IMMUNOGLOBULIN G

Marina Cabasés, Laura Ciaran, Marta Díez, Laura Enriquez

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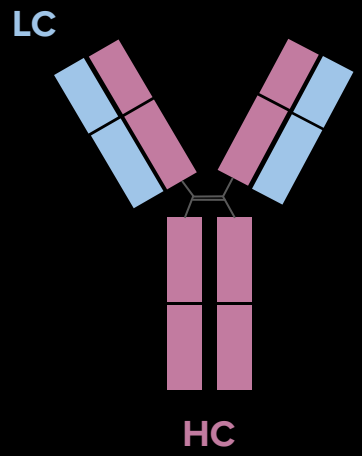
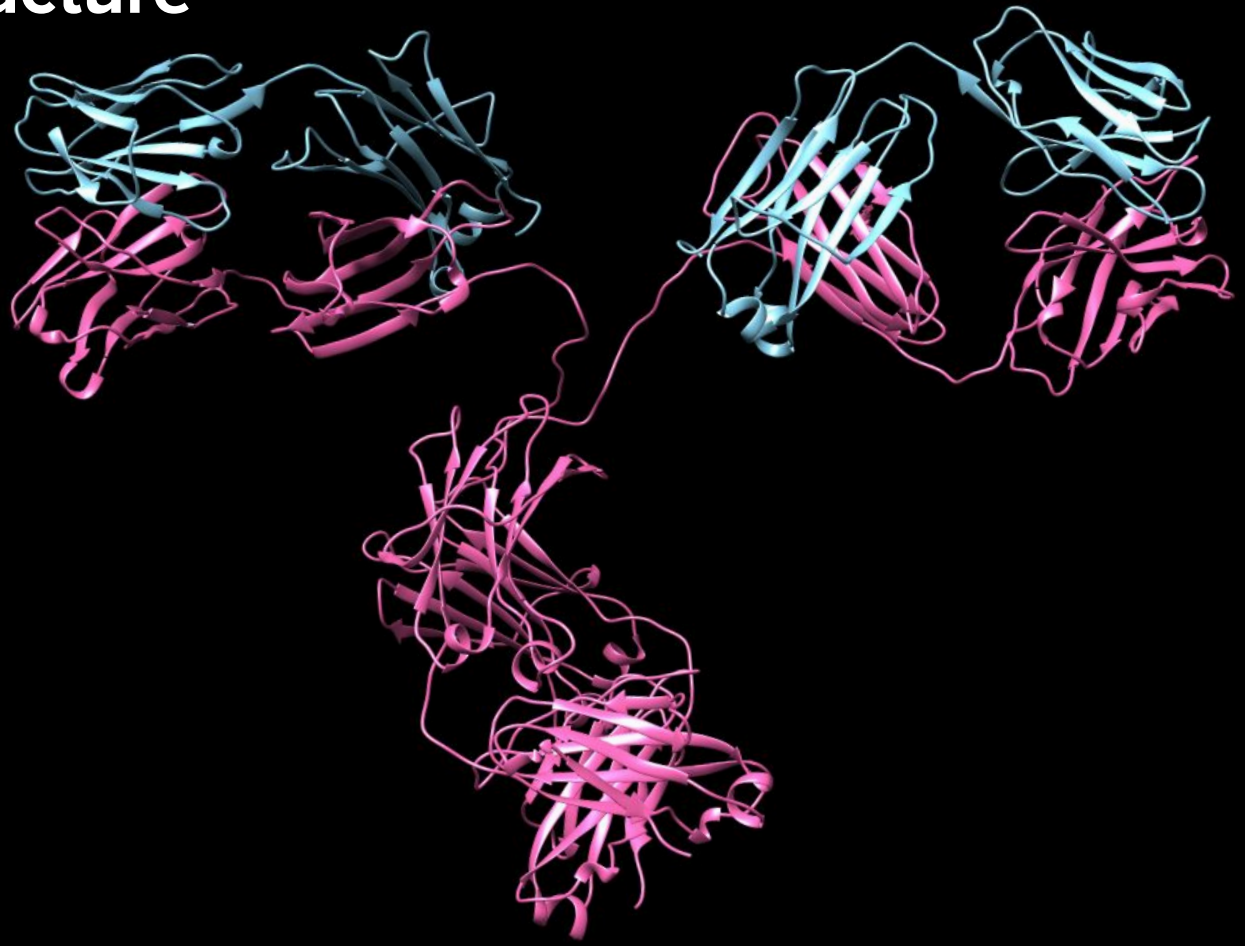
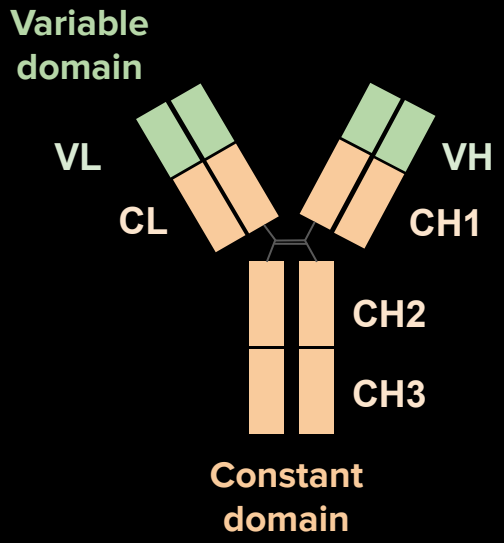
BASIC CONCEPTS

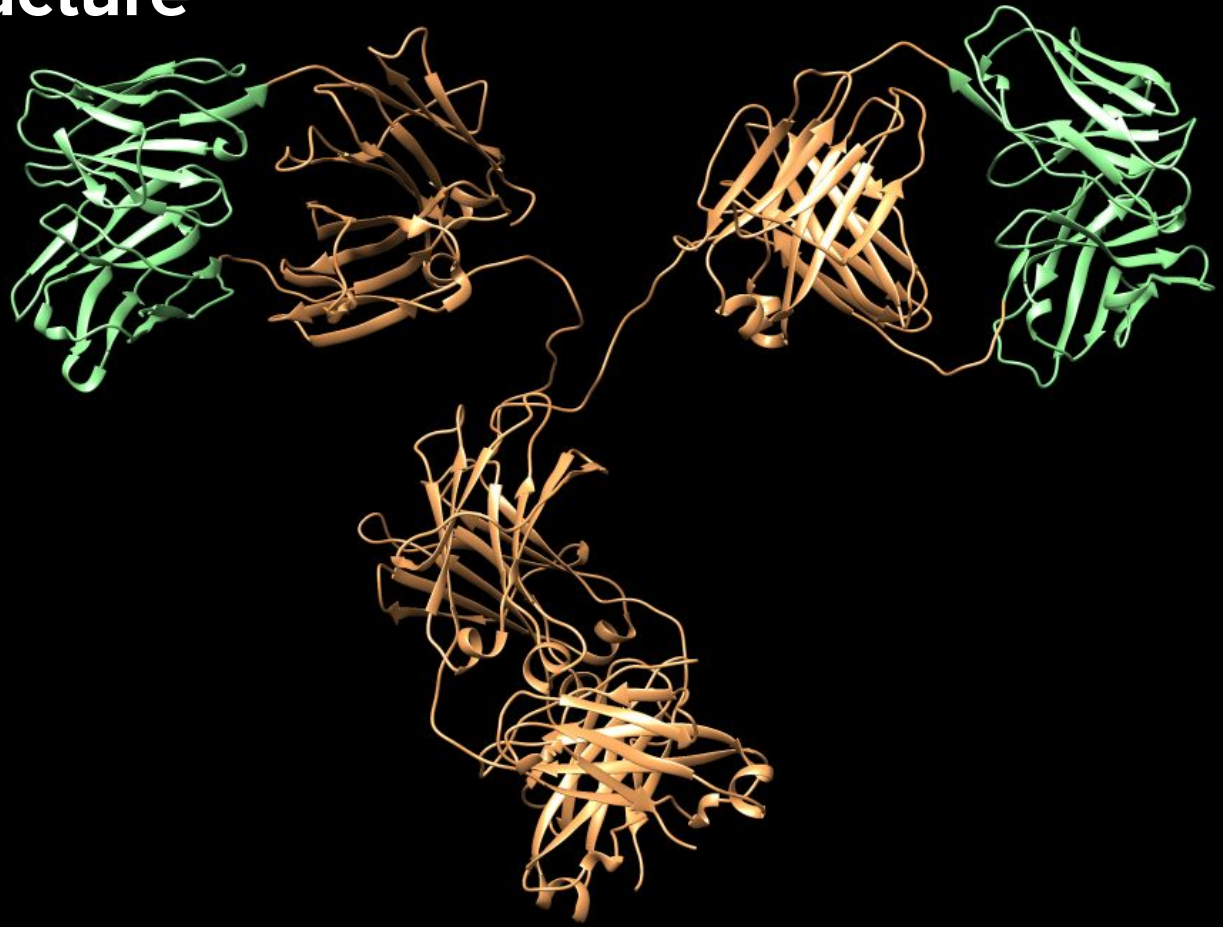
Introduction: Structure



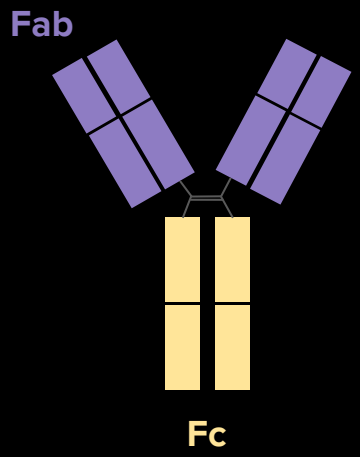


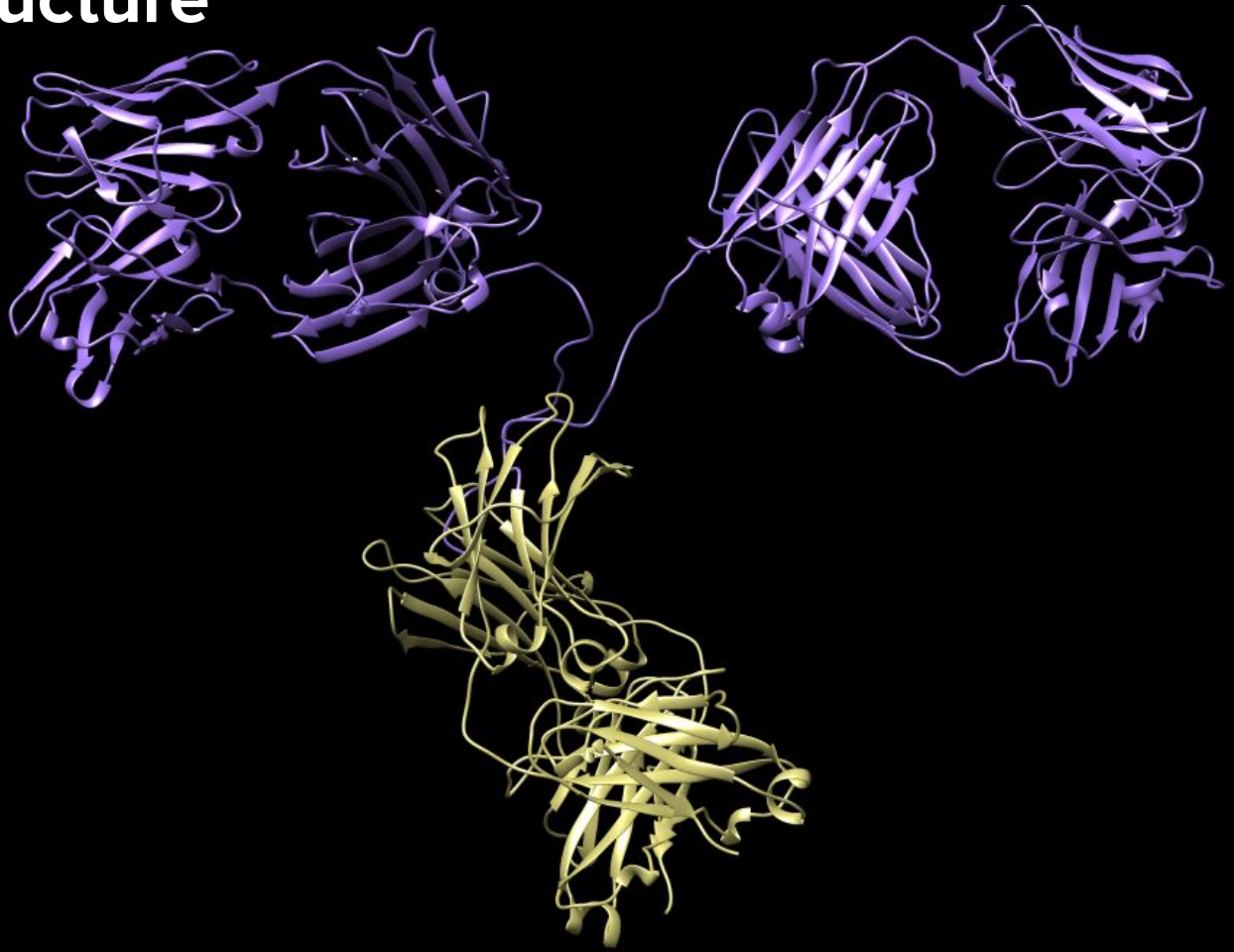
Introduction: Structure





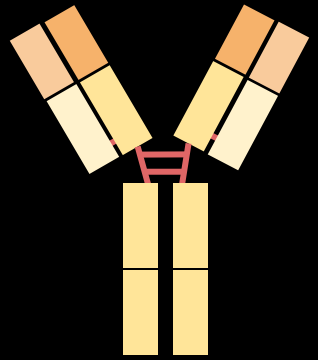
Introduction: Structure



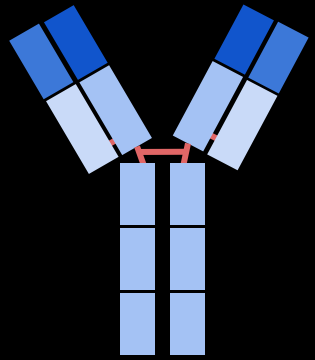


Introduction: Isotypes

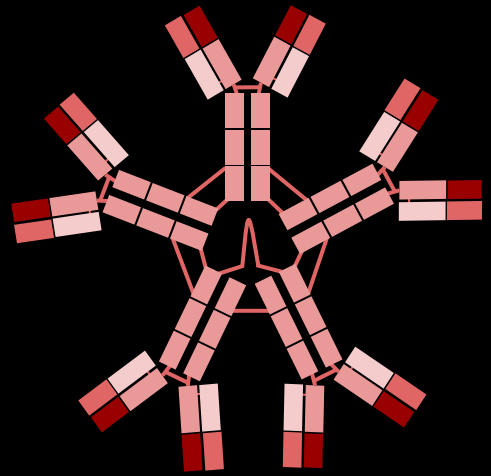
IgG



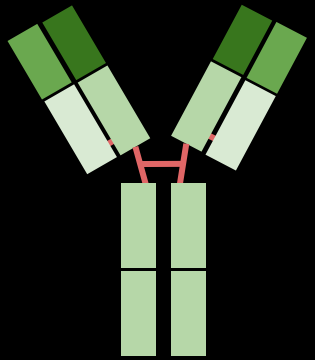
IgE



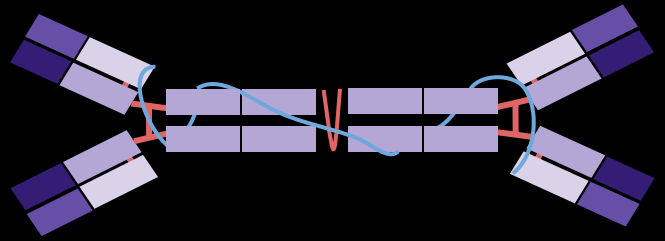
IgM



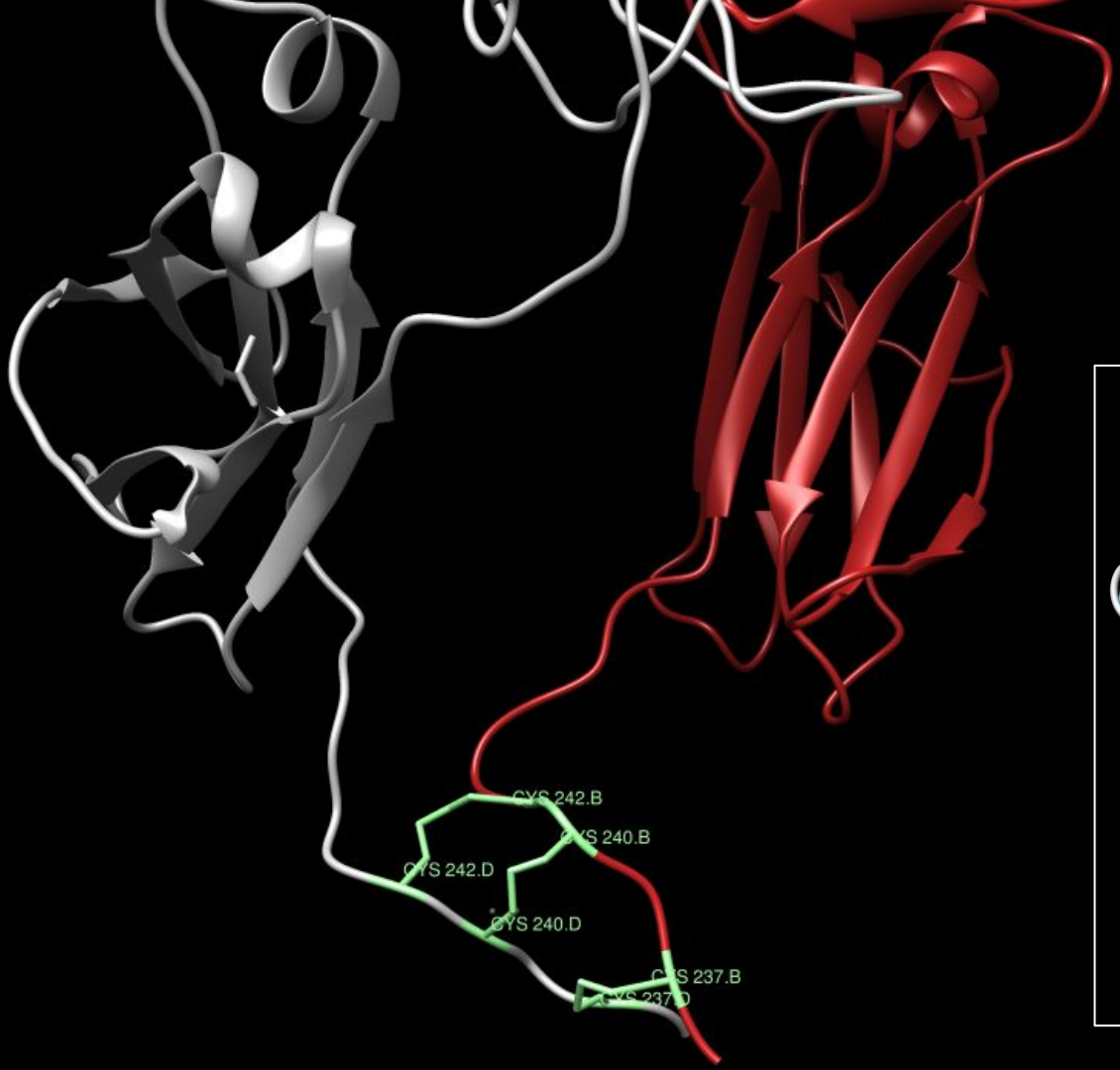
IgD

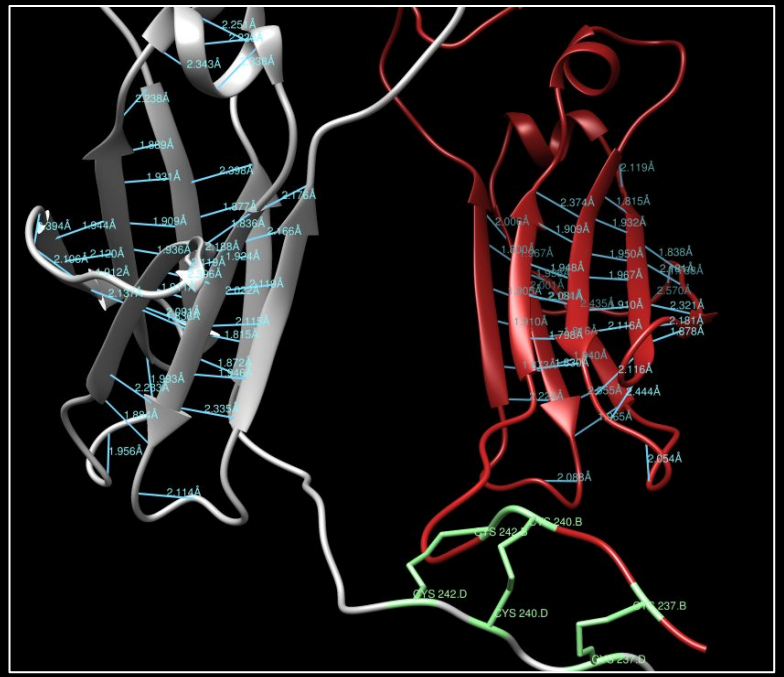


IgA

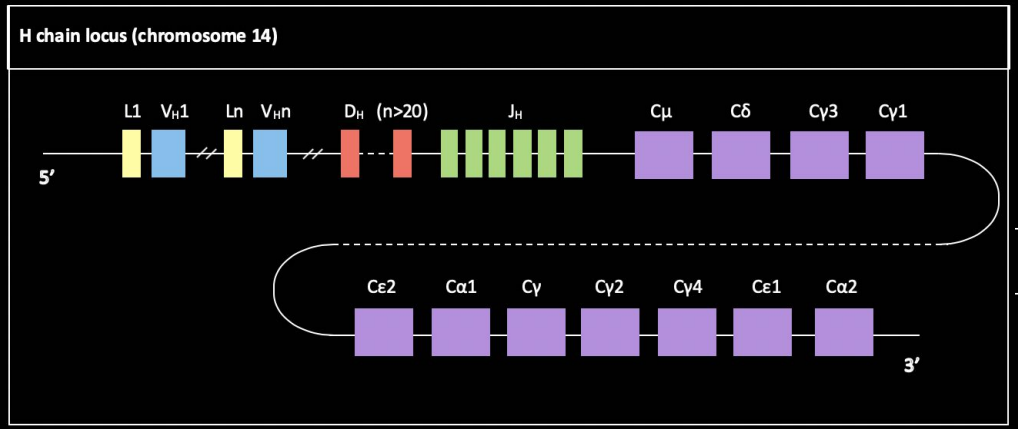


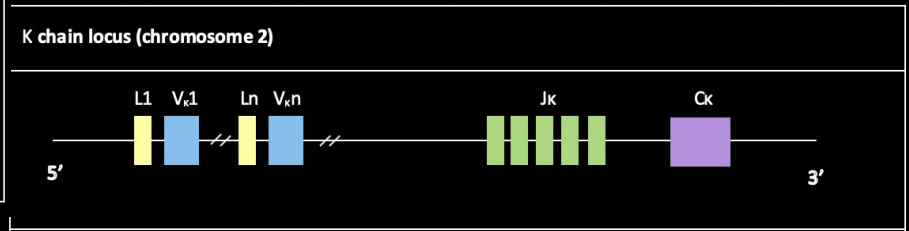
Introduction: Stabilization

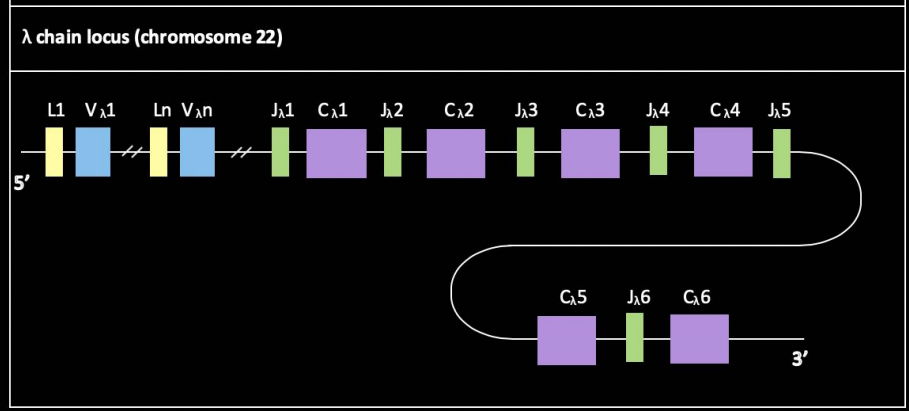




Introduction: Immunoglobulin rearrangement







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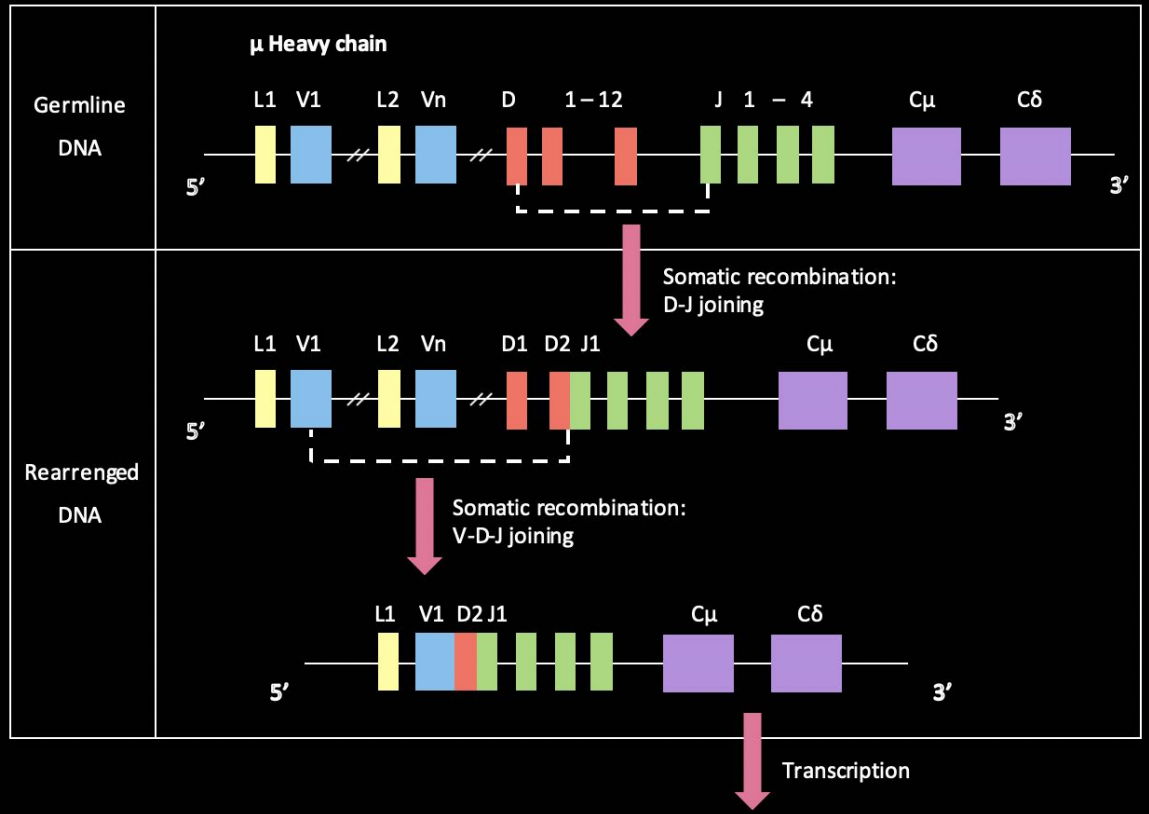
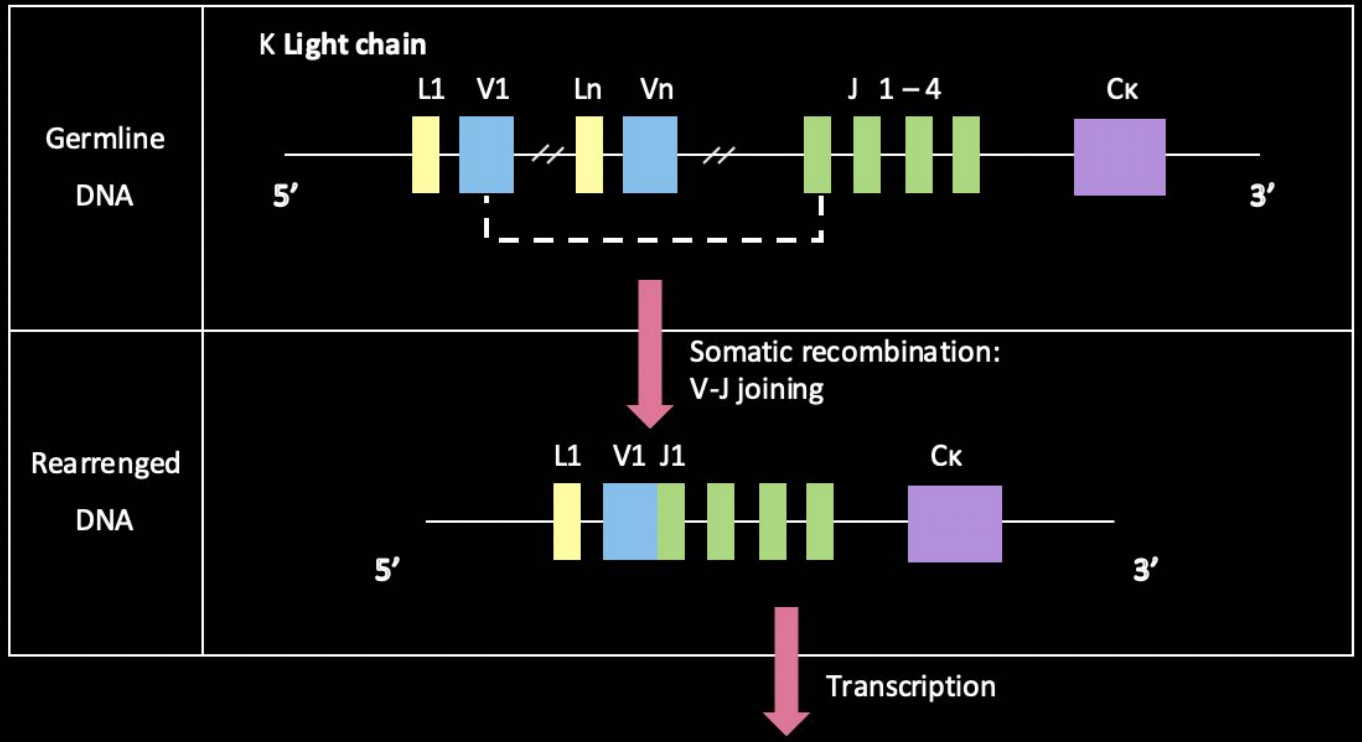
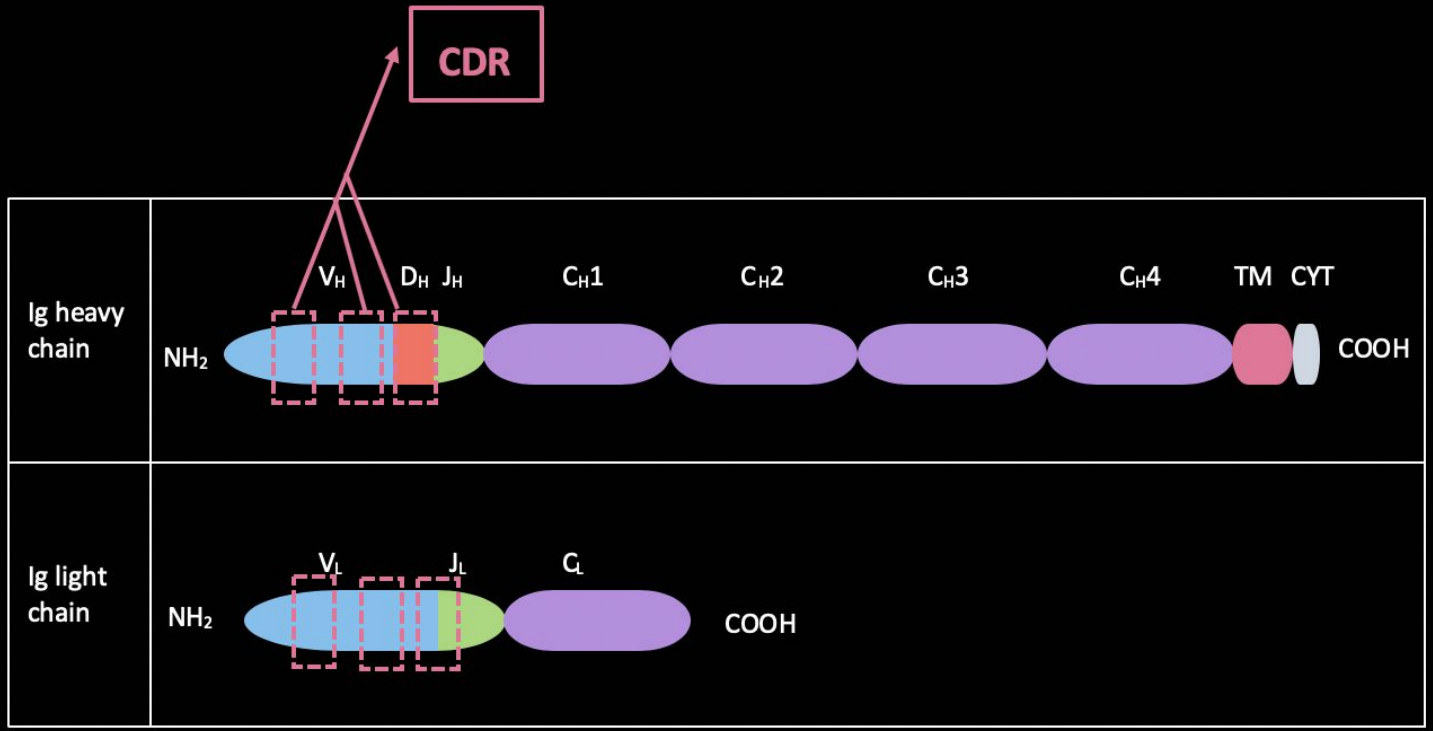


Introduction: Immunoglobulin rearrangement



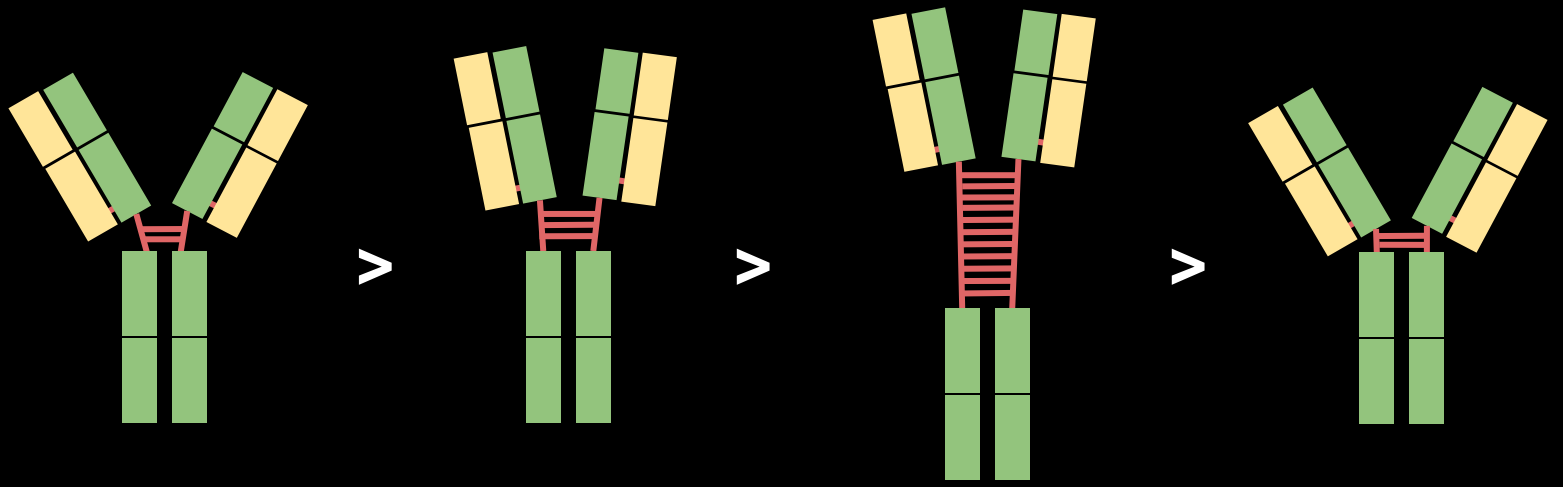
Introduction: Immunoglobulin rearrangement



IMMUNOGLOBULIN G

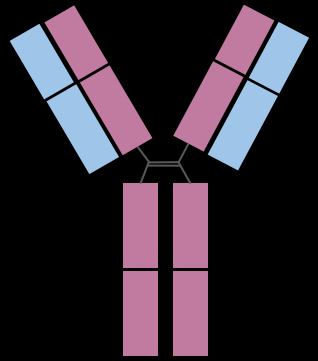
Immunoglobulin G: subclasses

The four subclasses of IgG differ in the structure of the hinge



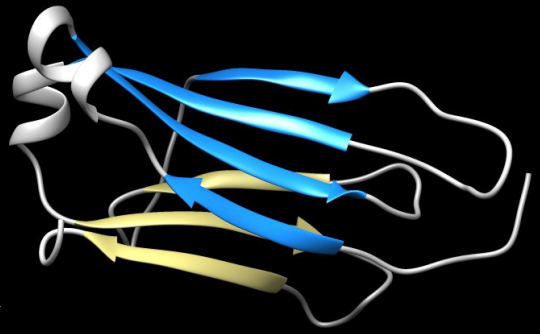
IgG1 IgG2 IgG3 IgG4

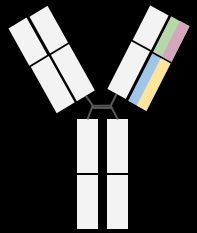
Immunoglobulin G: SCOP classification

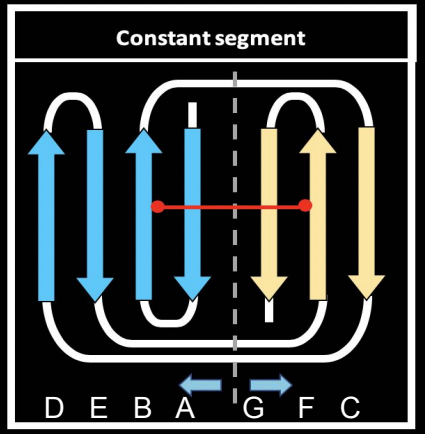


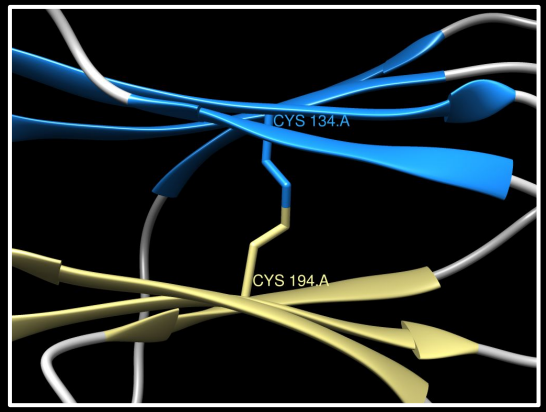
- Class ➜ All-ß proteins
- Fold ➜ Immunoglobulin like
- Superfamily ➜ Immunoglobulin
- Family:
  - **V set domains: antibody variable domain-like**
  - I set domains: are found in several cell adhesion molecules.
  - **C1 set domains: antibody constant domain-like**
  - C2 set domains: Ig-like domains resembling the antibody constant domain

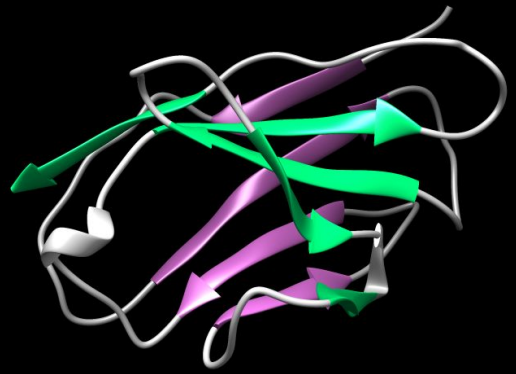
Immunoglobulin G: Fold

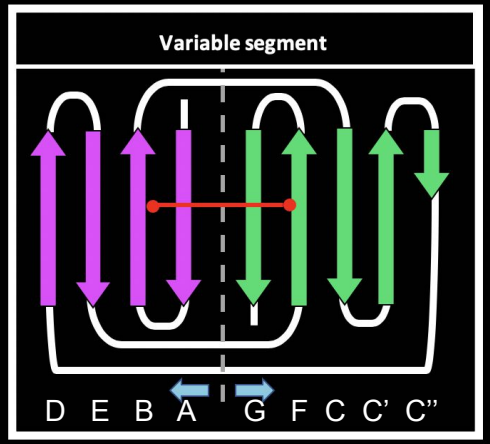


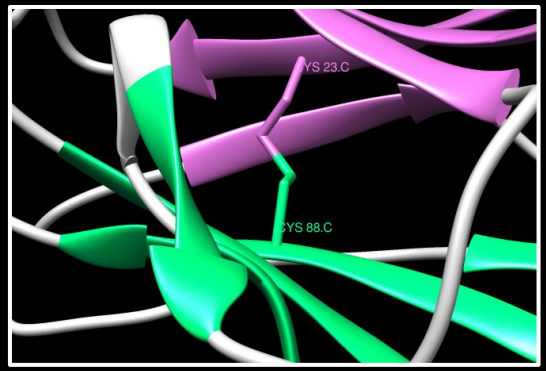




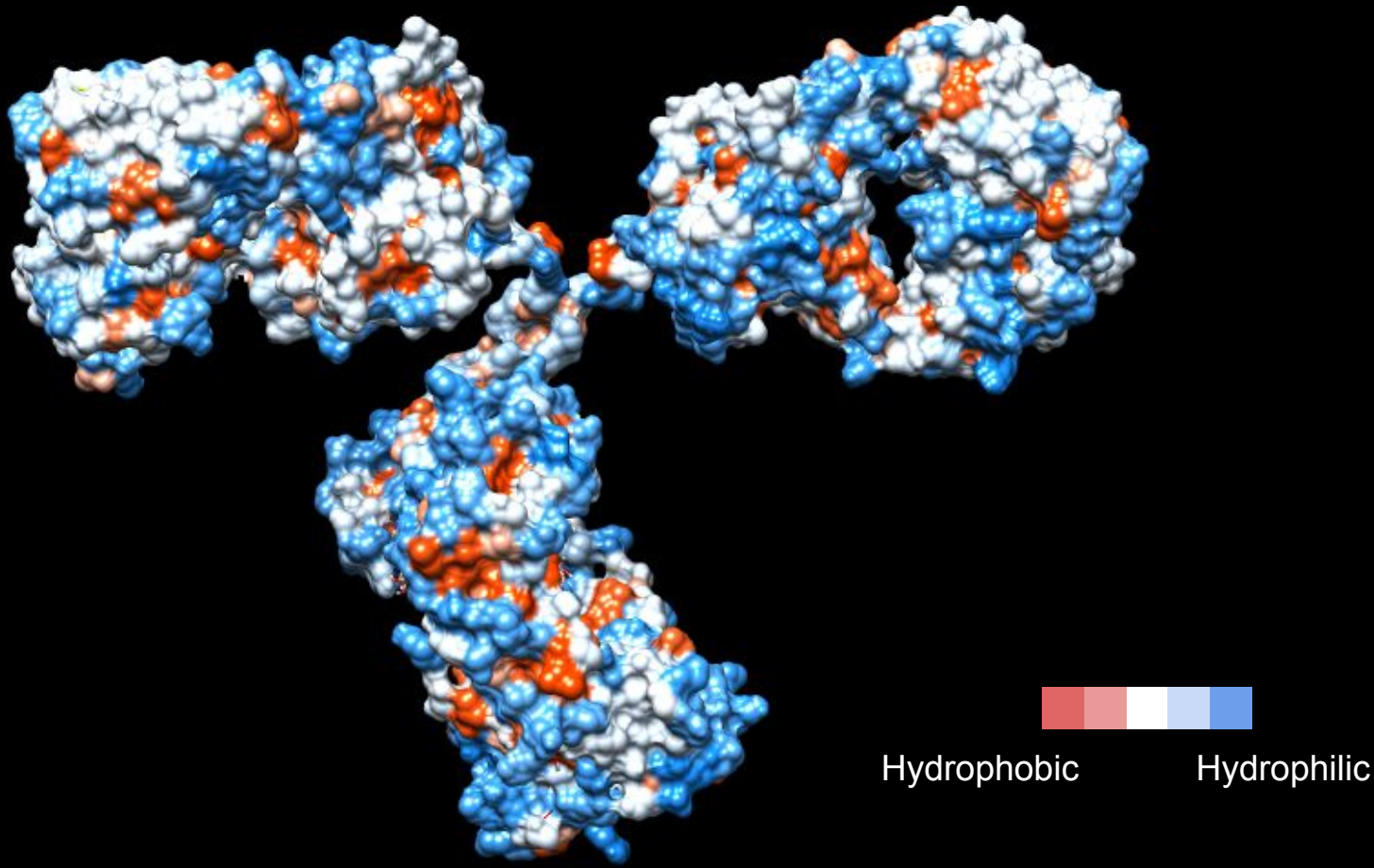








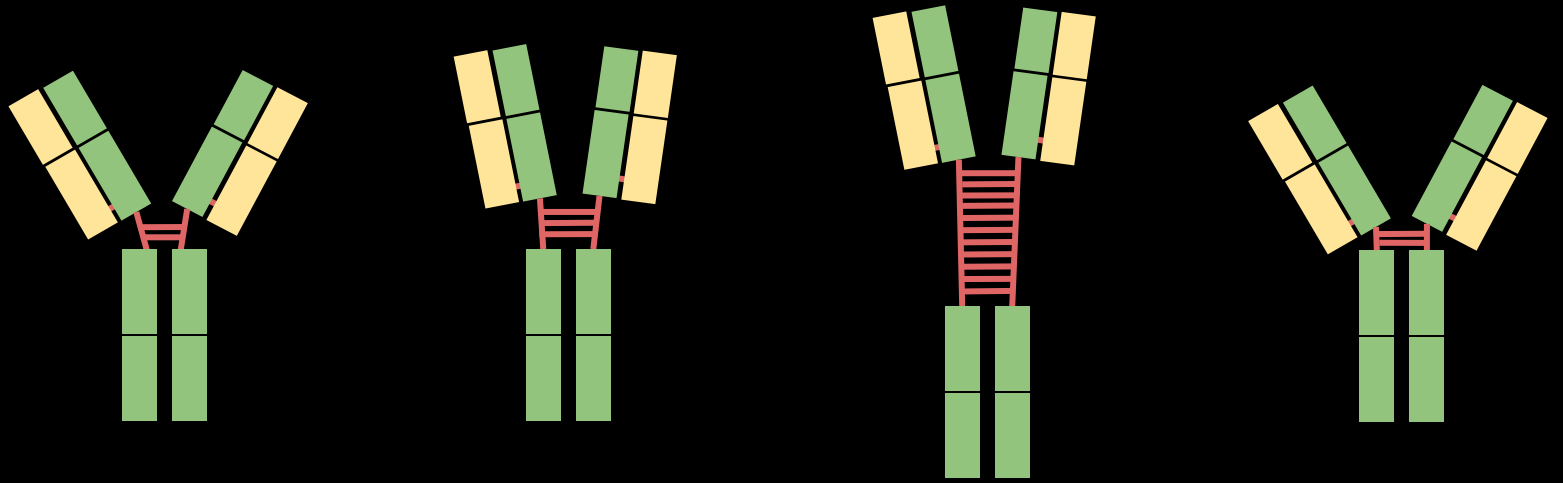
Immunoglobulin G: Architecture



Immunoglobulin G: Hinge

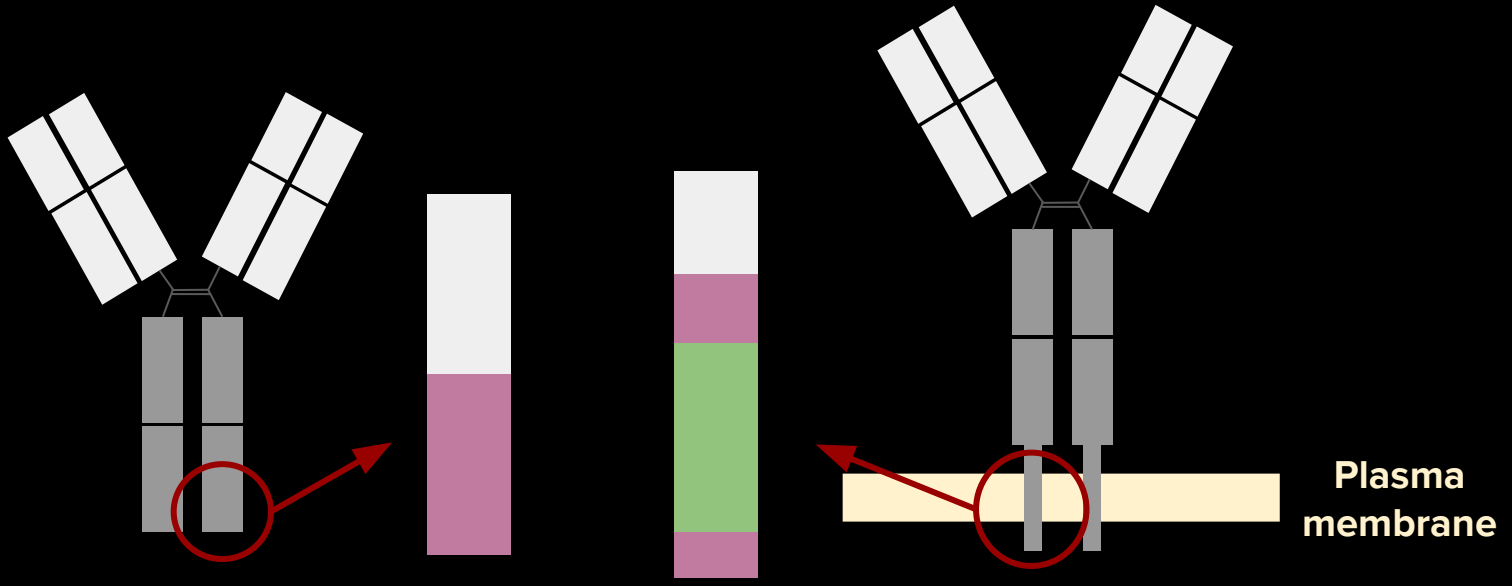
Immunoglobulin G: hinge

The four subclasses of IgG differ in the structure of the hinge



IgG FRAGMENT CRYSTALLIZABLE REGION (Fc)

Fc: Soluble vs Surface



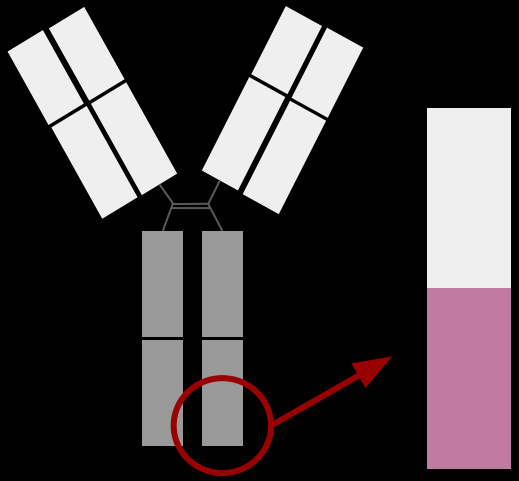
Soluble antibody

Hydrophilic

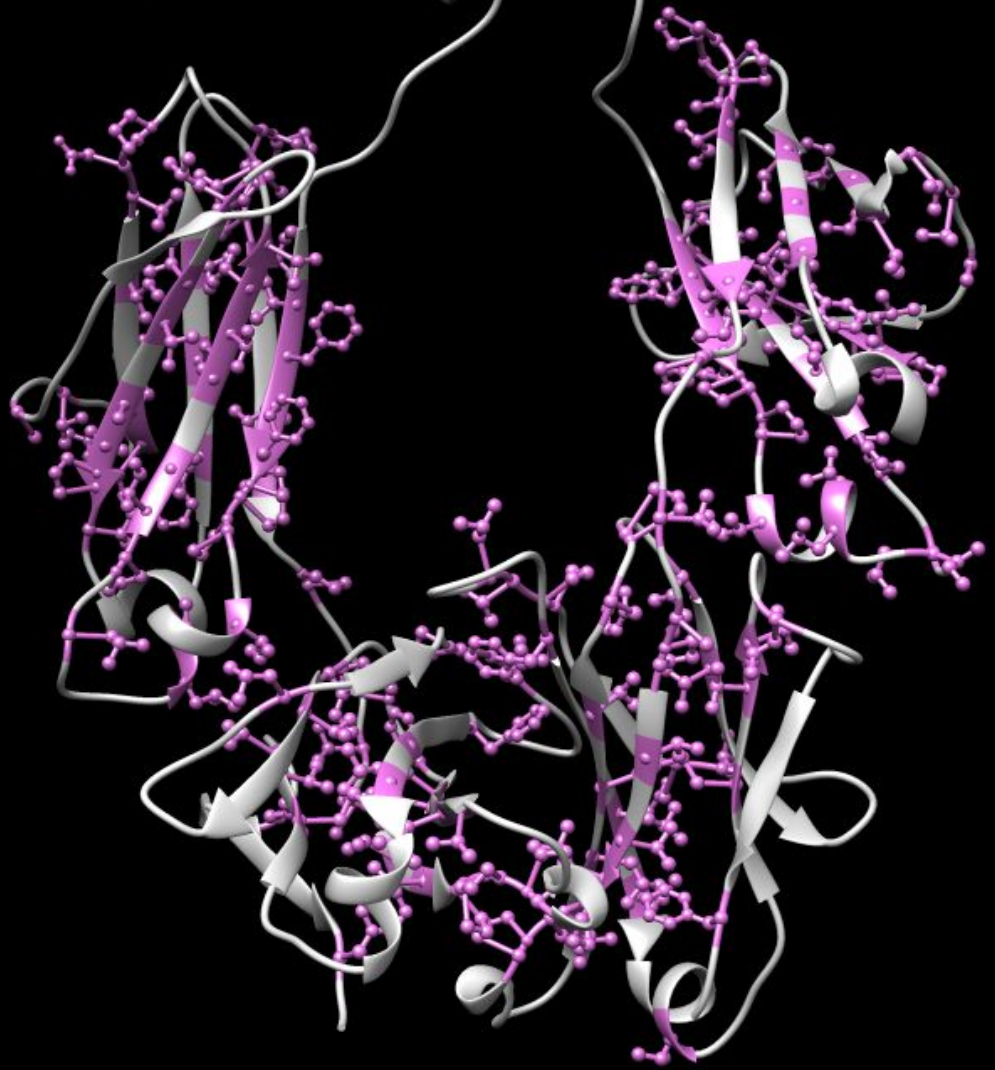
Hydrophobic

Surface antibody

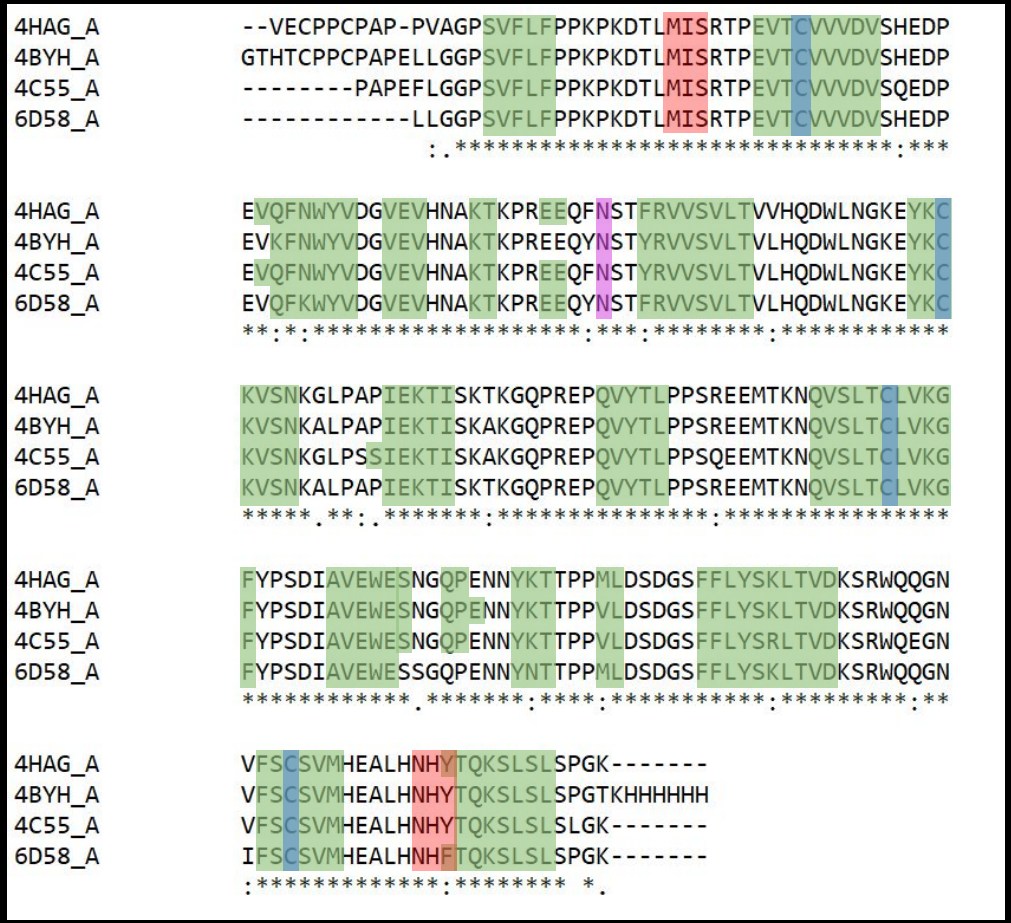
Fc: Soluble IgG



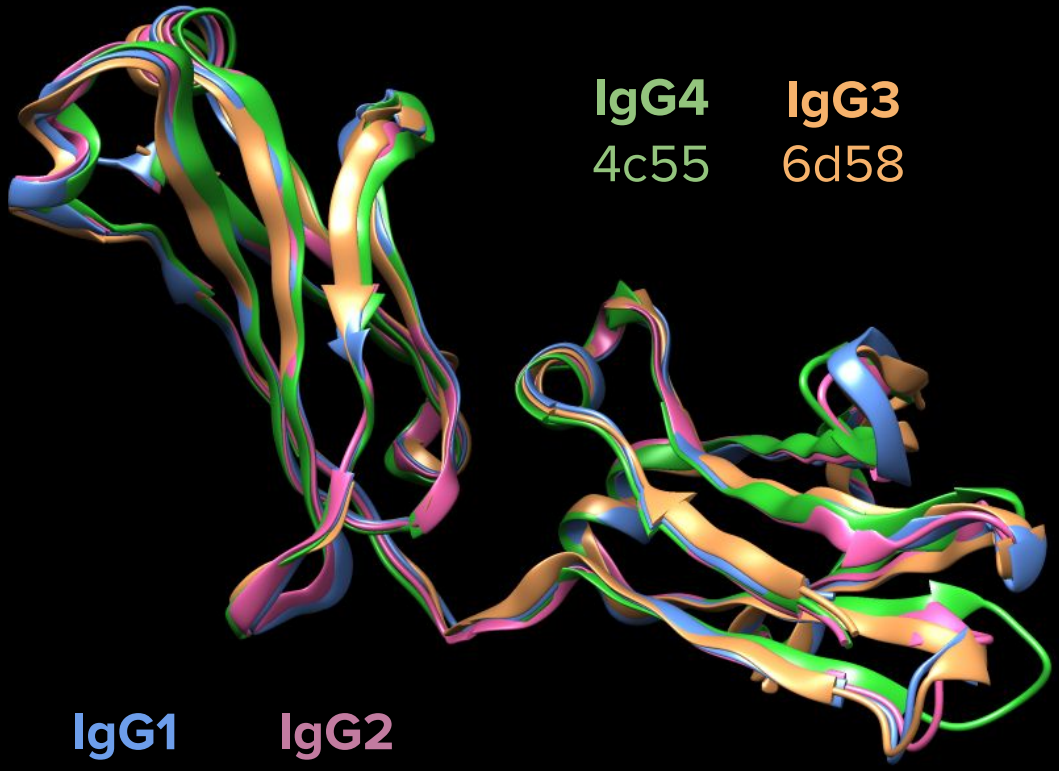
Soluble antibody

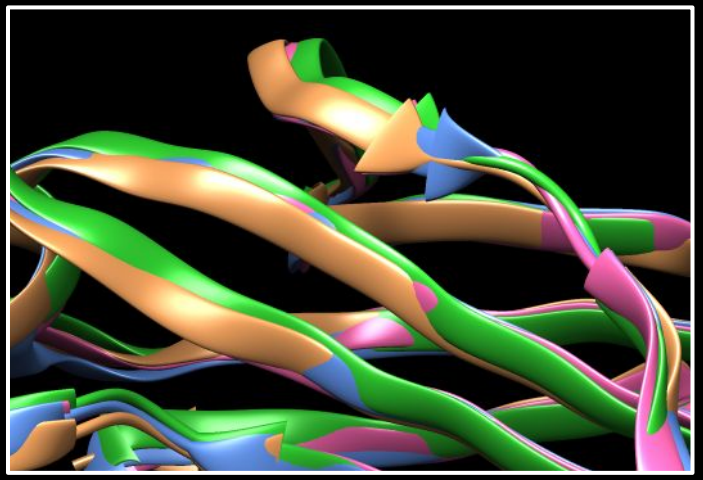


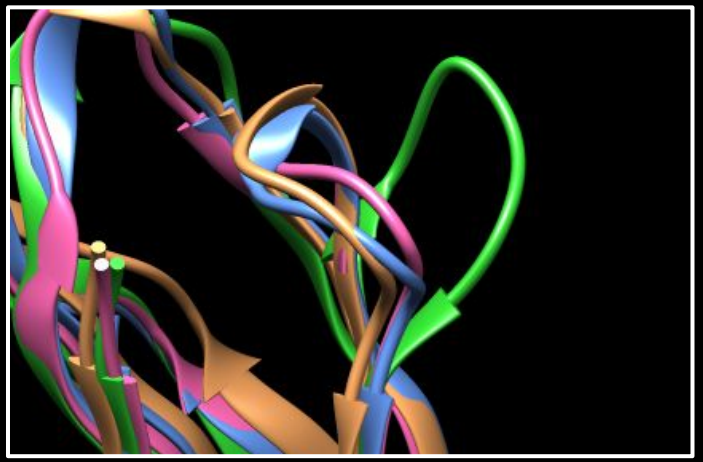
Fc: Subtypes comparison



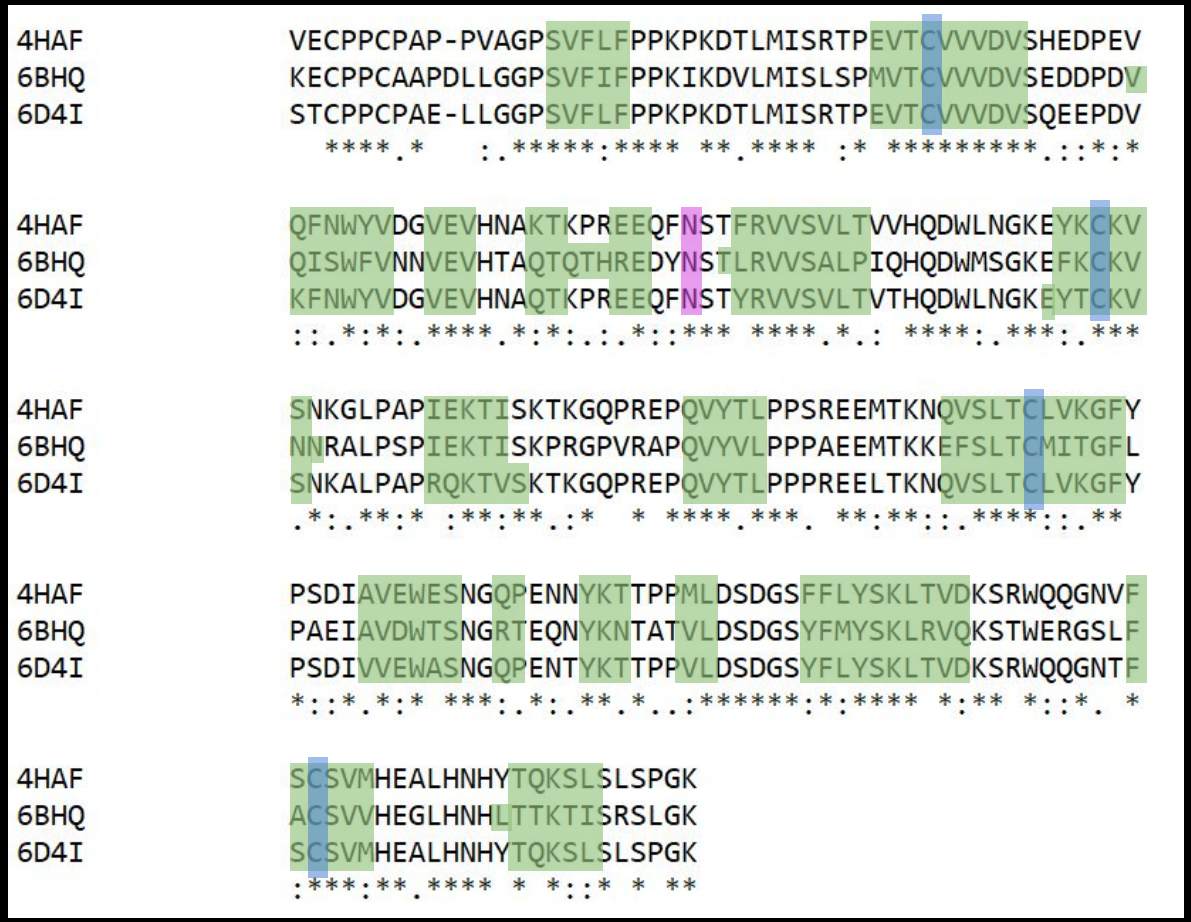
Fc: Subtypes comparison



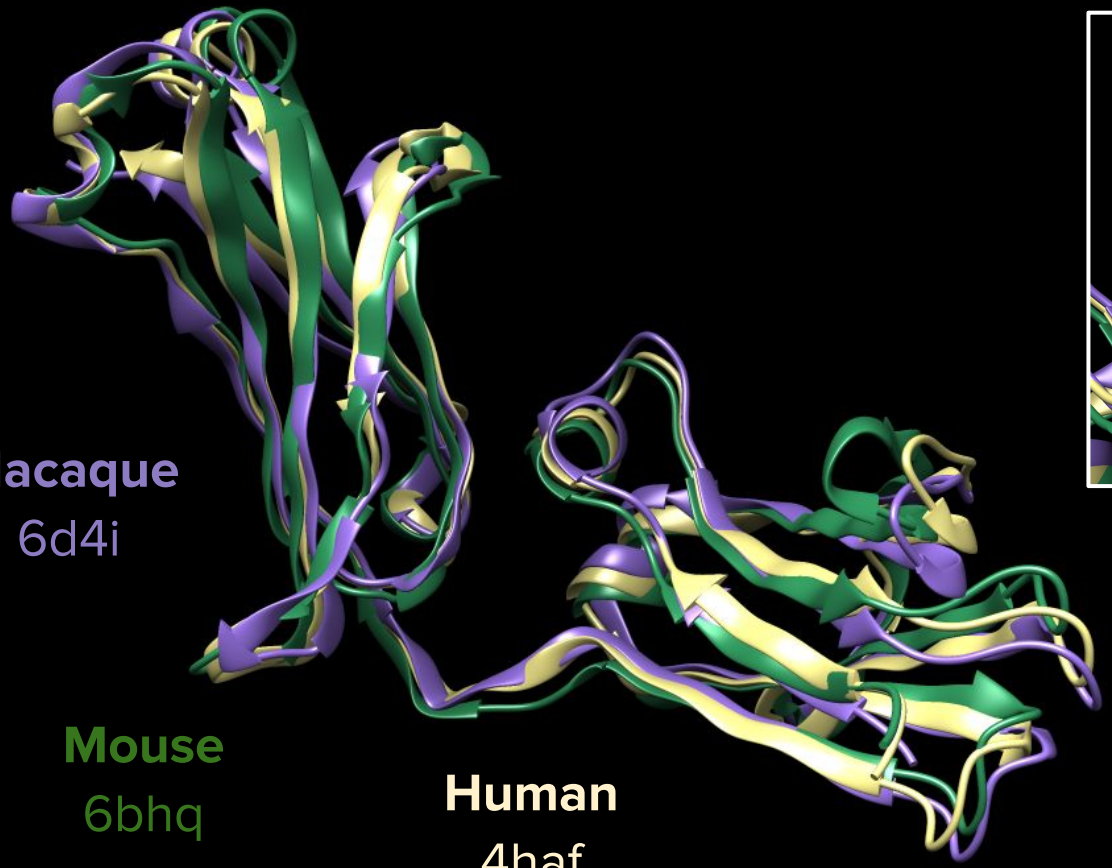




Fc: IgG2 among species



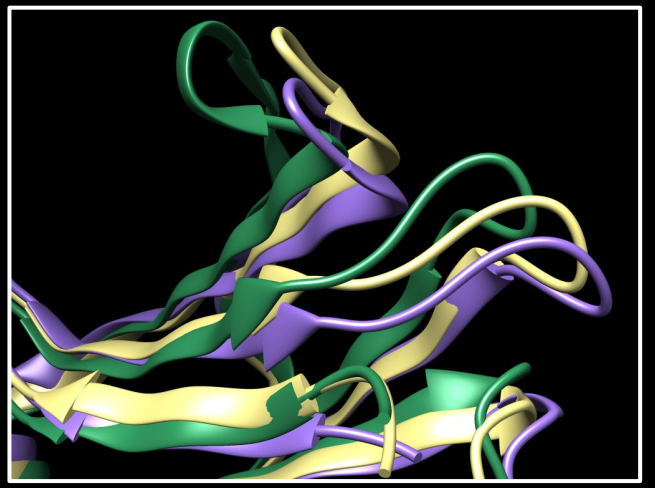
Fc: IgG2 superimposition



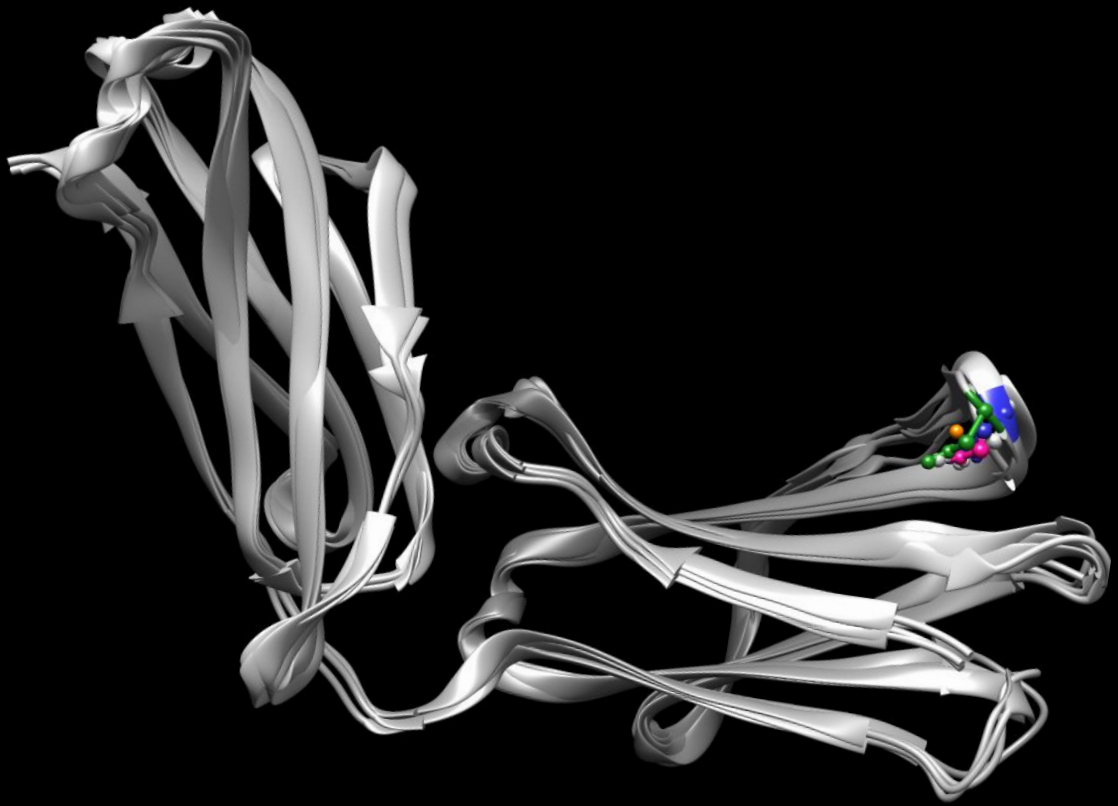
Macaque
6d4i

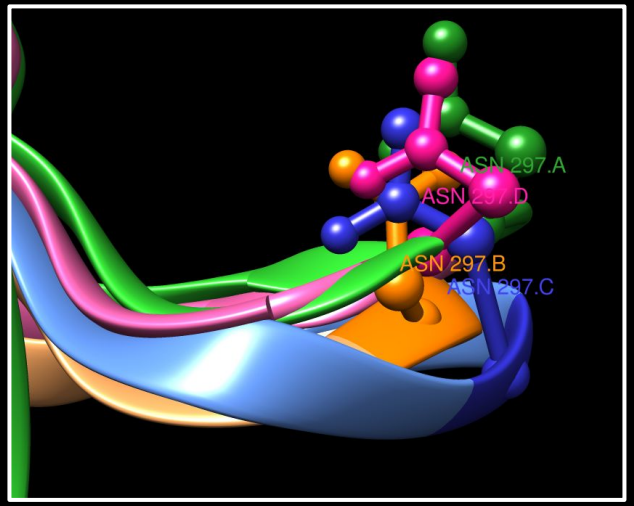
Mouse
6bhq

Human
4haf



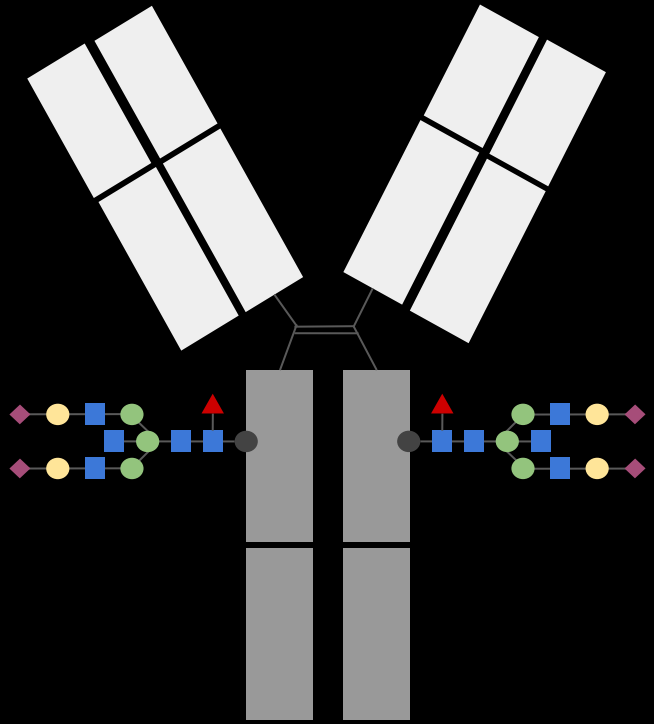
Fc: N-glycosilation

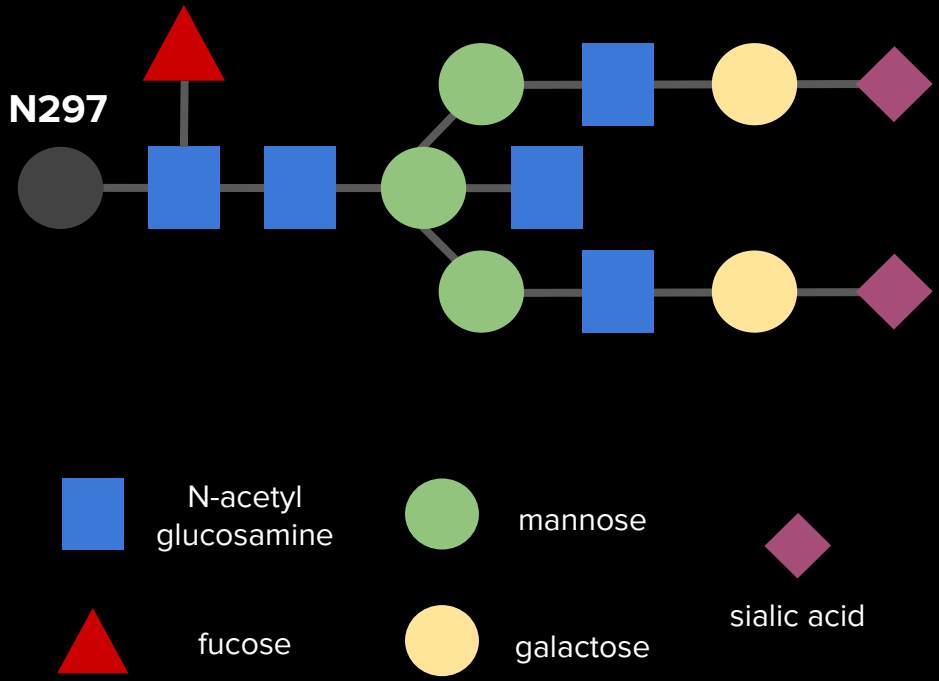




Asn 297

Fc: N-Glycosylation

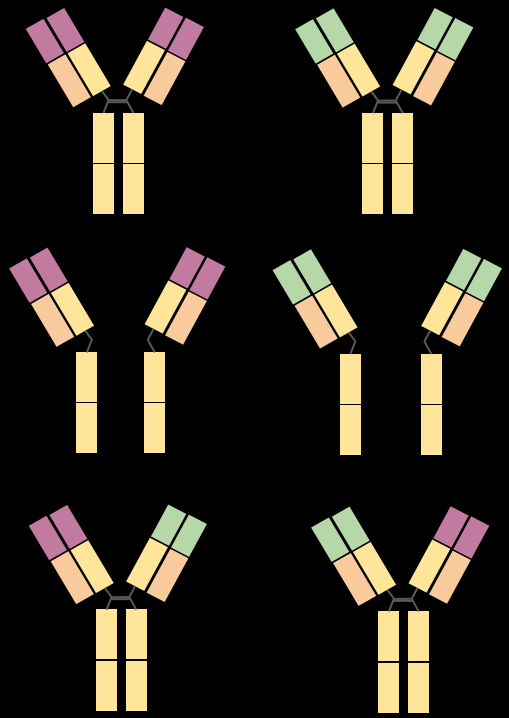




IgG4 Fab-ARM EXCHANGE (FAE)

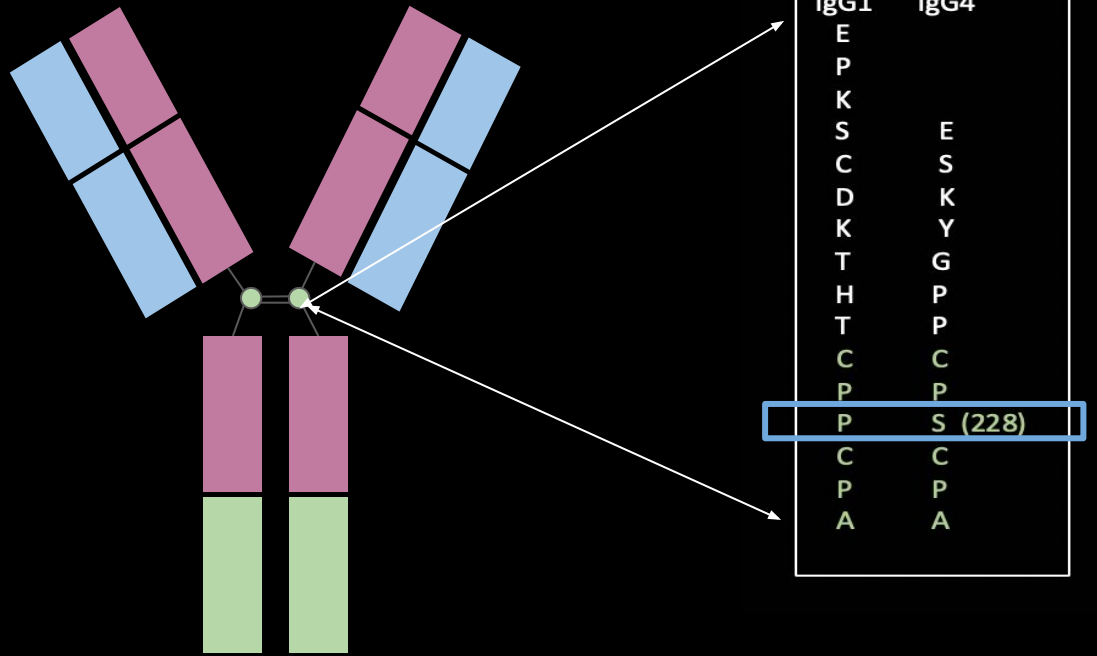
FAE

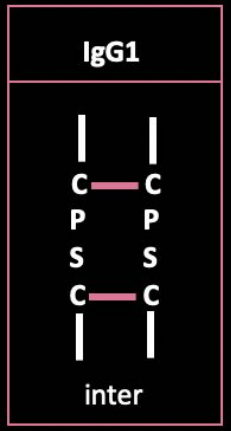
- Separation of the 2 IgG4 heavy chains to form ‘half-molecules’ comprising just 1 heavy and light chain.
  They recombine to create bispecific antibodies.
- Two determinants enable IgG4 to undergo FAE, namely the **core hinge** and the **Cγ3–Cγ3 domain interface**
- Imply both covalent and non-covalent interactions

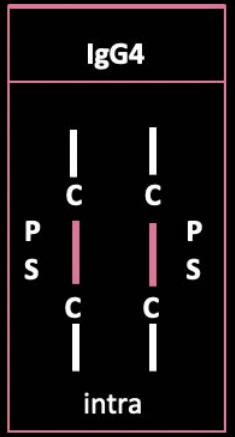


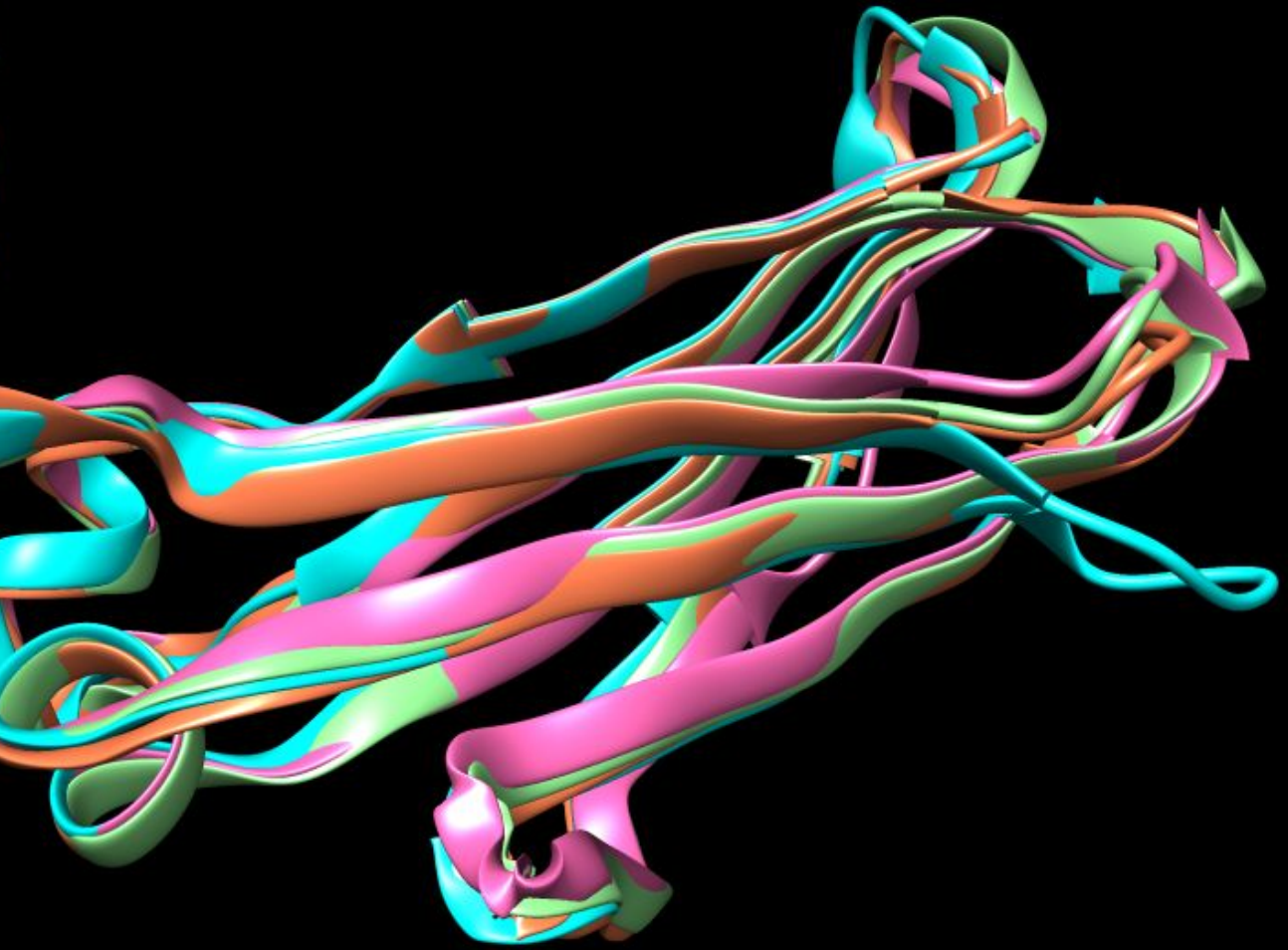
	228
IgG1	E P K S C D K T H T C P P C P A P E L L G G P
IgG4	E S K Y G P P C P S C P A P E L L G G P

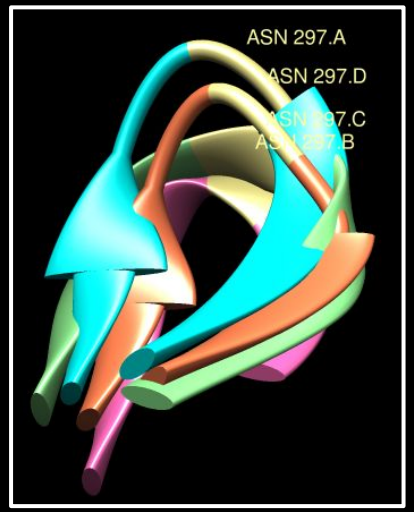
FAE

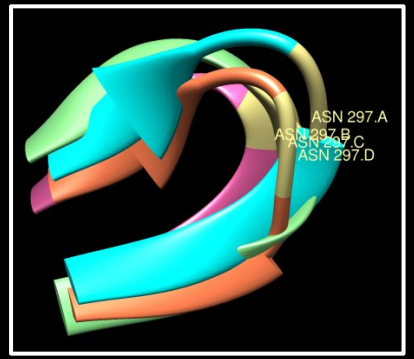

IgG1 IgG4
E
P
K
S E
C S
D K
K Y
T G
H P
T P
C C
P P
P S (228)
C C
P P
A A


IgG1
C C
P P
S S
C C
inter


IgG4
C C
P P
S S
C C
intra

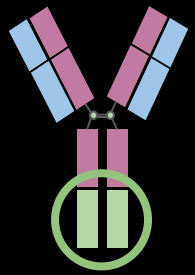



ASN 297.A
ASN 297.D
ASN 297.C
ASN 297.B


ASN 297.A
ASN 297.B
ASN 297.C
ASN 297.D

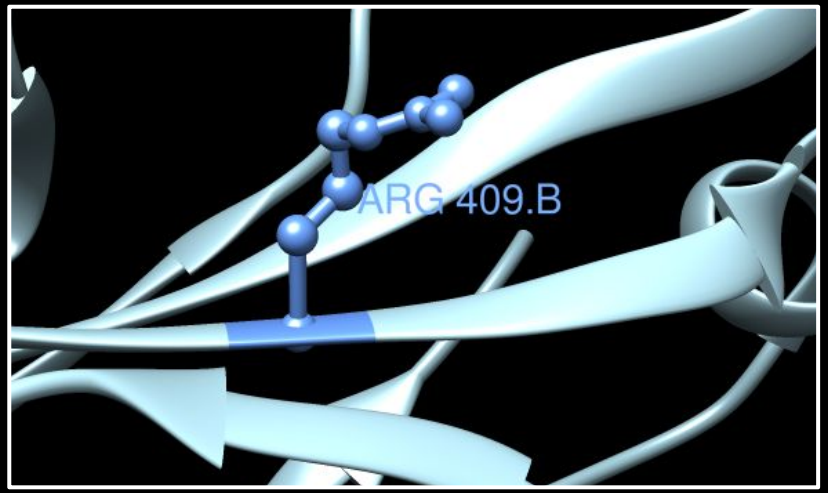
IgG4

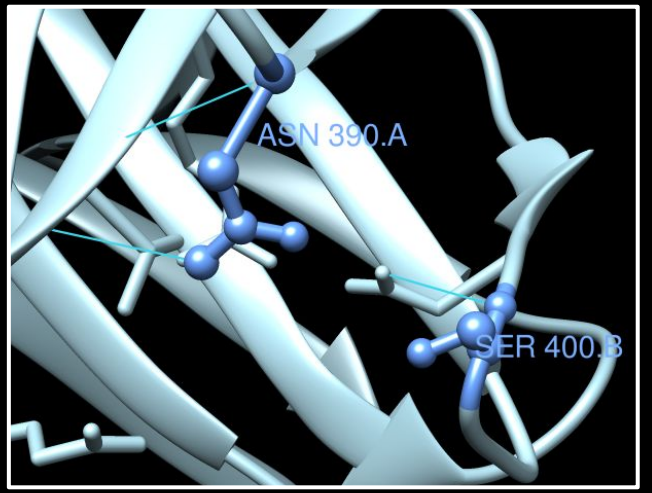
4b53

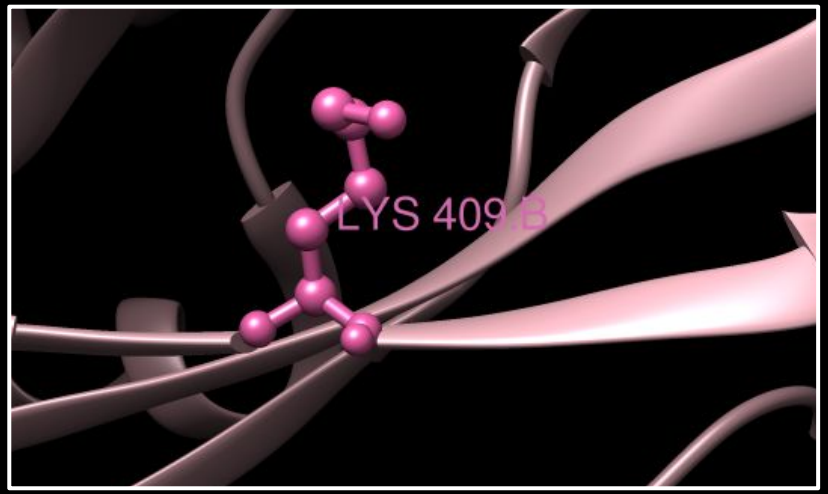


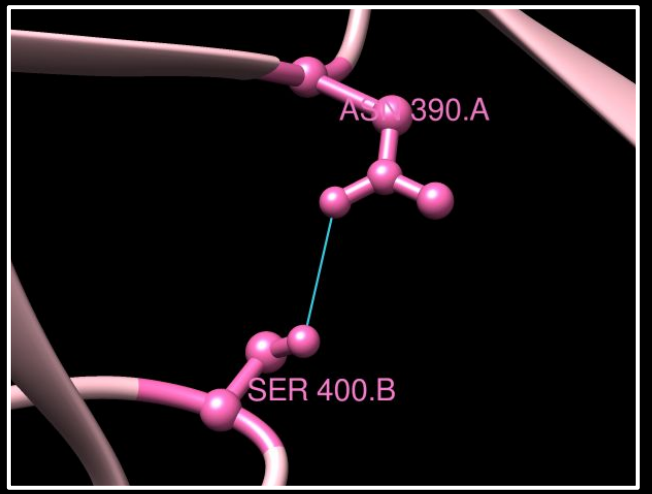
IgG1

3ave



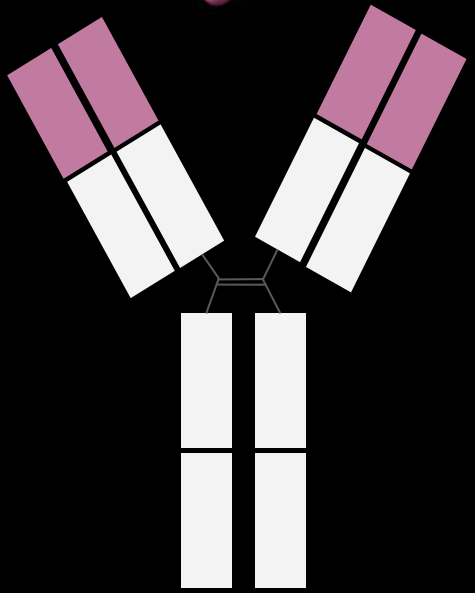






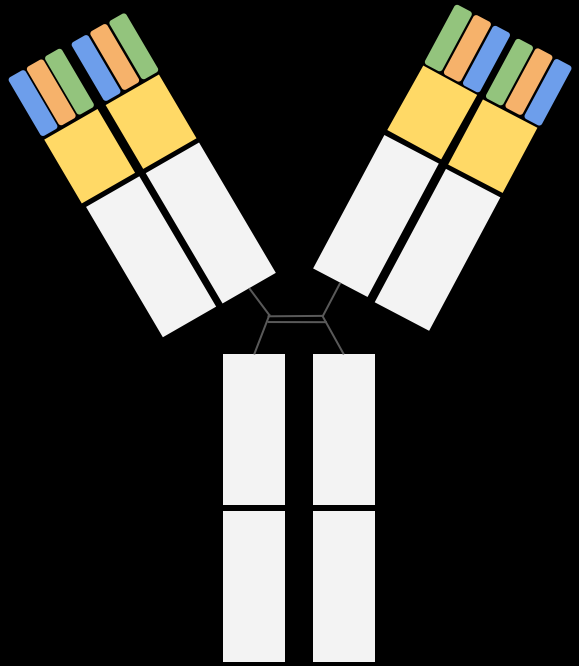
IgG VARIABLE DOMAIN

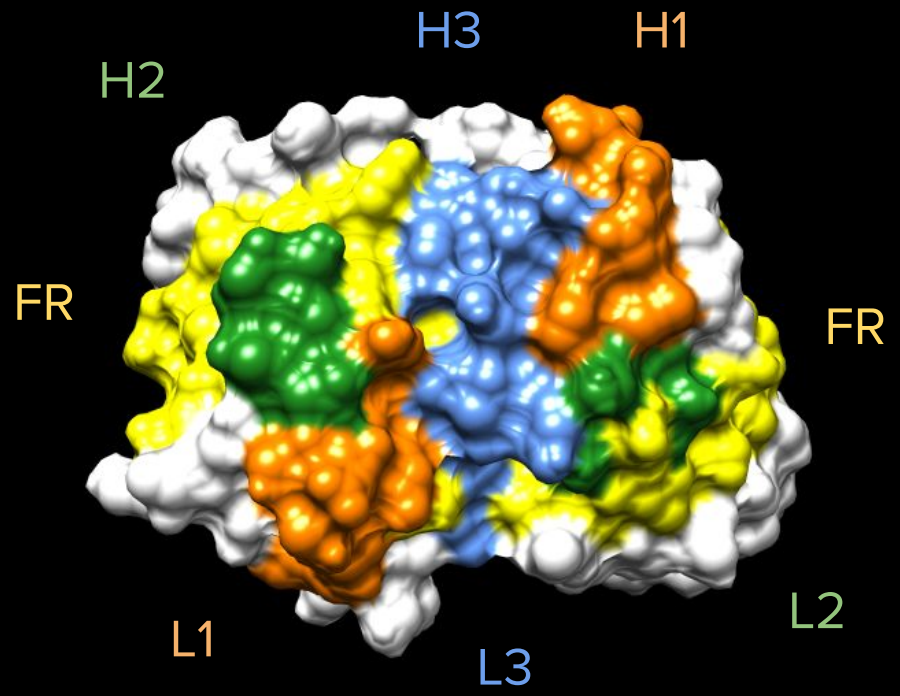
Variable domain



HYPERVARIABILITY

Complementarity-determining regions (CDRs)





HYPERVARIABILITY

Light chains
(lambda type)

Framework

L1

L2

L3

HYPERVARIABILITY

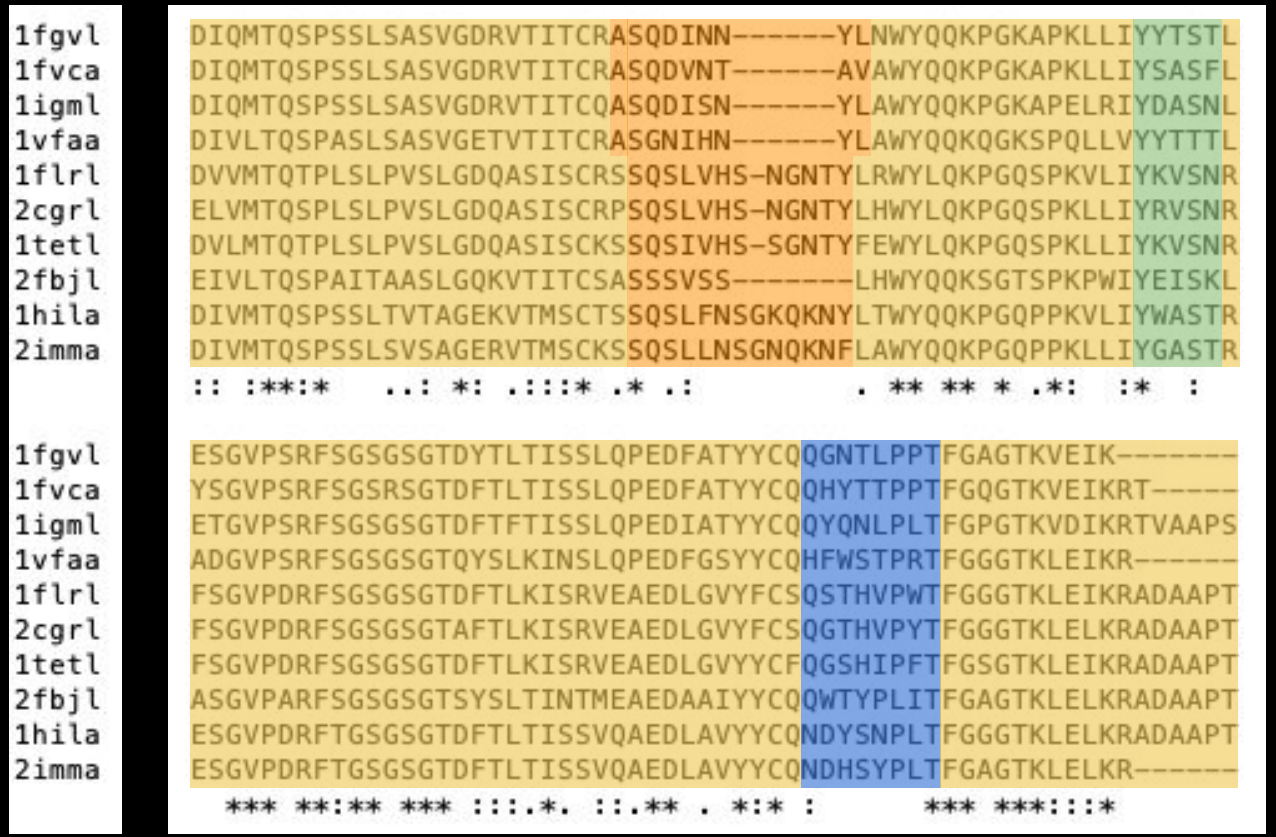
Light chains
(kappa type)

Framework

L1

L2

L3



HYPERVARIABILITY

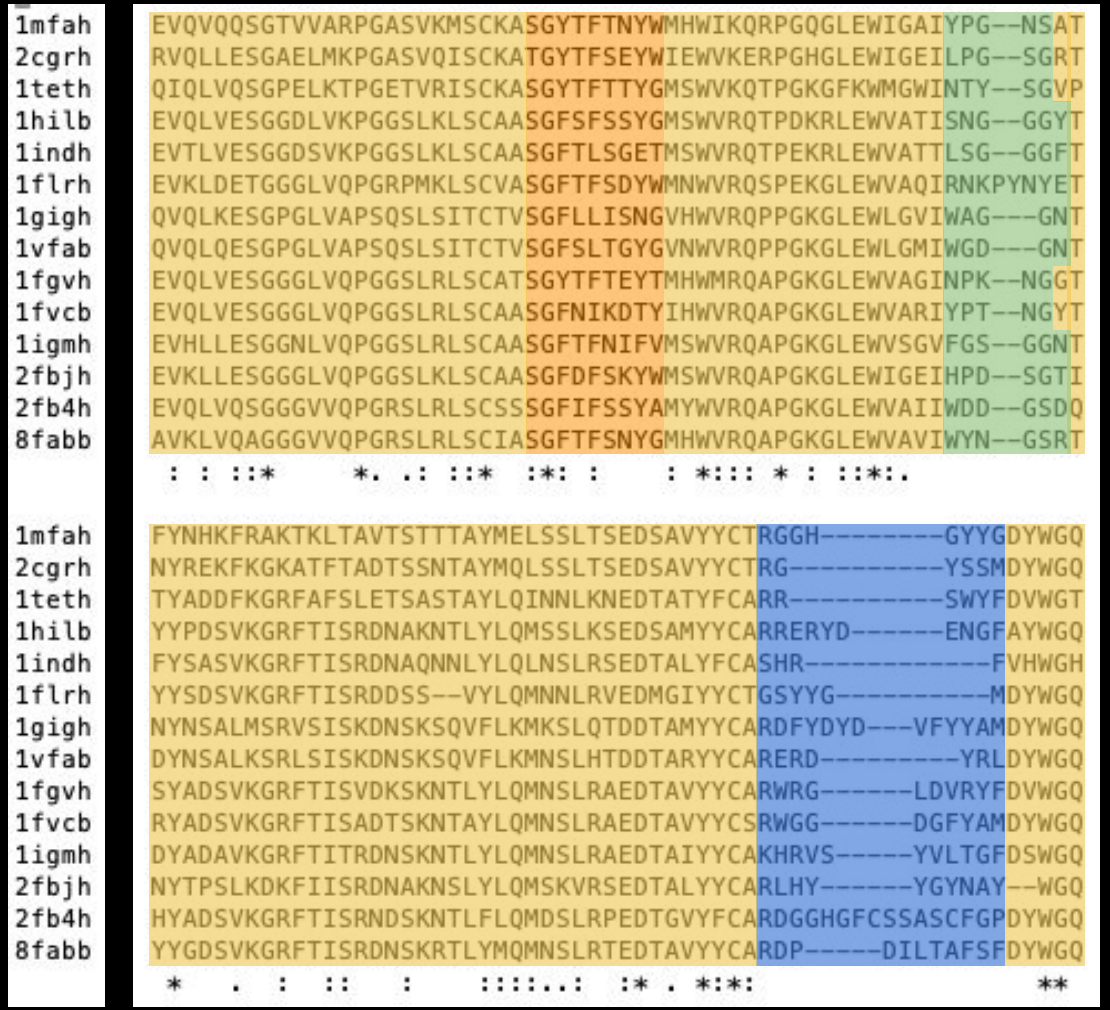
Heavy chains

Framework

H1

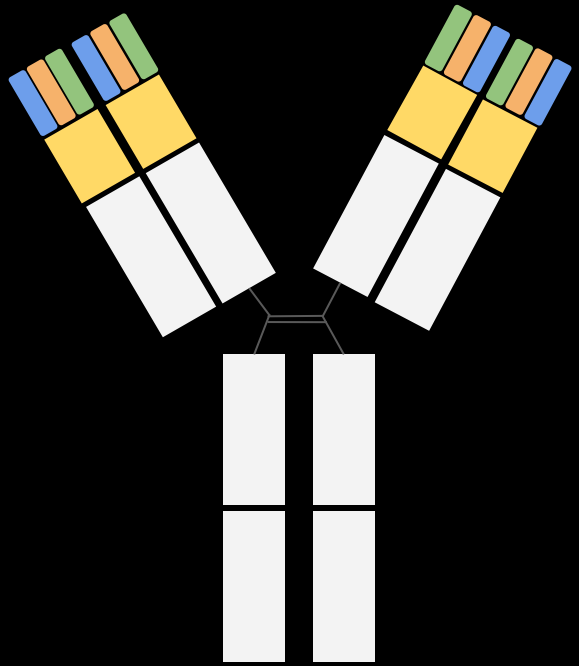
H2

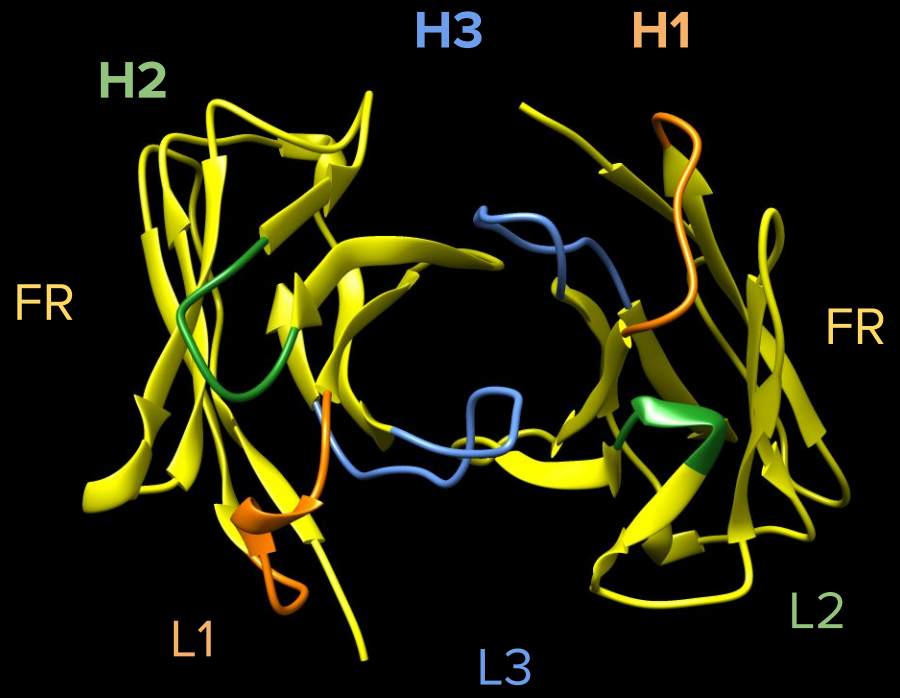
H3



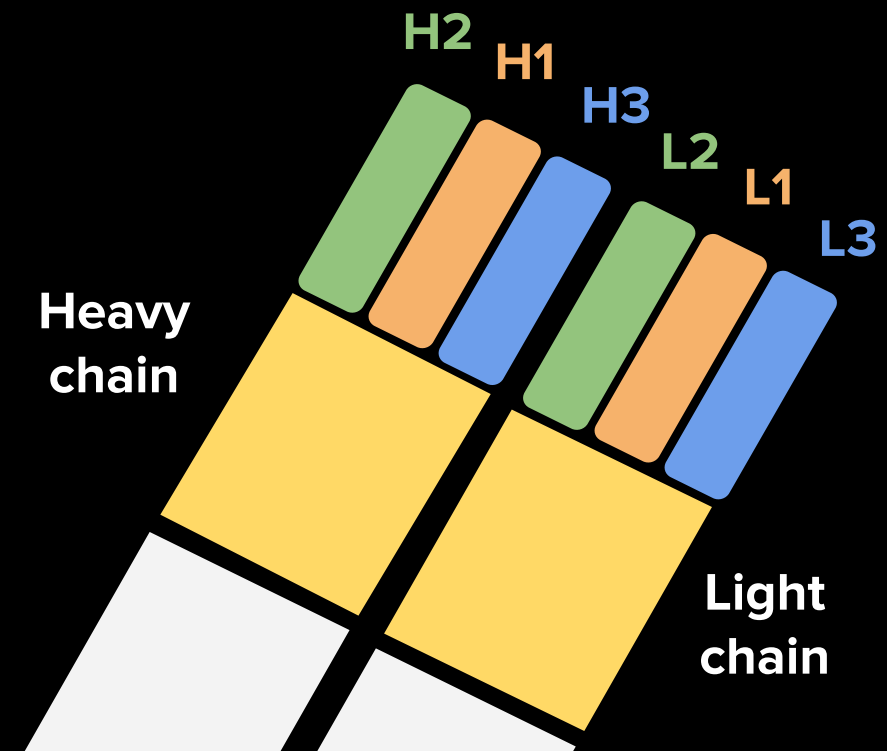
HYPERVARIABILITY

Complementarity-determining regions (CDRs)





CANONICAL STRUCTURES



H1

1 2 3

H2

1 2
3 4

H3

No canonical structure

L1

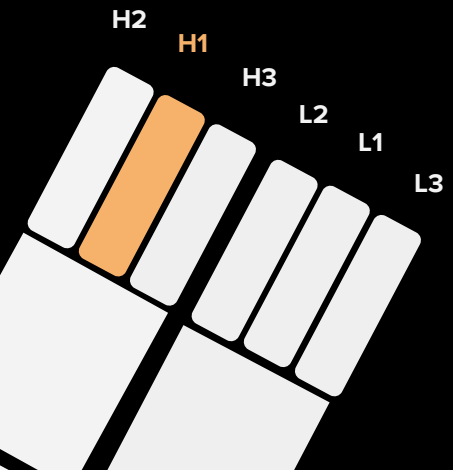
Kappa
1 2a 2b 3
4 5 6
Lambda
1 2 3 4

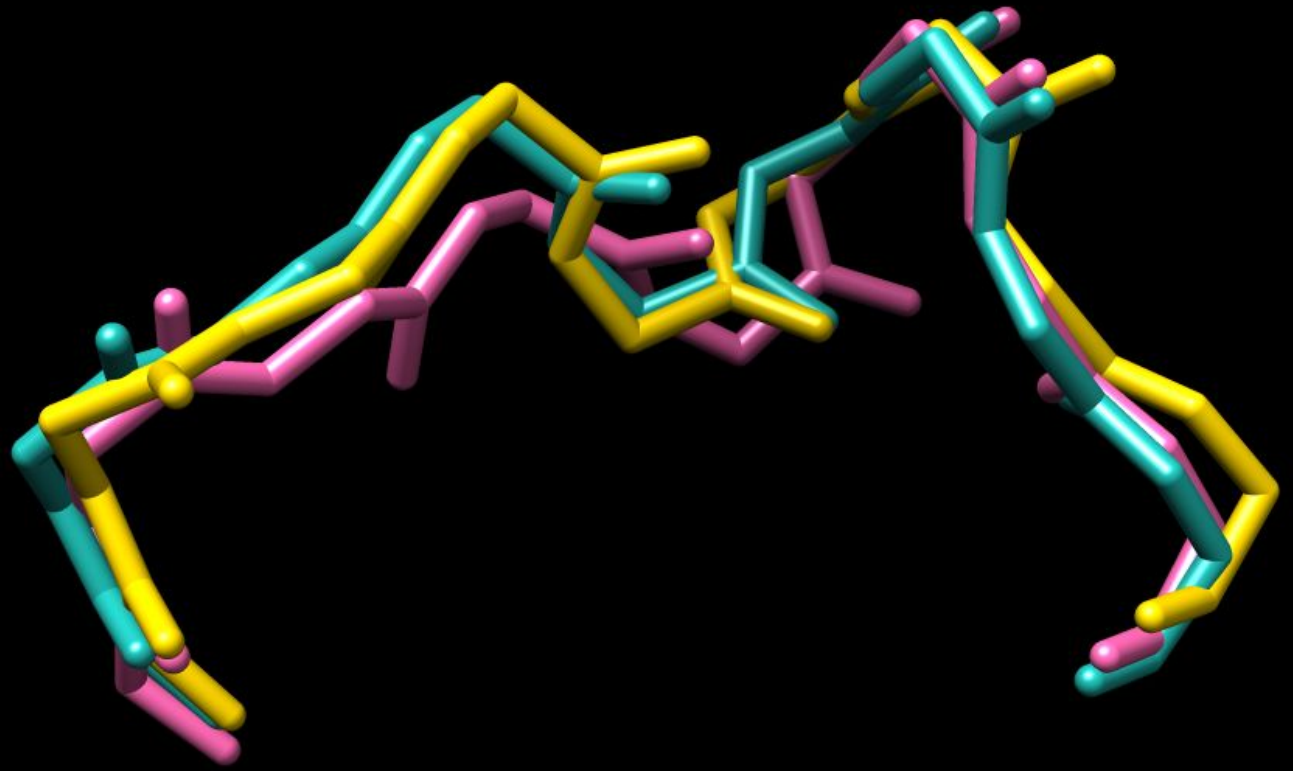
L3 **L2** Only 1

Kappa
1 2 3 4 5 6
Lambda
1 2

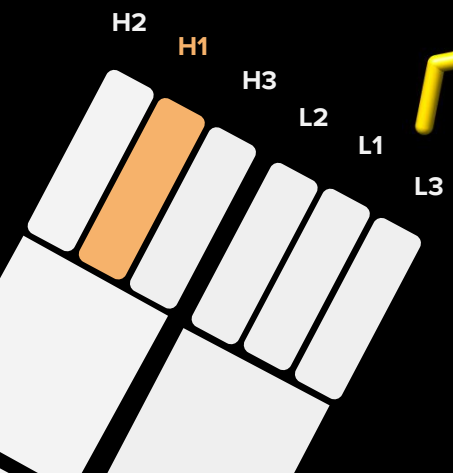
CANONICAL STRUCTURES

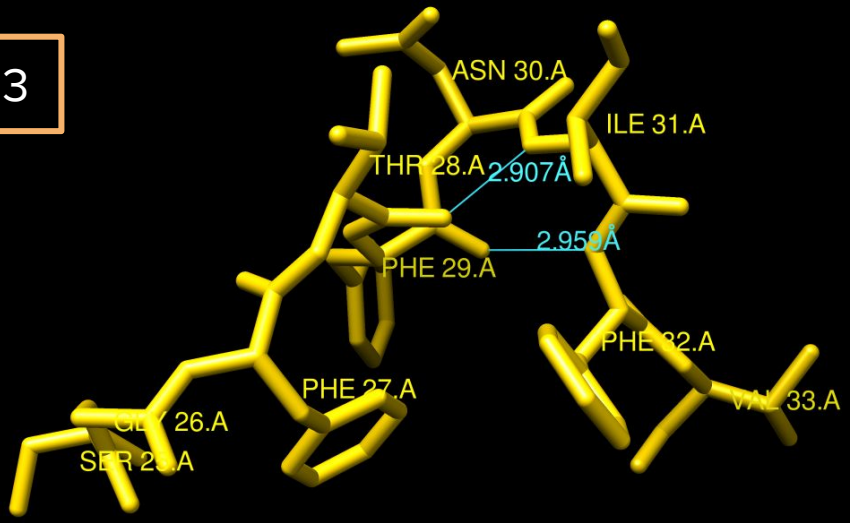
H1 (1) 2 3

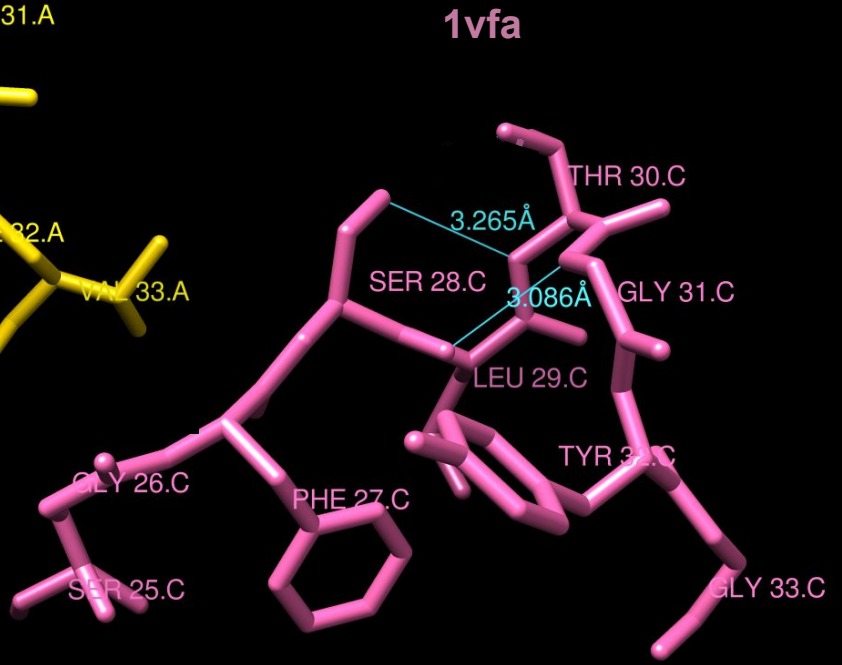




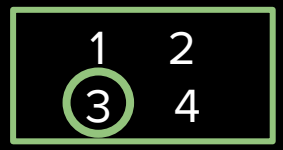
CANONICAL STRUCTURES

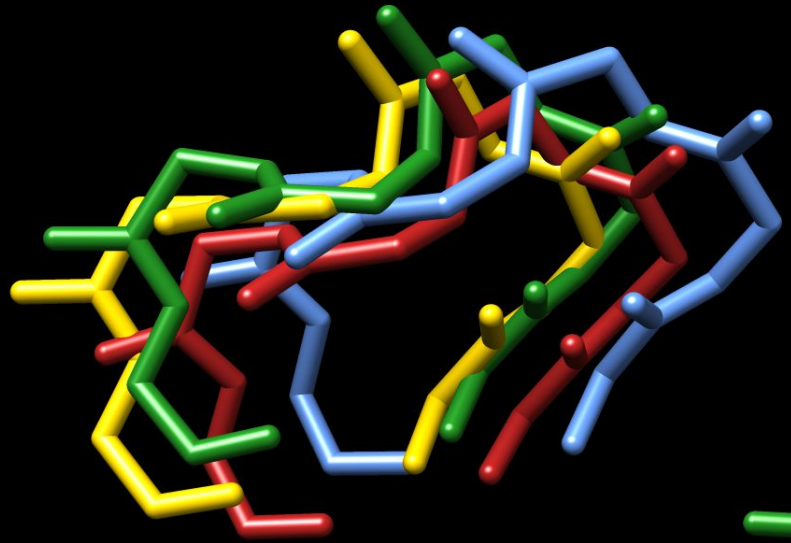






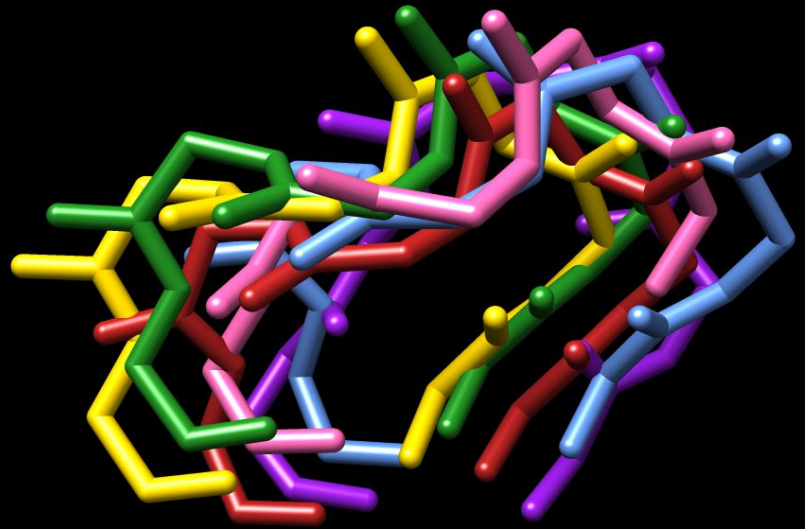
CANONICAL STRUCTURES

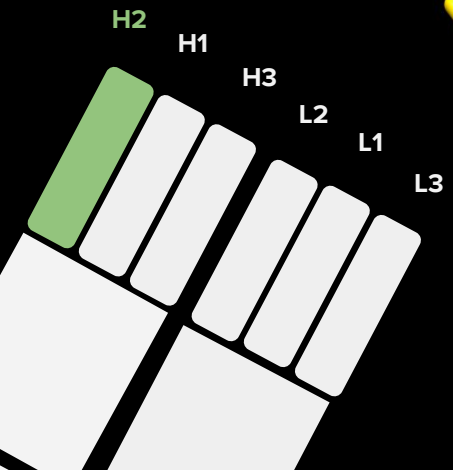




2fb4 1hil 2fbj 8fab

A





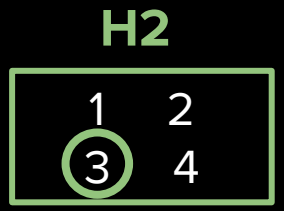
1ind

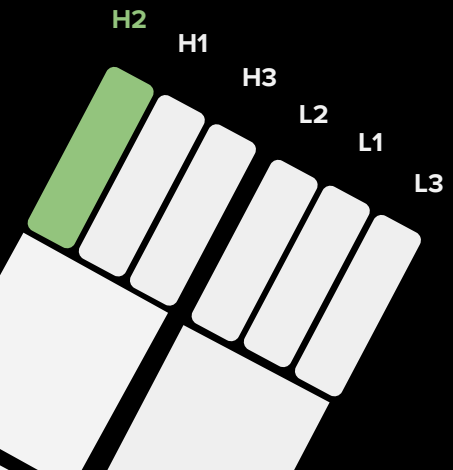
B

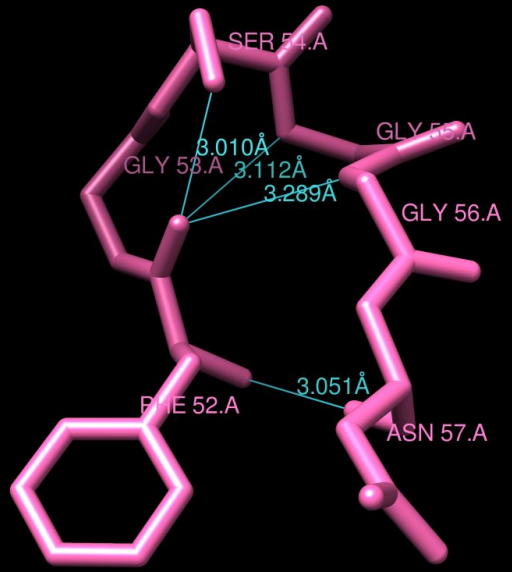
1igm

C

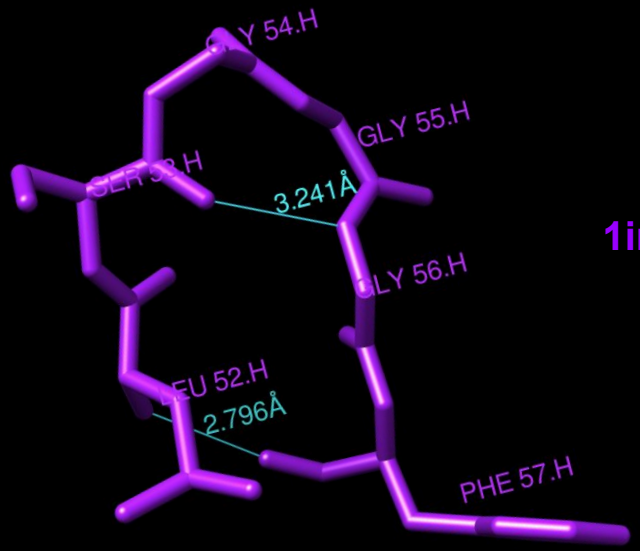
CANONICAL STRUCTURES



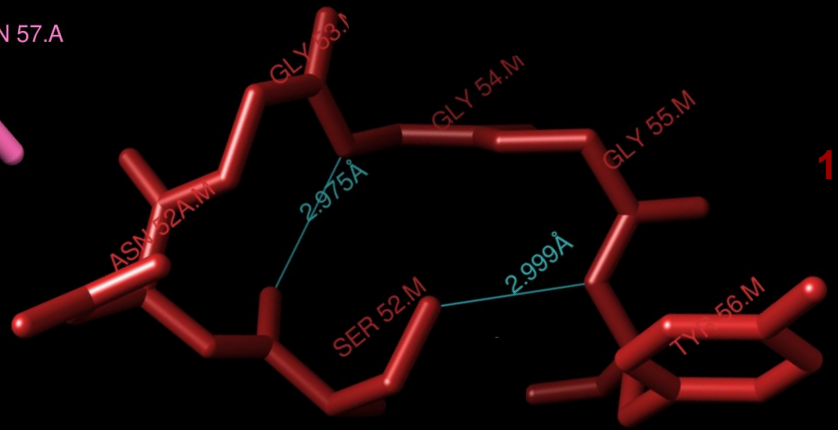




1igm



1ind

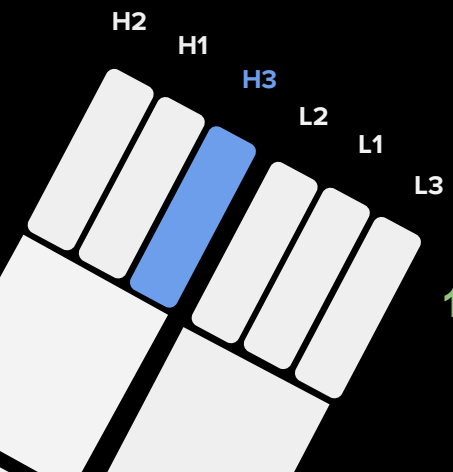


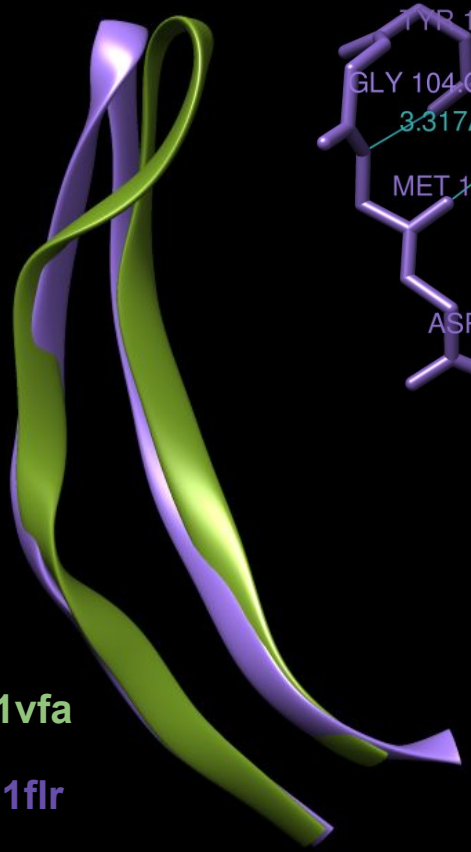
1hil

CANONICAL STRUCTURES

H3

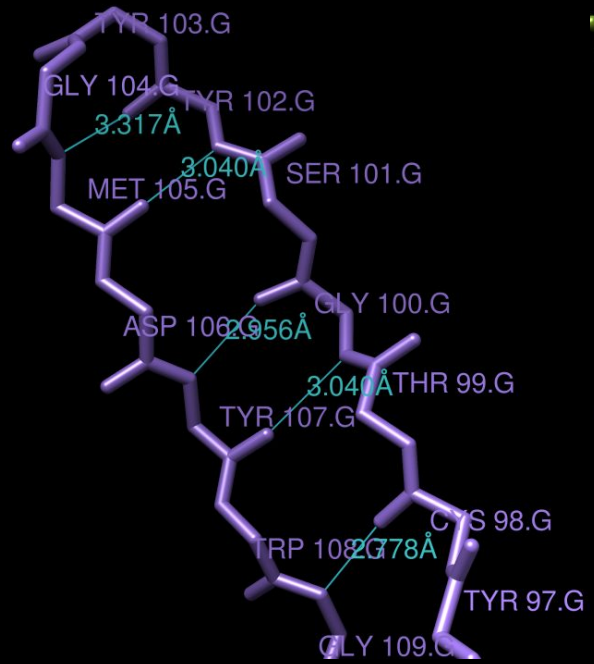
No canonical structure





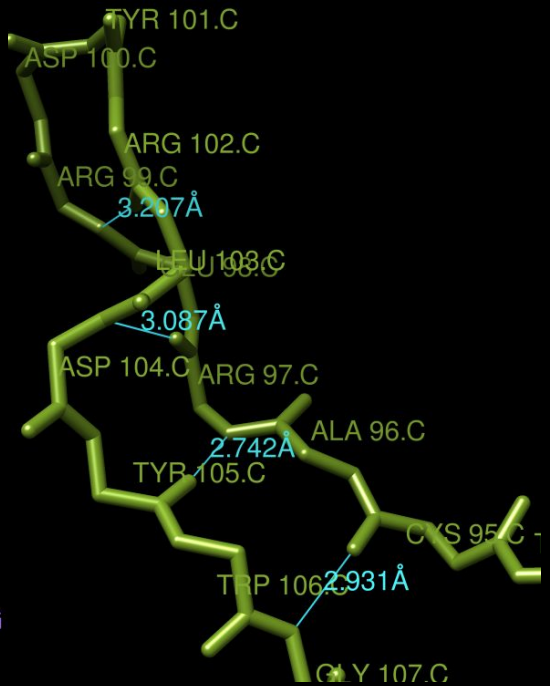
1vfa

1flr



Non-bulged

Extended



Bulged

Kinked

CANONICAL STRUCTURES

L1

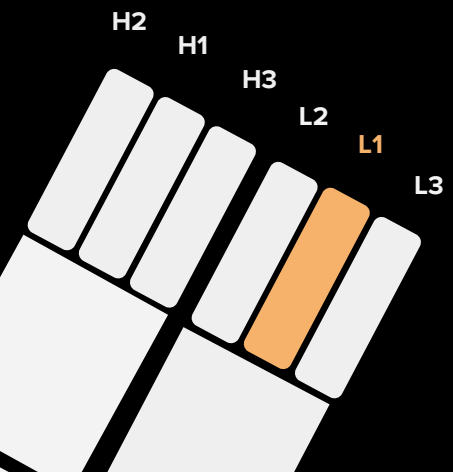
Kappa

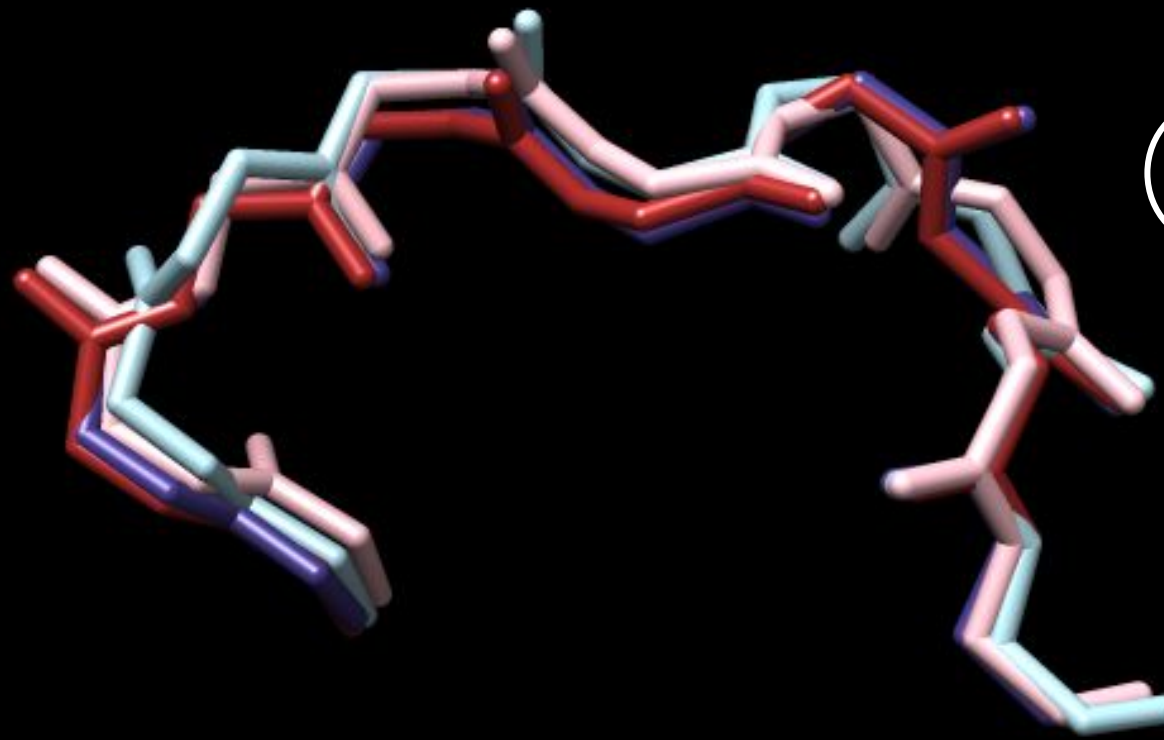
1 2 3

4 5 6

Lambda

1 2 3 4





1igm
1fvc

A

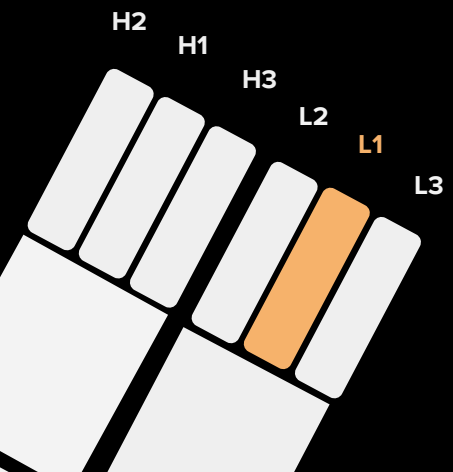
1fgv
1vfa

B

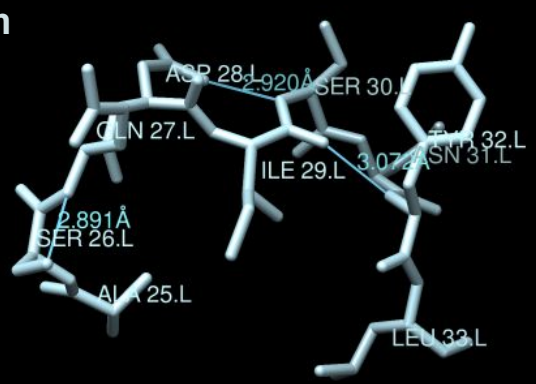
CANONICAL STRUCTURES

L1

Kappa		
1	2	3
4	5	6
Lambda		
1	2	3	4



1igm



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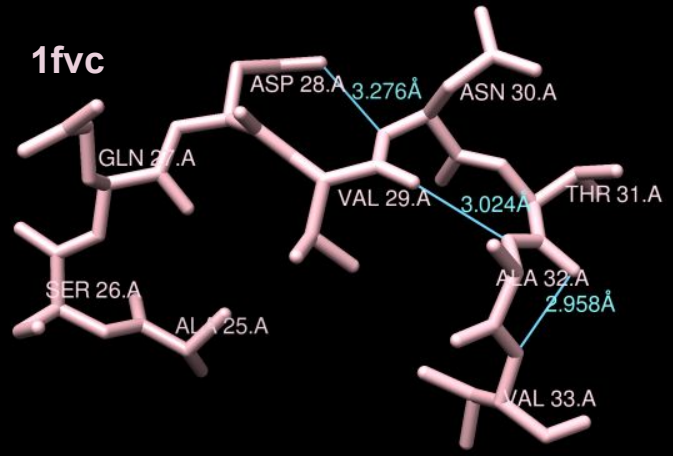

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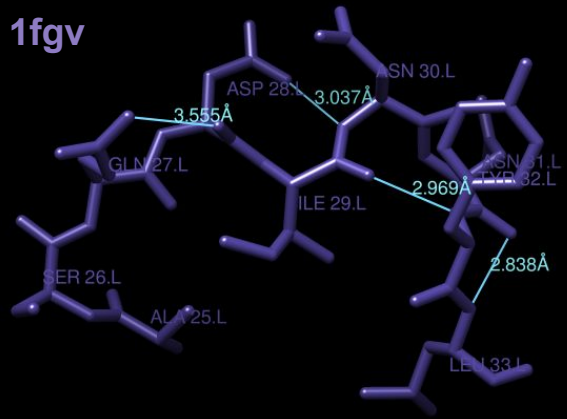

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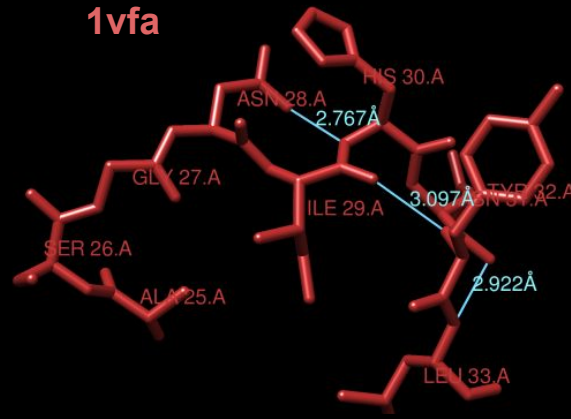
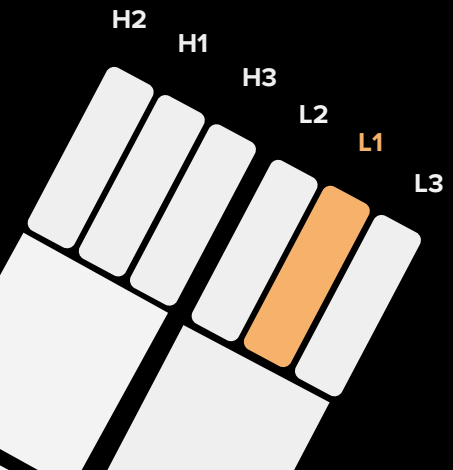
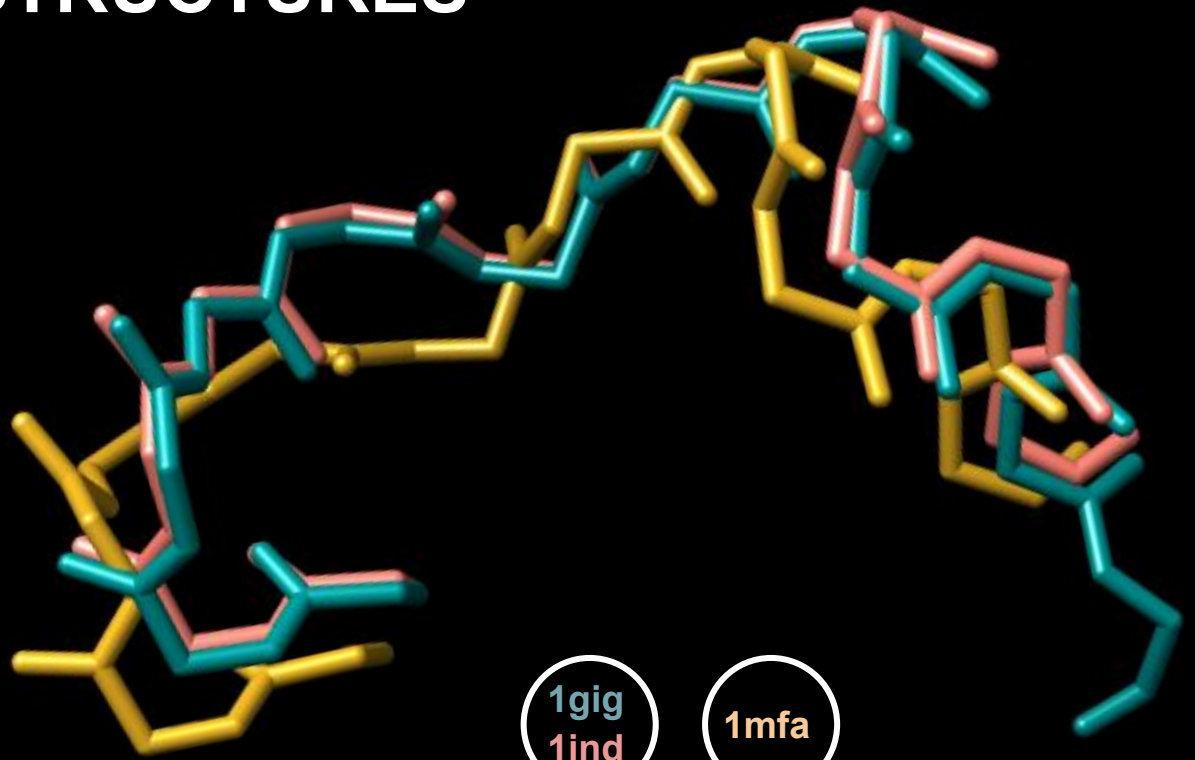


CANONICAL STRUCTURES

L1

Kappa		
1	2	3
4	5	6
Lambda		
1	2	**3**
4		





1gig
1ind
A

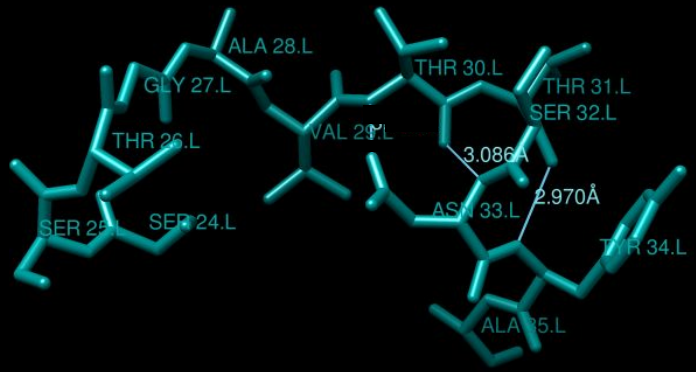
1mfa
B

CANONICAL STRUCTURES

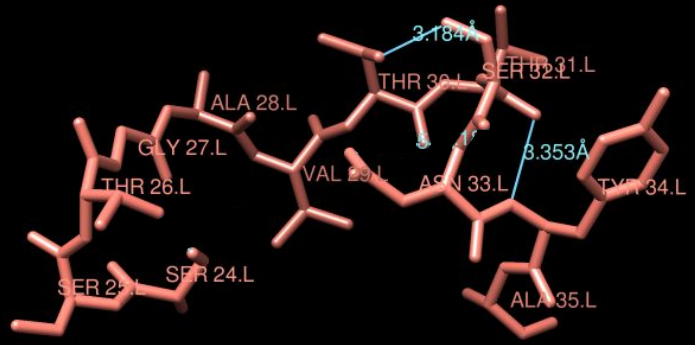
L1

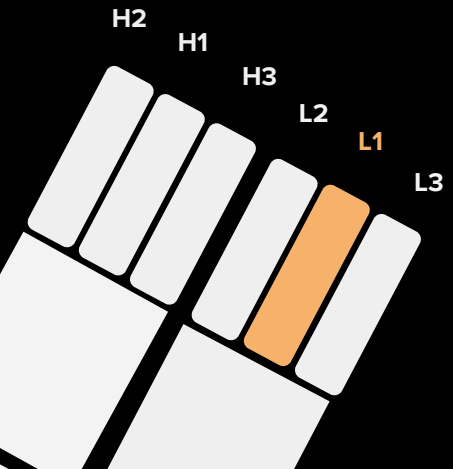
Kappa		
1	2	3
4	5	6
Lambda		
1	2	(3)
4		

1gig



1ind





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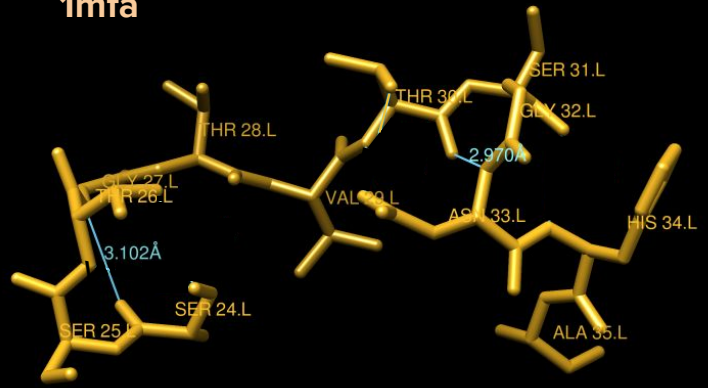
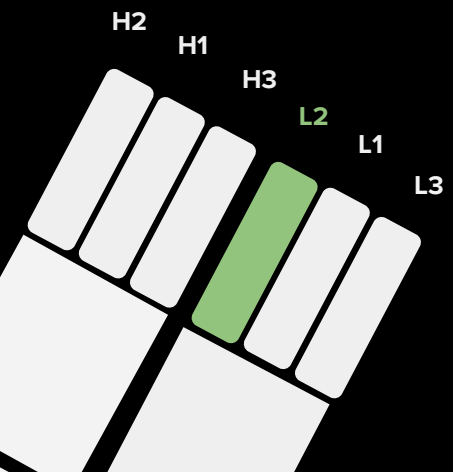
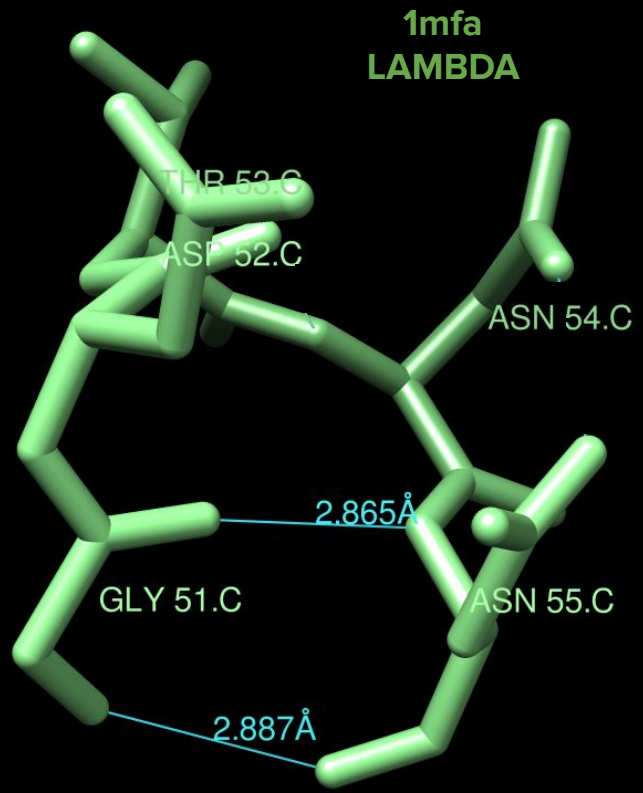
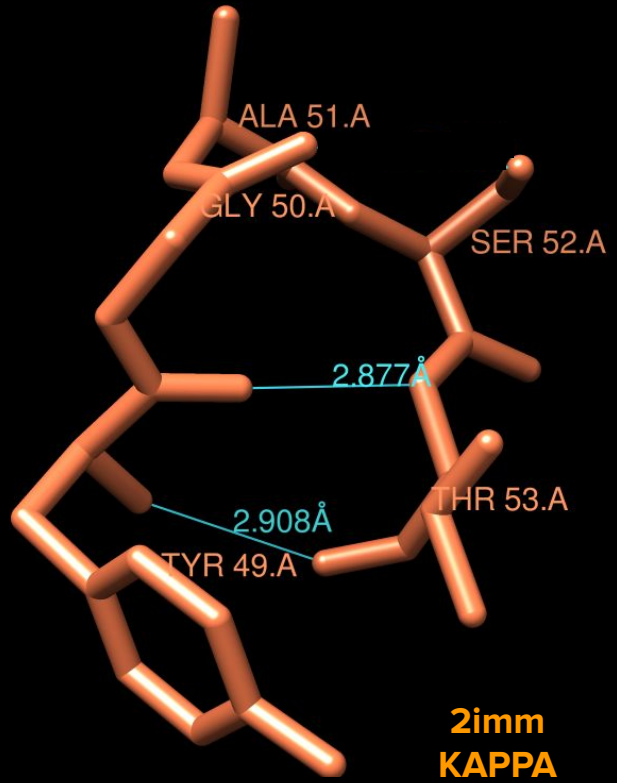


CANONICAL STRUCTURES

L2 Only 1







CANONICAL STRUCTURES

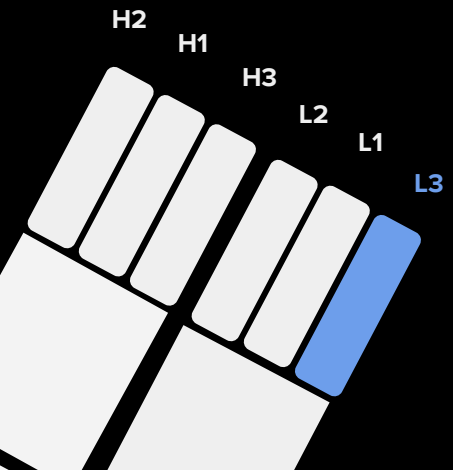
L3

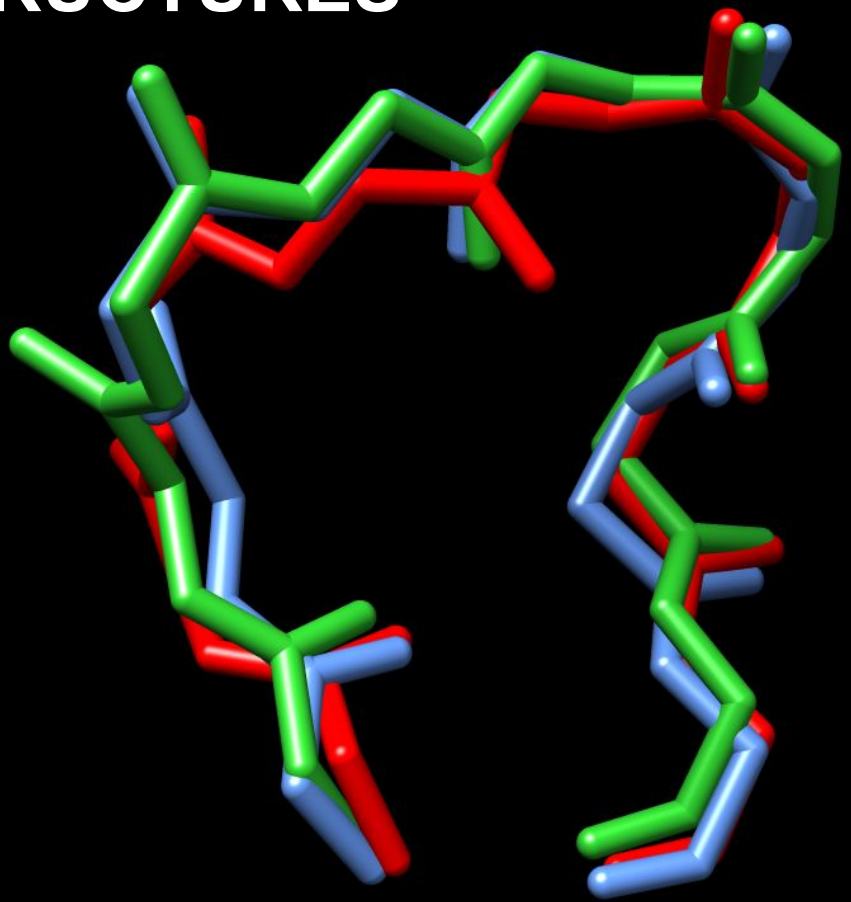
Kappa

1 2 3 4 5 6

Lambda

1 2





2imm

1igm

1fvc

CANONICAL STRUCTURES

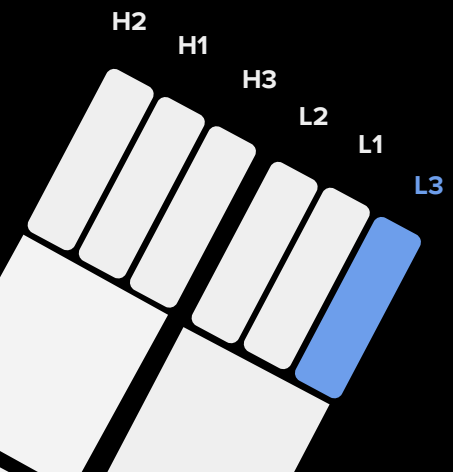
L3

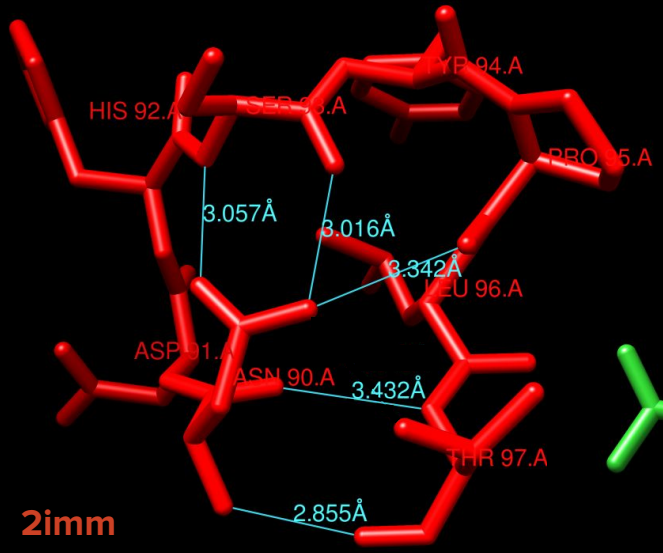
Kappa

1 2 3 4 5 6

Lambda

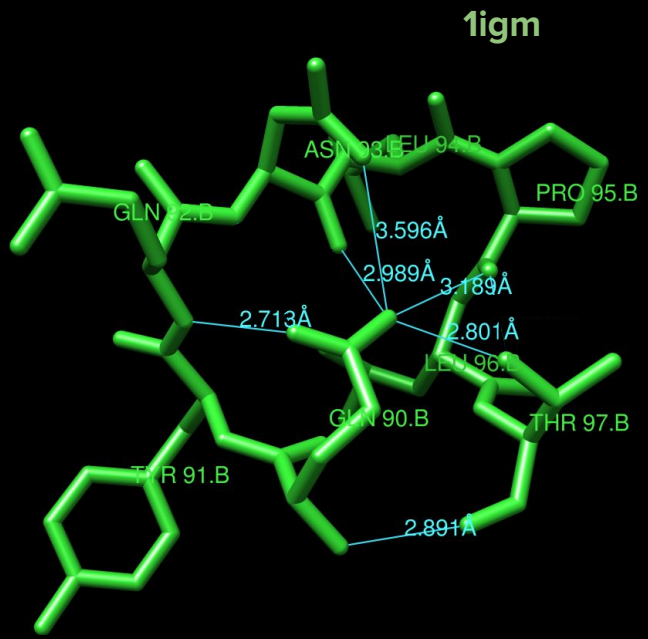
1 2





2imm

1igm



CANONICAL STRUCTURES

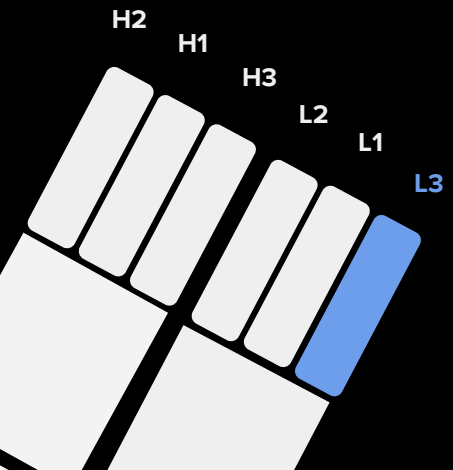
L3

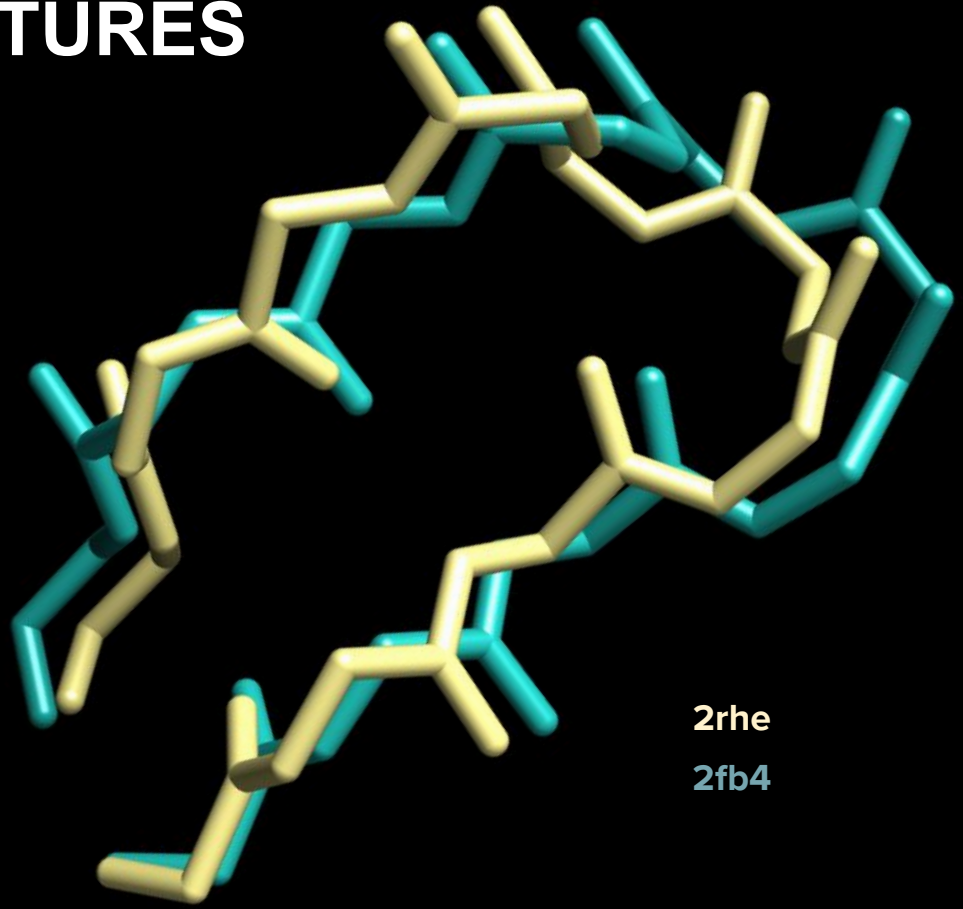
Kappa

1 2 3 4 5 6

Lambda

1 (2)





2rhe

2fb4

CANONICAL STRUCTURES

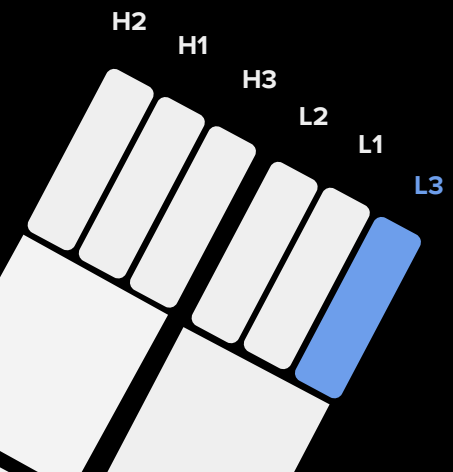
L3

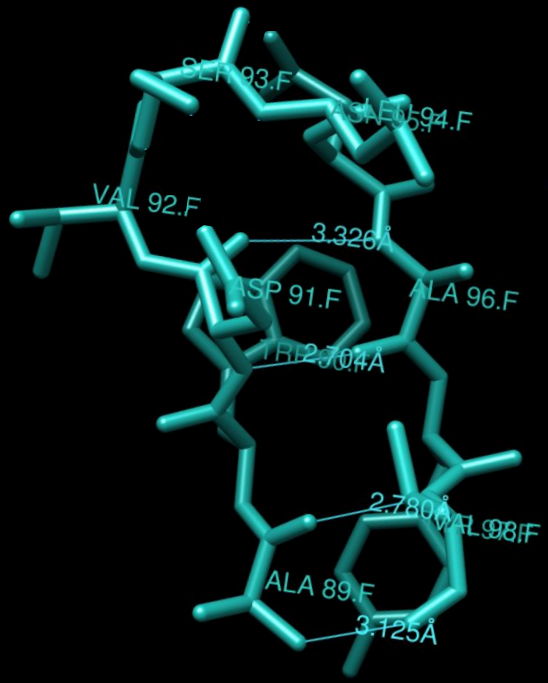
Kappa

1 2 3 4 5 6

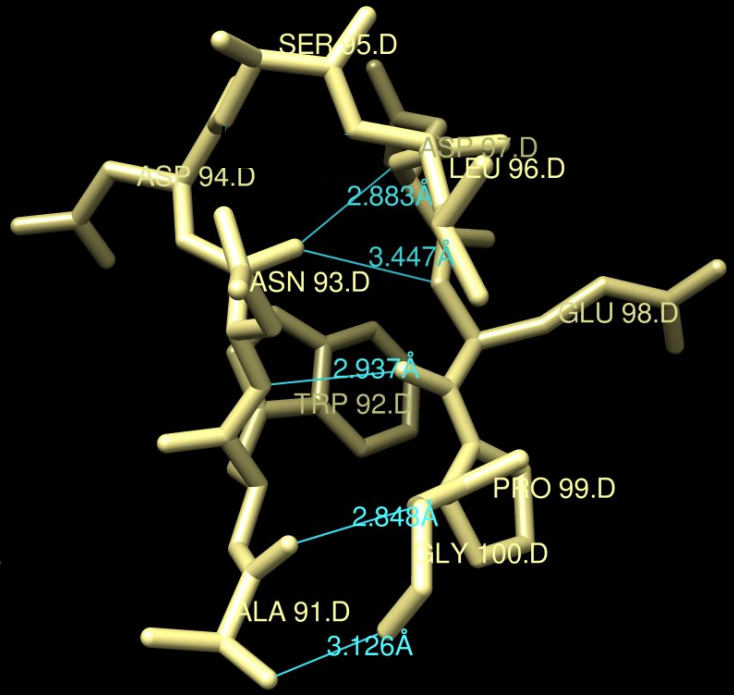
Lambda

1 (2)





2fb4



2rhe

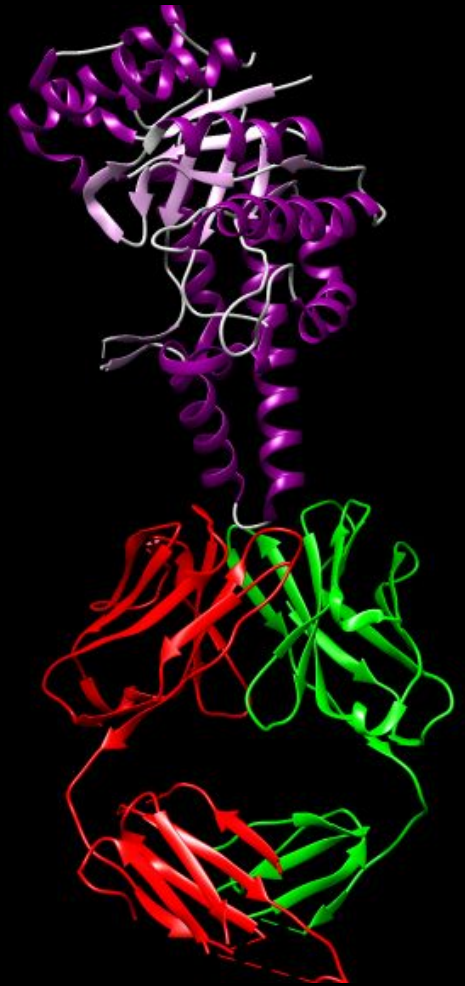
IgG - ANTIGEN INTERACTION

IgG-antigen

The forces involved in non covalent interactions are:

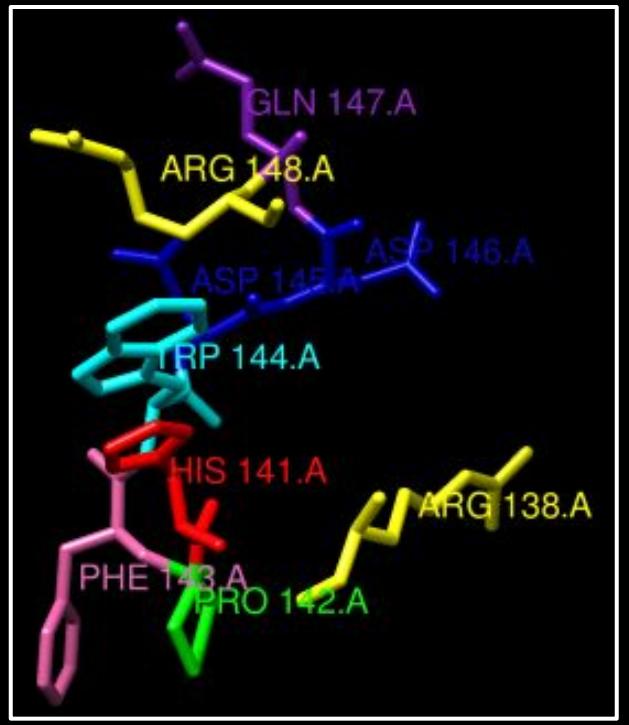
- Electrostatic forces
- Hydrogen bonds
- Van der Waals
- Hydrophobic forces
- Pi-cation interaction

The immunoglobulins have many aromatic aminoacids in antigen binding site which participate mainly in Van der Waals and hydrophobic interactions.

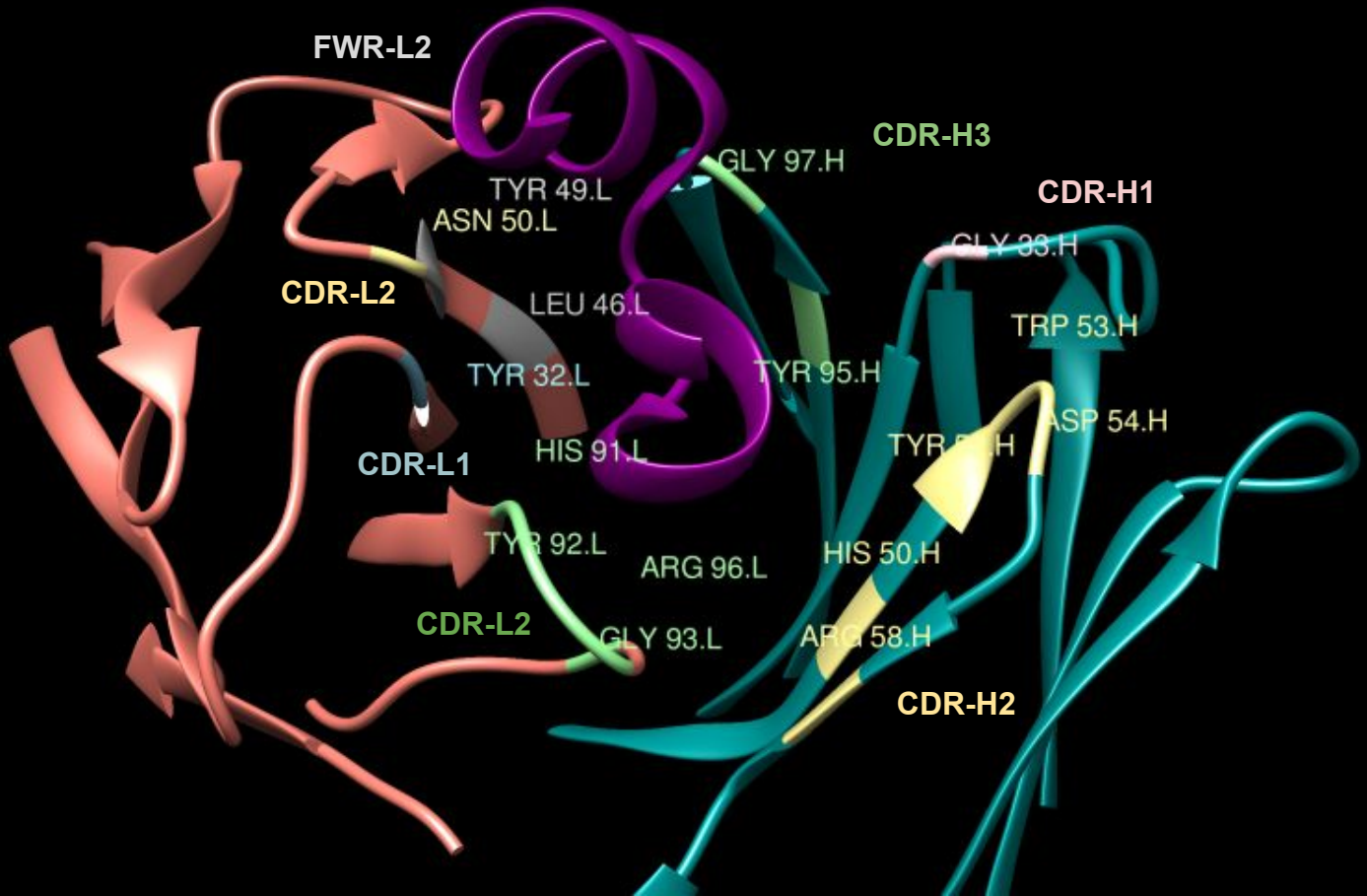


IgG-Hyaluronidase: Fold and Epitope

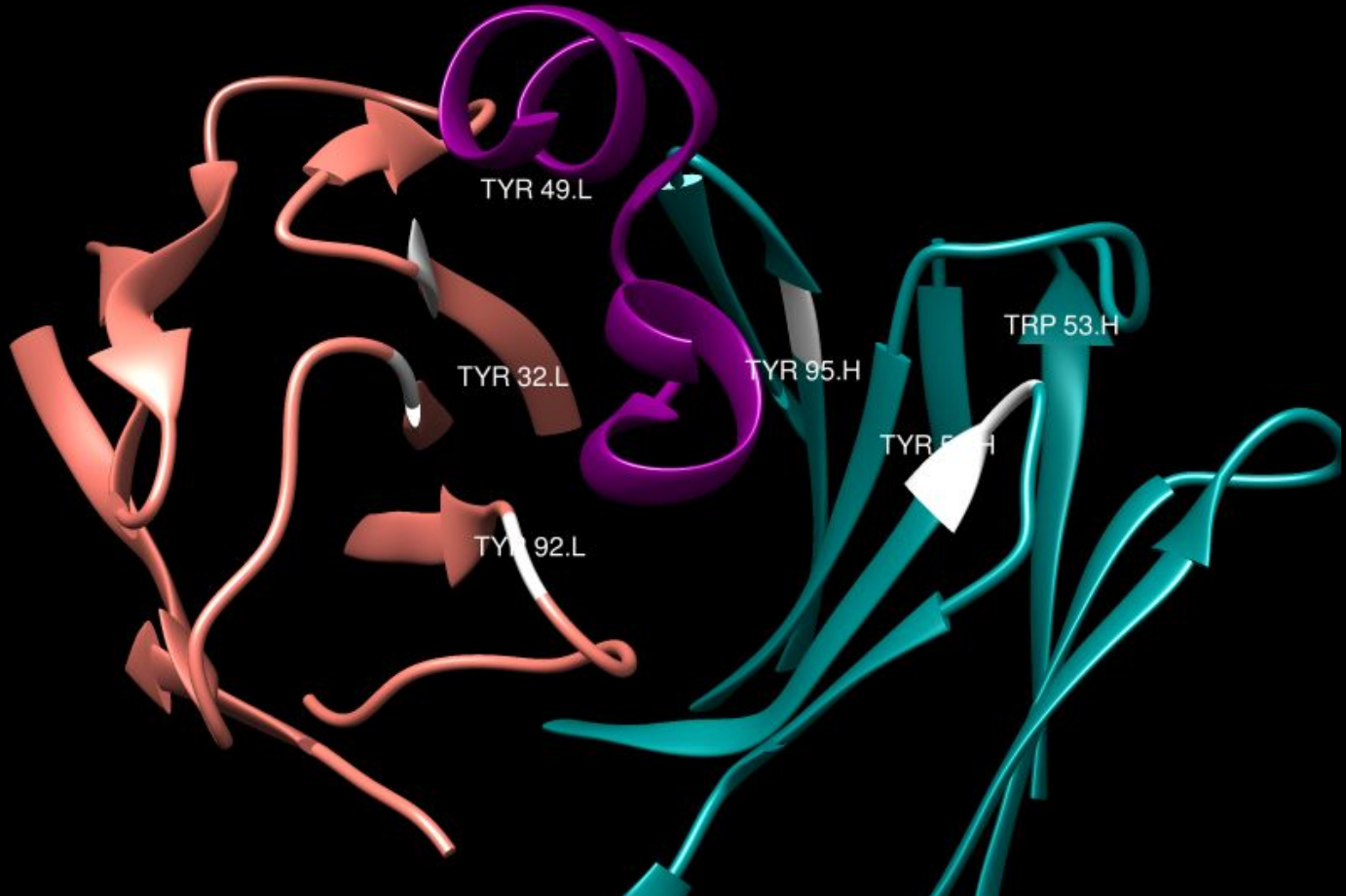
R+HPFWDDQR



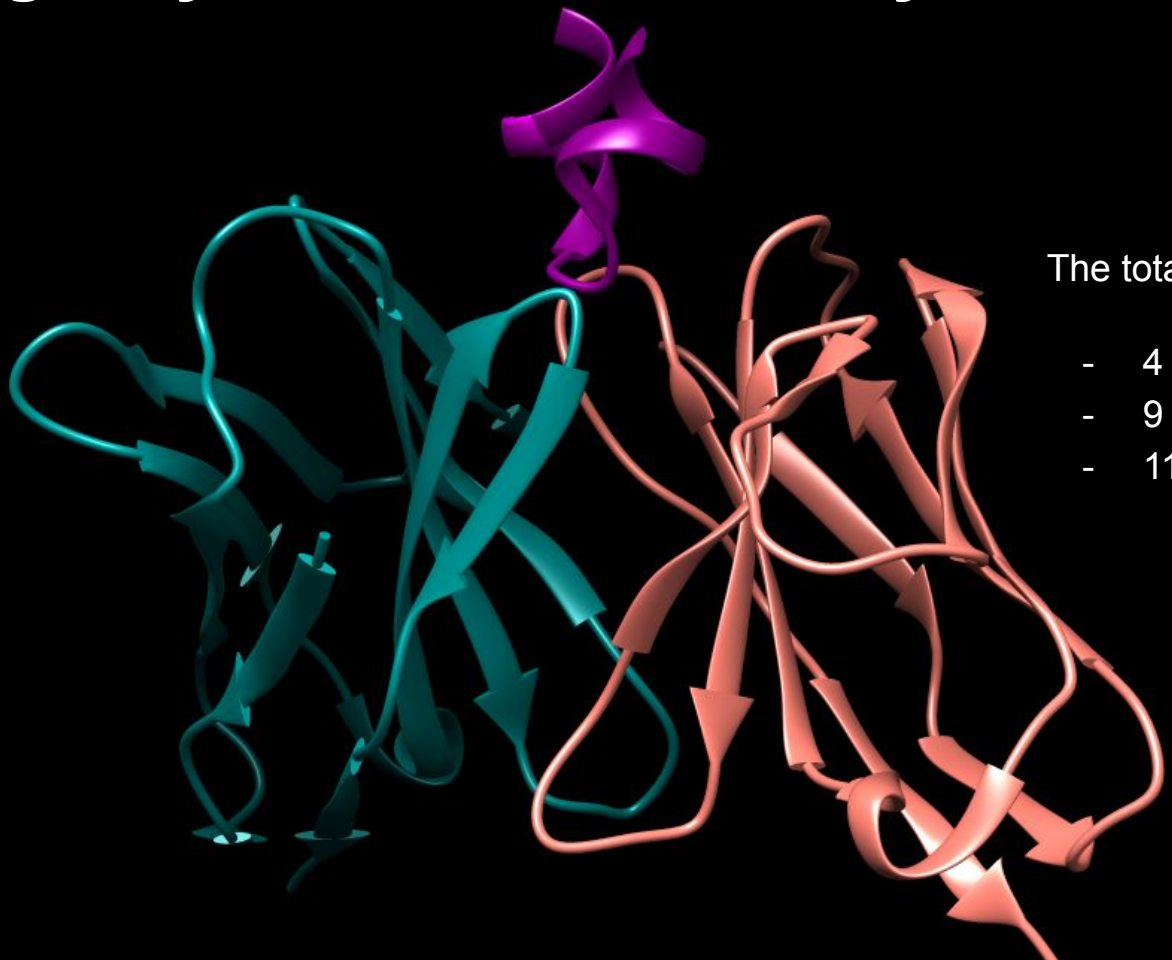
IgG-hyaluronidase: Fab-Hyal interaction



IgG-hyaluronidase: Aromatic aminoacids



IgG-hyaluronidase: Fab-Hyal interaction

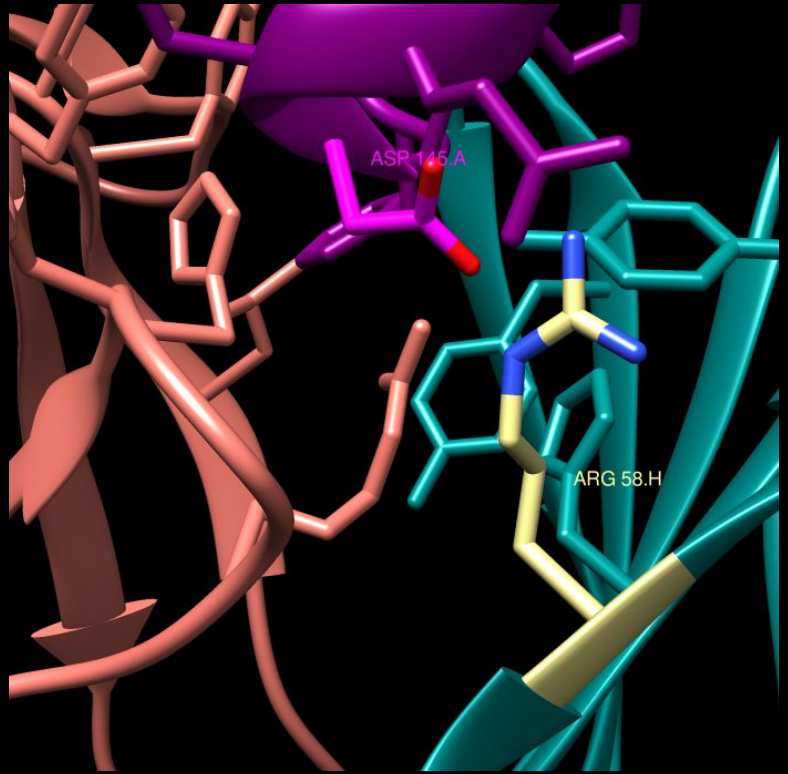


The total number of Hyal/Fab interactions:

- 4 salt bridges
- 9 hydrogen bonds
- 11 Van der Waals

IgG-hyaluronidase: Salt bridges

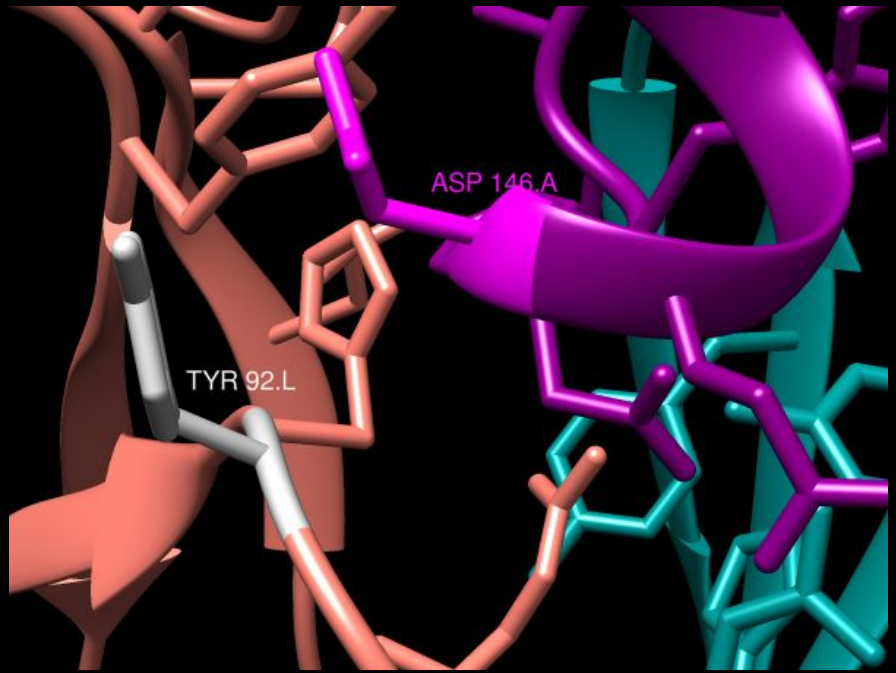
CDR-H2: ARG 58 - ASP 145, 3.2 Å



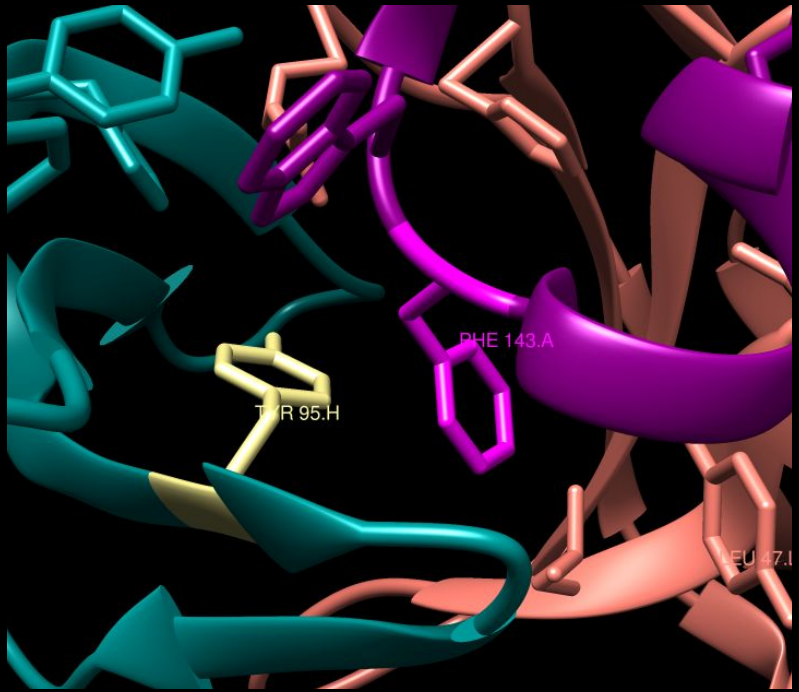
CDR-H2: ASP 54 - ARG 148, 3.2 Å

IgG-hyaluronidase: Van der Waals

CDR-L3: TYR 92 - ASP 146, 3.6 Å

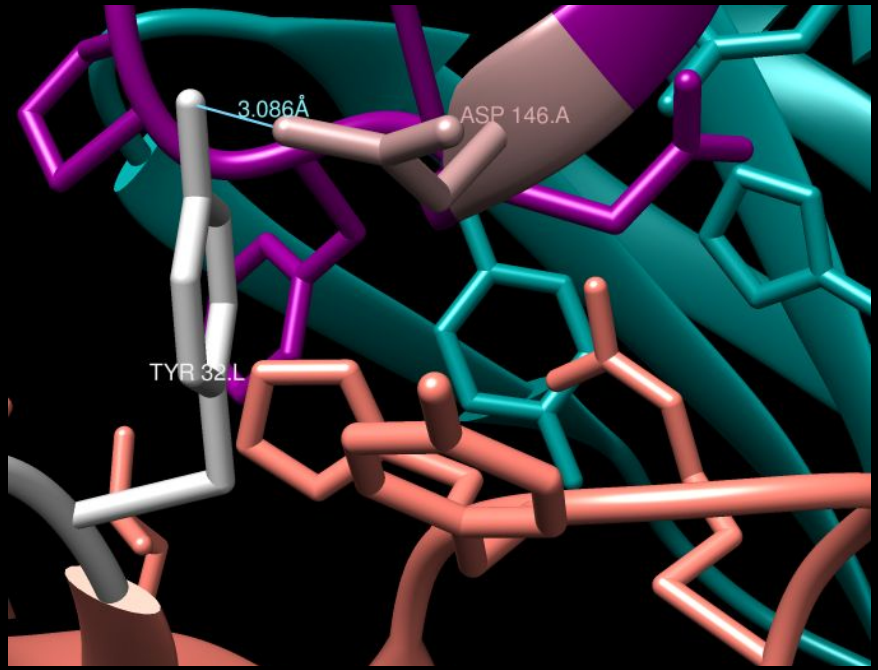


CDR-H3: TYR 95 - PHE 143, 3.5 Å

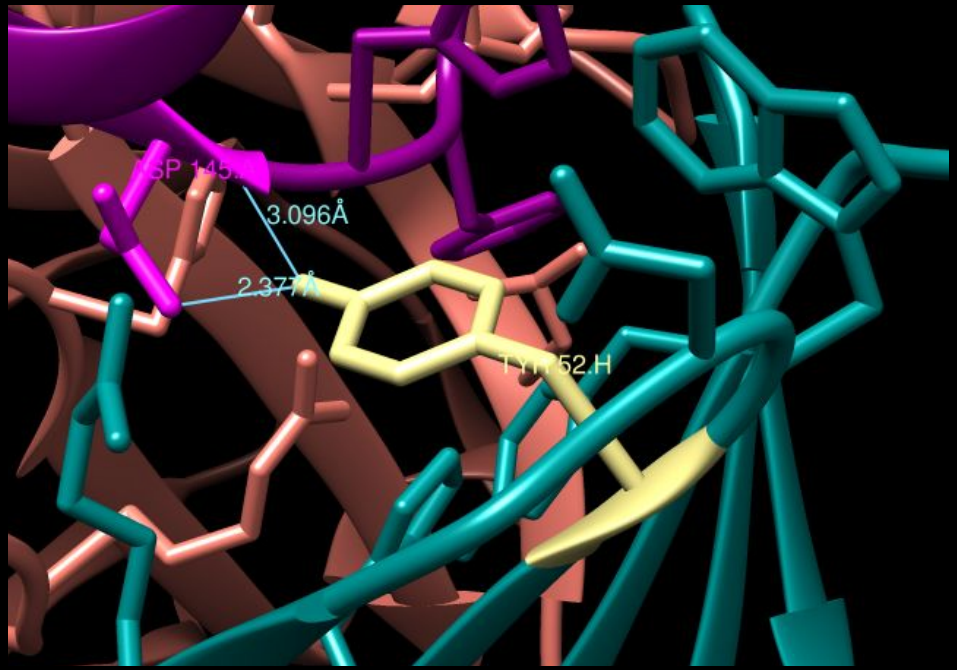


IgG-hyaluronidase: Hydrogen bonds

CDR-L1: TYR 32 - ASP 146, 3.1 Å



CDR-H2: TYR 52 - ASP 145, 3.1 Å

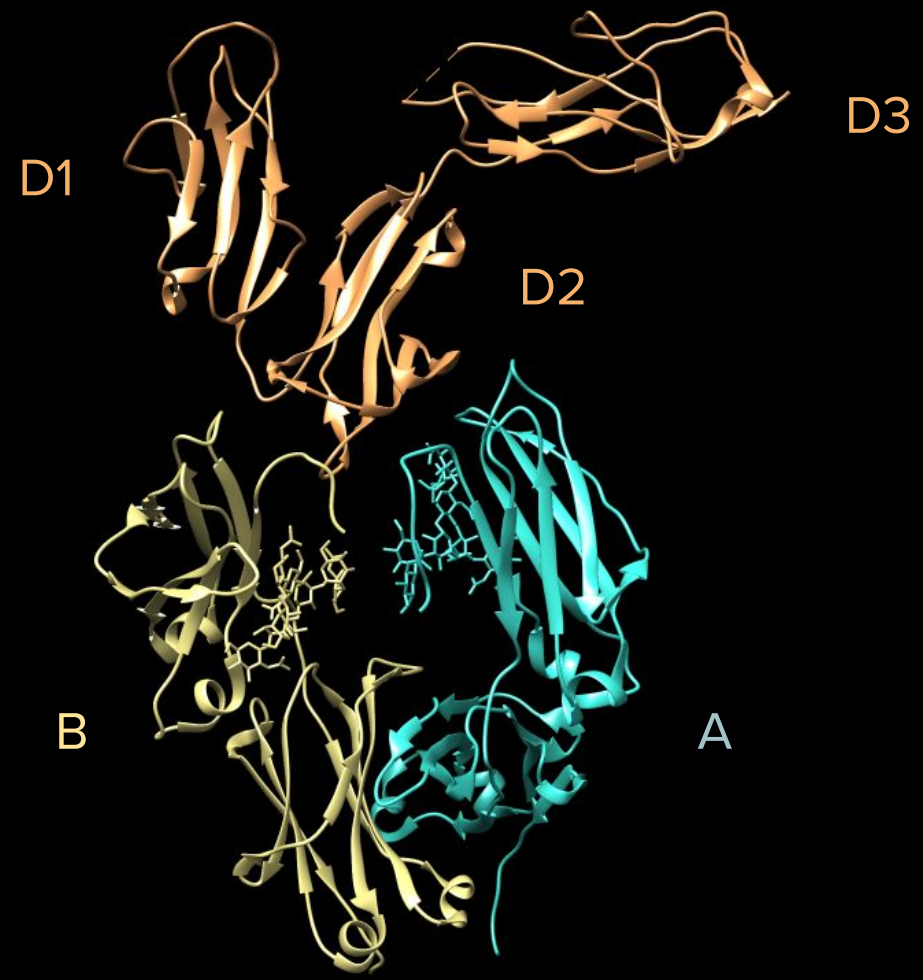


IgG - FcγRI INTERACTION

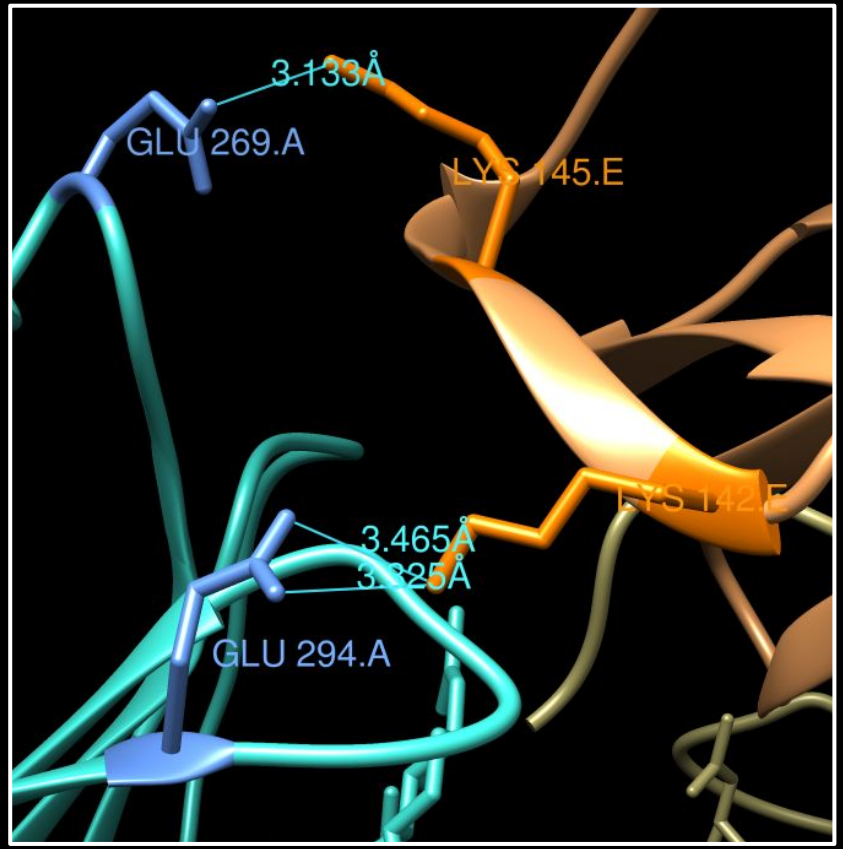
IgG-FcγRI

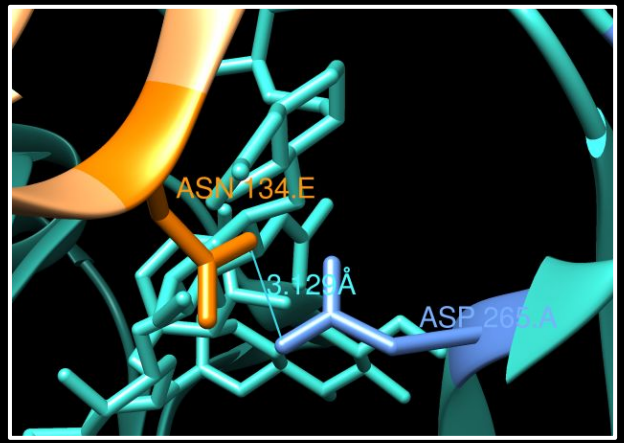
FcγRI

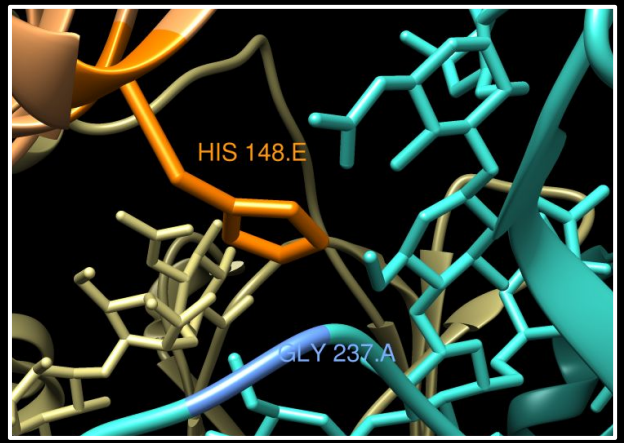
Fc IgG1



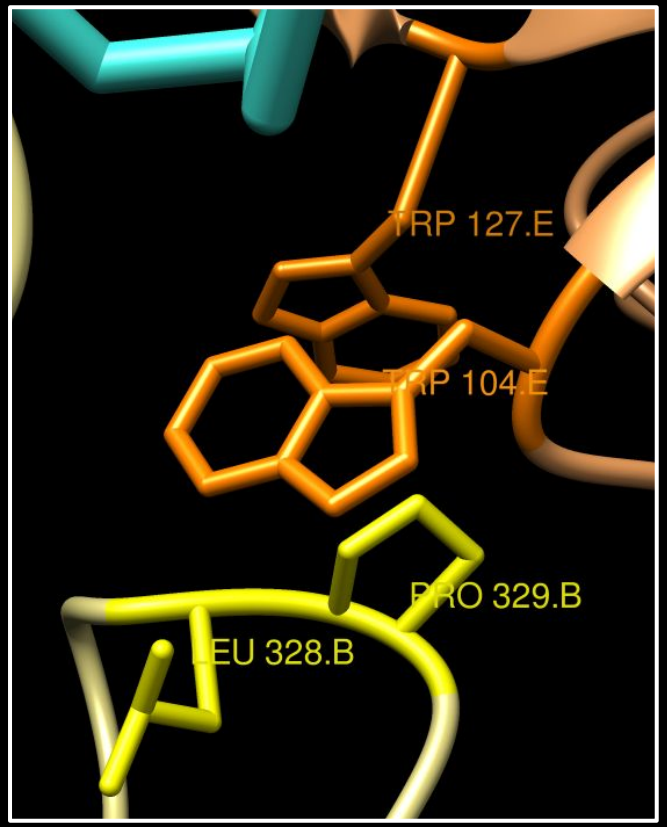
IgG-FcγRI: Region 1

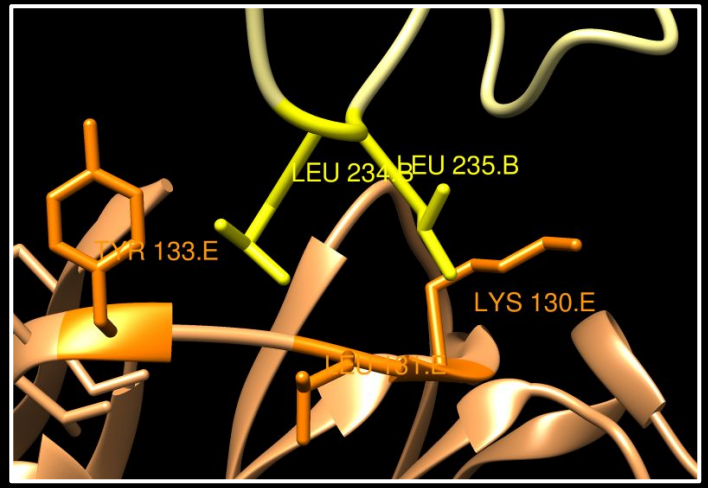


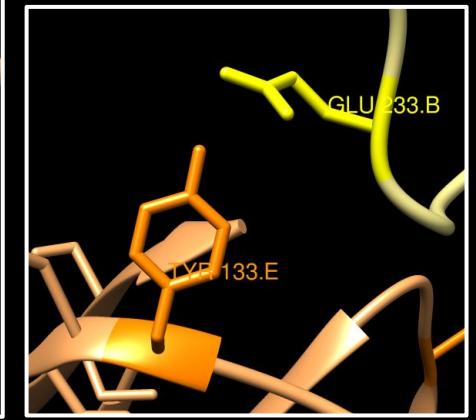


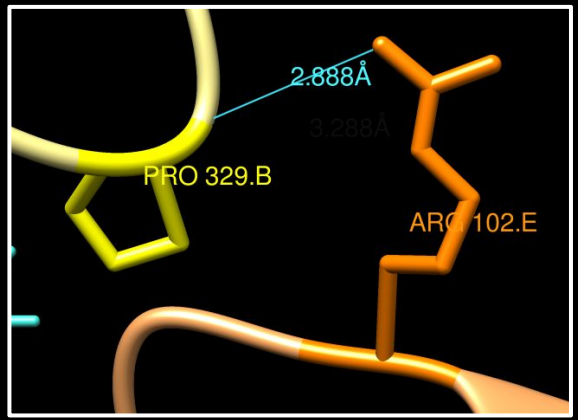


IgG-FcγRI: Region 2

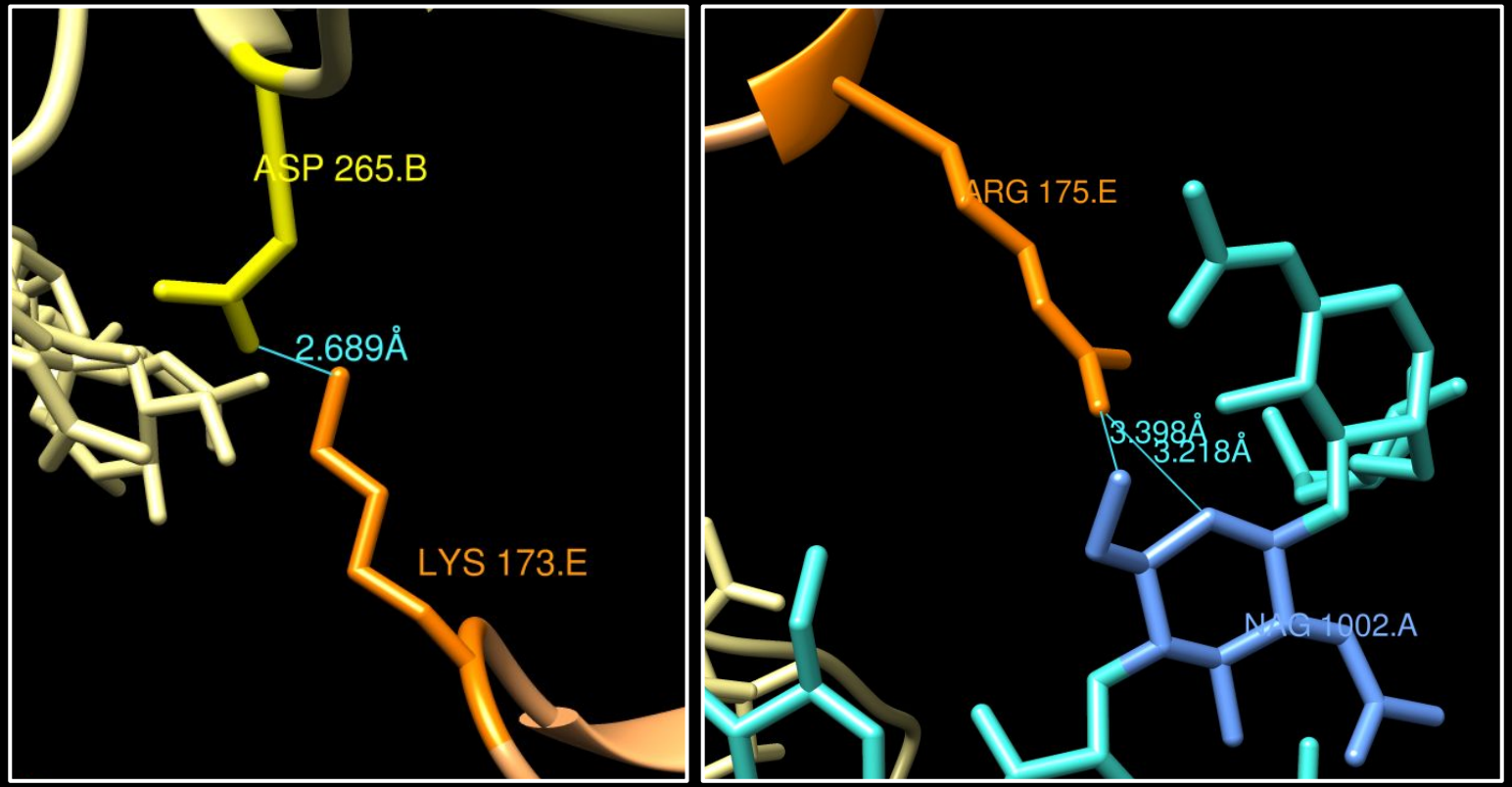








IgG-FcγRI: Region 3



CONCLUSIONS

- The immunoglobulins have an important function in the **immune system**.
- The **diversity** of immunoglobulins allows them to recognize specifically a great variety of **antigens**, as we have seen in hyaluronidase-IgG1 interaction.
- The Ig domain is formed by **two ß-sheets** built up with several **antiparallel ß-strands** with a **Greek key** topology. This ß-sheets are covalently linked with a **disulfide bond**.
- The flexibility of the immunoglobulin is conferred, in large part, by the **hinge region.**

- The heavy chain can exist in two forms: a soluble one and a surface one.
- The Fc of immunoglobulins is majorly conserved in the IgG.
- N-glycosylation plays a key role in the interactions with the effector cells.
- The **CDRs** are the hypervariable regions of the immunoglobulin. They contribute to its specificity and form the **antigen binding site**. There are 3 in the light chain (**L1**, **L2** and **L3**) and 3 in the heavy chain (**H1**, **H2** and **H3**).
- Although the great variation of the CDRs, they have small main chain conformations that are called **canonical structures**.

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Questions

About the basic concepts of immunoglobulins:

a) Structural differences in Fab fragment are the basis for five different isotypes classes of immunoglobulins.

b) In immunoglobulin G the disulfide bonds are formed between cysteine residues of constant domain 2 close to the hinge.

c) The Fc fragment are formed by the association of variable and constant domains of light chain, and the variable and constant 1 domains of heavy chain.

d) There are four different types of light chain.

e) There are three loci that encode for heavy chain.

The difference between the fold of the variable domain and the constant domain is:

a) The constant domain contains two extra ß-strands called C' and C''

b) The constant domains hasn't got a Greek Key topology

c) a and b are true

d) The variable domain contains two extra ß-strands called C' and C''

e) There is no difference between them

About the Fab arm exchange, which of the following is false:

a) It is characteristic from IgG3

b) Involves that the core hinge forms intra- rather than inter heavy chain disulfide bonds

c) It affects both, covalent and non covalent interaction

d) Half molecules can recombine to create bispecific immunoglobulines

e) All of them are true

The flexibility of the immunoglobulin is conferred in large part by:

a) The constant domain

b) The variable domain

c) The hinge

d) The light chain

e) The heavy chain

About the Fc from immunoglobulin G:

a) It is not mostly conserved between species

b) The cysteines that form the disulphide bonds vary between IgGs

c) There are two possible forms of the heavy chain that differ in the N-terminal end

d) There are few possible glycosylation patterns

e) When the glycosylation is removed the effector functions are impaired

Which determinant enables IgG4 to undergo Fab arm exchange?:

a) The hinge
b) CH3-CH3 domain interface
c) a and b are true
d) CDR L1
e) All of them are true

About the interaction between hyaluronidase and IgG1:

a) The hyaluronidase fold is a Beta-alfa-Beta motif.
b) The epitope is mostly continuous and composed of twenty consecutive residues.
c) The apolar residues form most of the contacts with Ag.
d) The hydrophobic residues are located predominantly at the periphery of Hyal binding surface.
e) The total number of polar Hyal/Fab interactions comprises 4 salt bridges and 9 hydrogen bonds.

About the Complementary Determining Regions (CDRs):

a) They are only found in the light chain and its function is to bind with the antigen
b) They are only found in the heavy chain and its function is to bind with the antigen
c) They are only found in the light chain and its function is to stabilize the immunoglobuline
d) They are found in both heavy and light chains and its function is to bind with the antigen
e) They are found in both heavy and light chains and its function is to stabilize the immunoglobuline

About the canonical structures of the Complementary Determining Regions (CDRs), mark the false sentence:

a) All of the CDRs have canonical structures
b) The canonical structures are small number of main chain conformations
c) L2 only have one canonical structure
d) The H3-CDR is the one with more variability
e) There are different canonical structures for the same CDR in lambda light chain and in kappa light chain

About the IgG1-FcgammaRI mark the correct answer:

1. There are no van der Waals interactions in any region
2. We can find three hydrophobic clusters
3. The interactions are with domains D1 and D2 from the receptor
4. The receptor also interacts with the glycan bound to the Fc of the immunoglobulin

a) 1, 2, 3
b) 1 i 3
c) 2 i 4
d) 4
e) 1, 2, 3, 4

Thank you for your attention!