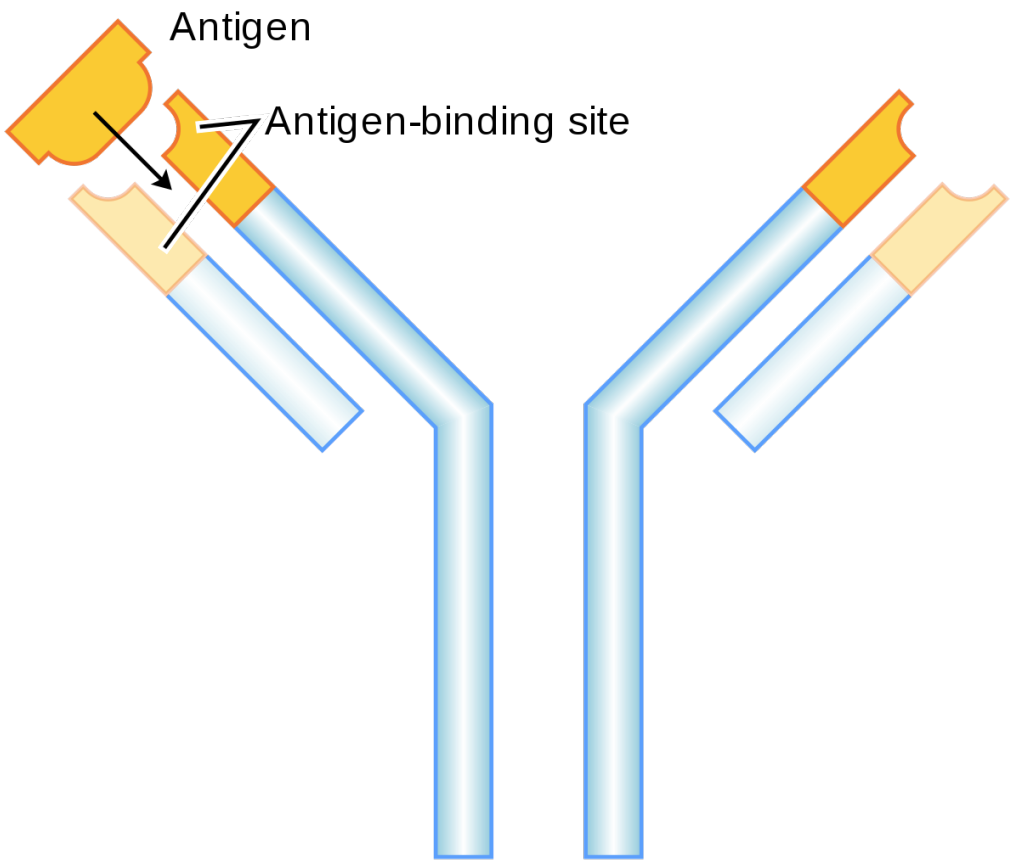
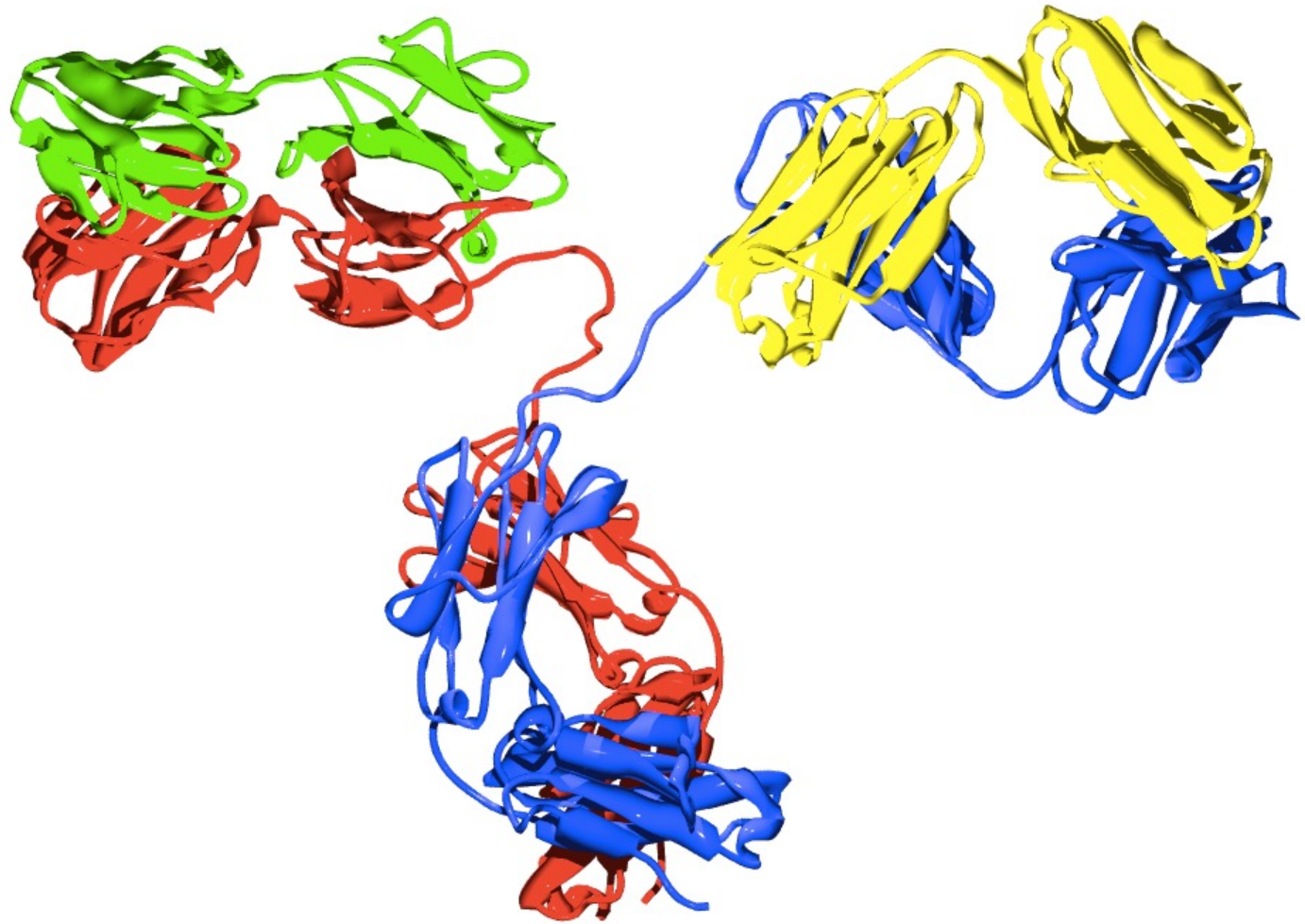

antigen
binding site
antigen
binding site
Variable region
Light chain
Light chain
Constant region
Heavy chain
Heavy chain

Antigens

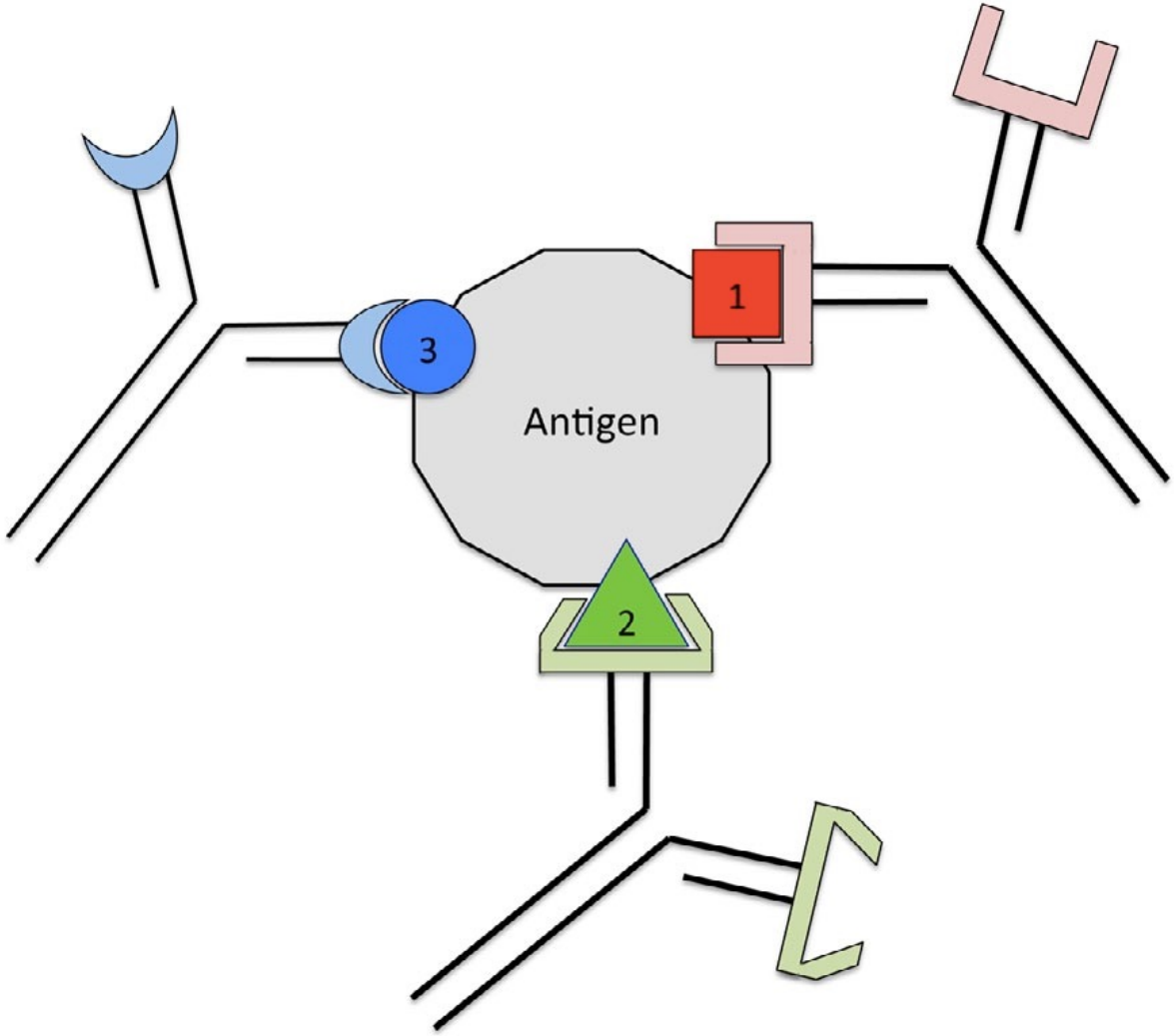




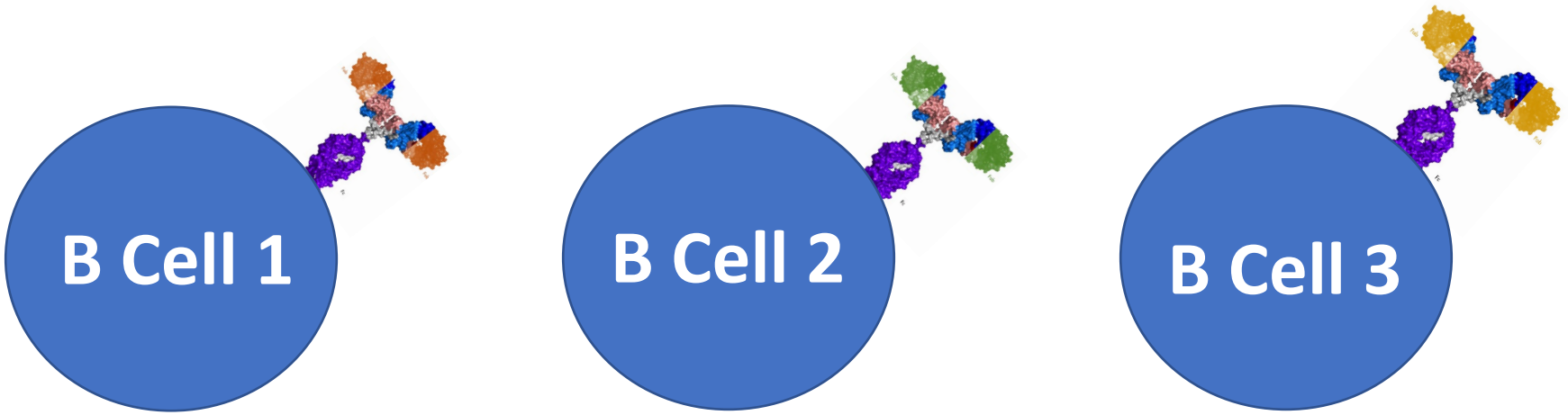
Antibody

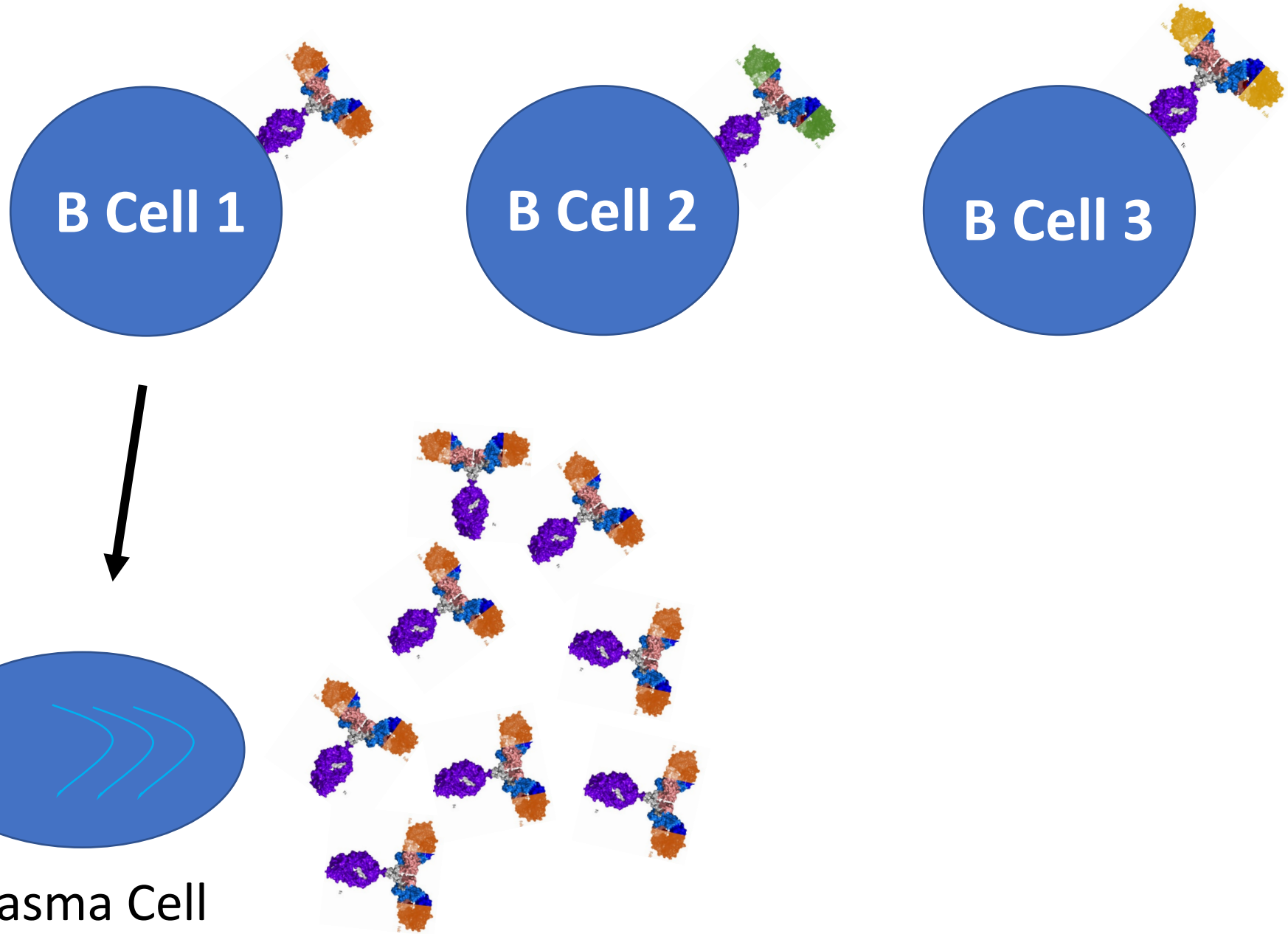


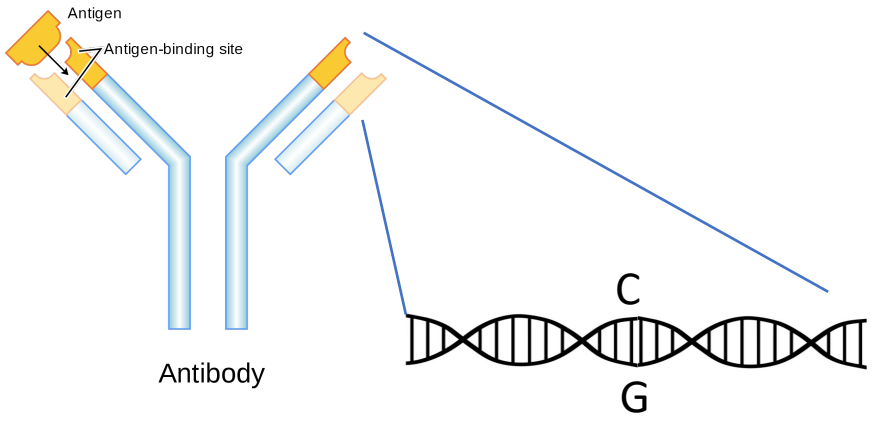
Three Different Epitopes



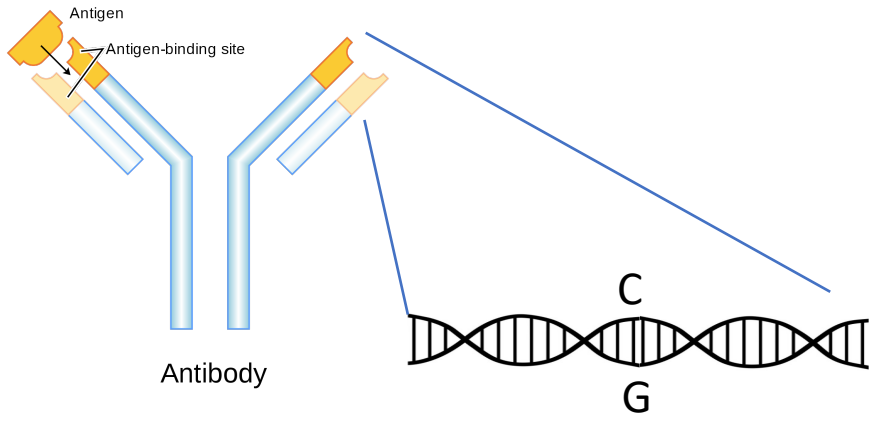
How does BER affect your Covid Booster Shot?


B Cell 1
B Cell 2
B Cell 3


B Cell 1
B Cell 2
B Cell 3
Plasma Cell



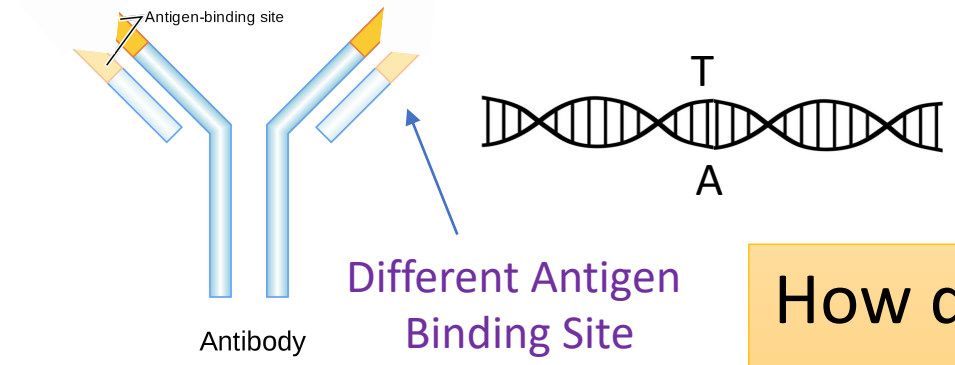
DNA that Codes for Antigen Binding Site



DNA that Codes for Antigen Binding Site

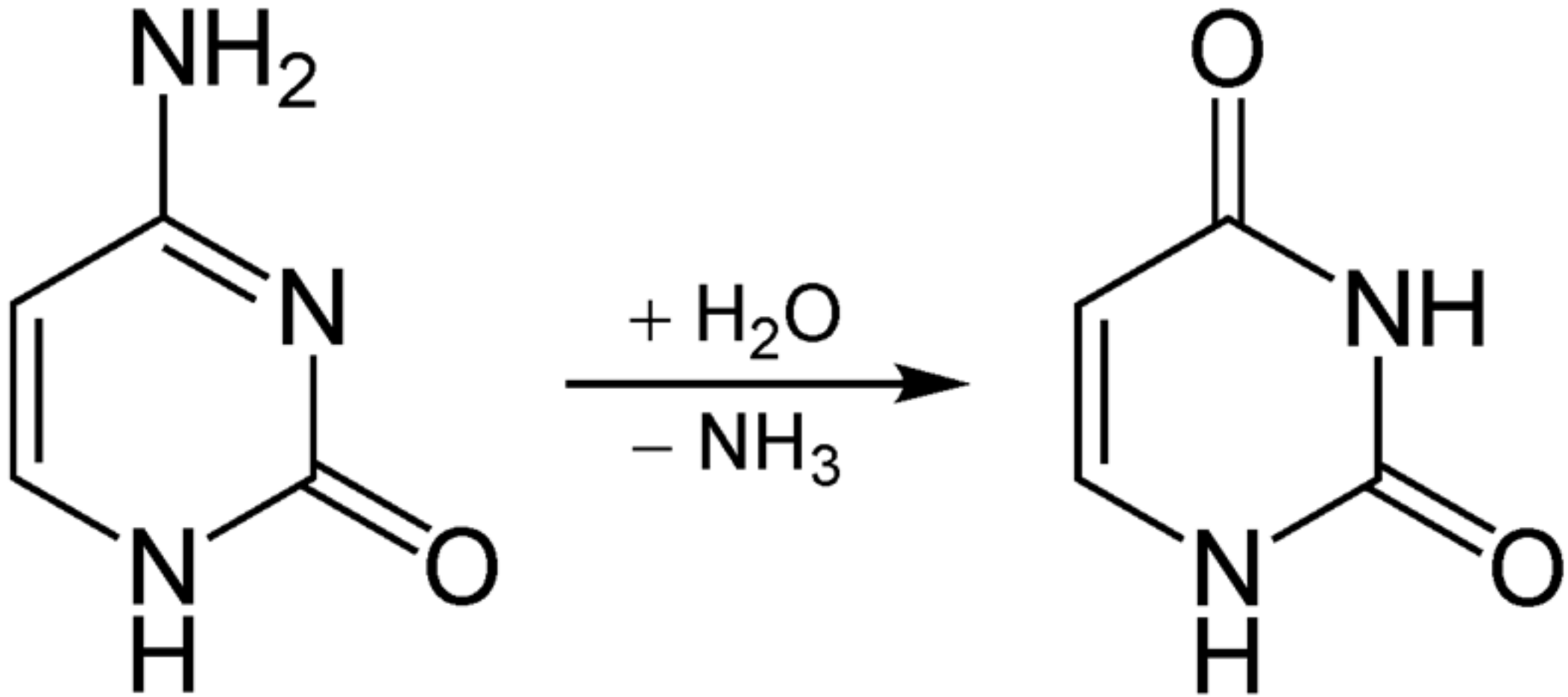
Create Mutations

To Make Different Antibodies



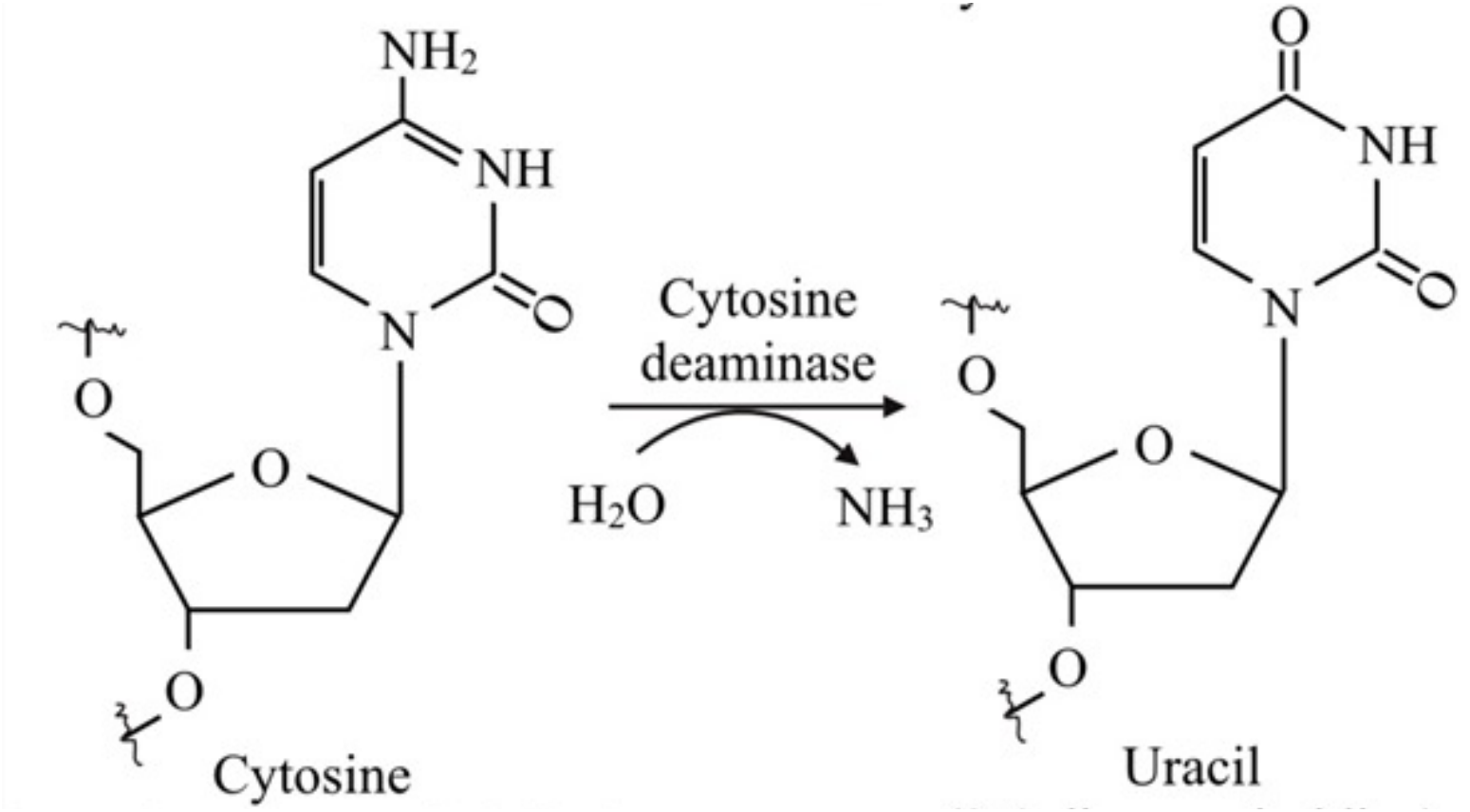
Different Antigen Binding Site

How do you change the sequence?
Introduce DNA damage!


NH2
N
N
H
O
+ H2O
– NH3
O
NH
N
H
O

How to Damage DNA on Purpose: Deaminate Cytosine

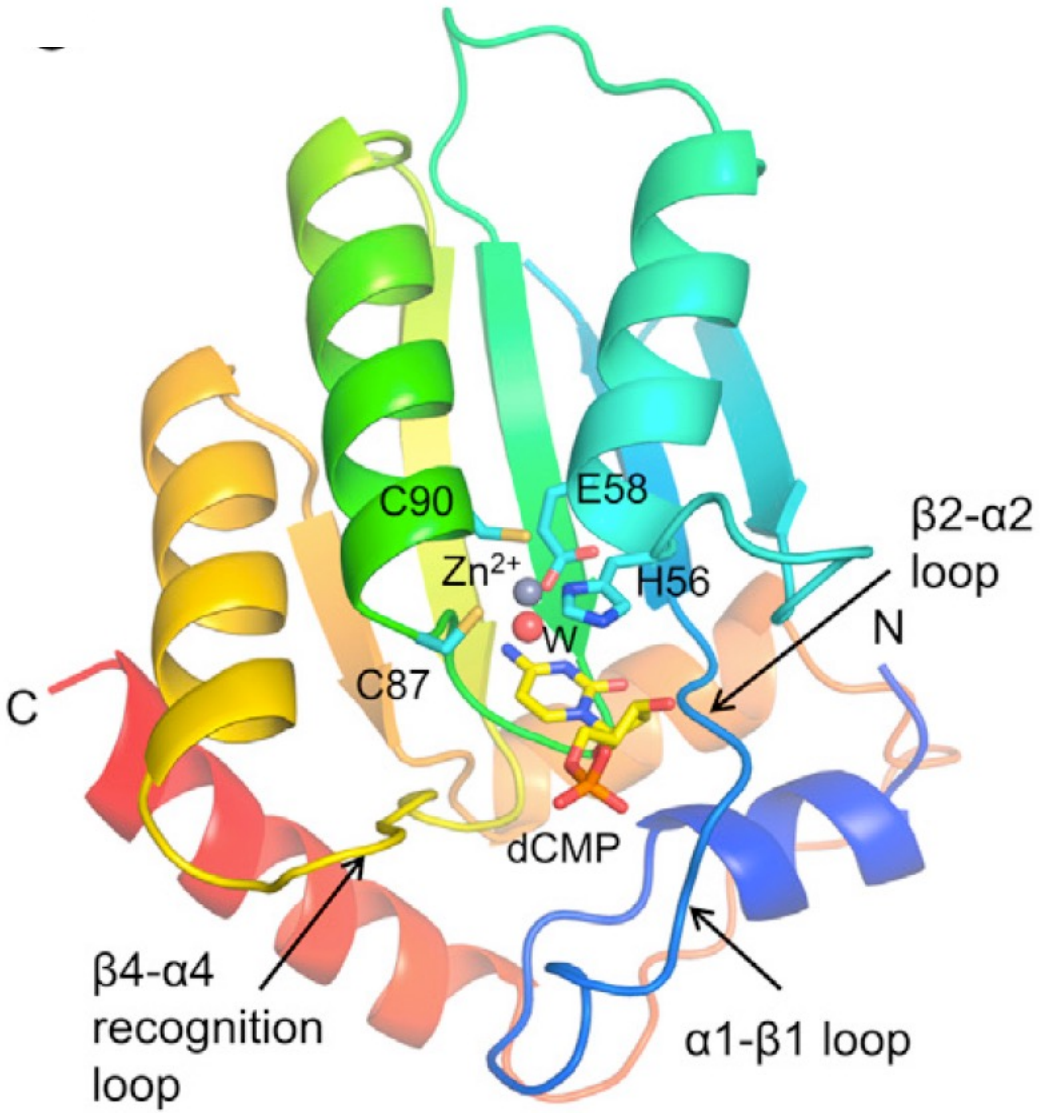
Activation Induced Cytidine Deaminase (AID)



Converts C to U in Certain Regions

Chegg

Activation Induced Cytidine Deaminase (AID)



C → U

G G

Qi Qiao... Hao Wu

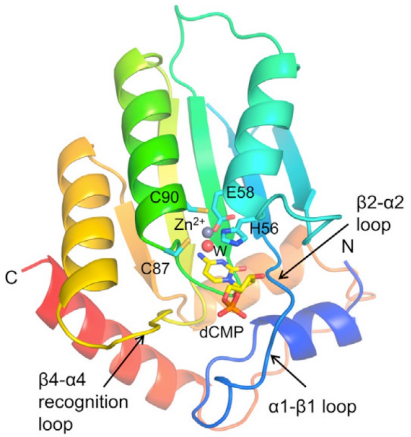
C

G

Activation Induced
Cytidine Deaminase (AID)

U

G



DNA REPLICATION

U

A

CG to TA

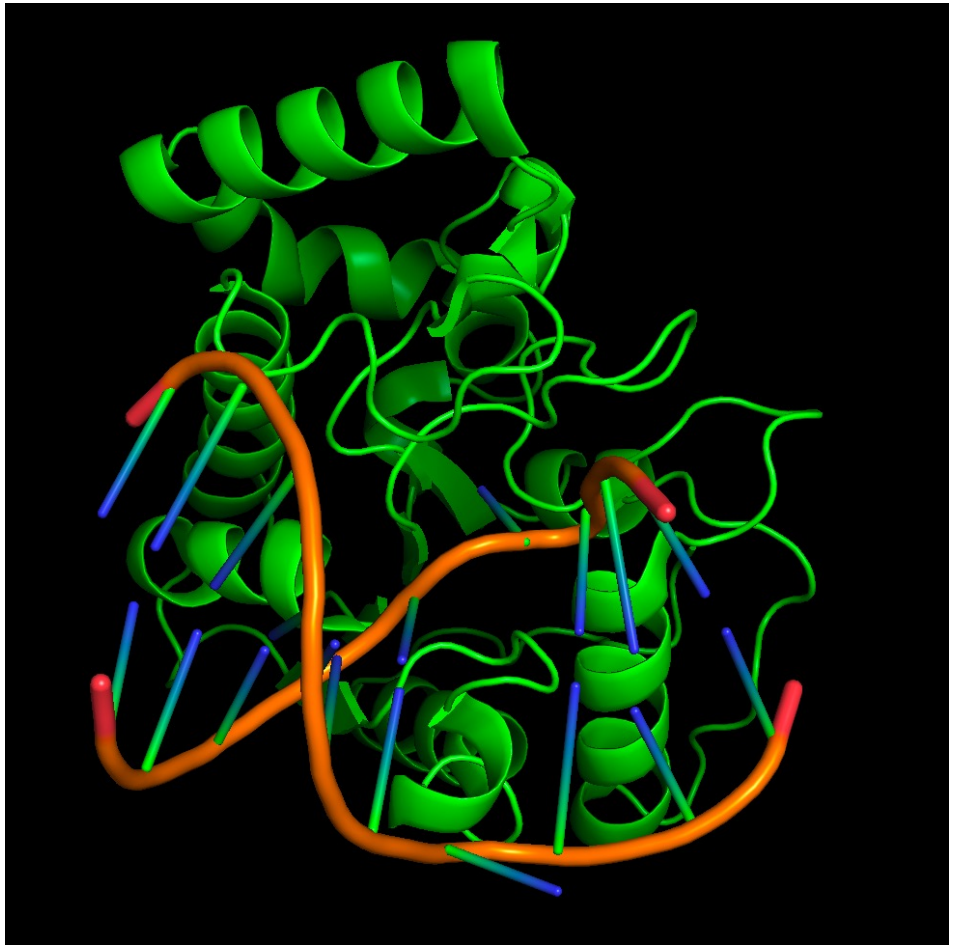
DNA REPLICATION

T

A

Direct conversion of C to T

Ung DNA Glycosylase Removes Uracil from the DNA

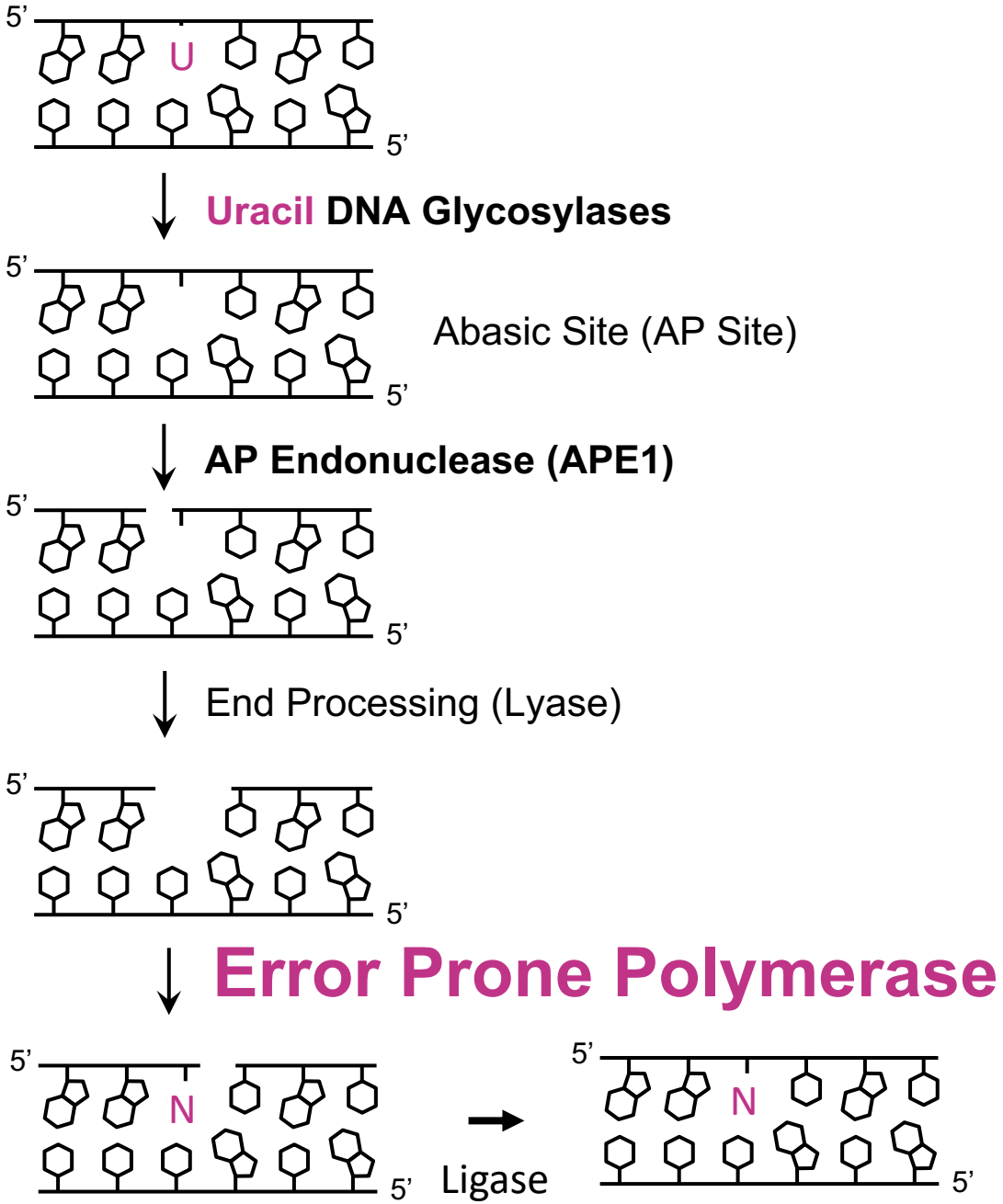


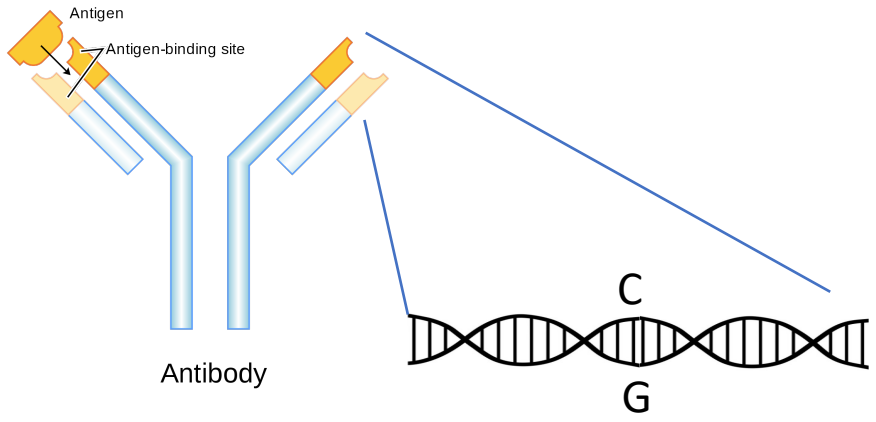
Parikh, S.S., Slupphaug, G., Krokan, H.E., Blackburn, G.M., Tainer, J.A.

Another way to change the DNA sequence is via Sloppy BER

Special Polymerase That is Error Prone

Makes Errors during BER





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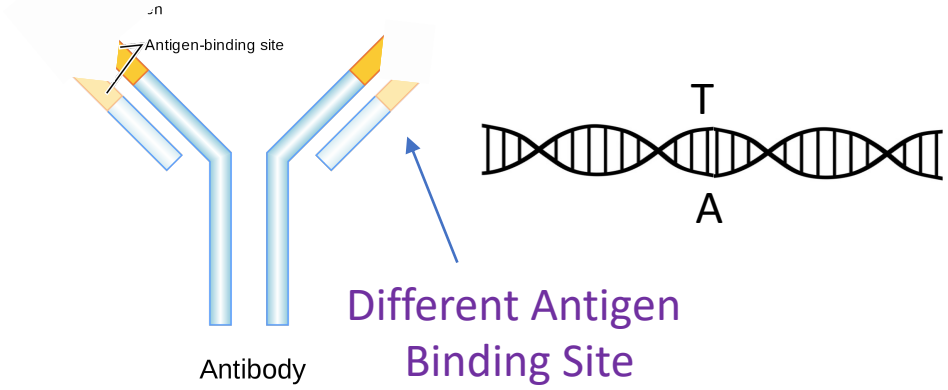
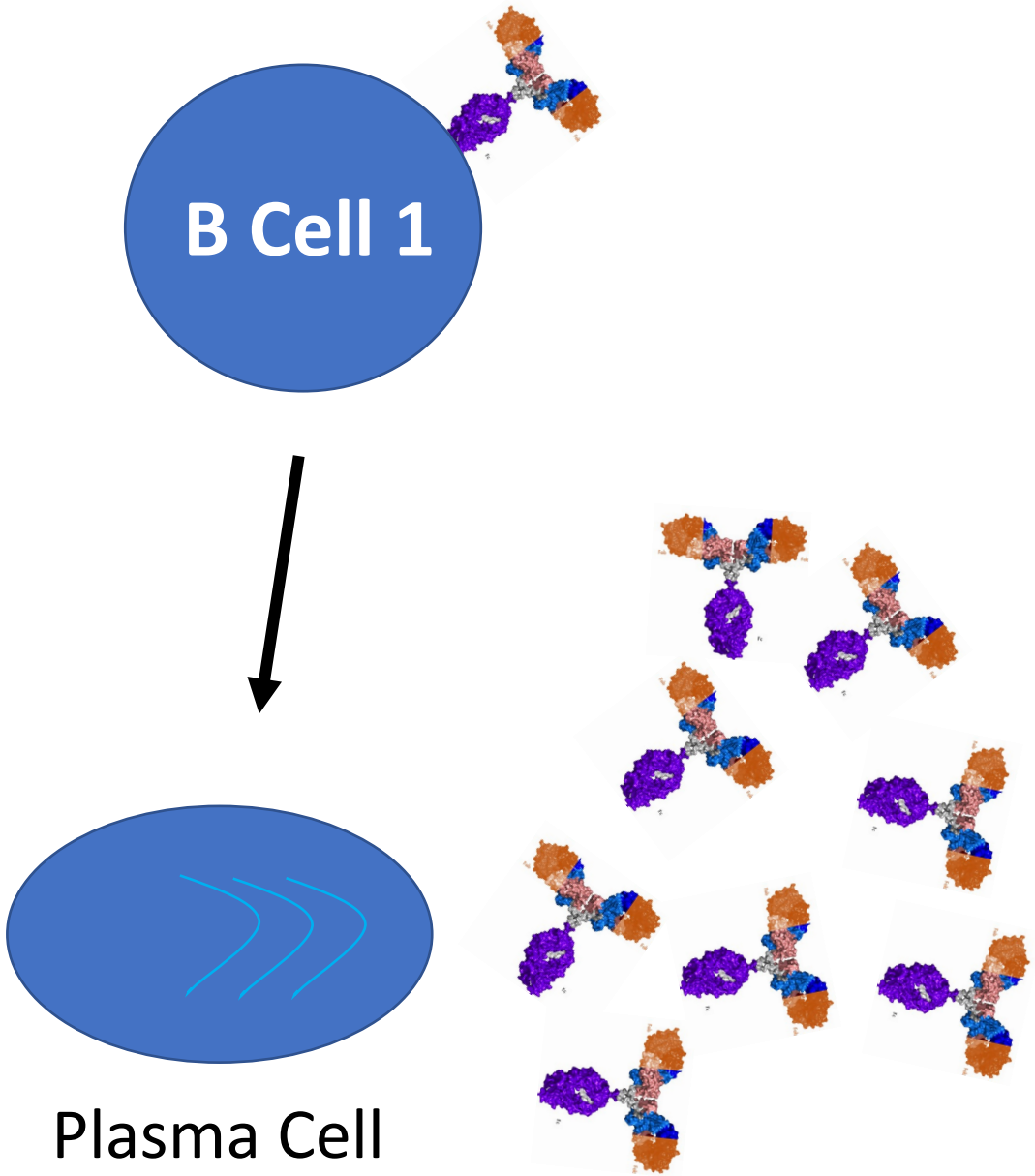
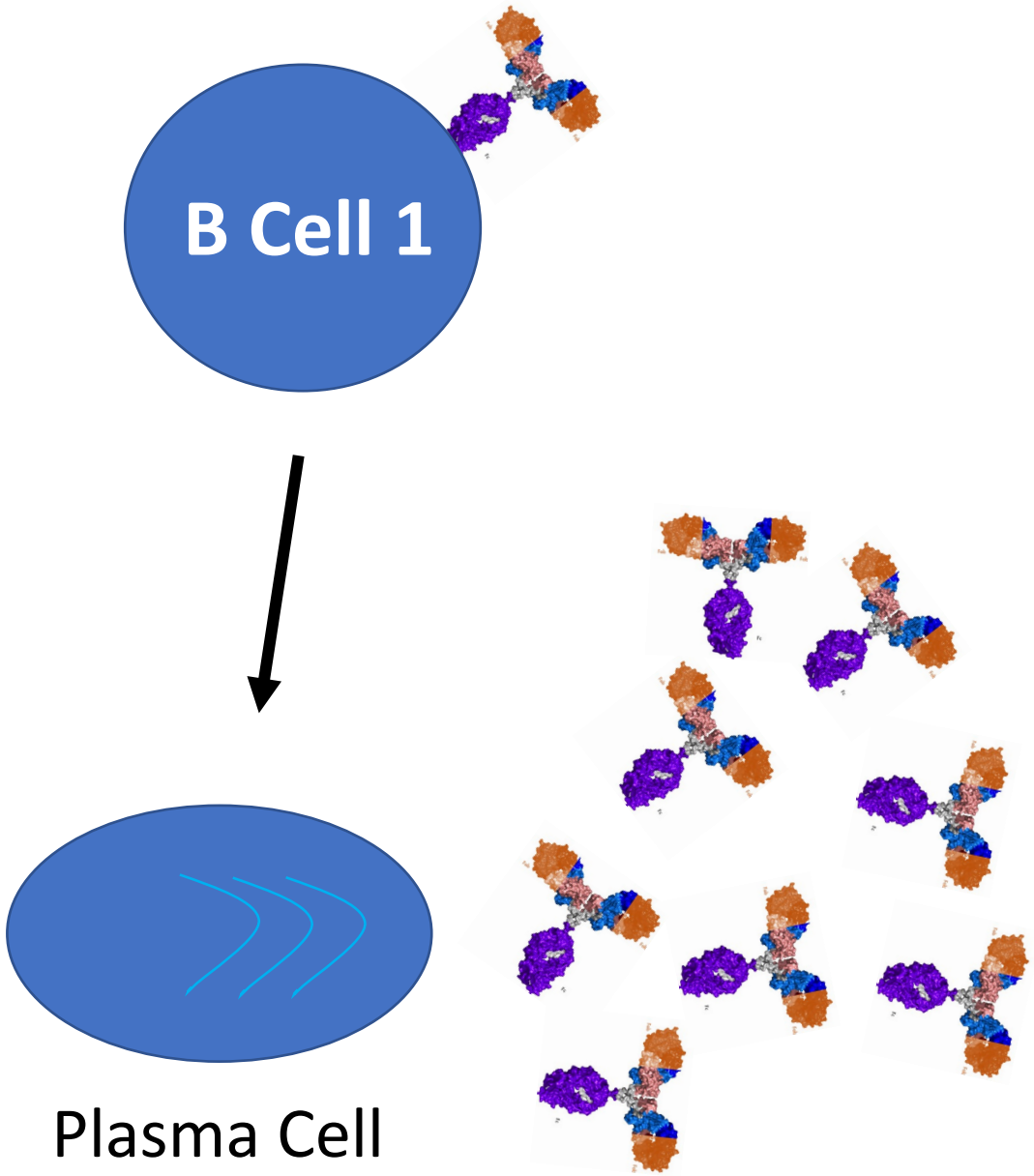
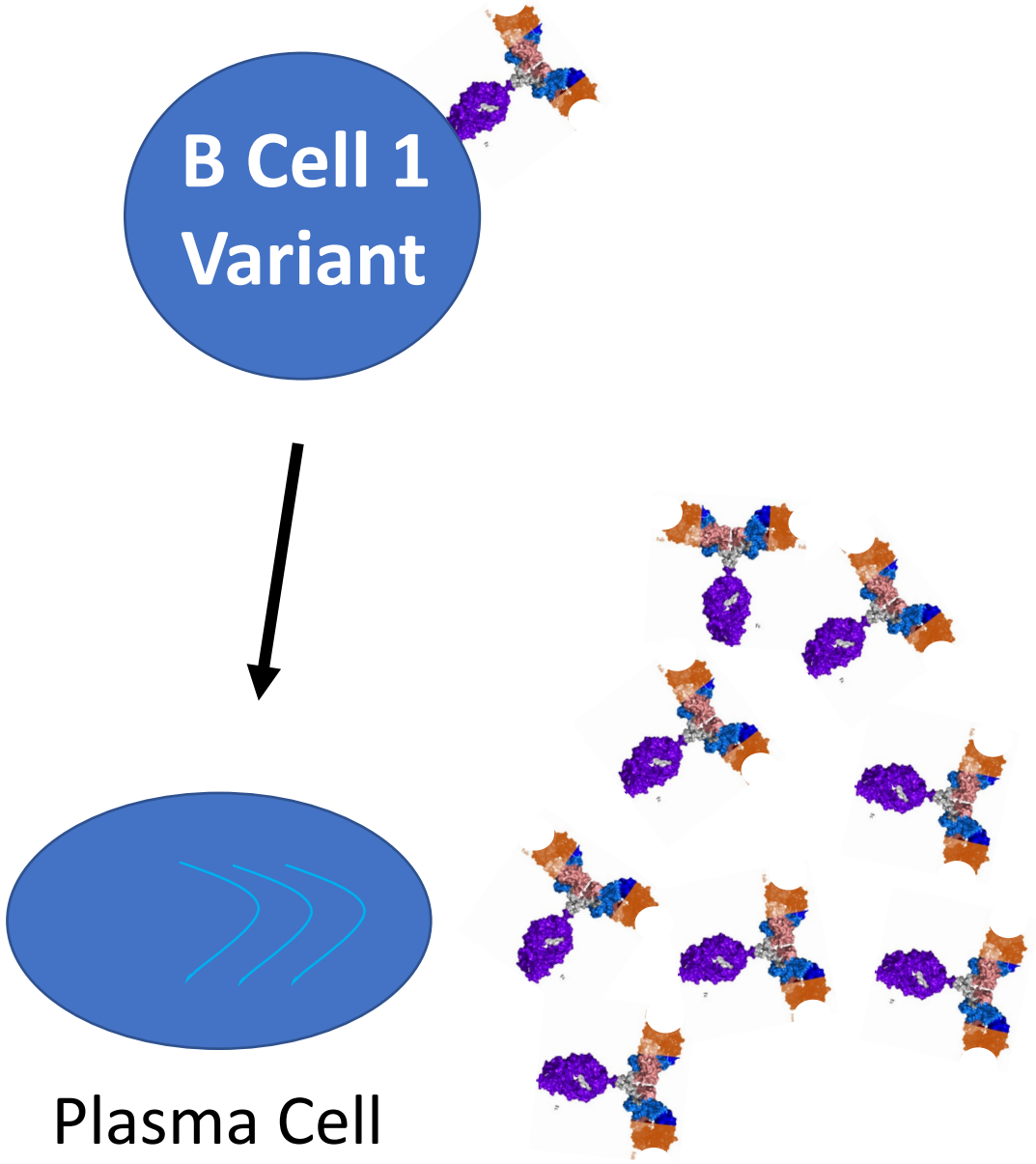
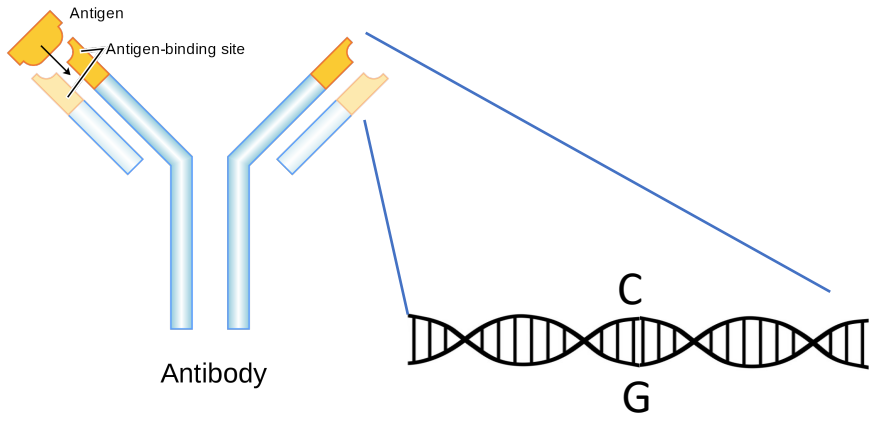



B Cell 1
Plasma Cell

Might have better affinity.







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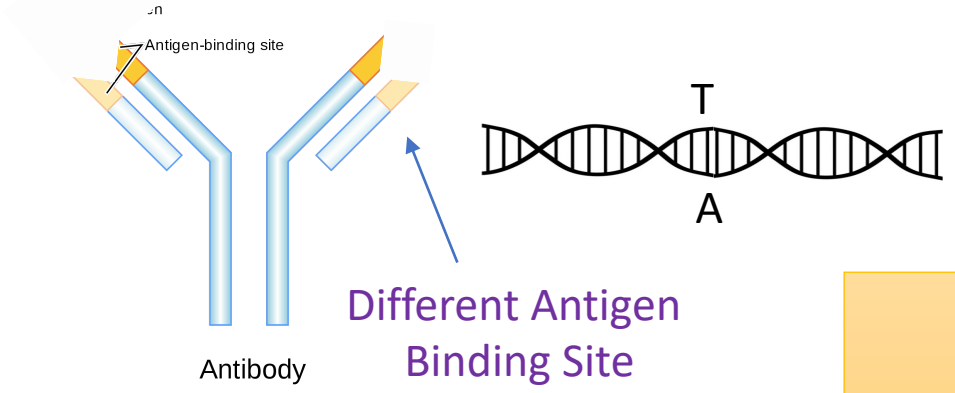

Different Antigen Binding Site

BER Promotes Antibody Diversification

Your booster shot wouldn’t work without BER!

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