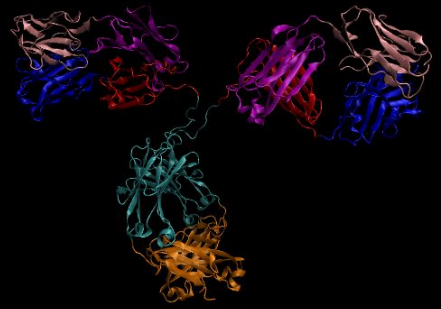
Universitat Pompeu Fabra Barcelona



Immunoglobulins G

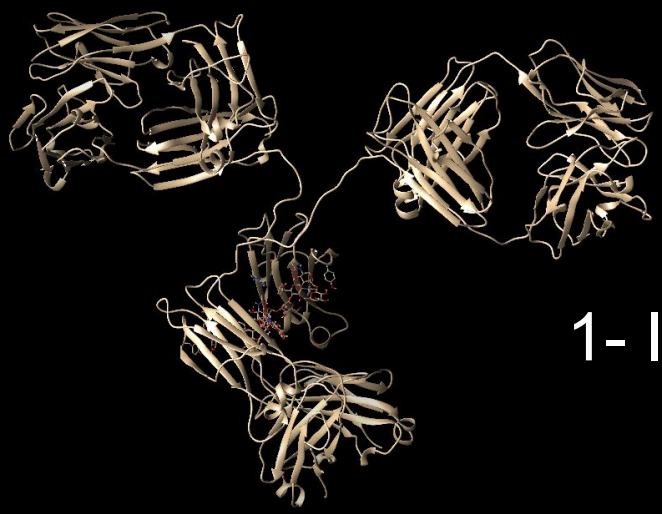
Structural biology

4th Human Biology

Aina de Manuel, Antònia Escanellas, Nuria Mei Barbero.

Outline

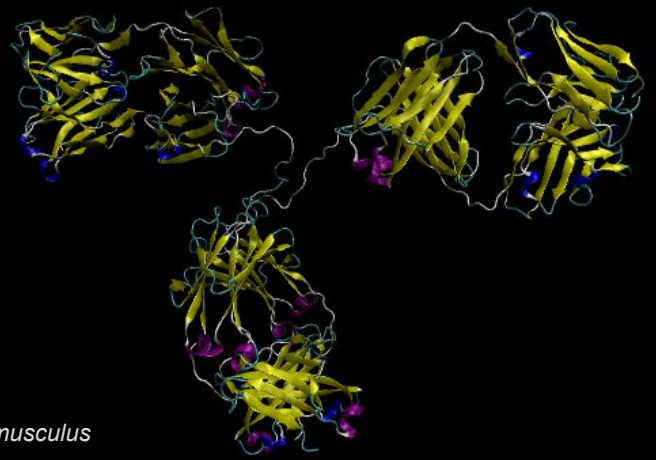
1. Introduction
2. Immunoglobulin fold
3. Immunoglobulins G
    - Constant region
    - Variable region
4. Conclusions
5. Bibliography
6. Multiple choice questions



1- INTRODUCTION

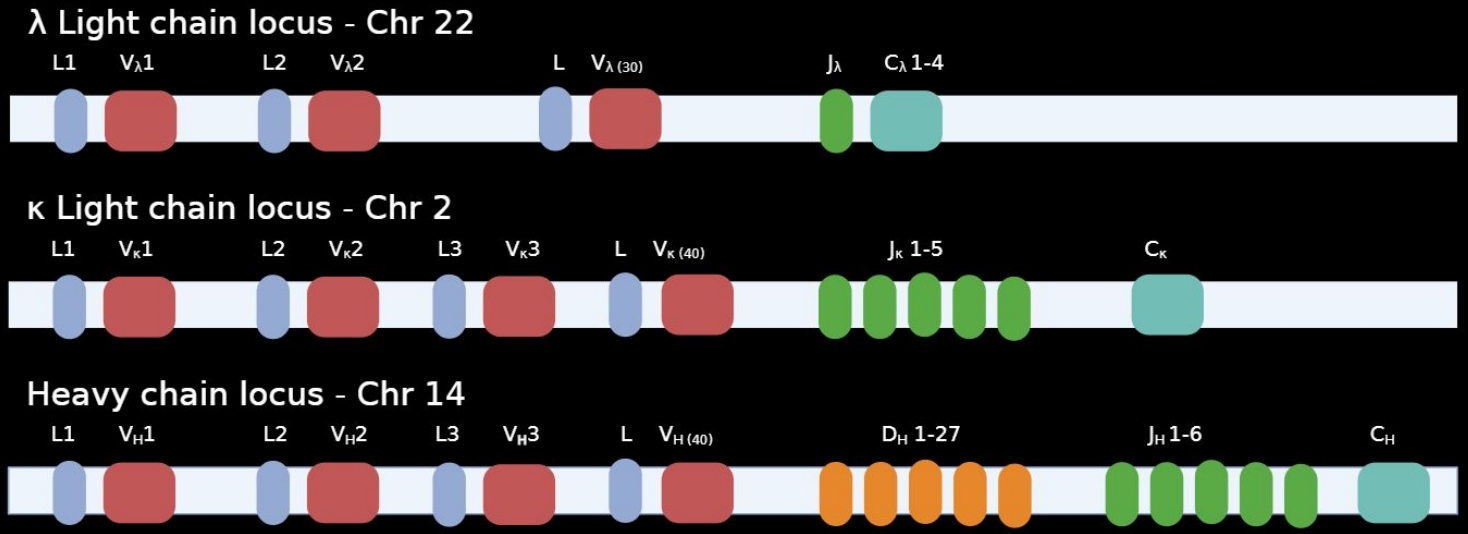
Immunoglobulins

- Glycoprotein molecules produced by plasma cells
- Main function: recognition and binding to specific antigens
  - Responsible for the adaptive immune response
- Structure:
  - 2 identical heavy chains and 2 identical light chains
  - Y - shaped: variable and constant regions



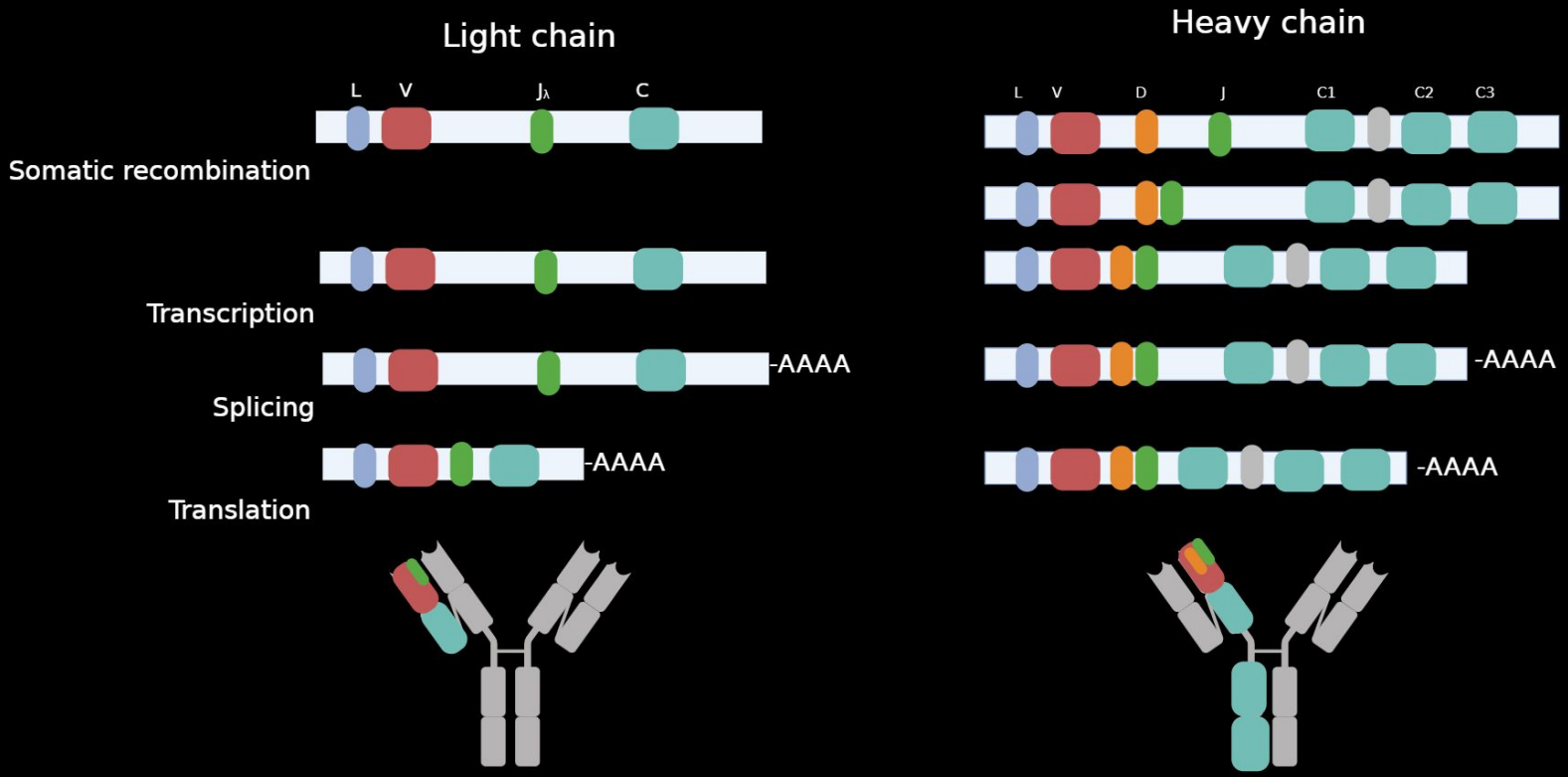
1IGT 2.8 Å, *Mus musculus*

Diversity

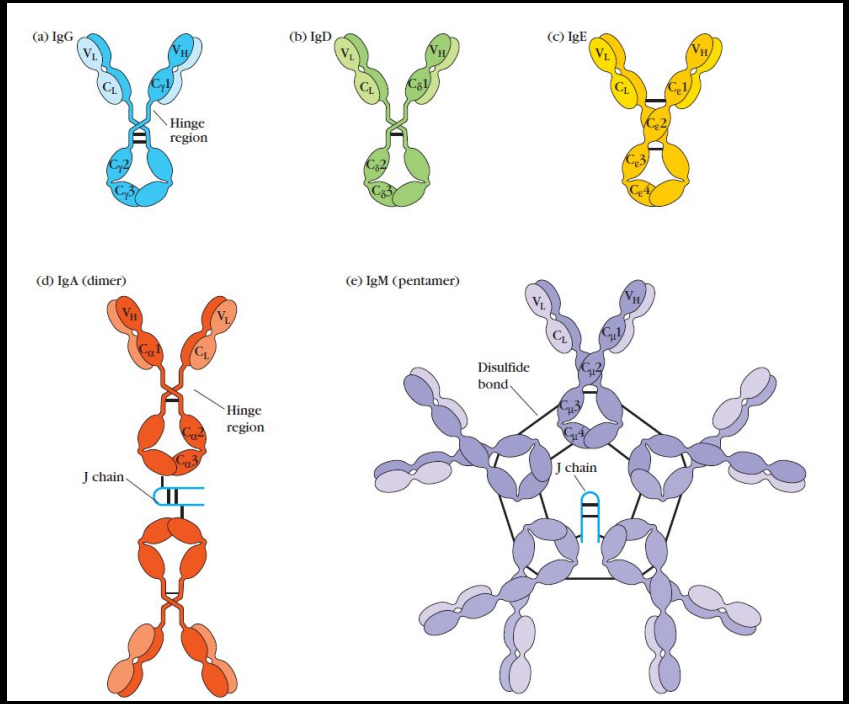


*Chromosomes 16, 6 and 12 respectively in mice

Diversity


Light chain
Heavy chain
L
V
J_λ
C
L
V
D
J
C1
C2
C3
Somatic recombination
Transcription
Splicing
Translation
-AAAA
-AAAA
-AAAA
-AAAA

Immunoglobulin isotypes

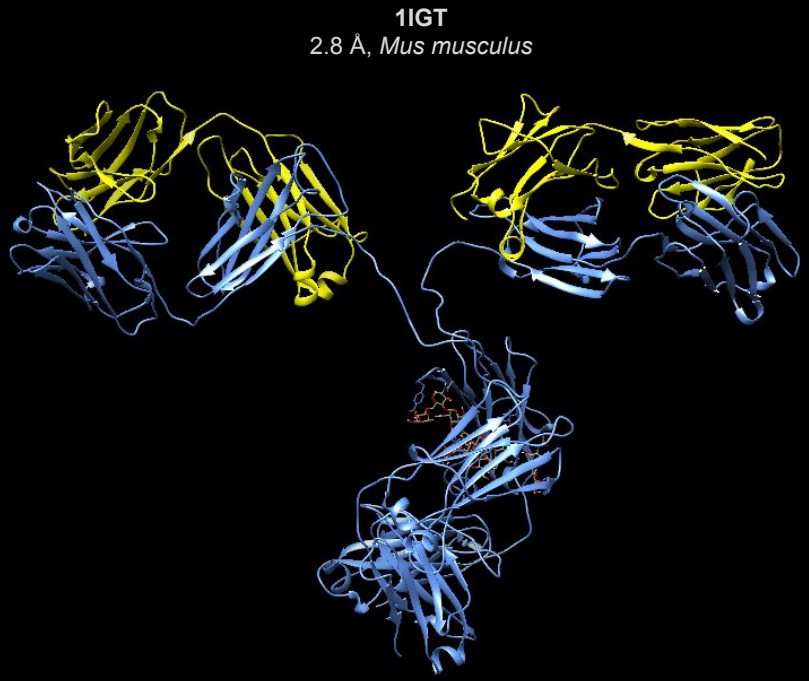


Stranford, S.A. *et al.* (2022) *Kuby Immunology*. Austin: Macmillan Learning.

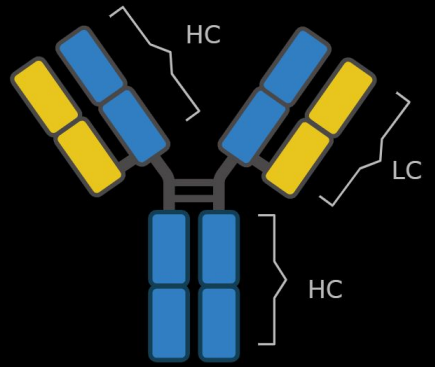
Immunoglobulin isotypes

Class Ig	Structure	Heavy chain	Number of CH Ig domains	Subclasses	Light chain	J chain	Functions	Location
Ig G	Monomer	γ	3	γ1, γ2, γ3, γ4 (humans) γ1, γ2a, γ2b, γ3 (mouse)	κ or λ	None	Complement activation, agglutination, opsonization and neutralization, crosses placenta to protect foetus.	Serum and intercellular fluid
Ig M	Pentamer	μ	4	-	κ or λ	Yes	Complement activation, opsotnization, agglutination, and neutralization	Serum
Ig A	Dimer	α	3	**α1, α2**	κ or λ	Yes	Agglutination and neutralization	Mucous membrane secretion, gut
Ig E	Monomer	ε	4	-	κ or λ	None	Triggers release of histamine from basophils and mast cells	Serum, mast cell surfaces
Ig D	Monomer	δ	4	-	κ or λ	None	Antigen receptor	B cell surface

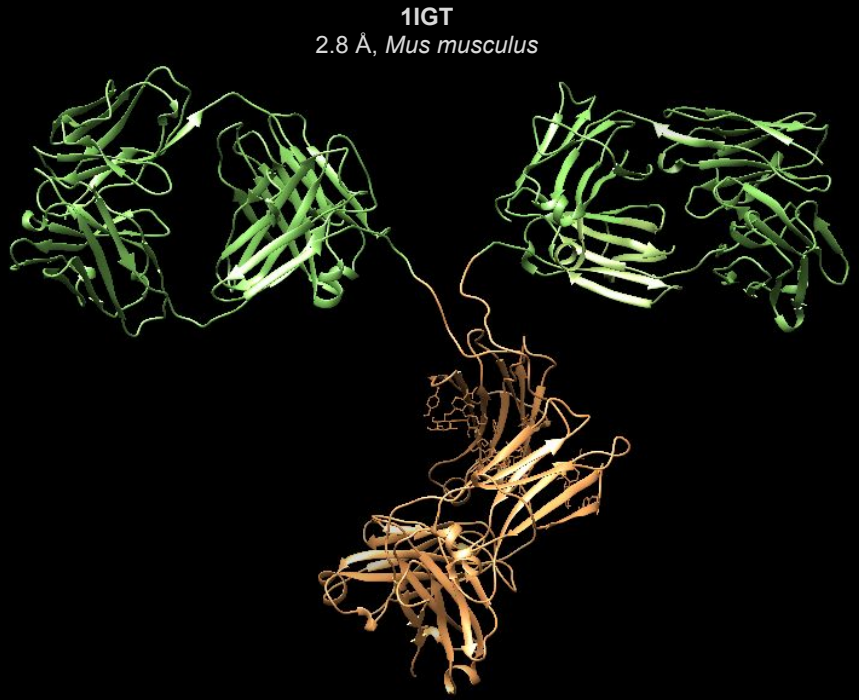
IgG structure

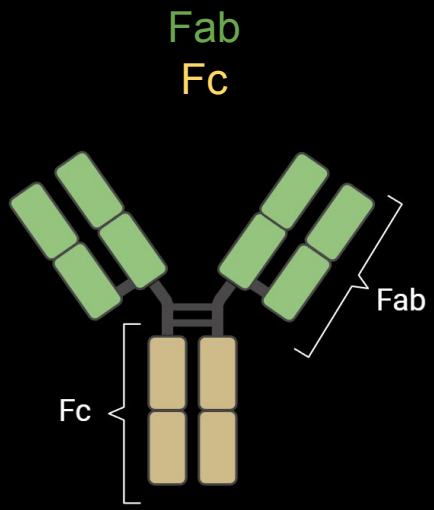


Heavy chains
Light chains

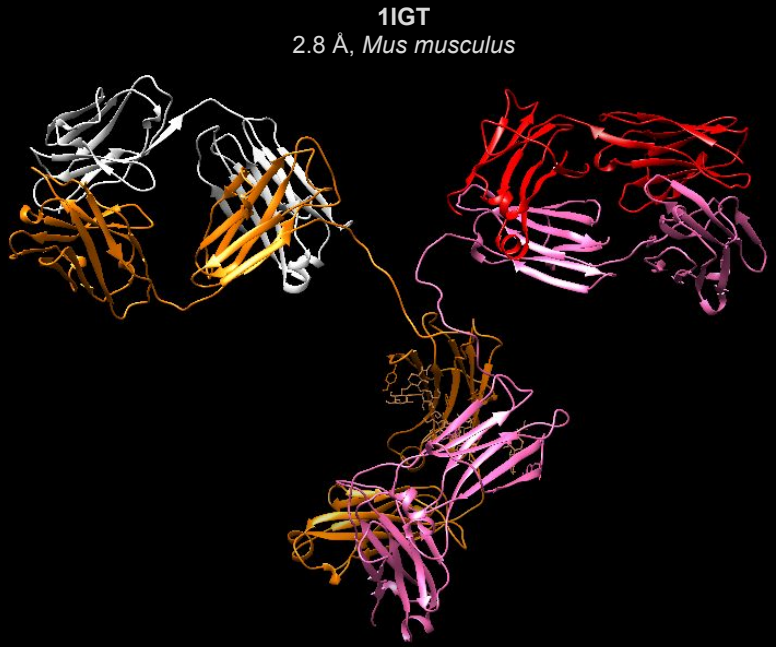


IgG structure

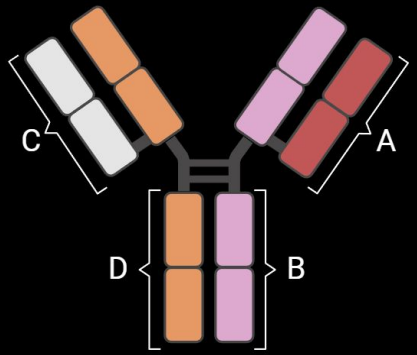




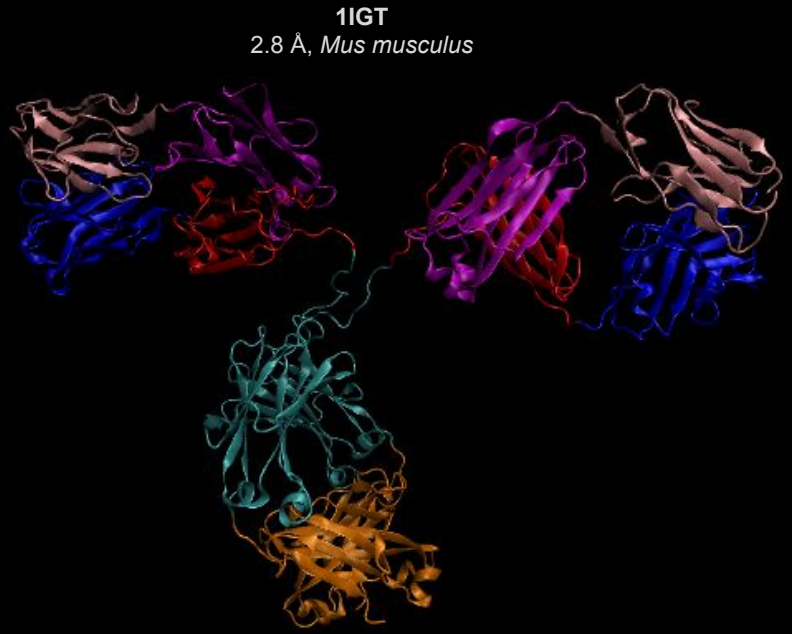
IgG structure

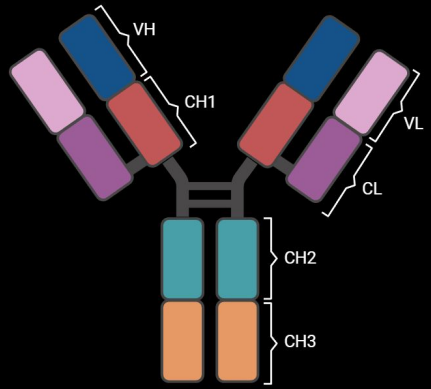


Chain A (light chain)
Chain B (heavy chain)
Chain C (light chain)
Chain D (heavy chain)



IgG structure

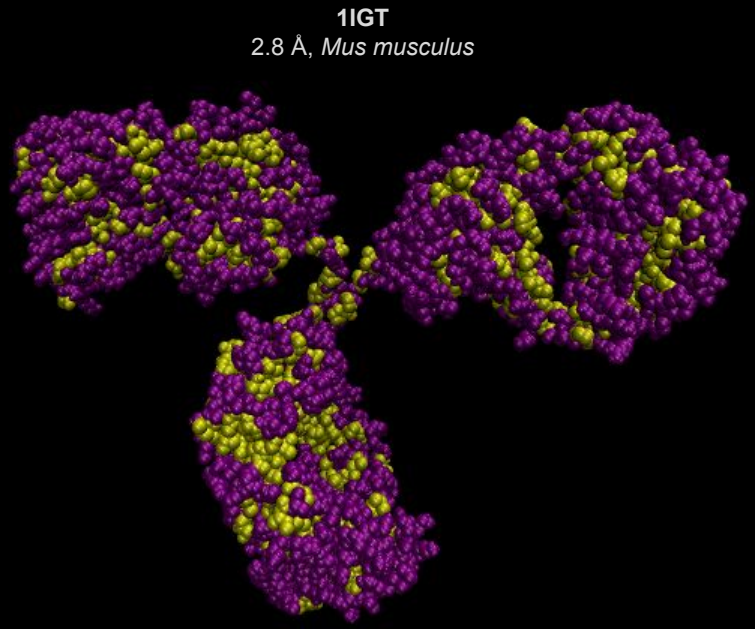




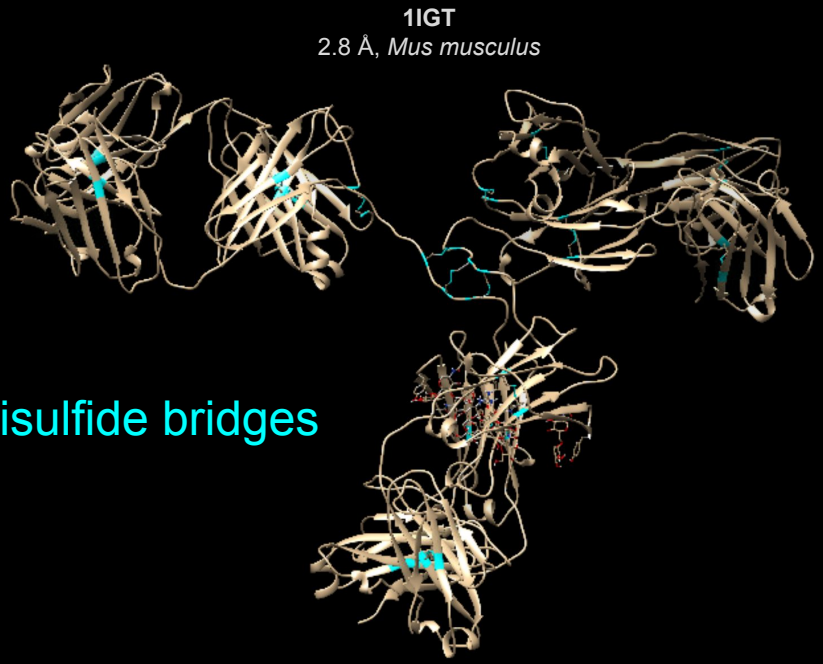
Hydrophobicity and hydrophilicity

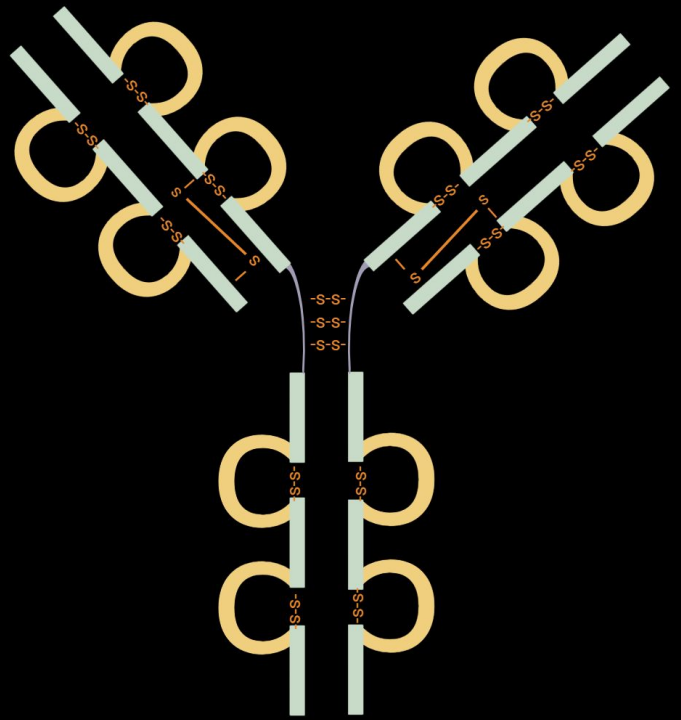
Hydrophobic

Hydrophyllic

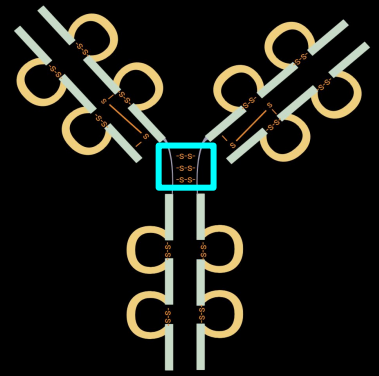


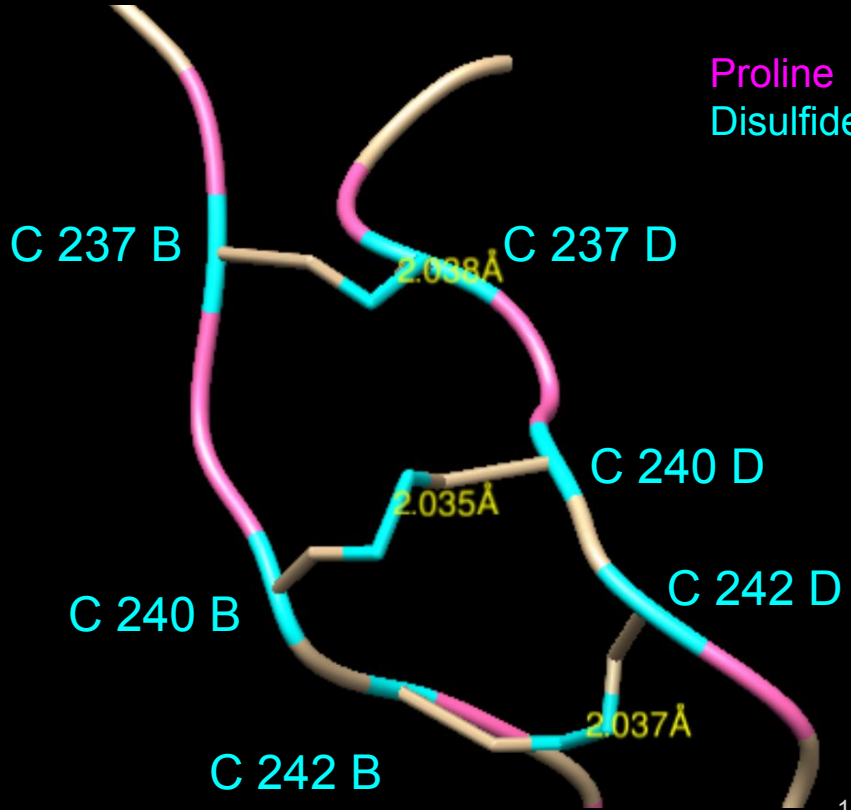
Disulfide bridges





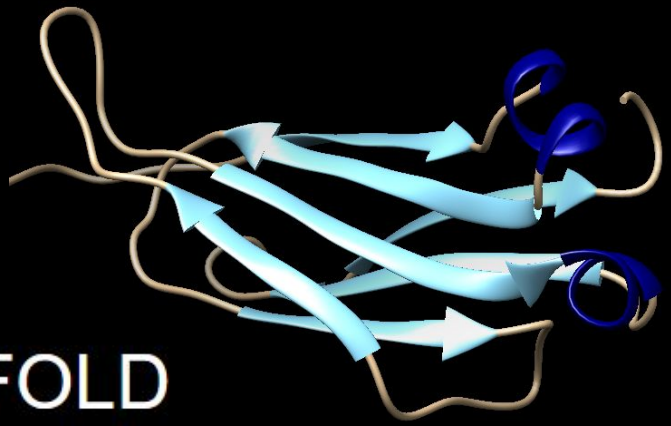
Disulfide bridges (Hinge region)





Residue chain B - D	Distance
C 237C 237 B - C237 D	2.038 Å
C 240 B - C 240 D	2.035 Å
C 242 B - C 242 D	2.037 Å

1IGT 2.8 Å, *Mus musculus*



2- IG FOLD

SCOP

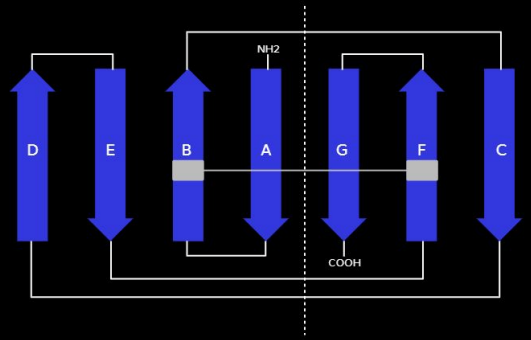
class:
All beta proteins

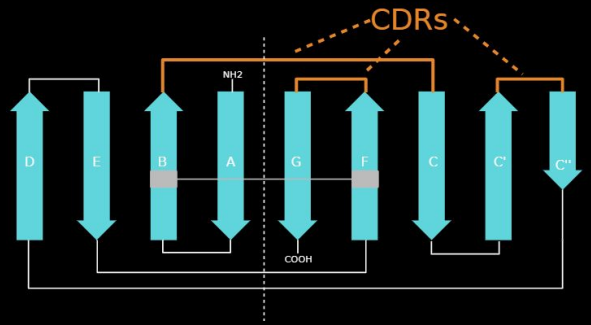
fold:
Immunoglobulin-like beta-sandwich

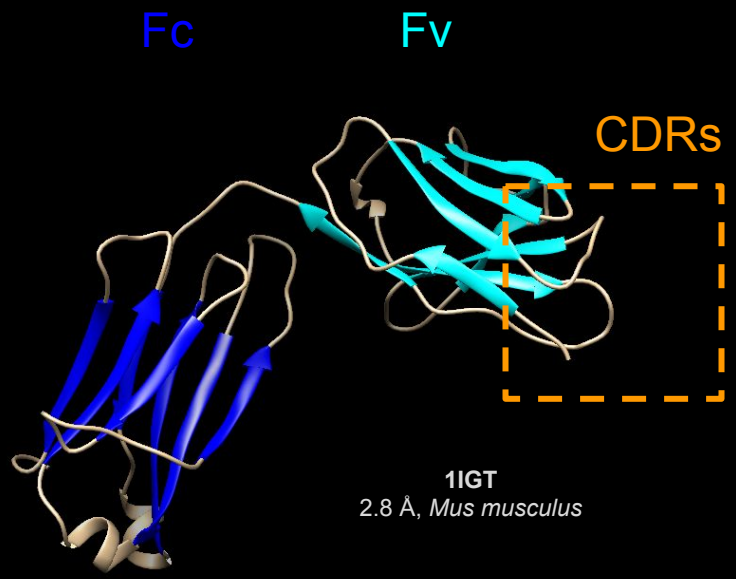
superfamily:
Immunoglobulin domain-like

families

Ig-like beta-sandwich



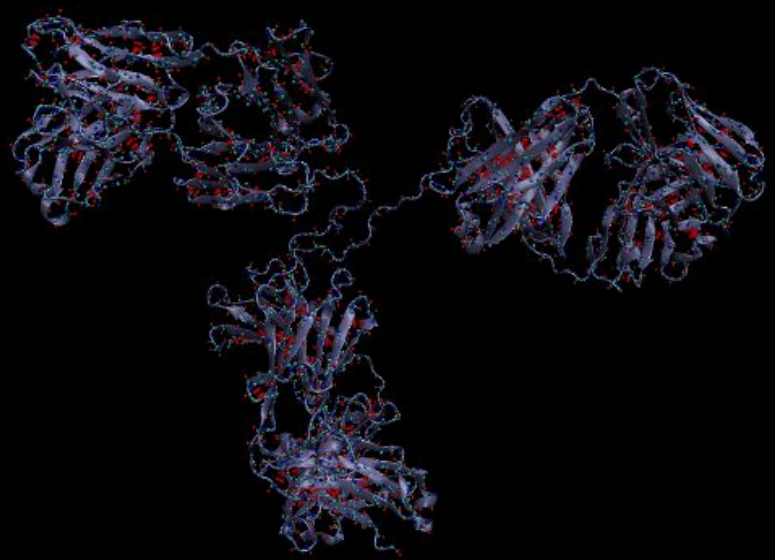


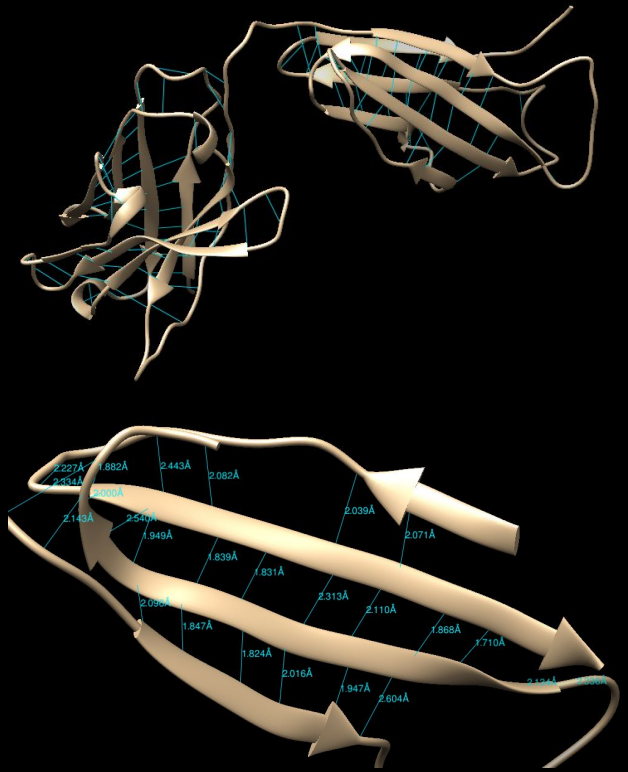


1IGT
2.8 Å, *Mus musculus*

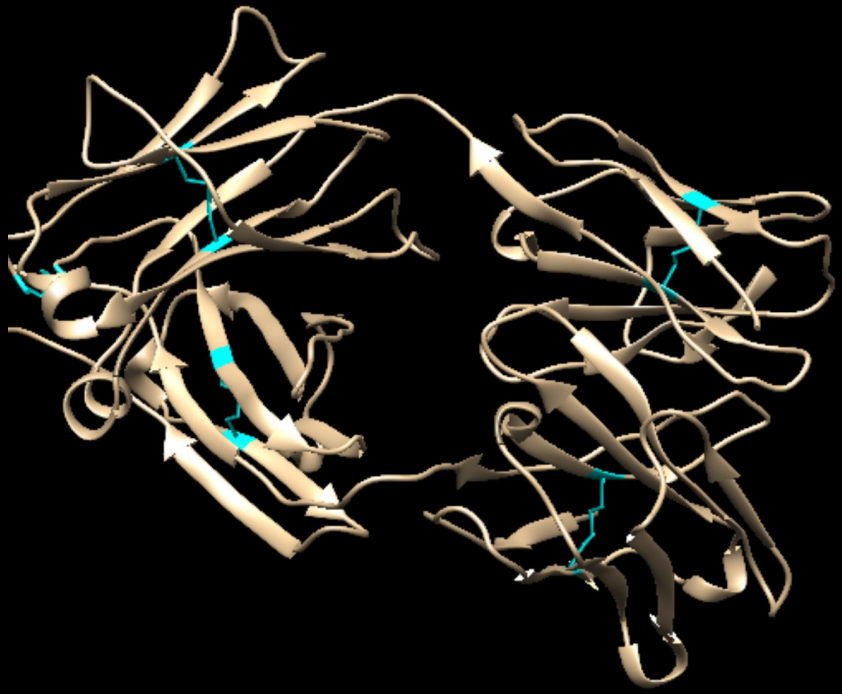
Hydrogen bonds

1IGT
2.8 Å, *Mus musculus*

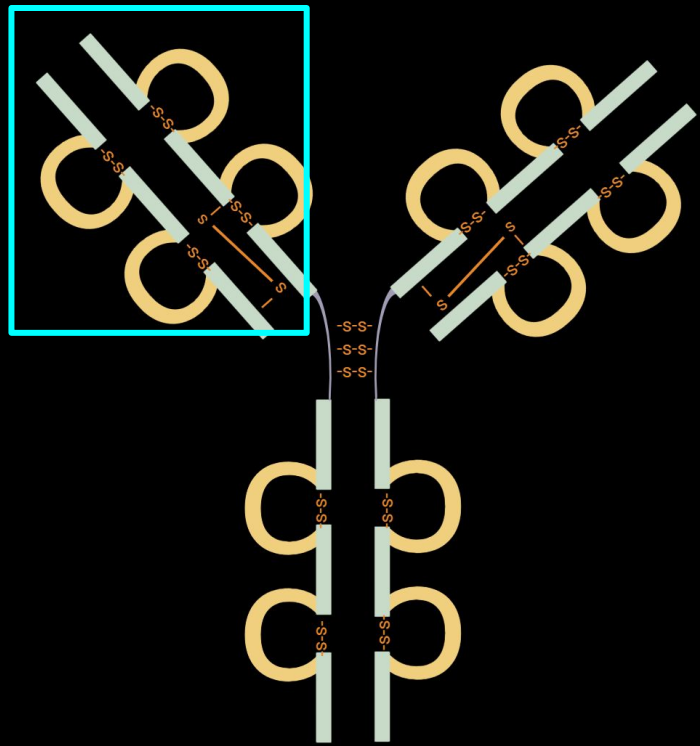




Disulfide bridges (Fab region)



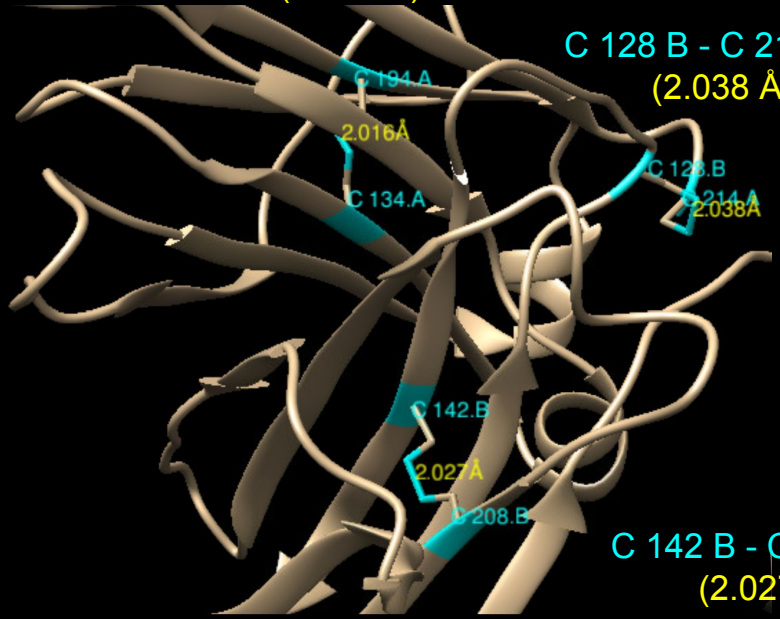
1IGT
2.8 Å, *Mus musculus*



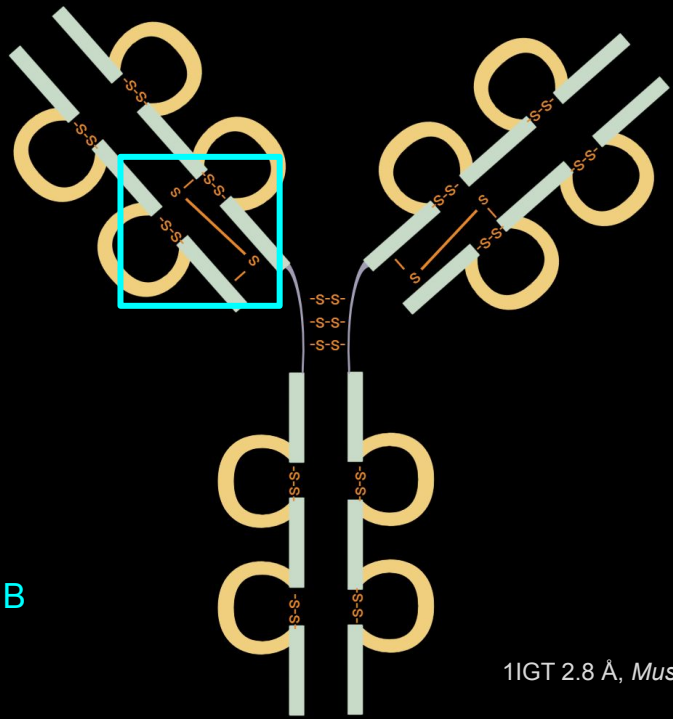
Disulfide bridges (Fab region)

C 194 A - C 134 A
(2.016 Å)

C 128 B - C 214 A
(2.038 Å)

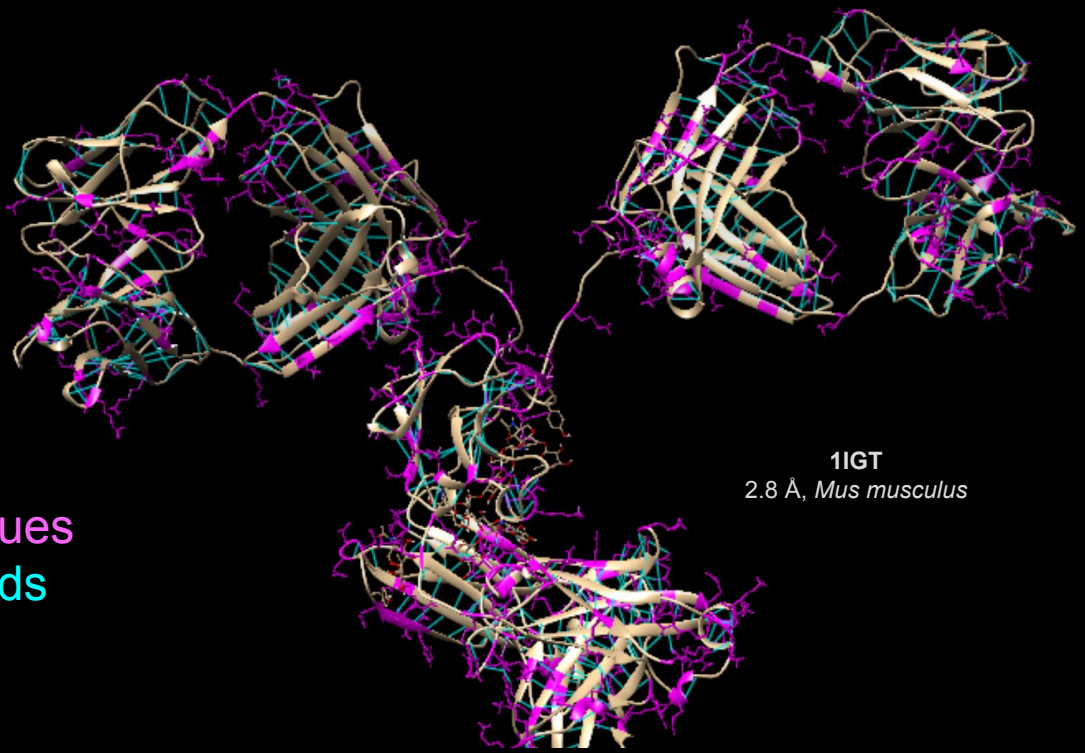


C 142 B - C 208 B
(2.027 Å)



1IGT 2.8 Å, *Mus musculus*

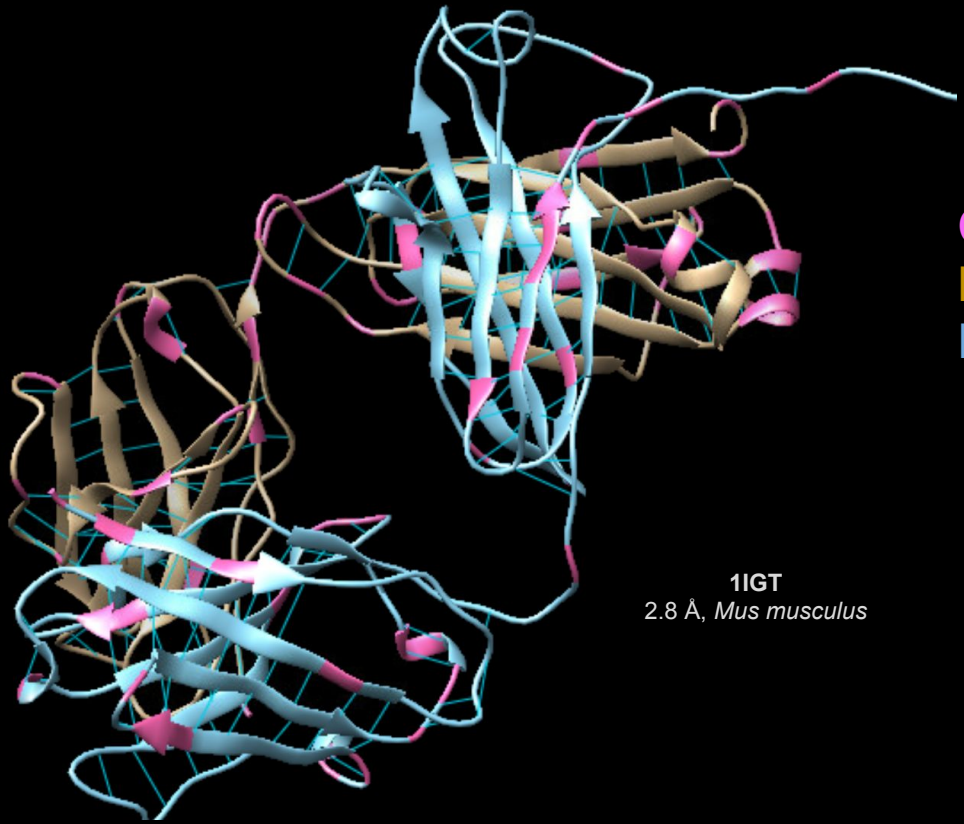
Salt bridges



1IGT
2.8 Å, *Mus musculus*

Charged residues
Hydrogen bonds

Salt bridges (Variable region)



Charged residues
Light chain
Heavy chain

SCOP

class:
All beta proteins

fold:
Immunoglobulin-like beta-sandwich

superfamily:
Immunoglobulin domain-like

families

Superfamily

- Distantly related or unrelated proteins
- Eukaryotes and prokaryotes
- Sequence identity < 10%
- Greek-key β-sandwich structure
- Common hydrophobic core

Domains:

C1 set

C2 set

V set

I set

Conservation

1fc1 (2.90 Å) → Human Fc fragment *(Homo sapiens)*

1hla (3.50 Å) → Human class I histocompatibility antigen *(Homo sapiens)*

1bec (1.70 Å) → Beta chain of T-cell antigen receptor *(Mus musculus)*

3cd4 (2.20 Å) → Human CD4 *(Homo sapiens)*

1hnf (2.50 Å) → Human CD2 *(Homo sapiens)*

1tnm (NMR) → Muscle protein titin *(Homo sapiens)*

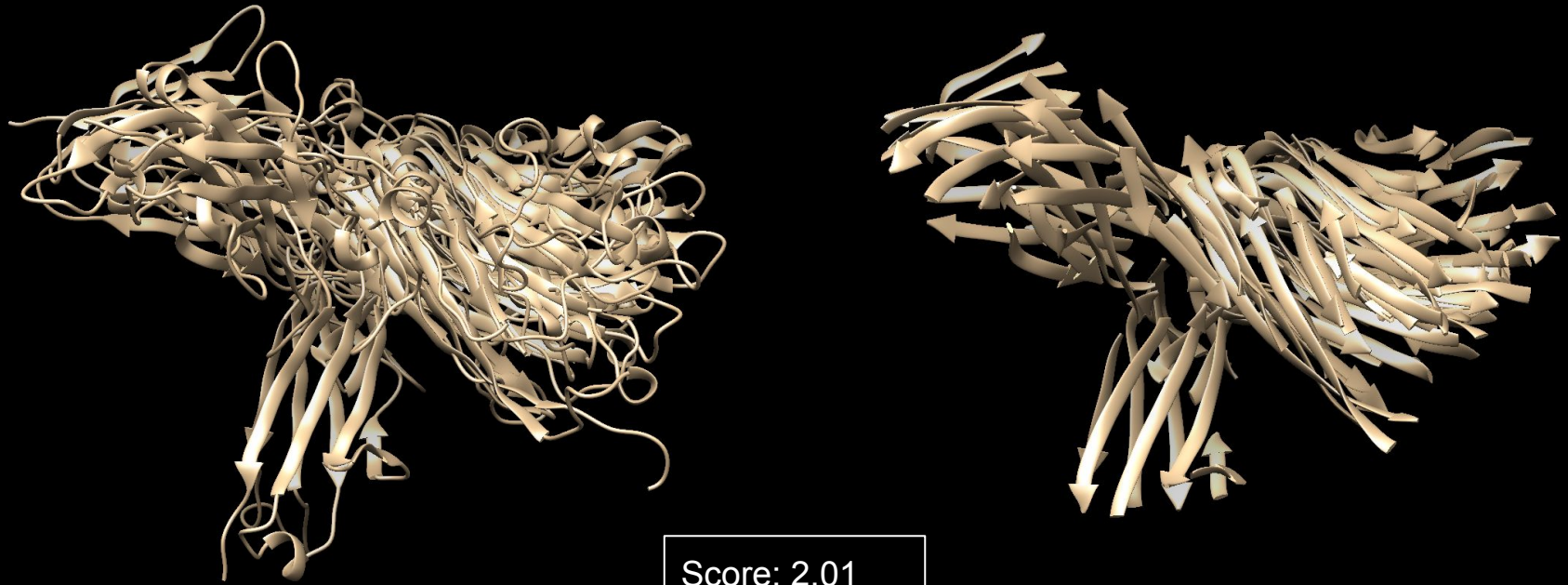
1nci (2.10 Å) → N-cadherin *(Mus musculus)*

2mcm (1.50 Å) → Macromomycin *(Streptomyces macromomyceticus)*

3hhr (2.80 Å) → Human GH receptor *(Homo sapiens)*

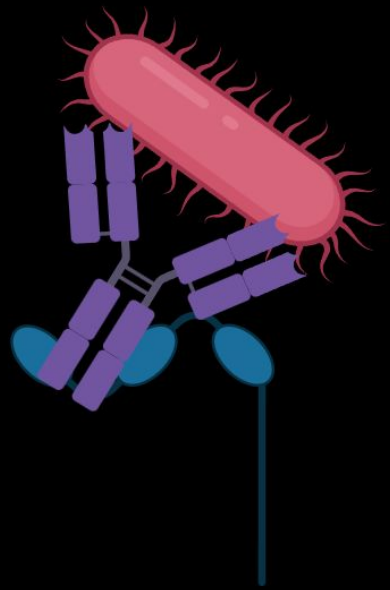
Conservation

Conservation

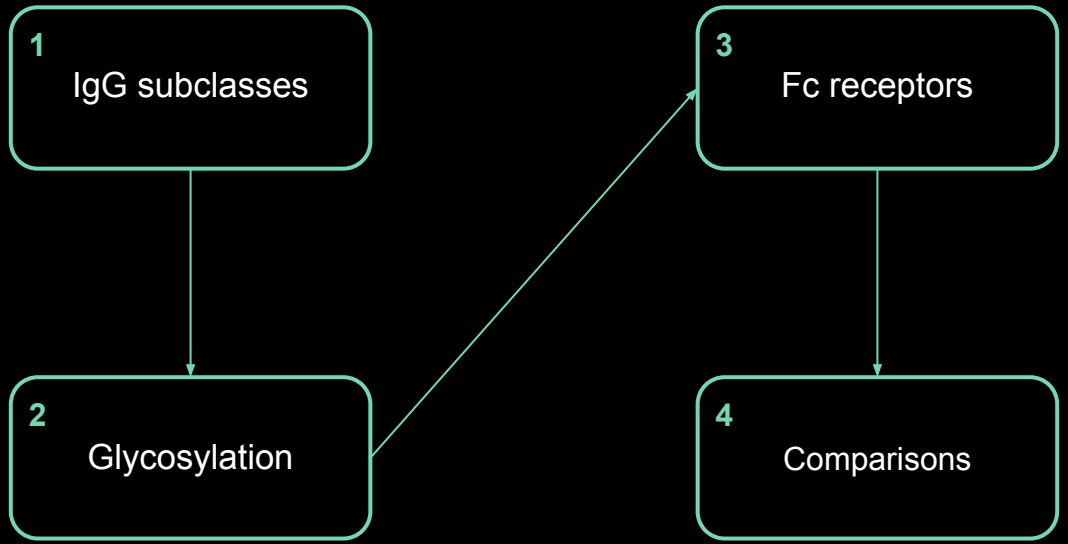


Score: 2.01
RMSD: 4.66

3- IMMUNOGLOBULIN G

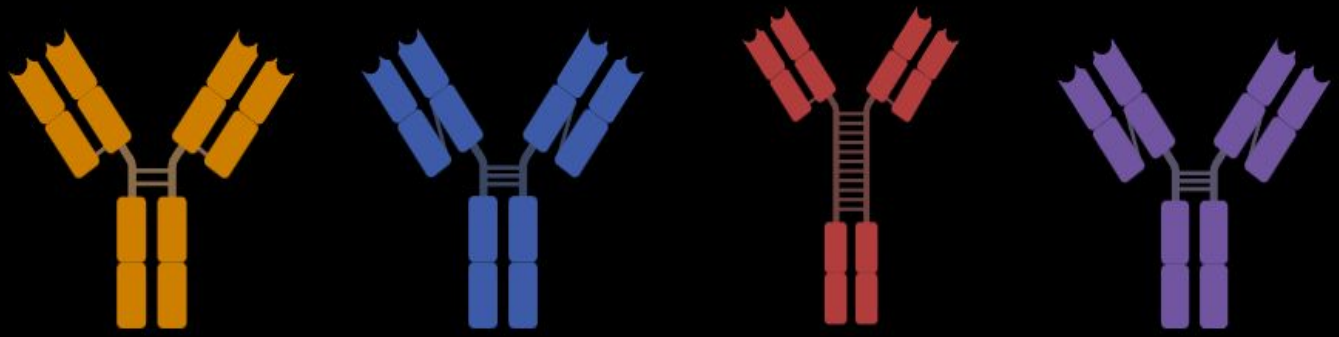


CONSTANT REGION



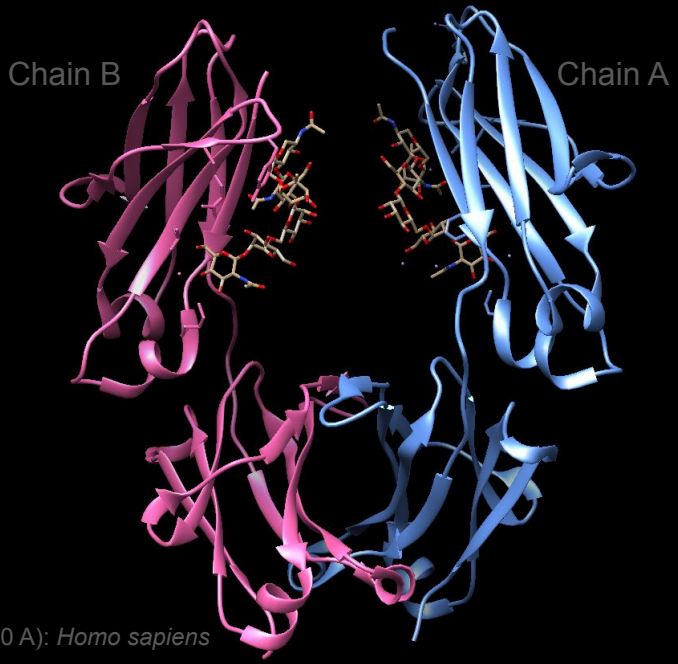
- Sequence alignments
- Superimpositions

IgG subclasses

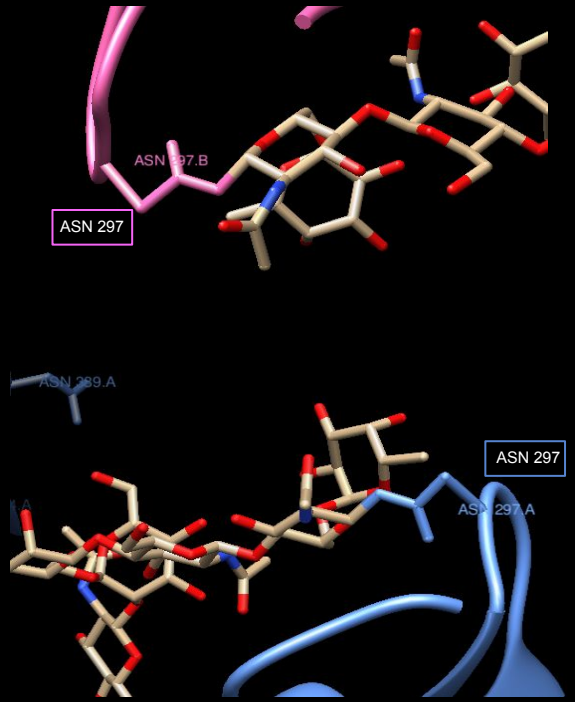


IgG1 IgG2 IgG3 IgG4

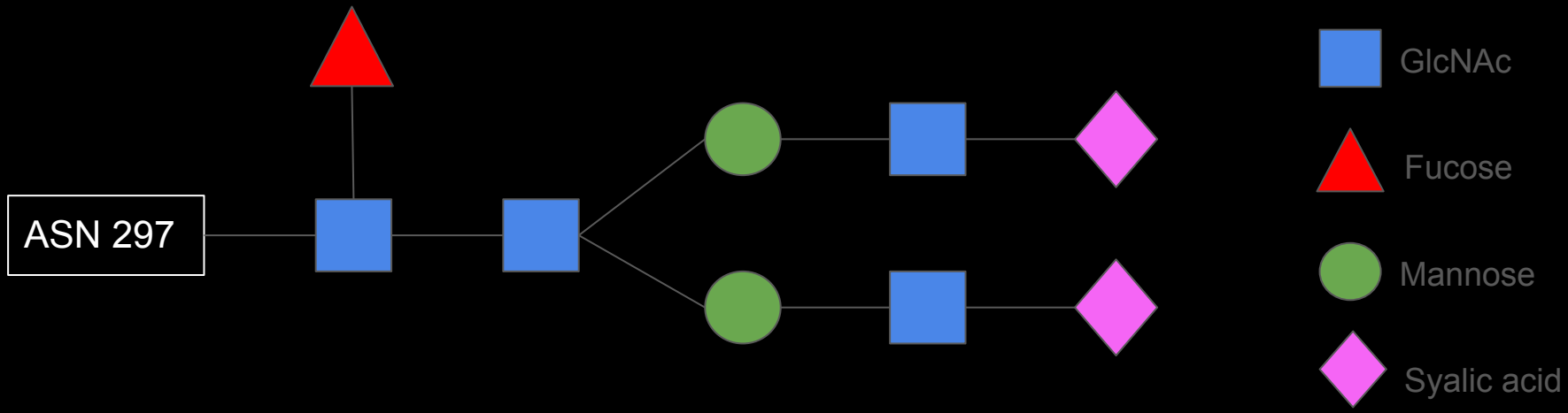
Glycosylation



3ave (2.0 A): *Homo sapiens*



Glycosylation



Fc receptors

High affinity → FcγRI

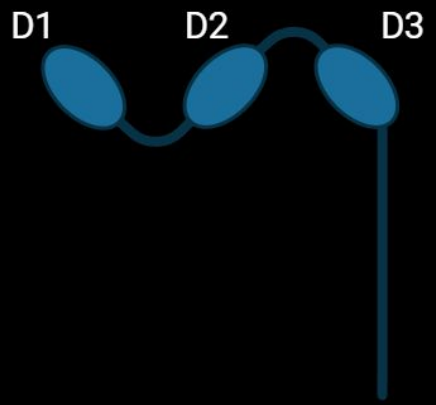
Low affinity → FcγRIII

Low affinity → FcγRIIA/C

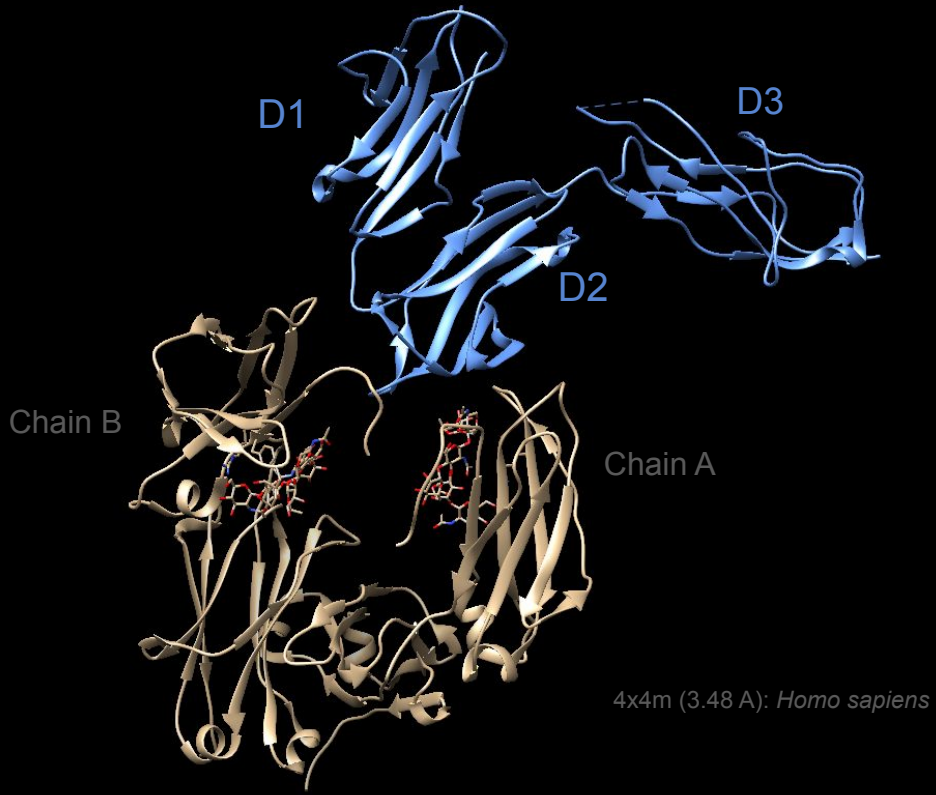
FcγRI, FcγRIII, FcγRIIA/C → Activation

Low affinity → FcγRIIB → Inhibitory

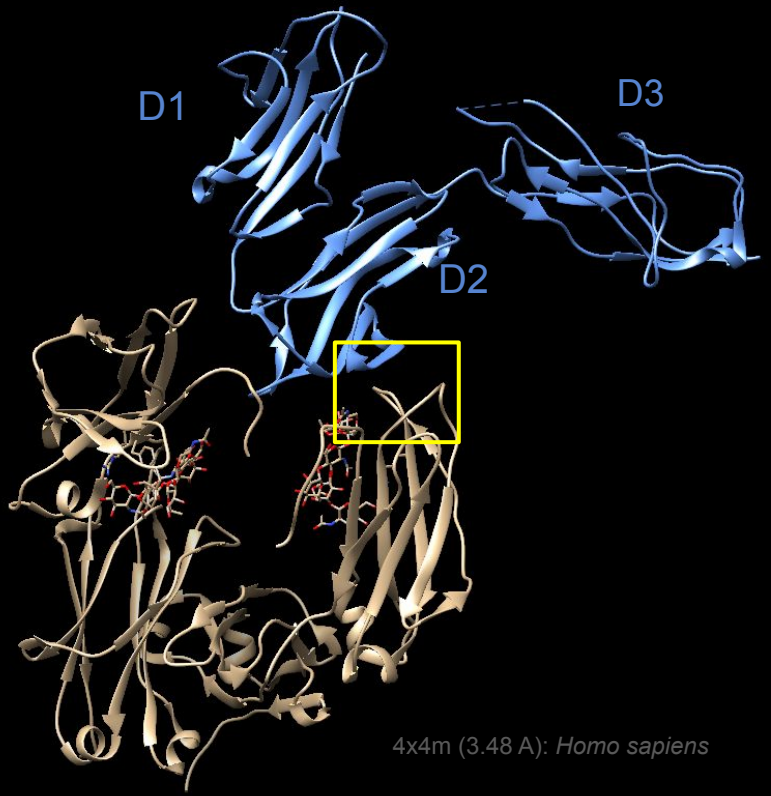
pH dependent → FcRn



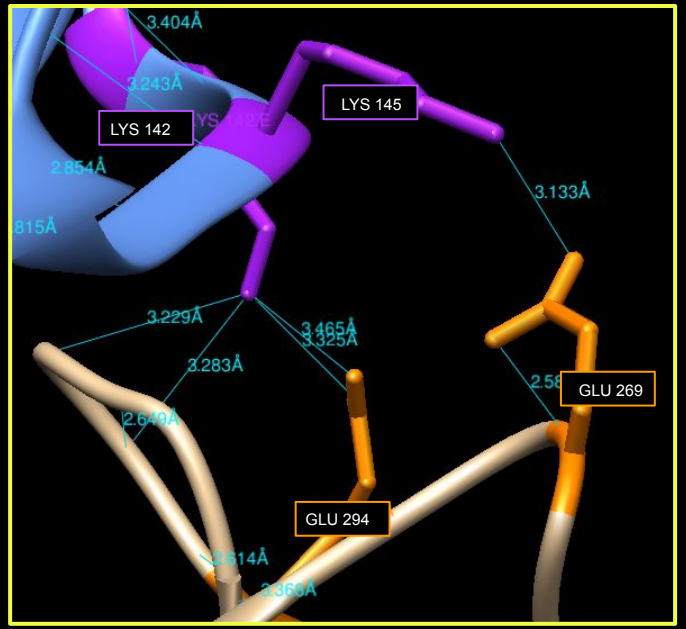
Fc receptors



Fc receptors

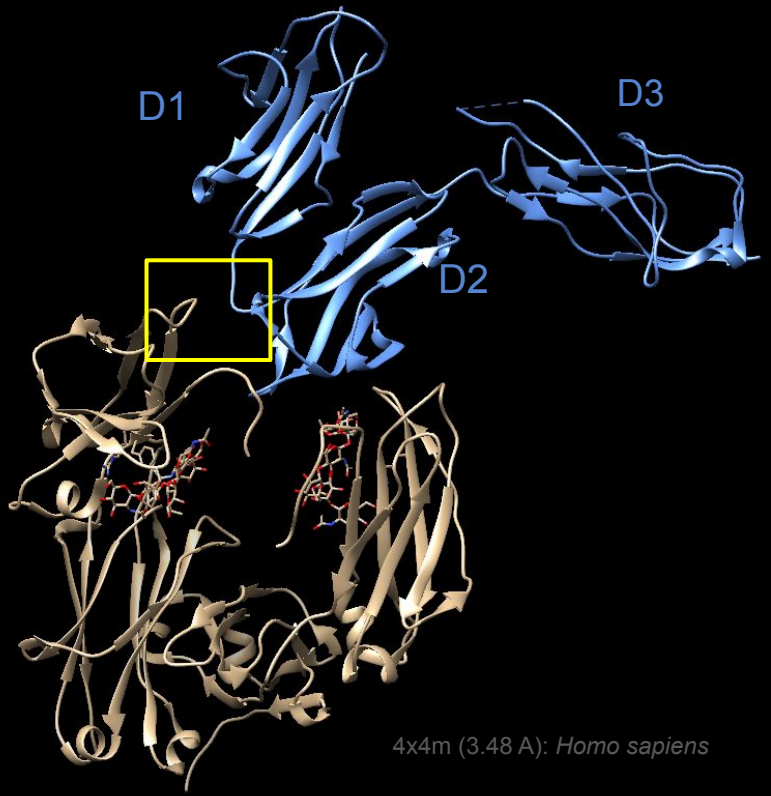


4x4m (3.48 A): *Homo sapiens*

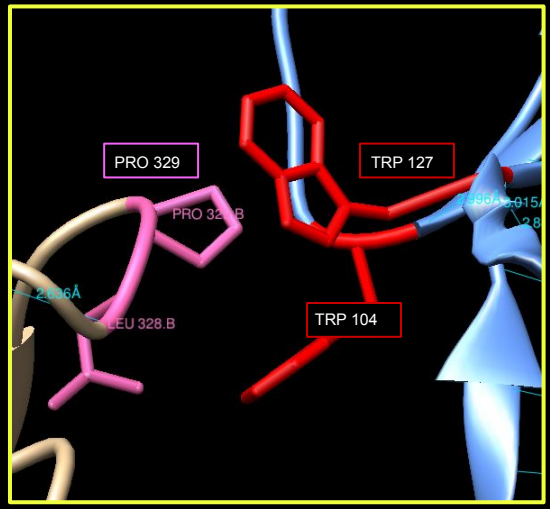


Salt Bridges:
Lys142 - Glu294
Lys 145 - Glu269

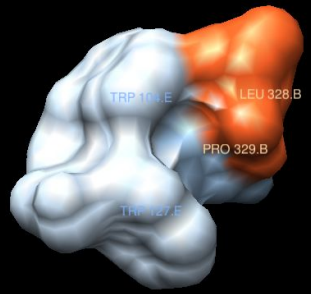
Fc receptors



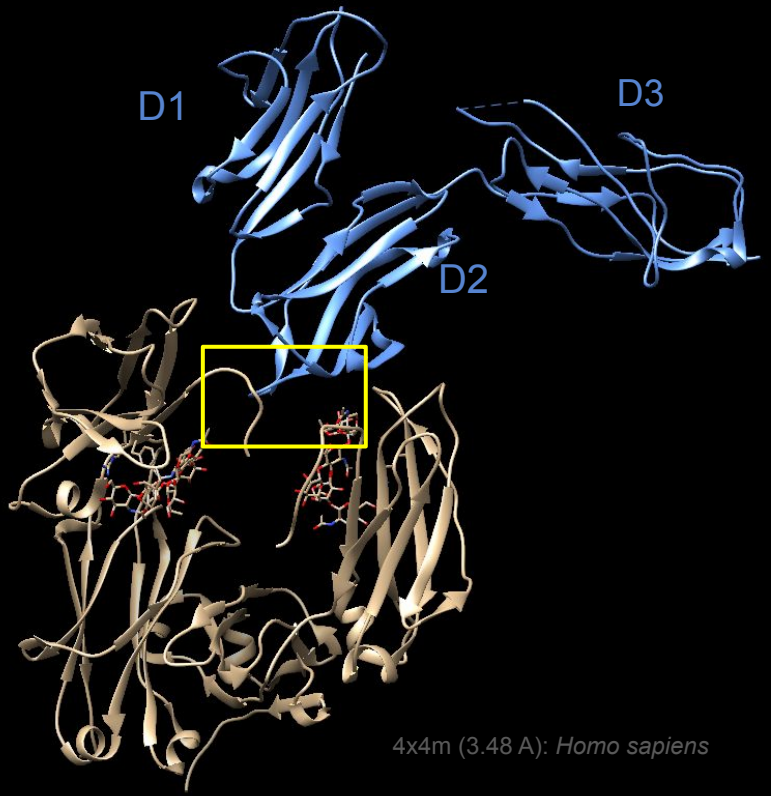
4x4m (3.48 A): *Homo sapiens*



Trp Pro Trp sandwich

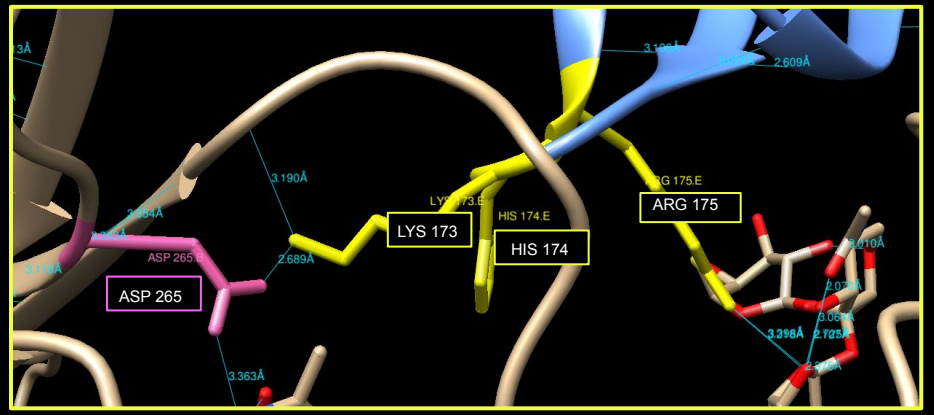


Fc receptors

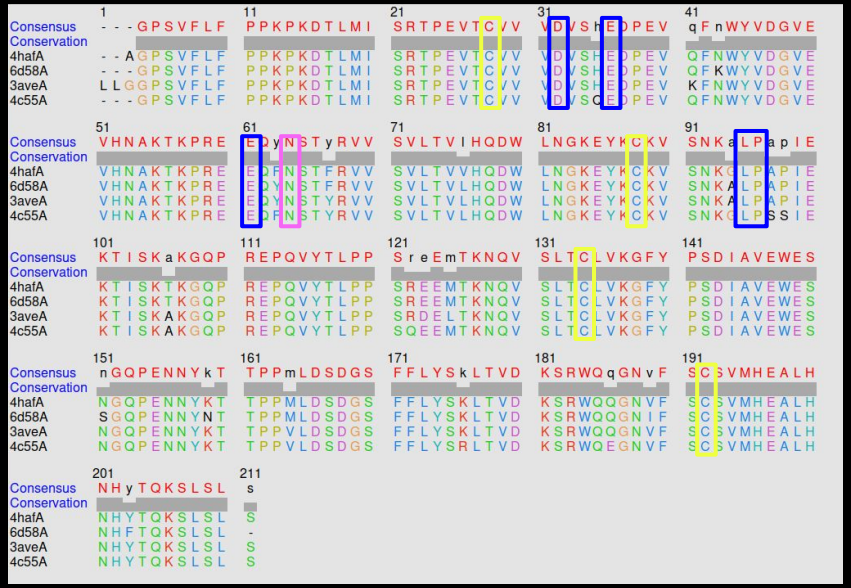


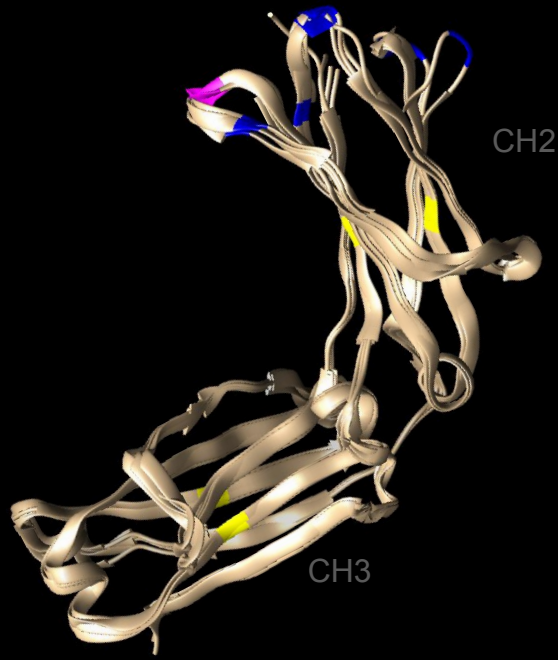
4x4m (3.48 A): *Homo sapiens*

The FG loop has contact with both chains



IgG subclasses

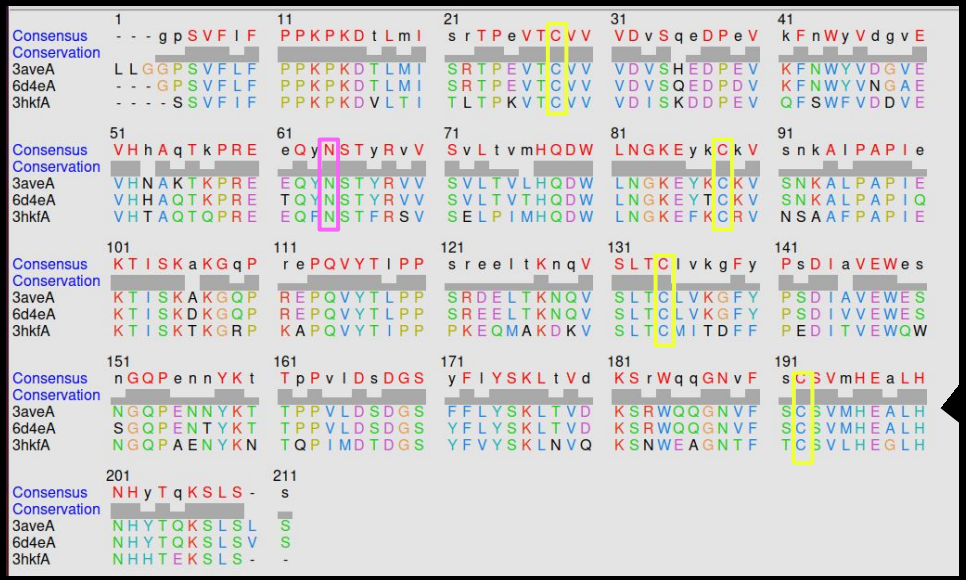


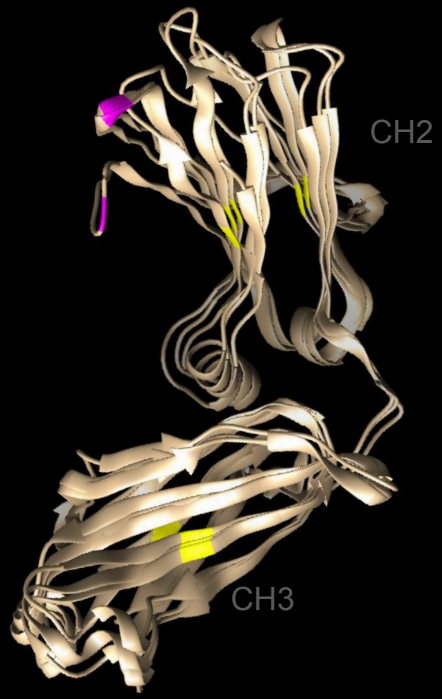


4haf (2.04 A): *Homo sapiens* IgG2
6d58 (2.39 A): *Homo sapiens* IgG3
3ave (2.0 A): *Homo sapiens* IgG1
4c55 (2.35 A): *Homo sapiens* IgG4

Score: 9.64
RMSD: 0.86

Different species





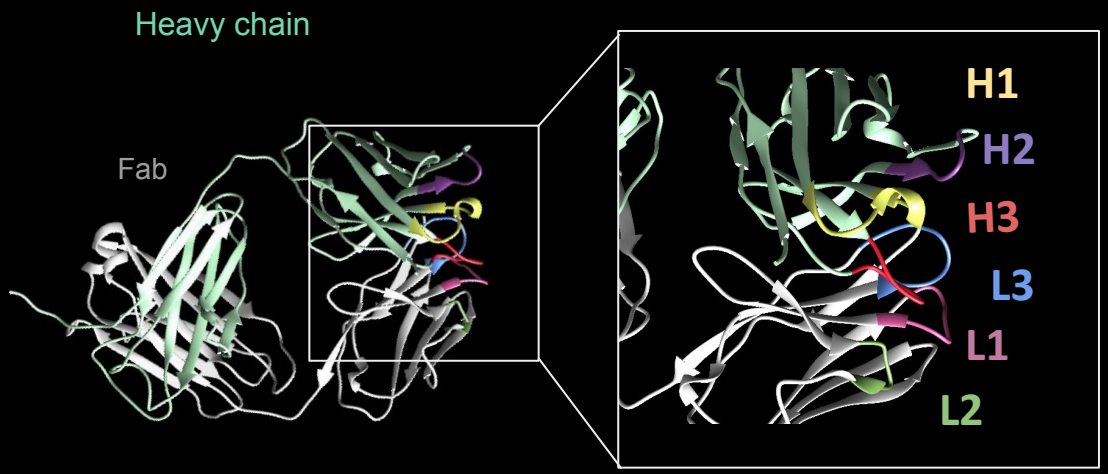
3ave (2.0 A): *Homo sapiens*
6d4e (2.80 A): *Macaca mulatta*
3hkf (2.50 A): *Mus musculus*

Score: 8.62
RMSD: 2.23

VARIABLE REGION

Complementary Determining Regions (CDR)

CDRs are the regions that directly interact with **antigens**!



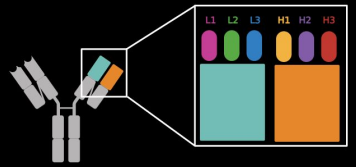
Framework regions vs CDRs

Chothia *et al.* classification

Heavy chain		Canonical structure
H1		1, 2, 3
H2		1, 2a, 2c, 3a, 3b, 3c, 4
H3		-
Light chain		**Canonical structure**
L1	Lambda (λ)	1λ, 2λ, 3λ, 4λ
	Kappa (κ)	1κ, 2κ, 3κ, 4κ
L2		I
L3	Lambda (λ)	1aλ, 1bλ, 1cλ, 2λ
	Kappa (κ)	1κ, 2κ

→ Loop length
→ Conformation of the loop
→ Conserved amino acid residues

Methodology



1 Studies on the classification of CDRs

2 Find homologs with **BLAST**

→ Light chain λ **8FAB**
→ Light chain κ **1FOR**
→ Heavy chain

3 Selection of PDB candidates

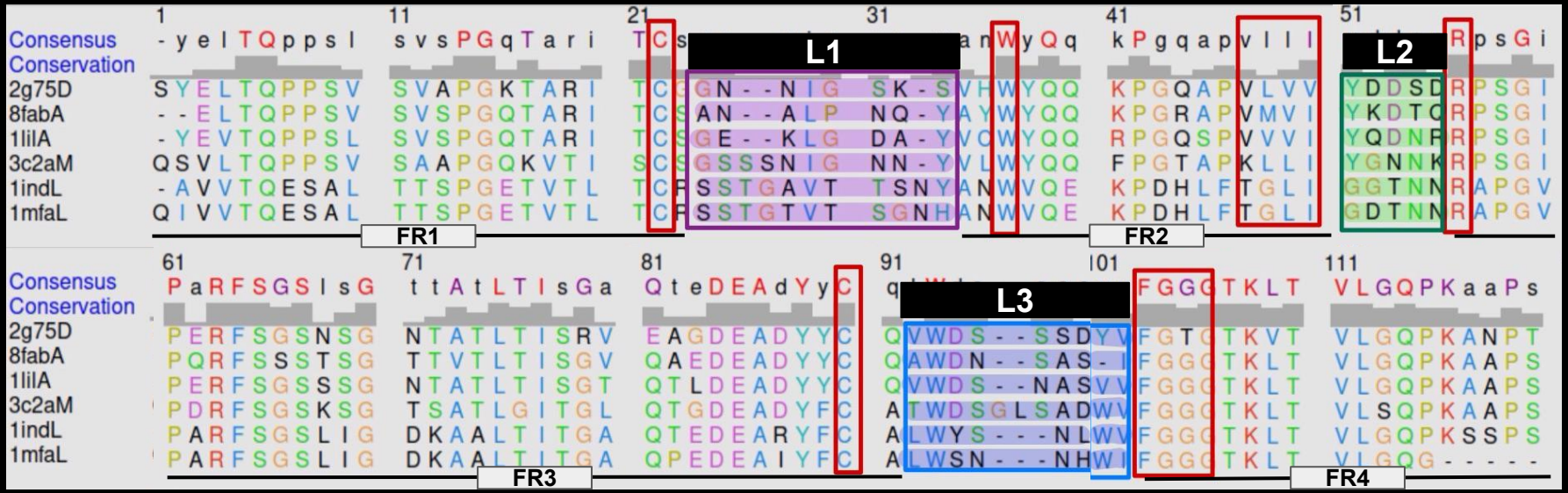
4 Sequence alignment with **ClustalW**

5 Identification of consensus sequences

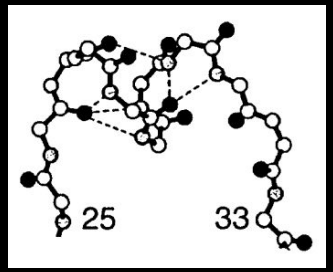
6 Structural superimposition with **STAMP**

7 Visualization of superimposition

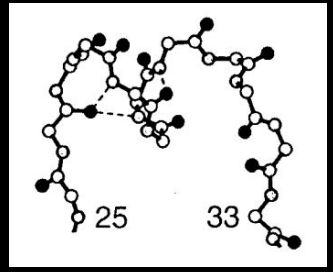
Light chain λ **Sequence alignment**



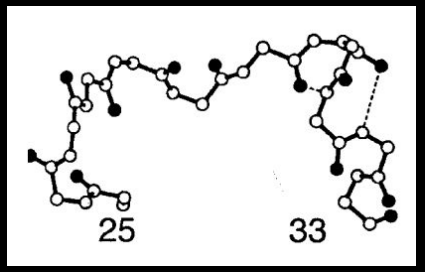
Light chain λ **Canonical structures**



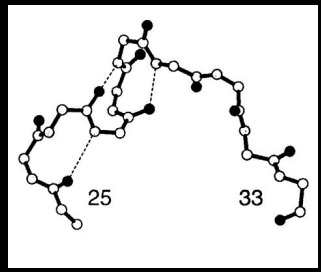
L1 1λ



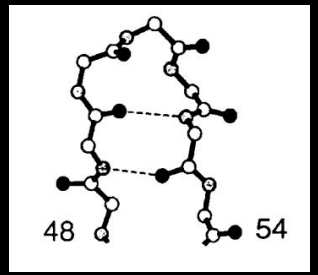
L1 2λ



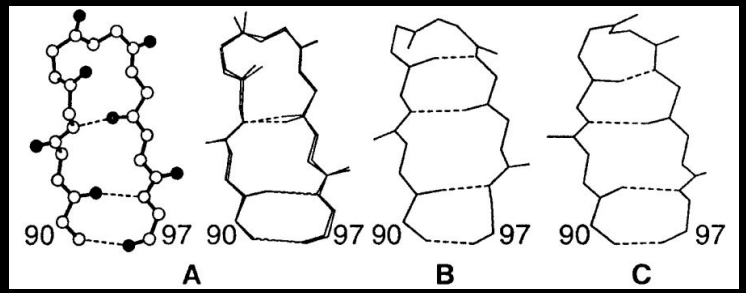
L1 3λ



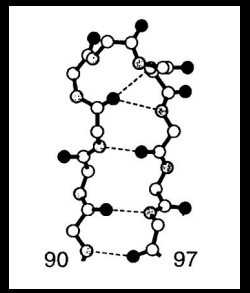
L1 4λ



L2

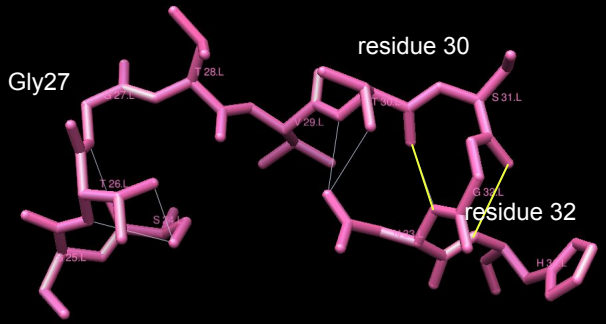


L3 1aλ, 1bλ, 1cλ



L3 2λ

Al-Lazikani B, Lesk AM, Chothia C. Standard conformations for the canonical structures of immunoglobulins. J Mol Biol.

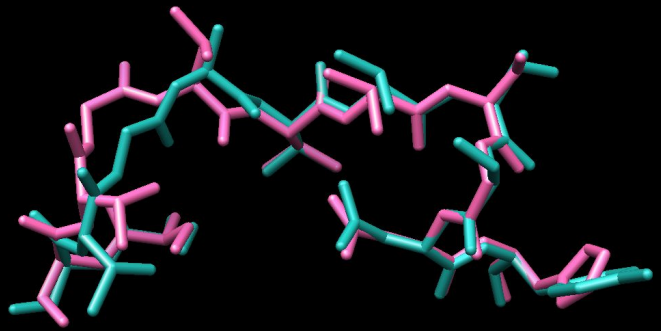


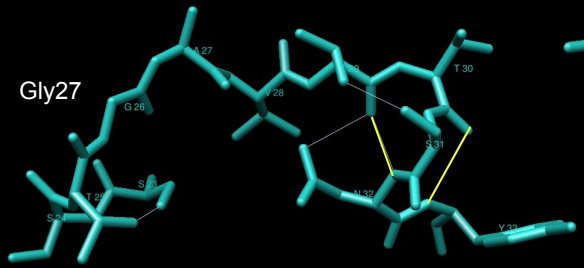
1MFA

IgG Fv at 1.7 Å

Homo sapiens

L1 3λ

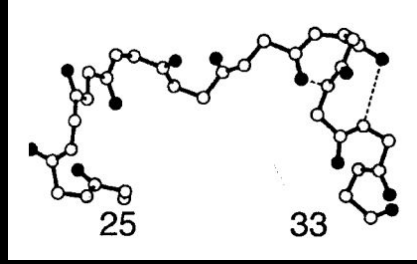




1IND

IgG Fab at 2.2 Å

Homo sapiens



→ Helical turn between residues 30 and 32, two hydrogen bonds

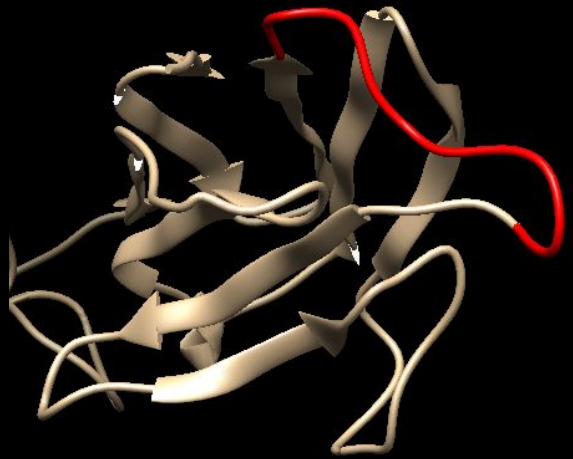
→ Gly27 orientation

30 32

1ind S S T G A V T T S N Y

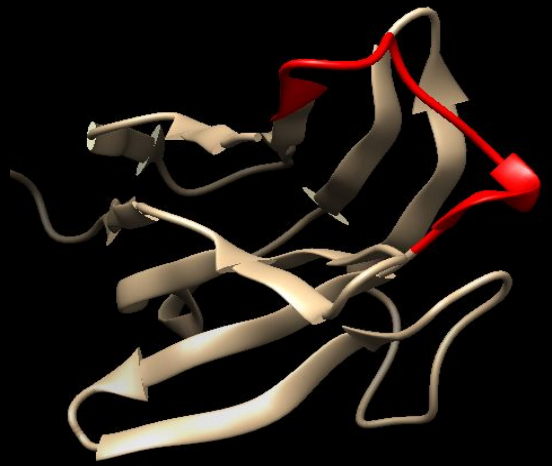
1mfa S S T G T V T S G N H

L1 κ is extended

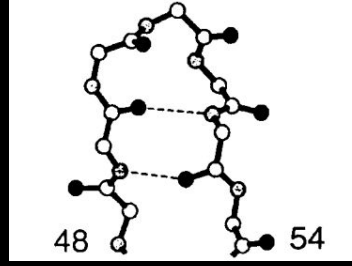


L1 κ
1BBD
2.80 Å, *Homo sapiens*

L1 λ is helical

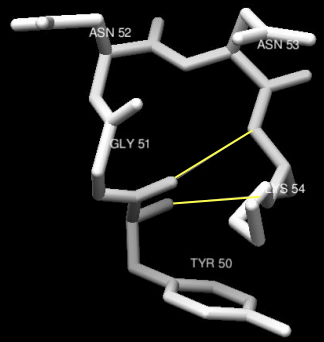


L1 λ
1IND
2.20 Å, *Homo sapiens*



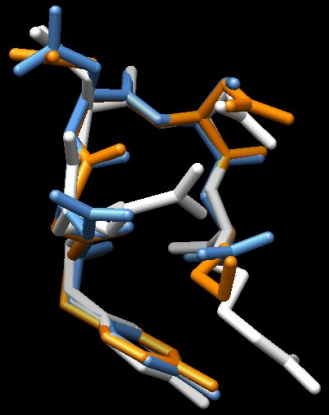
→ Hairpin loop formed by residues 49 to 52, two hydrogen bonds

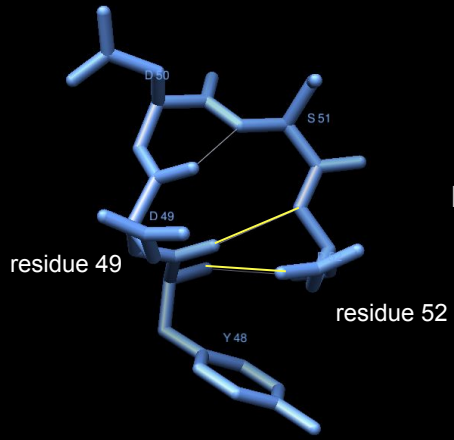
→ Tyr48 conserved



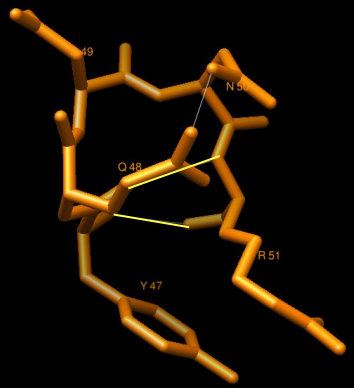
1LIL
IgG Fab at 2.65 Å
Homo sapiens

L2



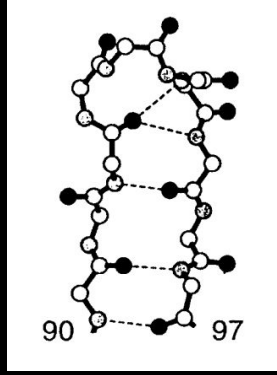


2G75
IgG Fab at 2.28 Å
Homo sapiens



3C2A
IgG Fab at 2.1 Å
Homo sapiens

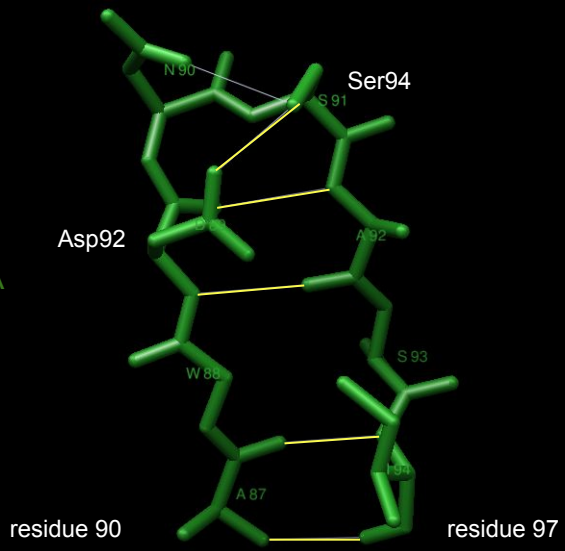
L3 2λ



8FAB

IgG Fab at 2.8 Å

Homo sapiens

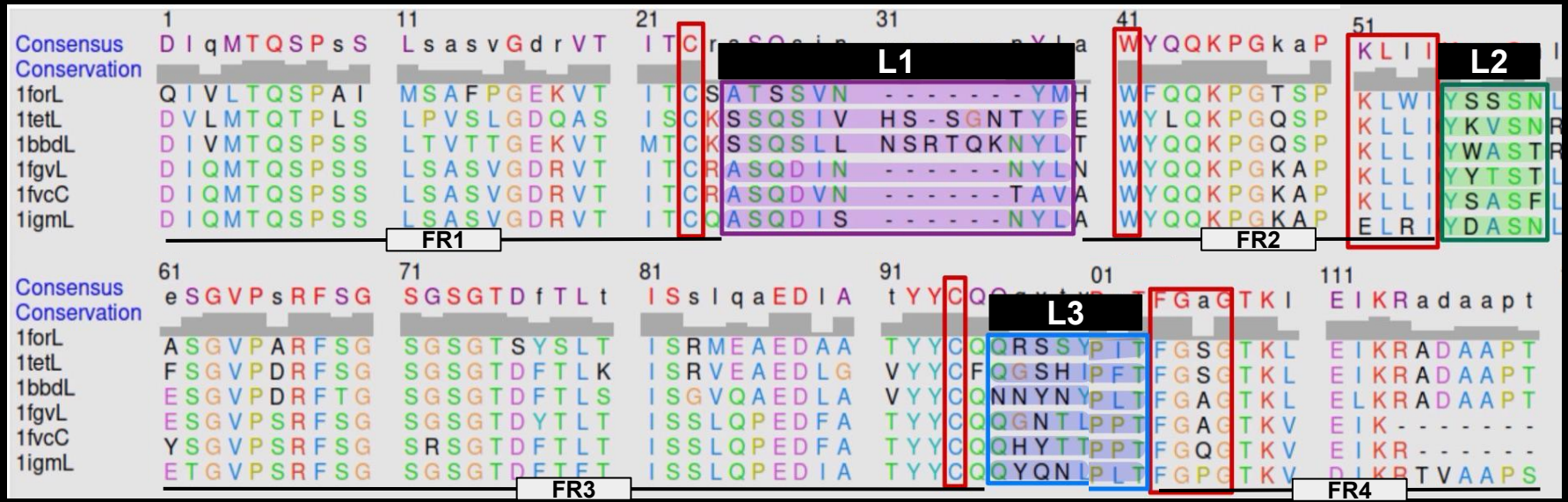


→ 8 residues in a hairpin

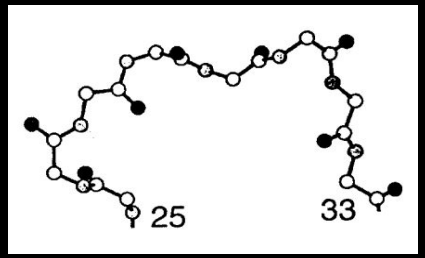
→ 4 residues at the top: turn

→ Ser94 and Asp92, H bond

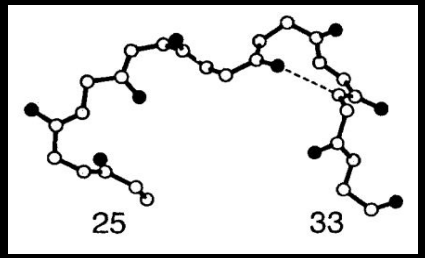
Light chain $\varkappa$ **Sequence alignment**



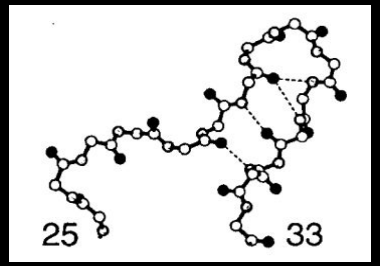
Light chain κ **Canonical structures**



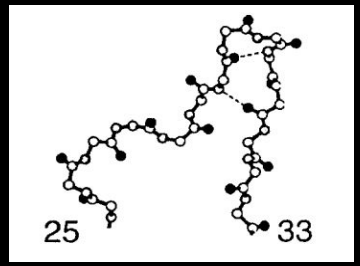
L1 1κ



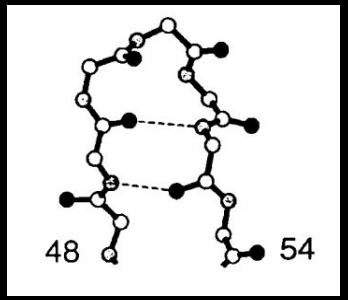
L1 2κ



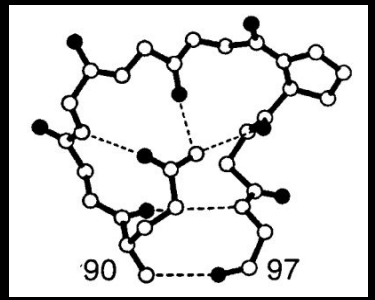
L1 3κ



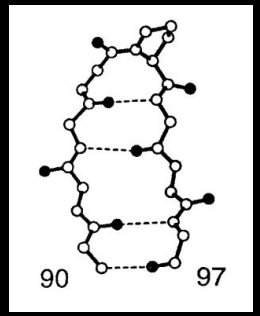
L1 4κ



L2



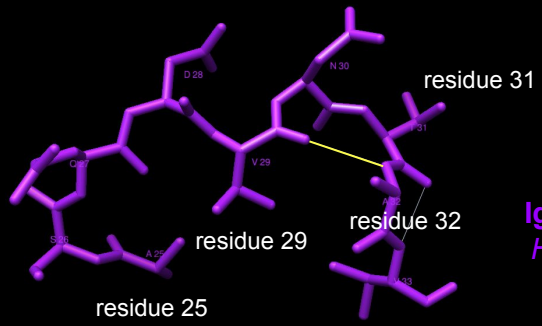
L3 1κ



L3 2κ

Al-Lazikani B, Lesk AM, Chothia C. Standard conformations for the canonical structures of immunoglobulins. J Mol Biol.

L1 2$\mathcal{H}$

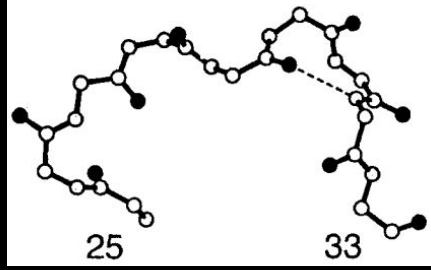


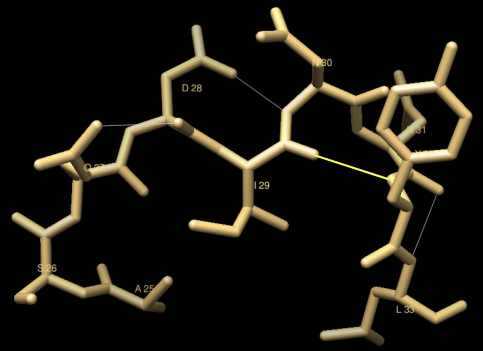
1FVC
IgG Fv at 2.2 Å
Homo sapiens

→ Residues 25 to 29: extended confirmation

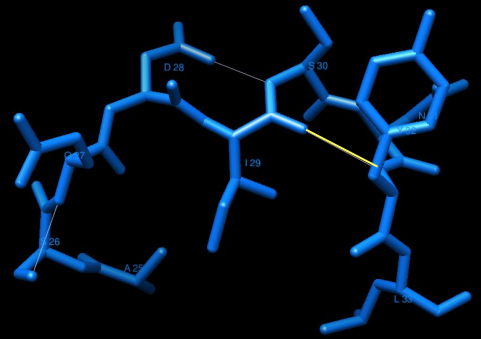
→ Residues 29 to 32: short links/hairpin loops

→ Residue 29 with 31: hydrogen bond (I and N)

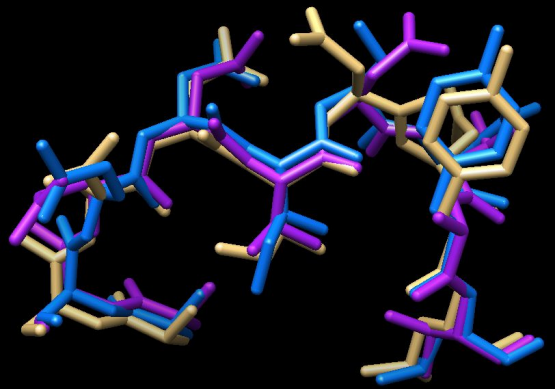




1FGV
IgG Fab at 1.9 Å
Homo sapiens



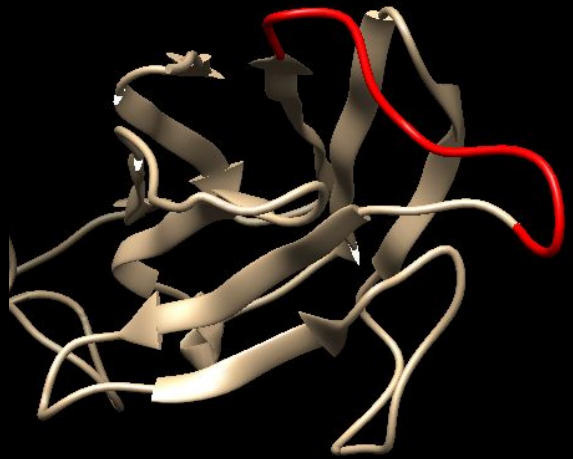
1IGM
IgG Fv at 2.3 Å
Homo sapiens



29 31

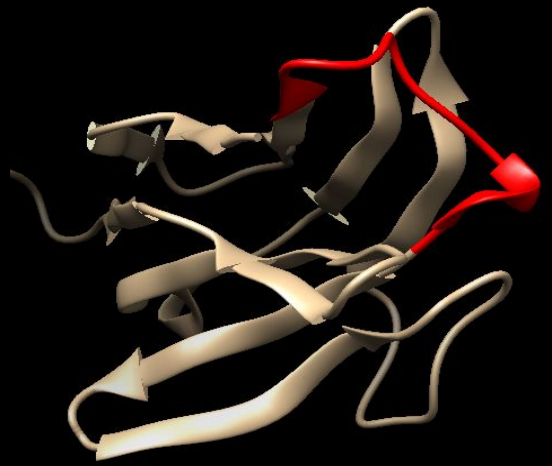
1fvc A S Q D I N N Y L
1fgv A S Q D V N T A V
1igm A S Q D I S N Y L

L1 κ is extended



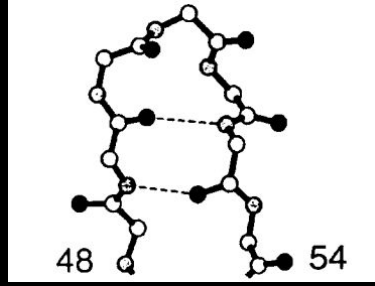
L1 κ
1BBD
2.80 Å, *Homo sapiens*

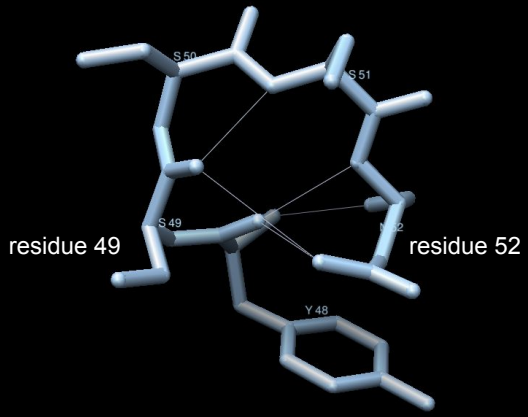
L1 λ is helical

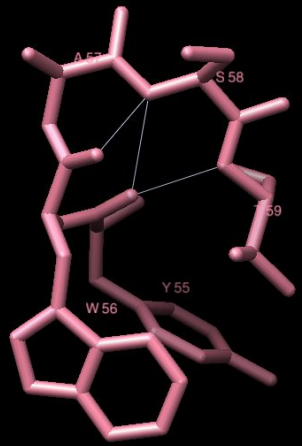


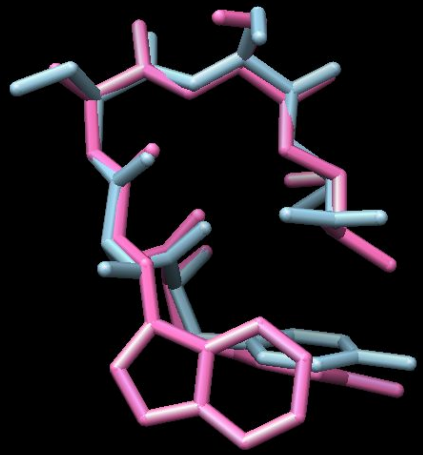
L1 λ
1IND
2.20 Å, *Homo sapiens*

L2









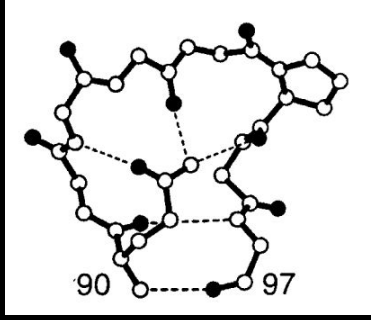
→ Hairpin loop formed by residues 49 to 52, two hydrogen bonds

→ Tyr48 conserved

1FOR
IgG Fab at 2.75Å
Homo sapiens

1BBD
IgG Fab at 2.8 Å
Homo sapiens

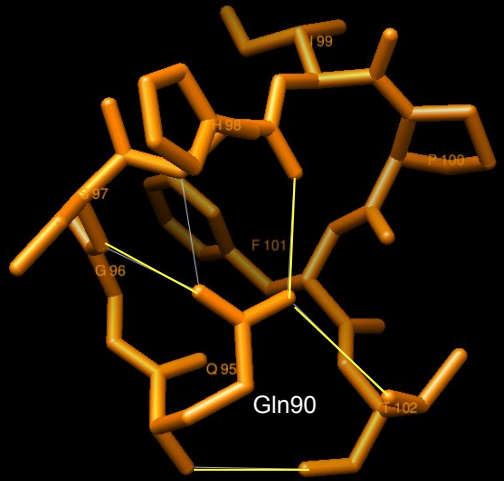
L3 1κ



1TET

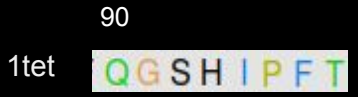
IgG1 Fab at 2.3 Å

Homo sapiens

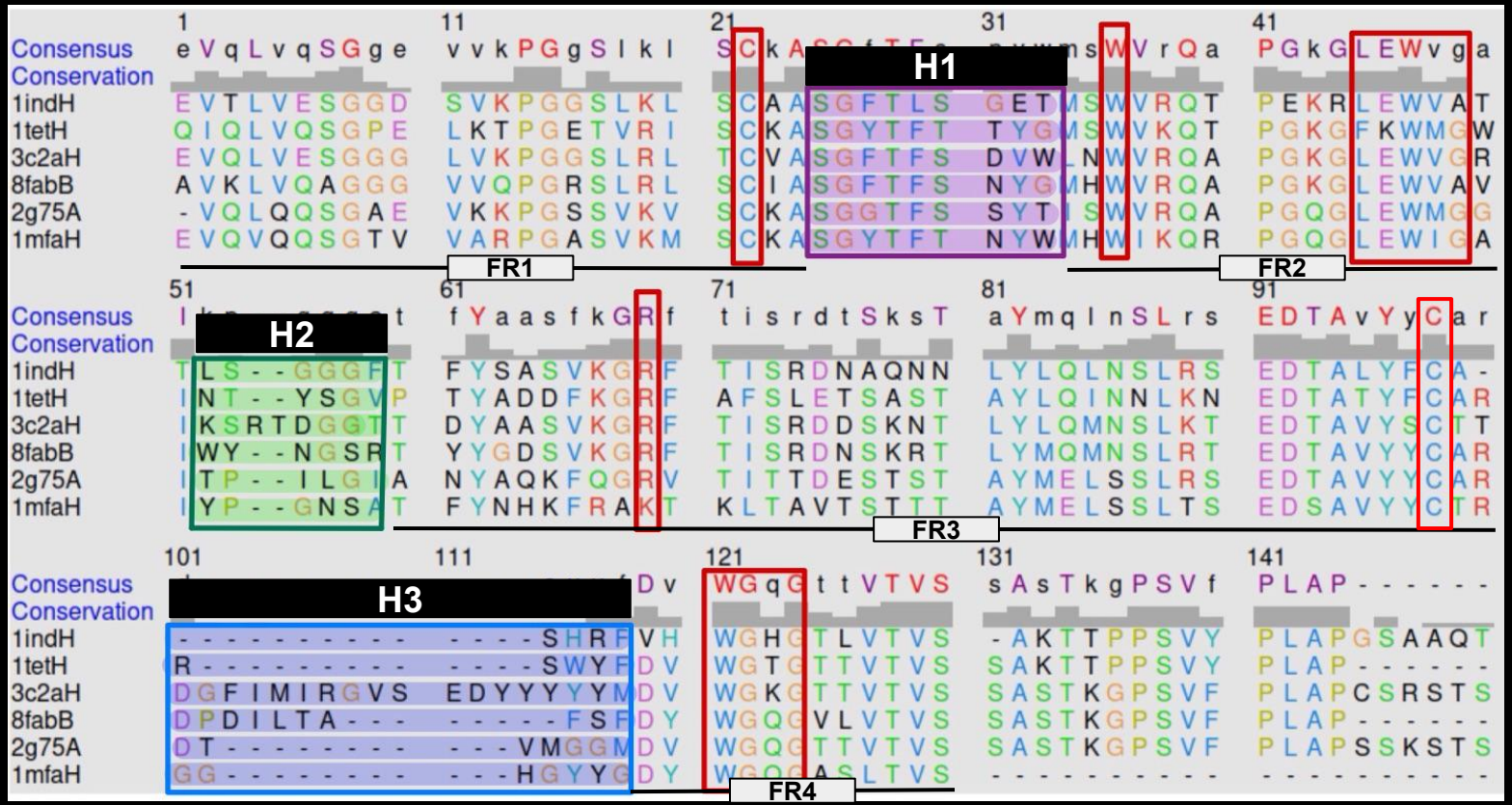


→ Most common in k L3

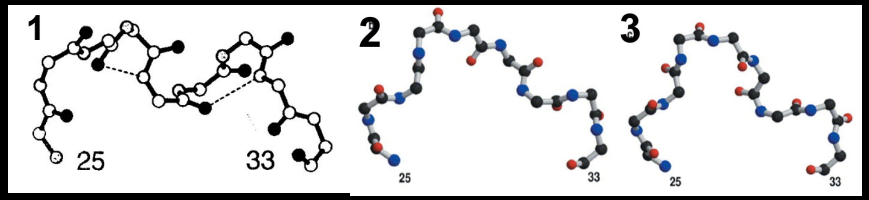
→ Gln90 conserved



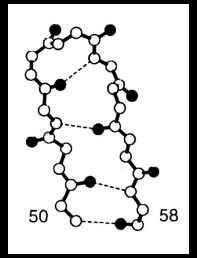
Heavy chain **Sequence alignment**



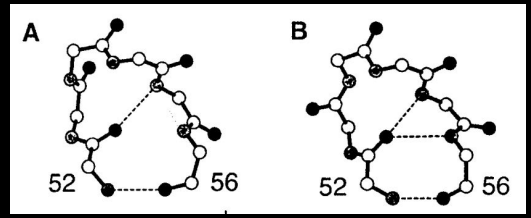
Heavy chain **Canonical structures**



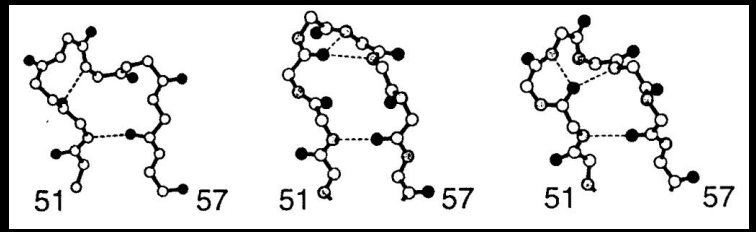
H1 1, 2, 3



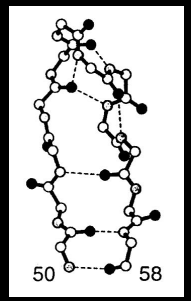
H2 1



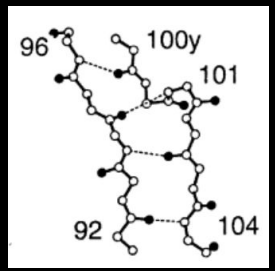
H2 2a, 2b



H2 3a, 3b, 3c

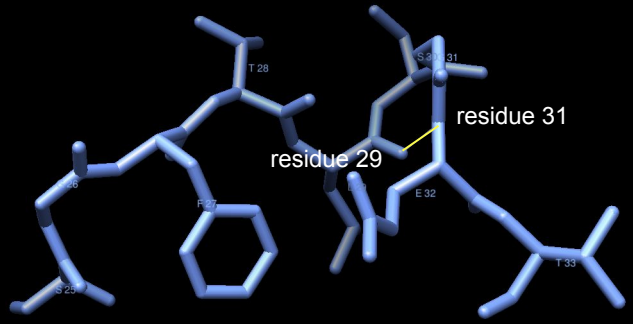


H2 4



H3 (base)

Al-Lazikani B, Lesk AM, Chothia C. Standard conformations for the canonical structures of immunoglobulins. J Mol Biol.

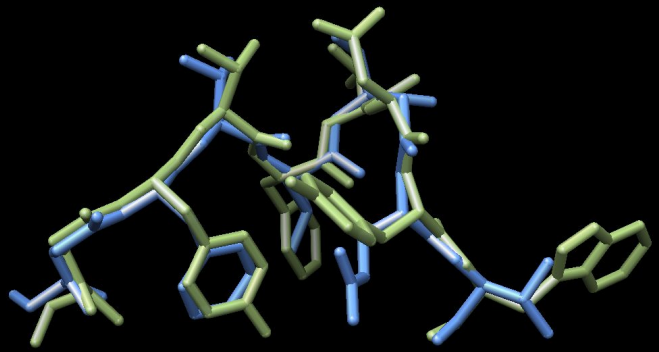


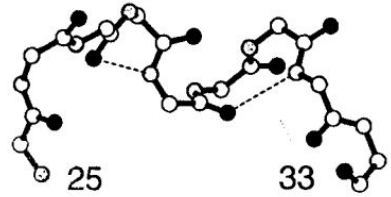
1IND

IgG Fab at 2.2 Å

Homo sapiens

H1 1



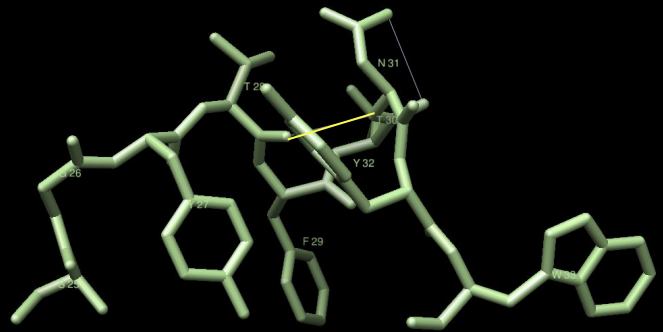


→ Helical turn: hydrogen bond residues 29 and 31

1MFA

IgG Fv at 1.7 Å

Homo sapiens



29 31

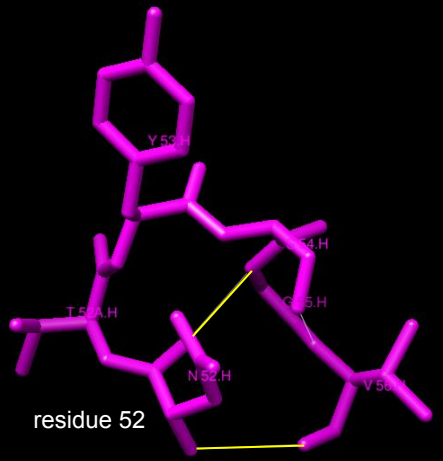
1ind S G F T L S G E T

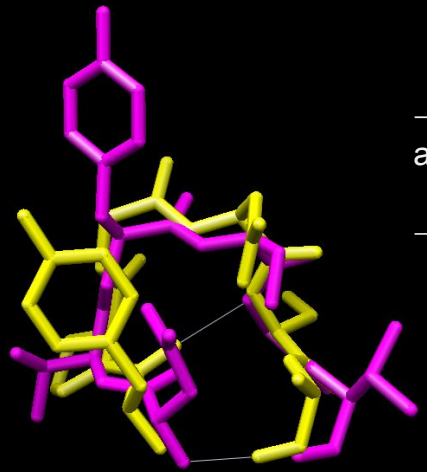
1mfa S G Y T F T N Y W

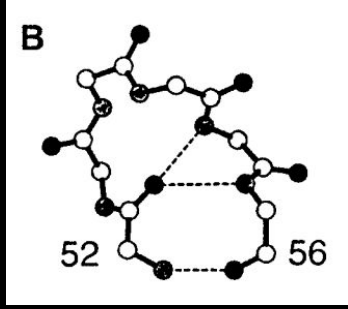
H2 2a

1MFA
IgG Fv at 1.7 Å
Homo sapiens

1TET
IgG1 Fab at 2.3 Å
Homo sapiens







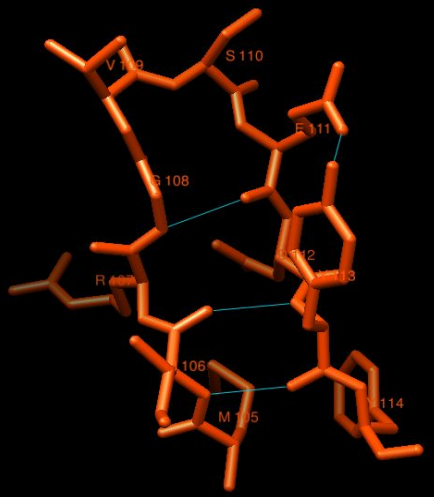
→ In our templates: all the amino acids are different!

→ Residue 52: H bonds

52

1tet N T Y S G
1mfa Y P G N S

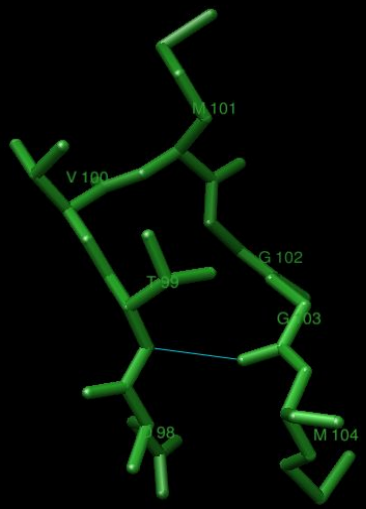
H3



3C2A

IgG Fab at 2.1 Å

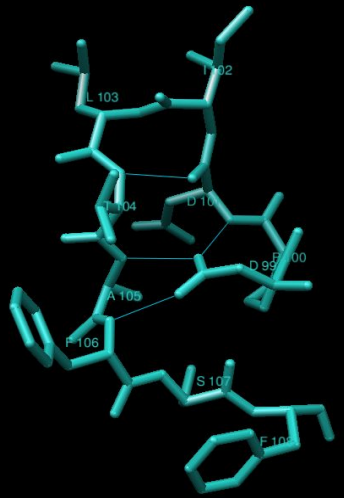
Homo sapiens



2G75

IgG Fab at 2.28 Å

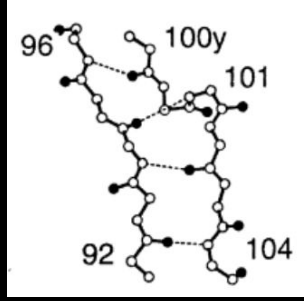
Homo sapiens



8FAB

IgG Fab at 2.8 Å

Homo sapiens

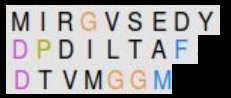


→ Huge length and sequence differences

→ Impact: antigen binding

→ Variability: full loop structure

3c2a
2g75
8fab

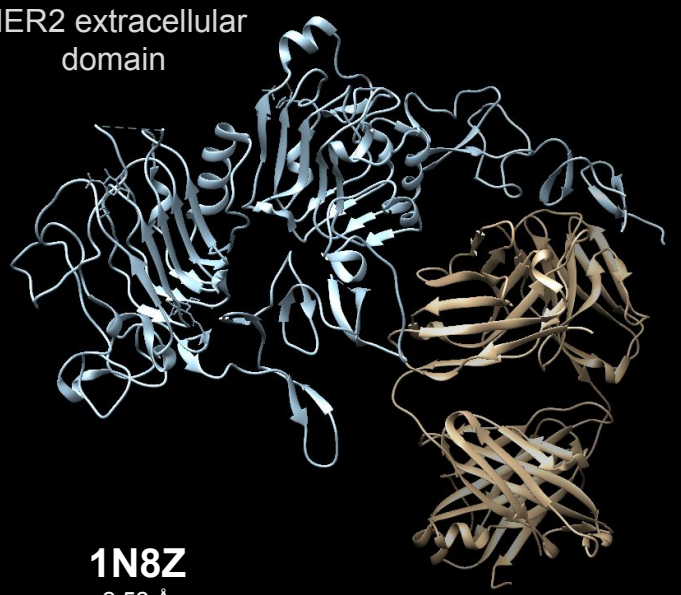


ANTIBODY-ANTIGEN INTERACTION

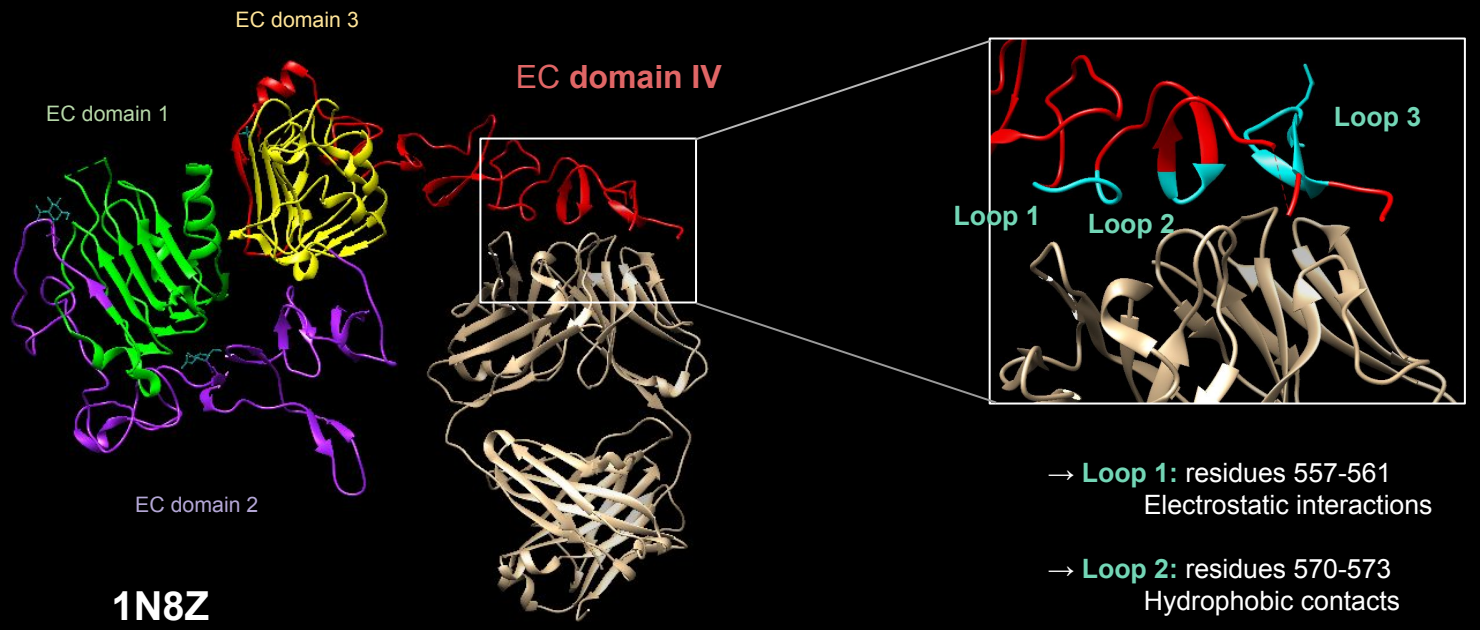
Herceptin-HER2 interaction

Reversible noncovalent interactions

- Electrostatic forces
- Hydrogen bonds
- Van der Waals forces
- Hydrophobic forces



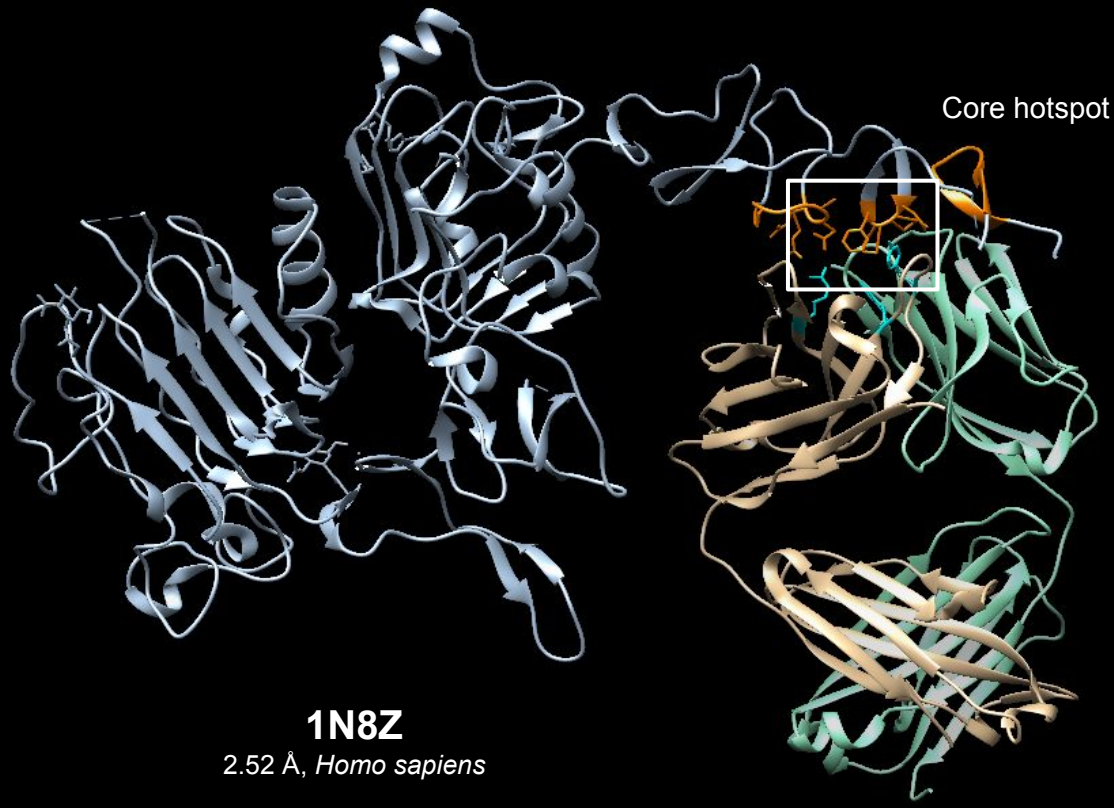
Herceptin binds HER2 on the C-terminal portion of domain IV



→ **Loop 1:** residues 557-561
Electrostatic interactions

→ **Loop 2:** residues 570-573
Hydrophobic contacts

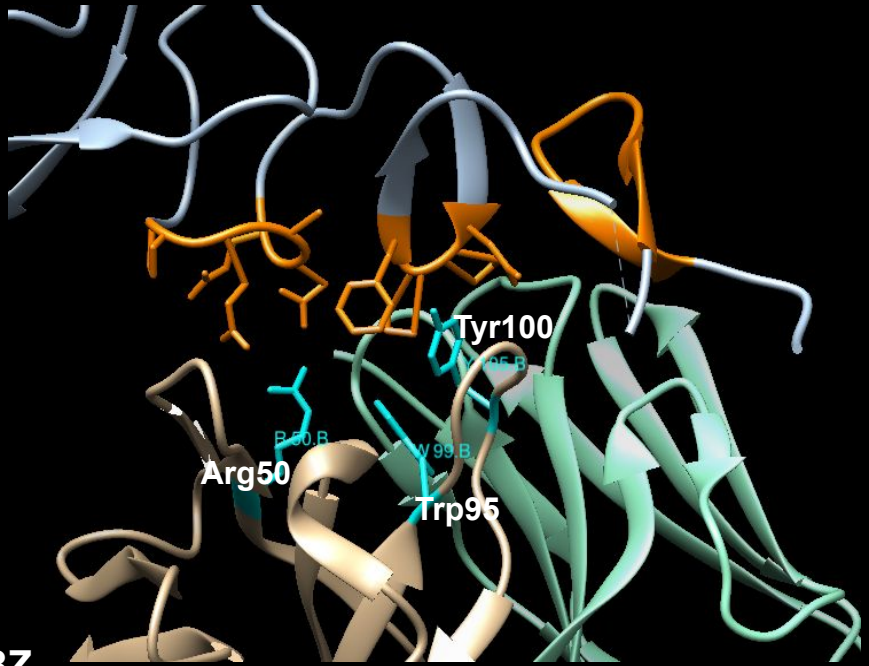
→ **Loop 3:** residues 593-603
Electrostatic interactions



1N8Z

2.52 Å, *Homo sapiens*

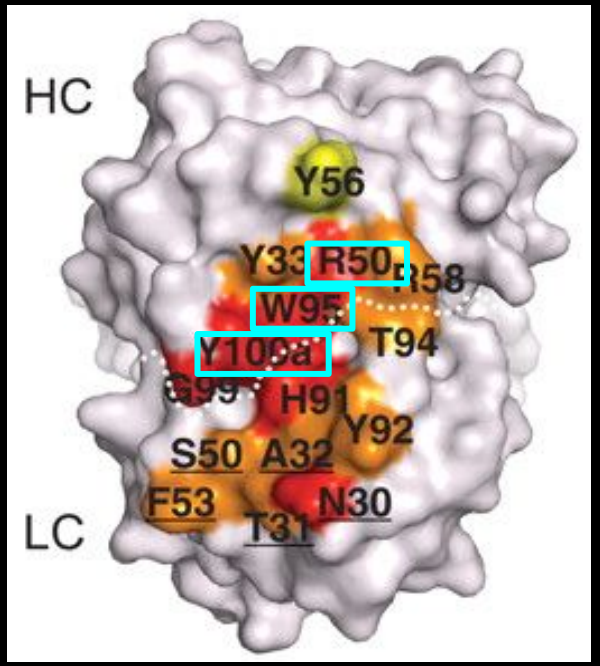
Herceptin's core hotspot residues for HER2 binding



1N8Z
2.52 Å

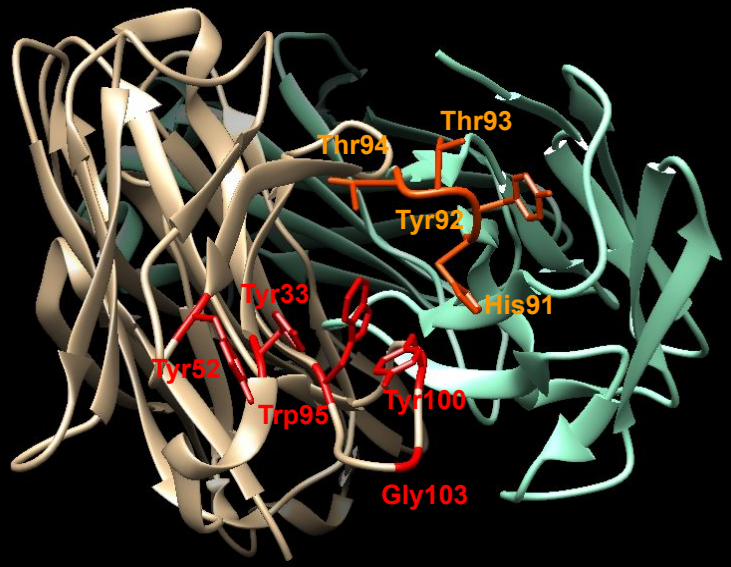
Heavy chain

Light chain



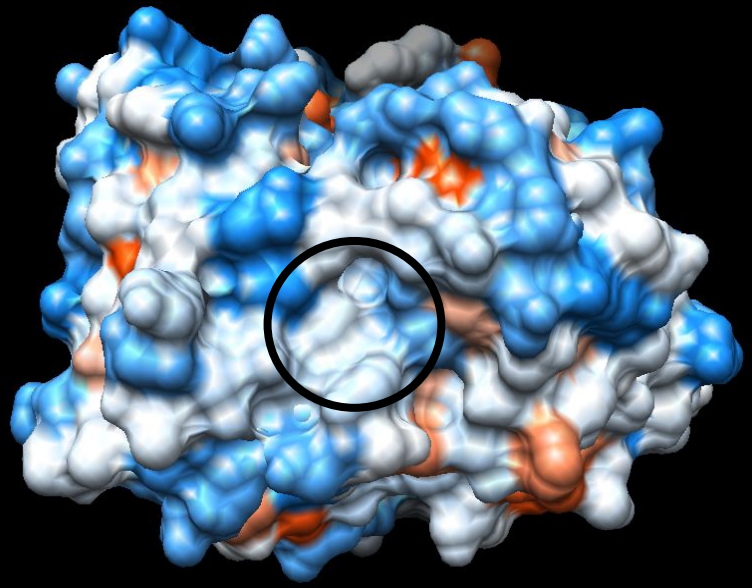
Sun, T.-Y.; Wang et al. Trastuzumab-Peptide Interactions: Mechanism and Application in Structure-Based Ligand Design. *Int. J. Mol. Sci.* Figure 4.

Hydrophobic groove is formed by residues in H3 and L3



Heavy chain

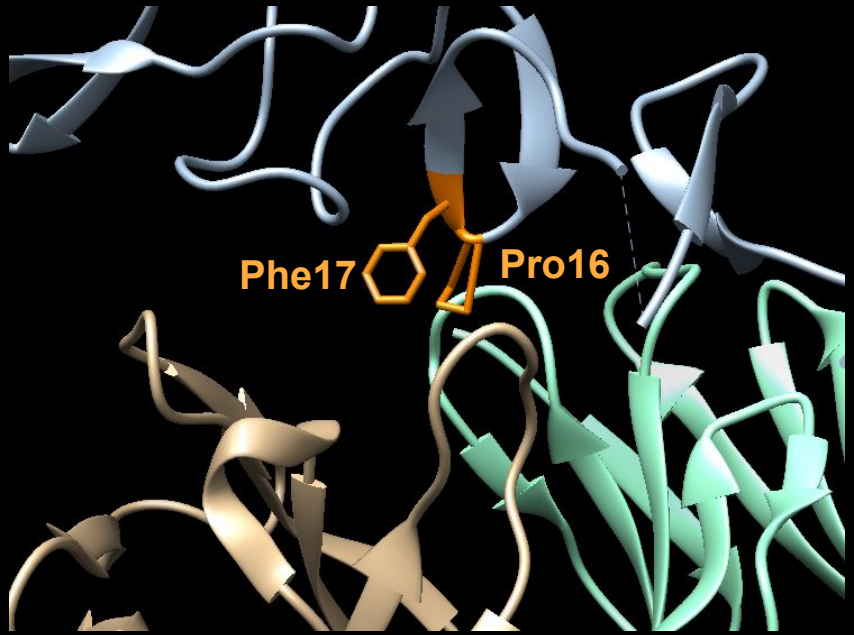
Light chain

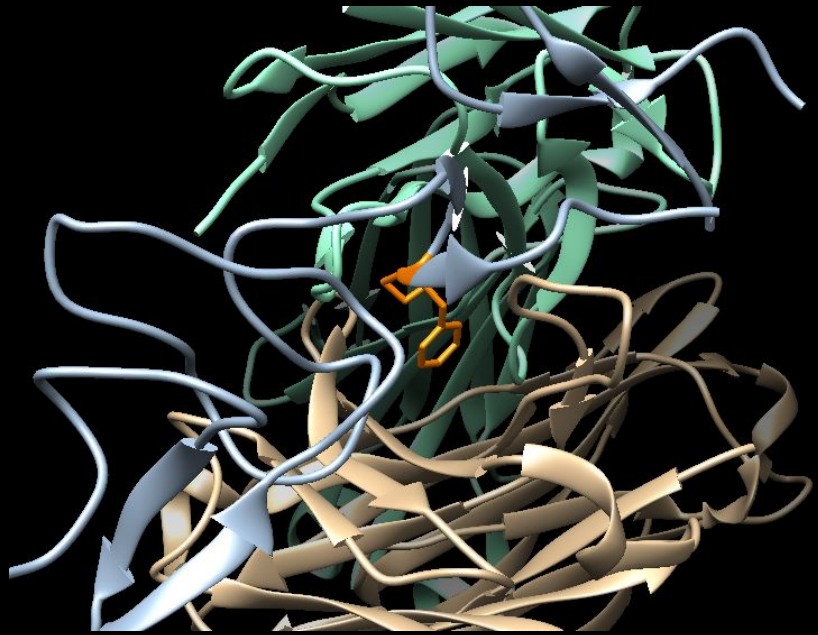


1N8Z

2.52 Å

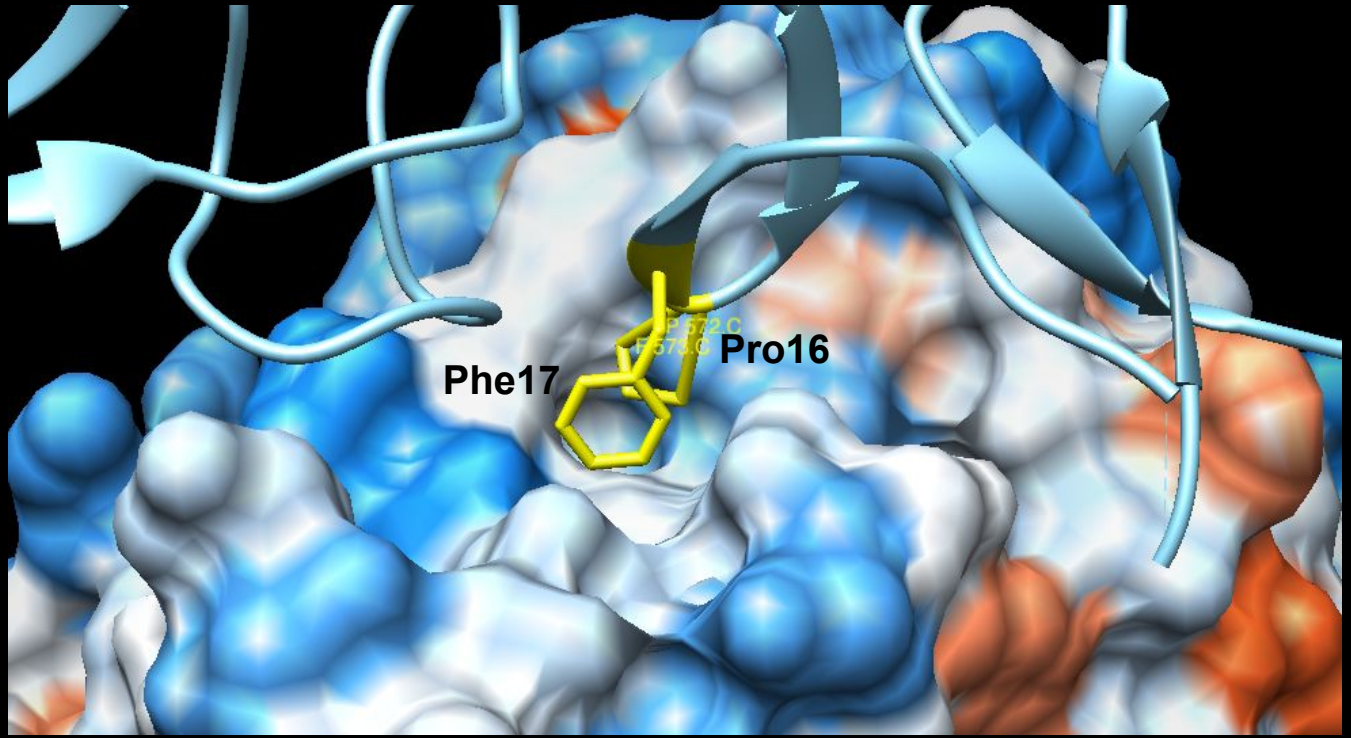
Phe17 and Pro16 bind to the hydrophobic groove





1N8Z

2.52 Å

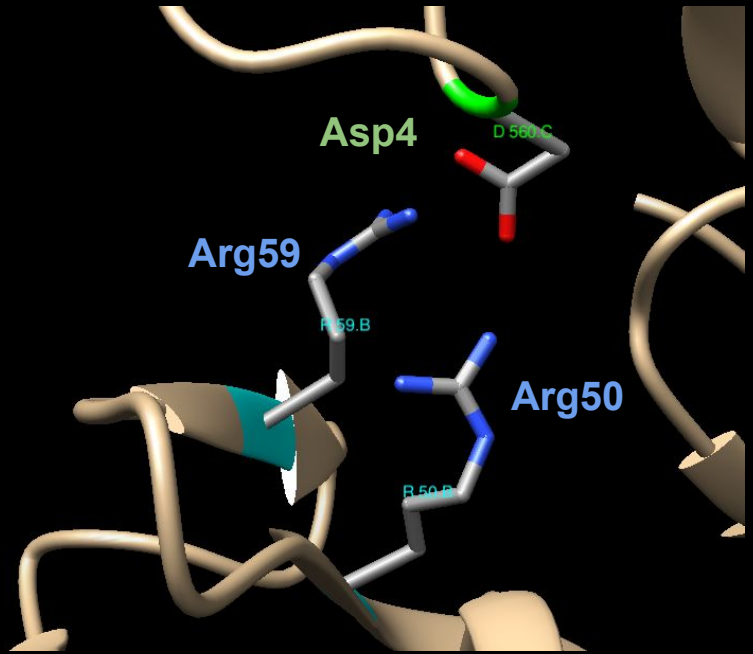


1N8Z

2.52 Å

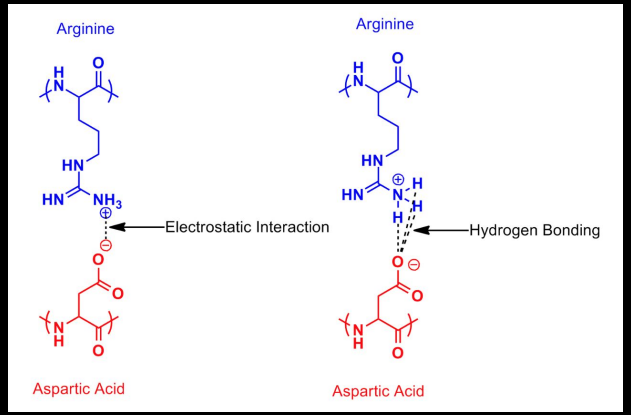
Strong electrostatic interaction between Asp4 and Arg50, Arg59 occur with remarkable hydrogen bond interactions

CDR H3
Herceptin



1N8Z
2.52 Å

Loop 1
EC domain IV



Arginine Aspartic Acid salt bridge. From Wikimedia Commons.

CONCLUSIONS

Conclusions

1. Immunoglobulins have a very stable and conserved structure, which is mediated by different bonds such as disulfide bridges and hydrogen bonds
2. The immunoglobulin fold provides a perfect example of how structure determines and/or facilitates function
3. The constant region of IgG is the most conserved in sequence and structure
4. Glycosylation is important for the open conformation of the Fc region and the interaction with the receptors
5. Despite CDRs being hypervariable regions, there are some chain conformations that are more frequently found, defining canonical structures
6. CDRs are a clear example of the fact that structure is generally more conserved than sequence
7. In the antigen-antibody binding, both hydrophobic interactions and hydrogen bonds are formed between the CDRs of the Fab and the epitope

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Multiple choice questions

1. How many light chains are encoded in the human genome?

a. 2 of them: lambda and kappa
b. 4 of them: mu, sigma, delta and alpha
c. 6 of them: mu, sigma, delta, lambda, kappa and alpha
d. All of the above
e. None of the classifications is correct

2. Mark the correct sentence about the Ig - like fold is…

a. On an immunoglobulin G there is only one Ig - like fold
b. Only found on proteins that belong to the immune system
c. The constant region and the variable region is structurally identical
d. It is a typical example of how function determines the structure
e. It is formed by beta and alpha helices

3. The structure of an immunoglobulin G …

a. Has an Y shape
b. It is formed by 2 heavy chains and 2 light chains
c. The variable domain is formed by light and heavy chains
d. The Fab and Fc fragment is obtained by the action of the enzyme papain
e. All of them are correct

Multiple choice questions

4. Which type of fold have immunoglobulins?

a) Ig-like alpha-helix
b) Ig-like beta-sandwich
c) Alpha + beta
d) Alpha / beta
e) None of them

5. IgG glycosylation:

a) Is important for the binding of the antigen
b) Is located in the hinge region
c) Can be located at any part of the Fc
d) Is important for the binding of the receptor
e) Is not important at all

6. About Fc receptors:

a) They bind the Fab part of IgGs
b) FcγRI is the one with less afinity
c) FcγRI is an inhibitory receptor
d) FcγRI has 2 loops
e) FcγRI has 3 loops, but only two of them interacts with IgG

7. Which is the most variable CDR?

a) L1
b) L3
c) H2
d) H1
e) H3

Multiple choice questions

8. About the canonical structures of CDRs:

a) Canonical structures are defined by the loop length, the conformation of the loop, and conserved residues
b) Canonical structures are defined by the loop length and by conserved residues
c) There are 10 canonical structures in total
d) There are canonical structures characterized for every CDR
e) L1 and H1 share the same canonical structure

9. About CDRs in immunoglobulins:

a) CDRs are not the most variable regions within immunoglobulins
b) CDRs of the same type can't share main chain conformations
c) CDRs confer specificity to antibodies by facilitating antigen recognition
d) Canonical structures for CDRs were initially characterized by Baldomero Oliva and Nuria Centeno
e) CDR H3 has several characterized canonical structures

10. About the interaction between HER2 and Herceptin:

a) No hydrogen bonds are formed in this interaction
b) Herceptin binds specifically to the extracellular domain IV of HER2
c) The interaction is uniquely based on electrostatic interactions
d) A hydrophobic groove is formed by residues present in CDRs L3 and H3
e) Answers a and c are correct

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