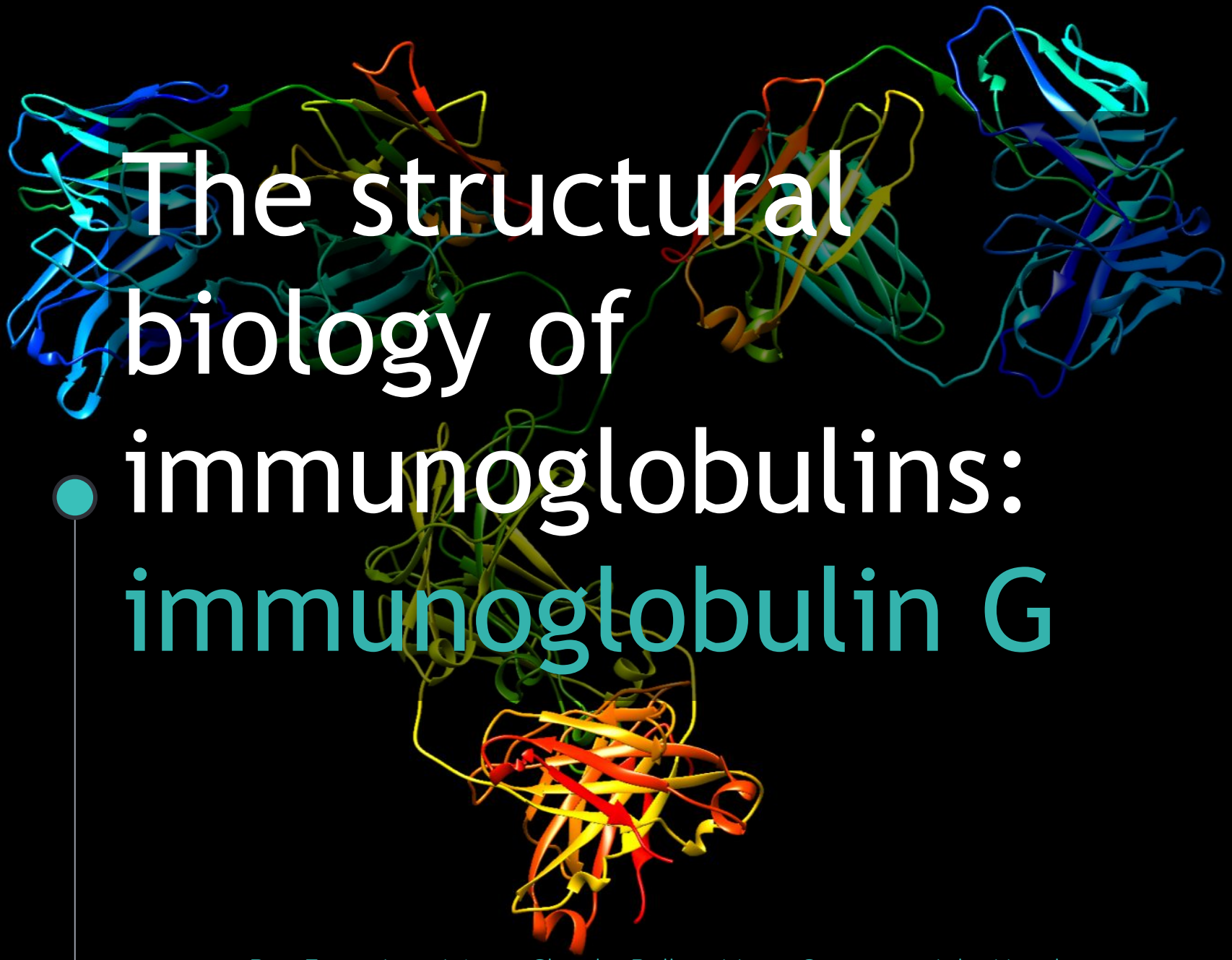
The structural biology of immunoglobulins: immunoglobulin G



Pau Forte, Laia López, Claudia Pallisé, Maria Quintana, Júlia Urgel

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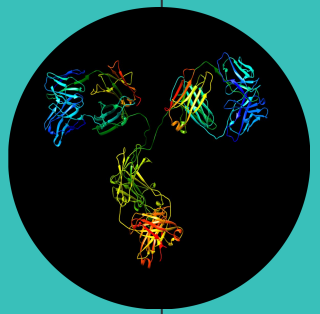


Basic concepts of immunoglobulins

Basic concepts: Introduction

Immunoglobulins

Globular proteins

1IGT

Functions

Conserved set of effector molecules

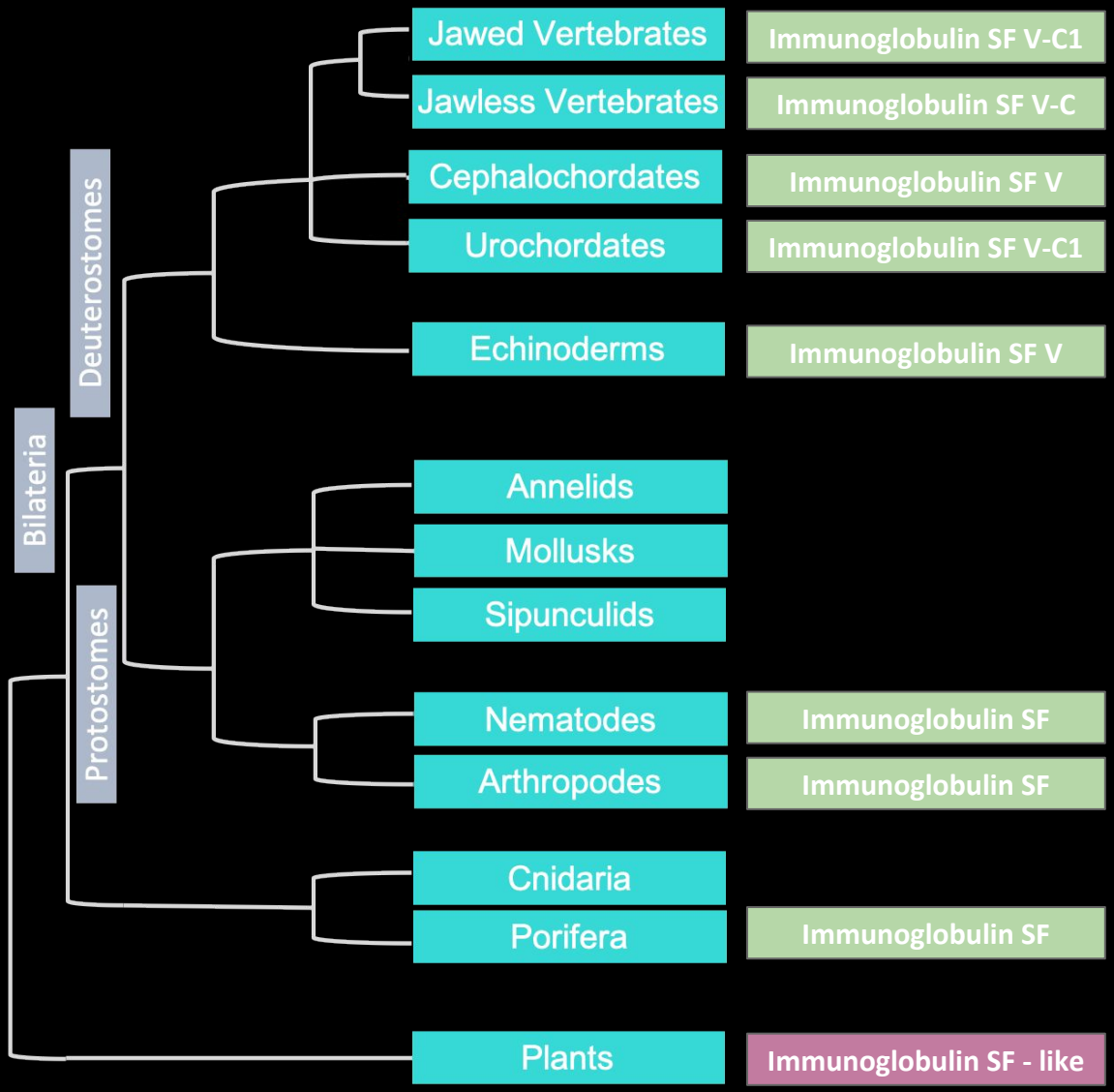
- **Activation** and **fixation** of the complement
- **Binding** to Fc receptors on the surface of granulocytes, monocytes, platelets and other components.

Inflammation

Policlonal set of diverse ligand binding sites

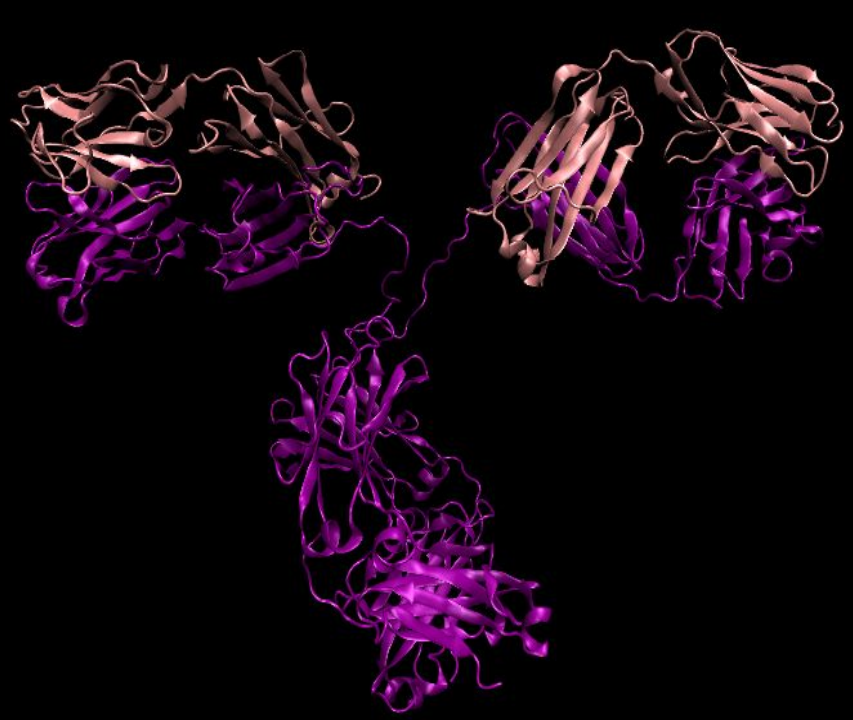
- Recognison of self ad non-self-antigens

Basic concepts: evolutionary origin

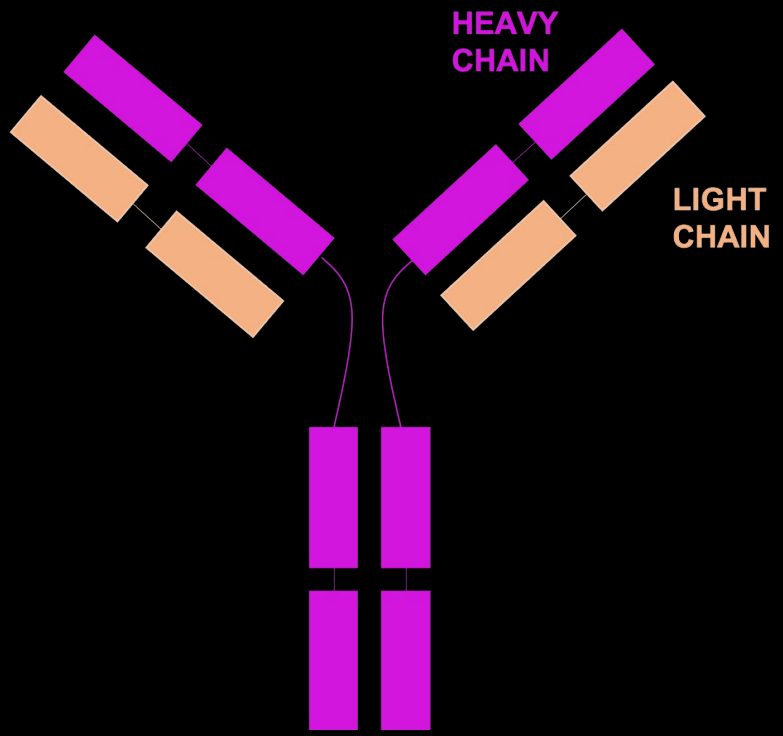


Special cases:
- Bacteria
- Plants

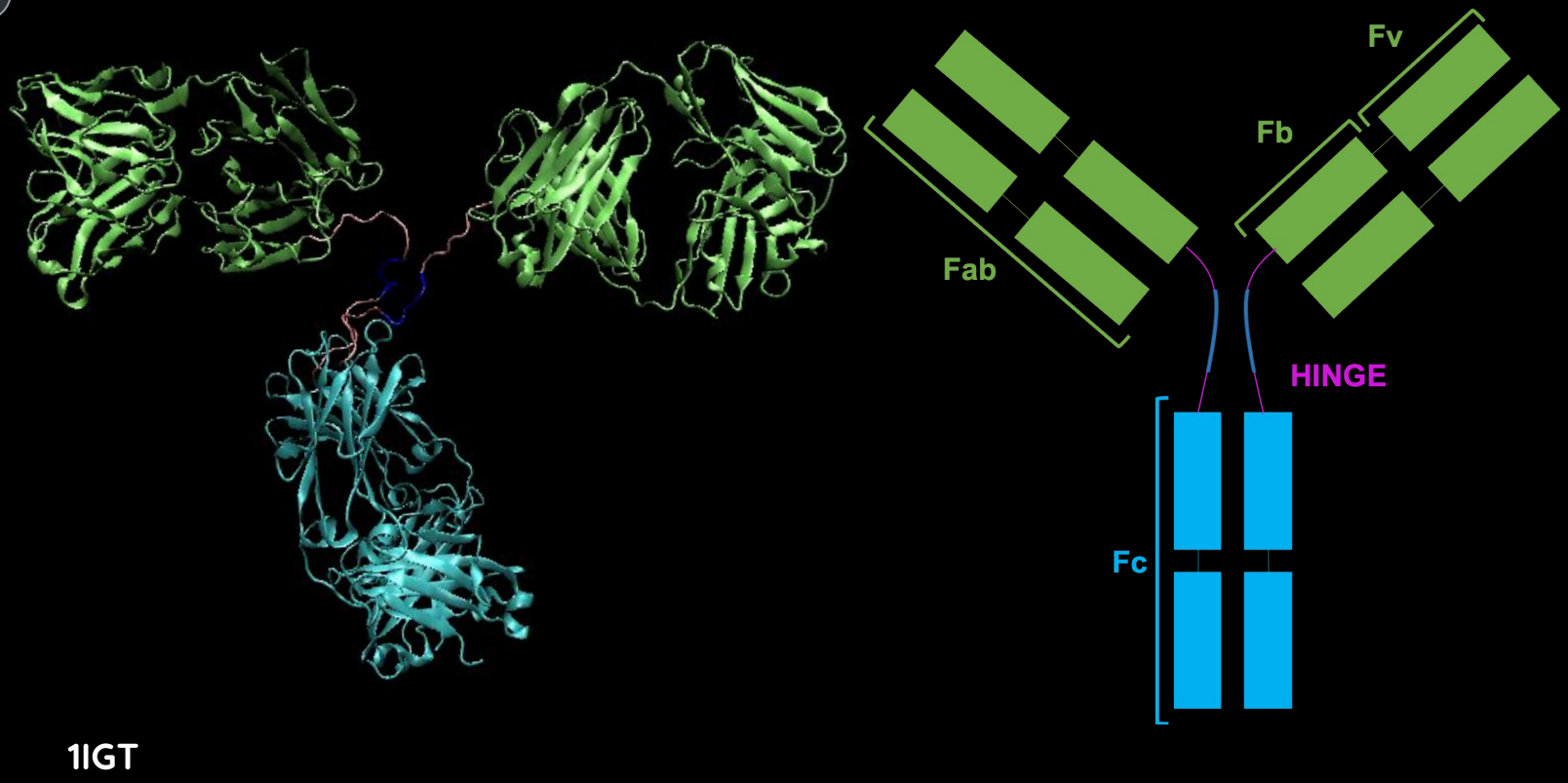
Basic concepts: Introduction - Immunoglobulins as Heterodimers



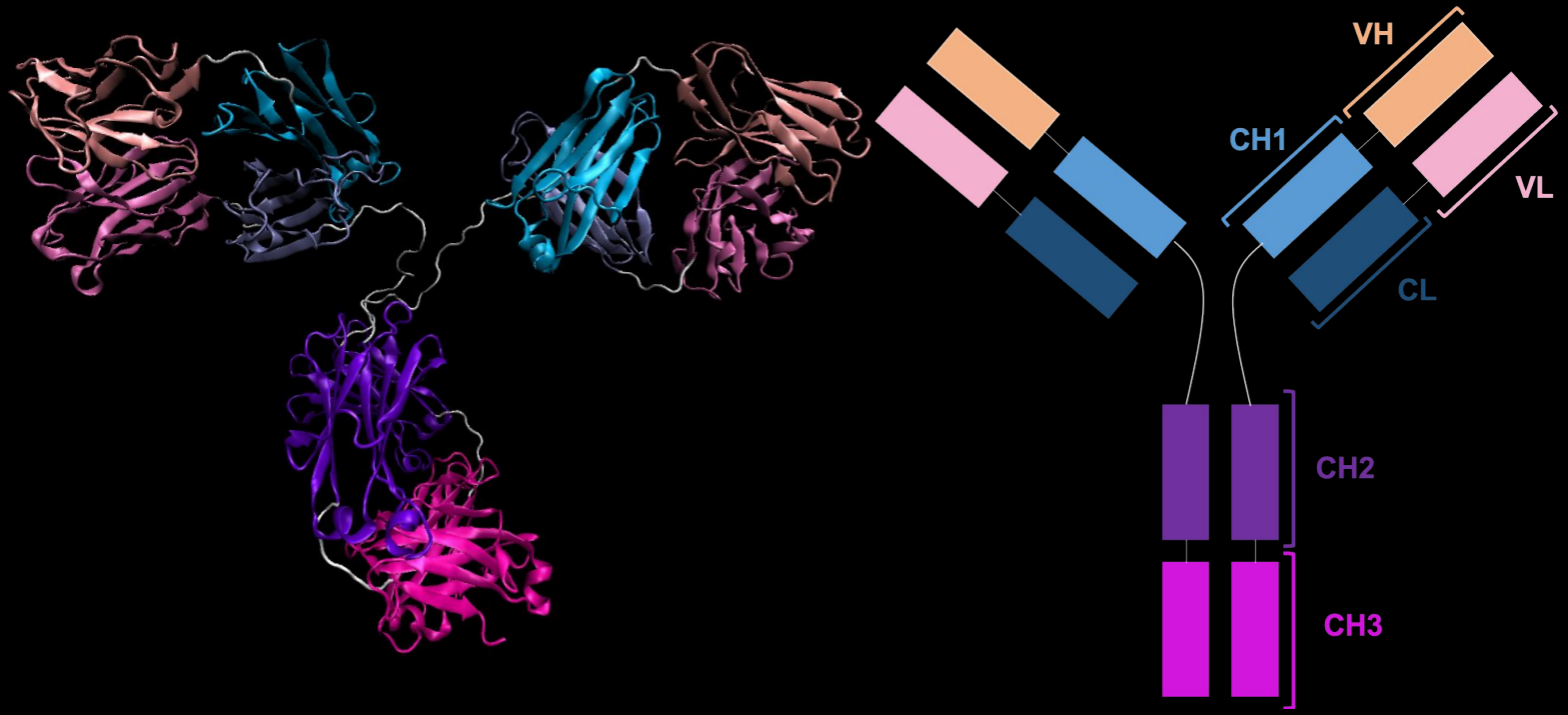
1IGT



Basic concepts: Introduction - Immunoglobulins as Heterodimers



Basic concepts: Introduction - Immunoglobulins as Heterodimers

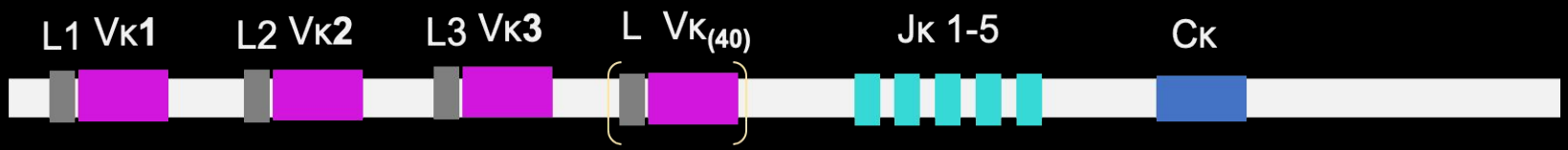


1IGT

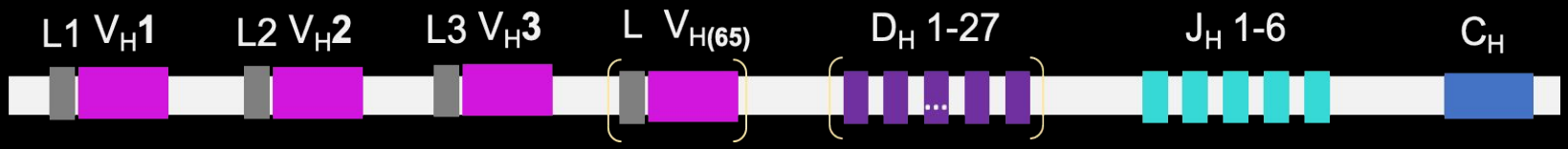
Basic concepts: Diversity

λ light chain locus - Chr 22

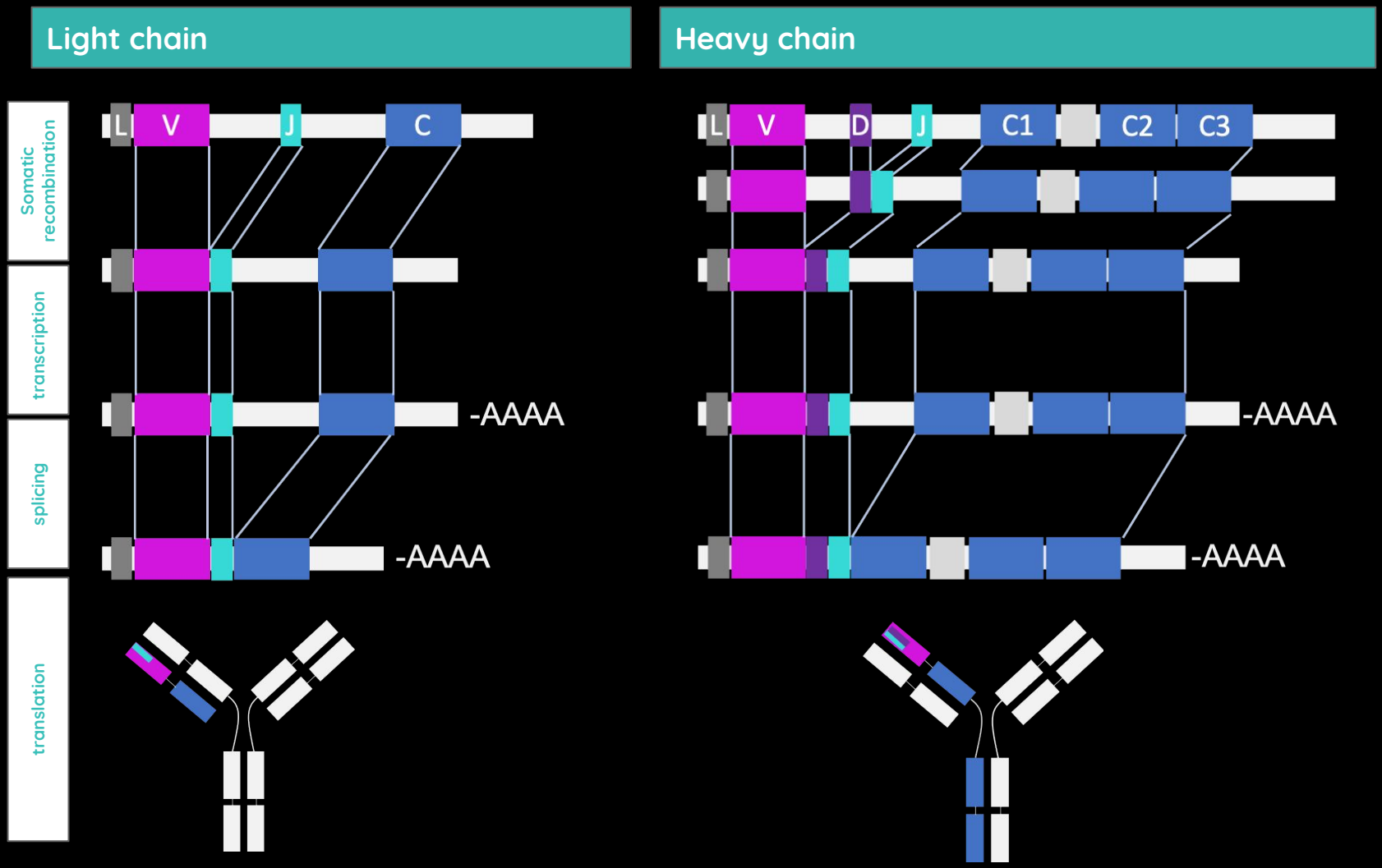
κ Light chain locus - Chr 2



Heavy chain locus -Chr 14



Basic concepts: Diversity



Basic concepts: Immunoglobulin Structure

SCOP Classification

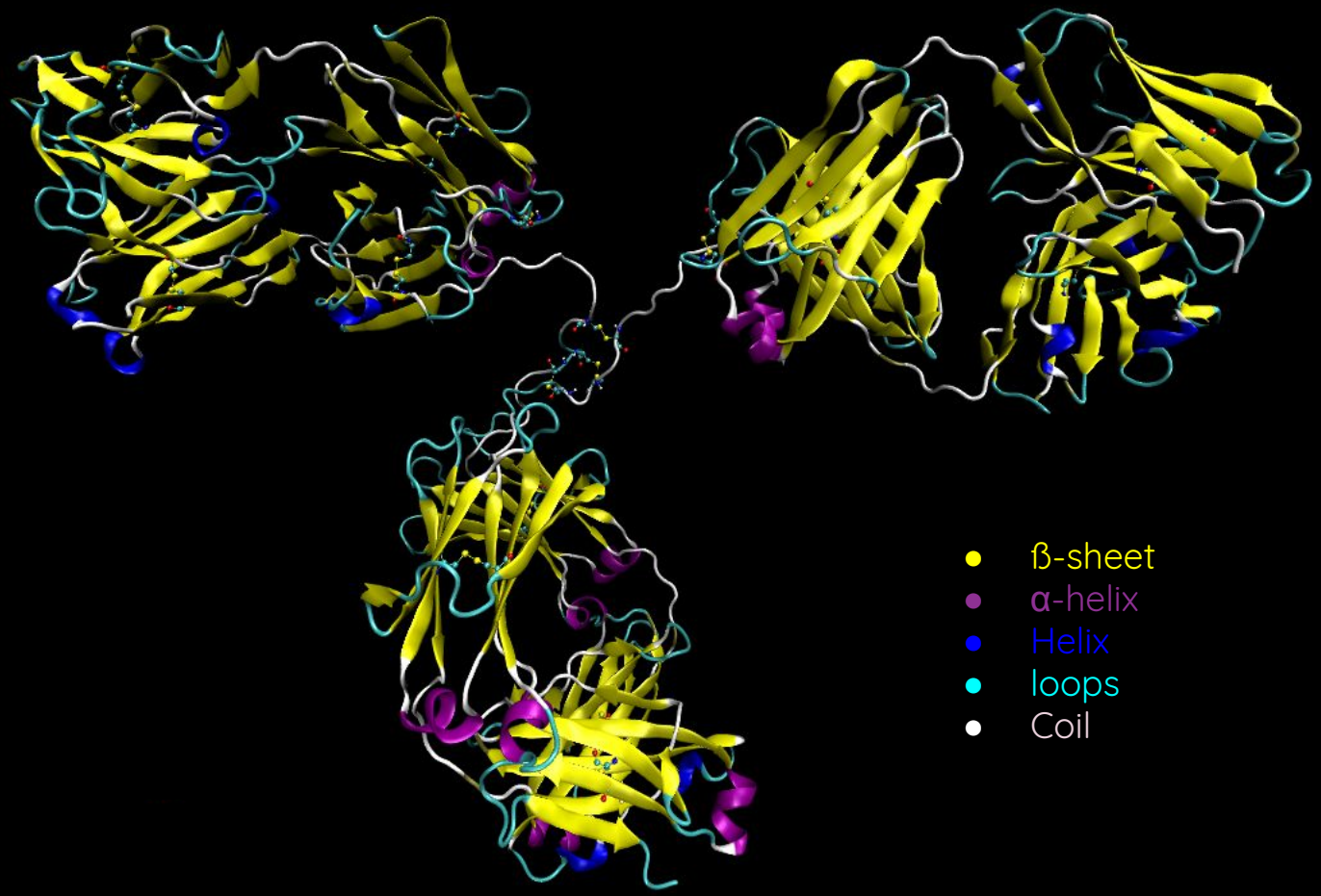
Lineage

- **Class:** All beta-proteins
- **Fold:** Immunoglibulin-like beta-sandwich
- **Superfamily:** Immunoglobulin

Families

- V set domains (antibody variable domain-like)
- C1 set domains (antibody constant domain-like)
- C2 set domains
- I set domains

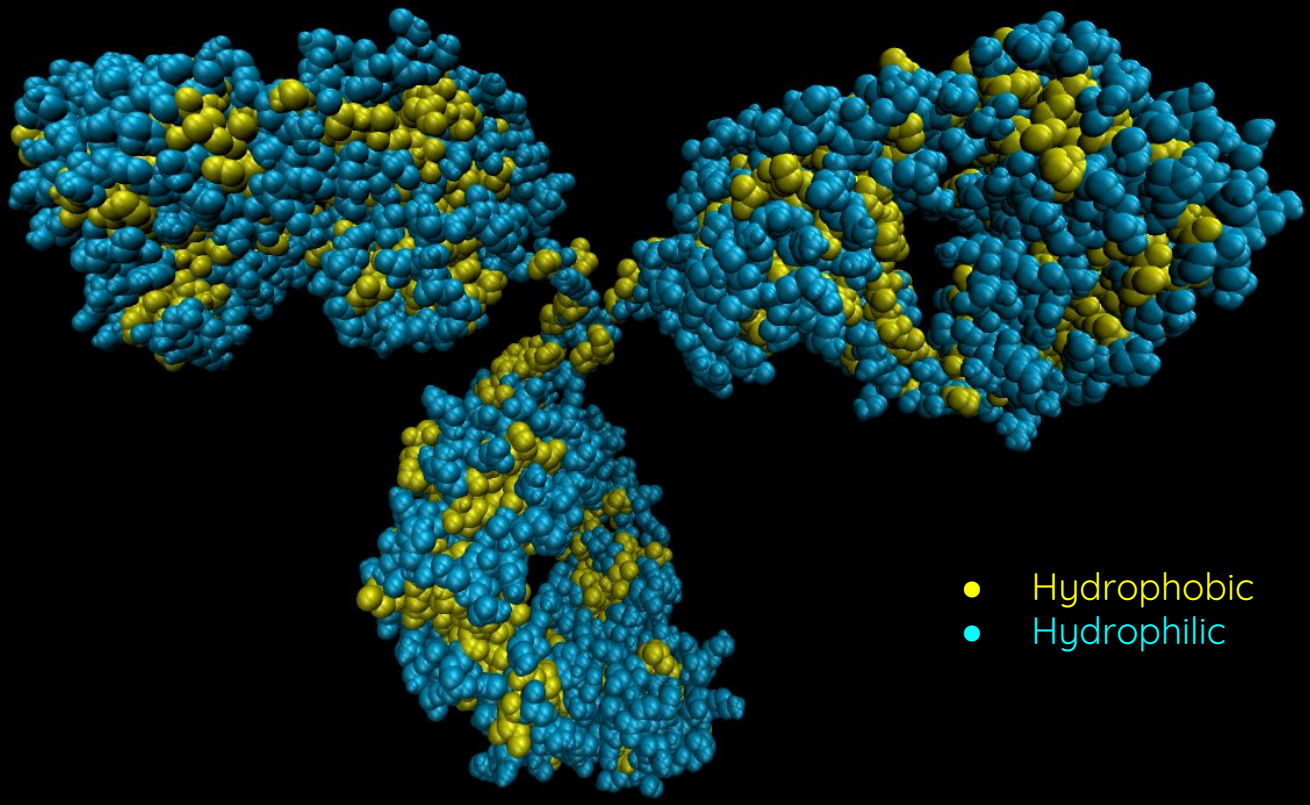
Immunoglobulin Structure: Class - *All beta proteins*



- ß-sheet
- α-helix
- Helix
- loops
- Coil

1IGT

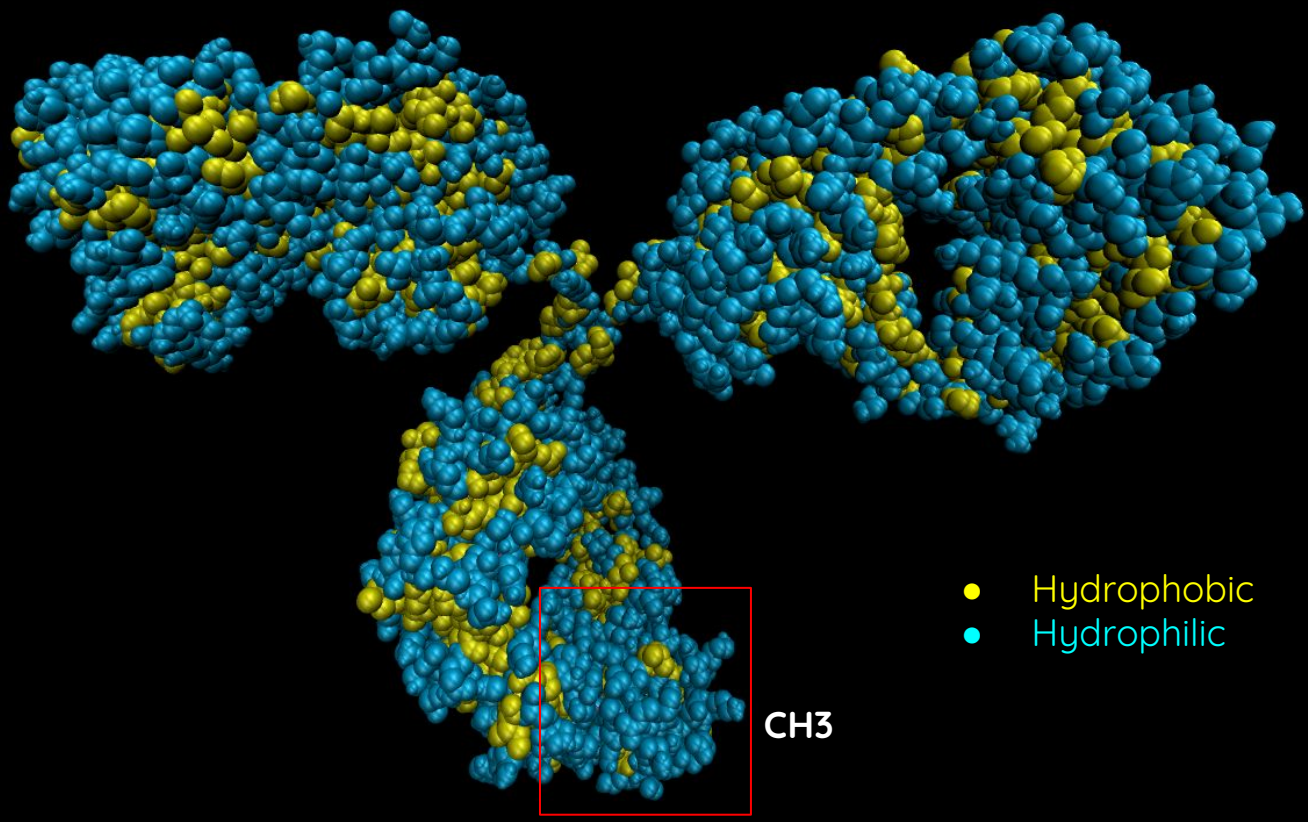
Immunoglobulin Structure: Class - *Hydrophobicity*



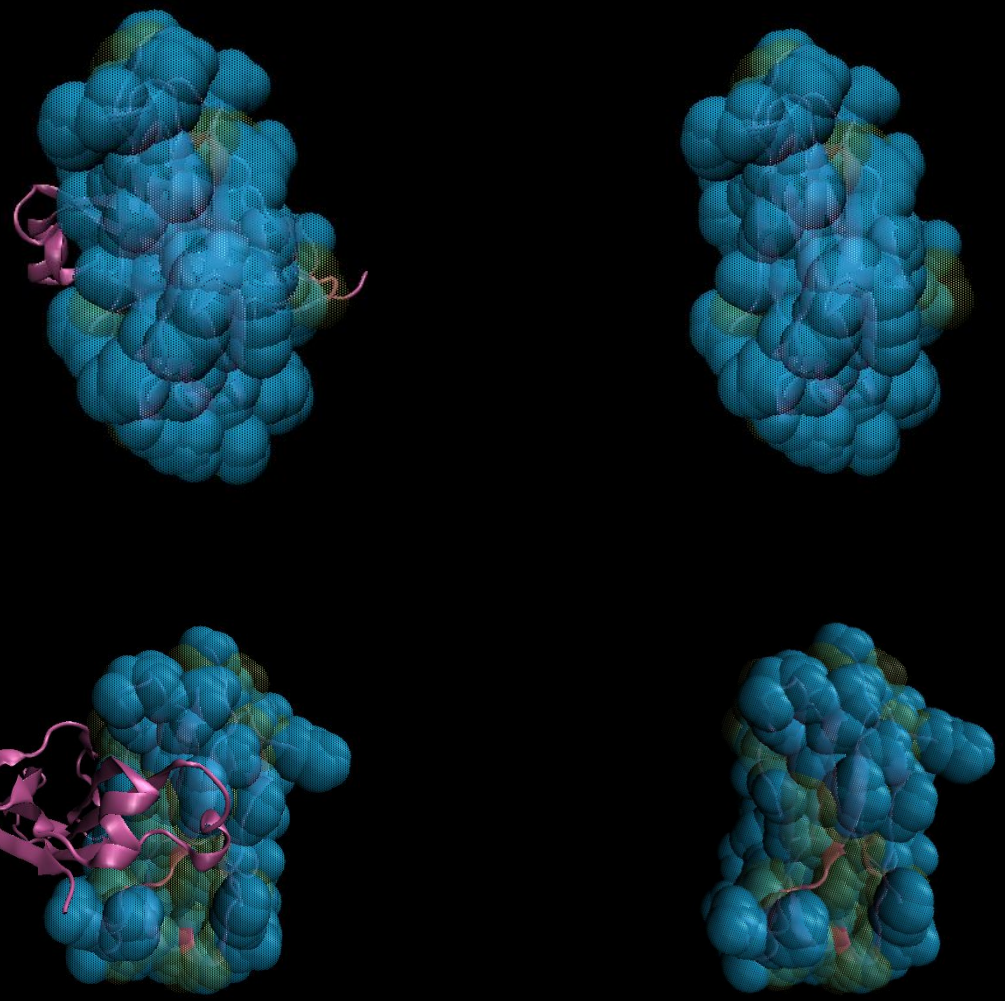
- Hydrophobic
- Hydrophilic

1IGT

Immunoglobulin Structure: Class - *Hydrophobicity*



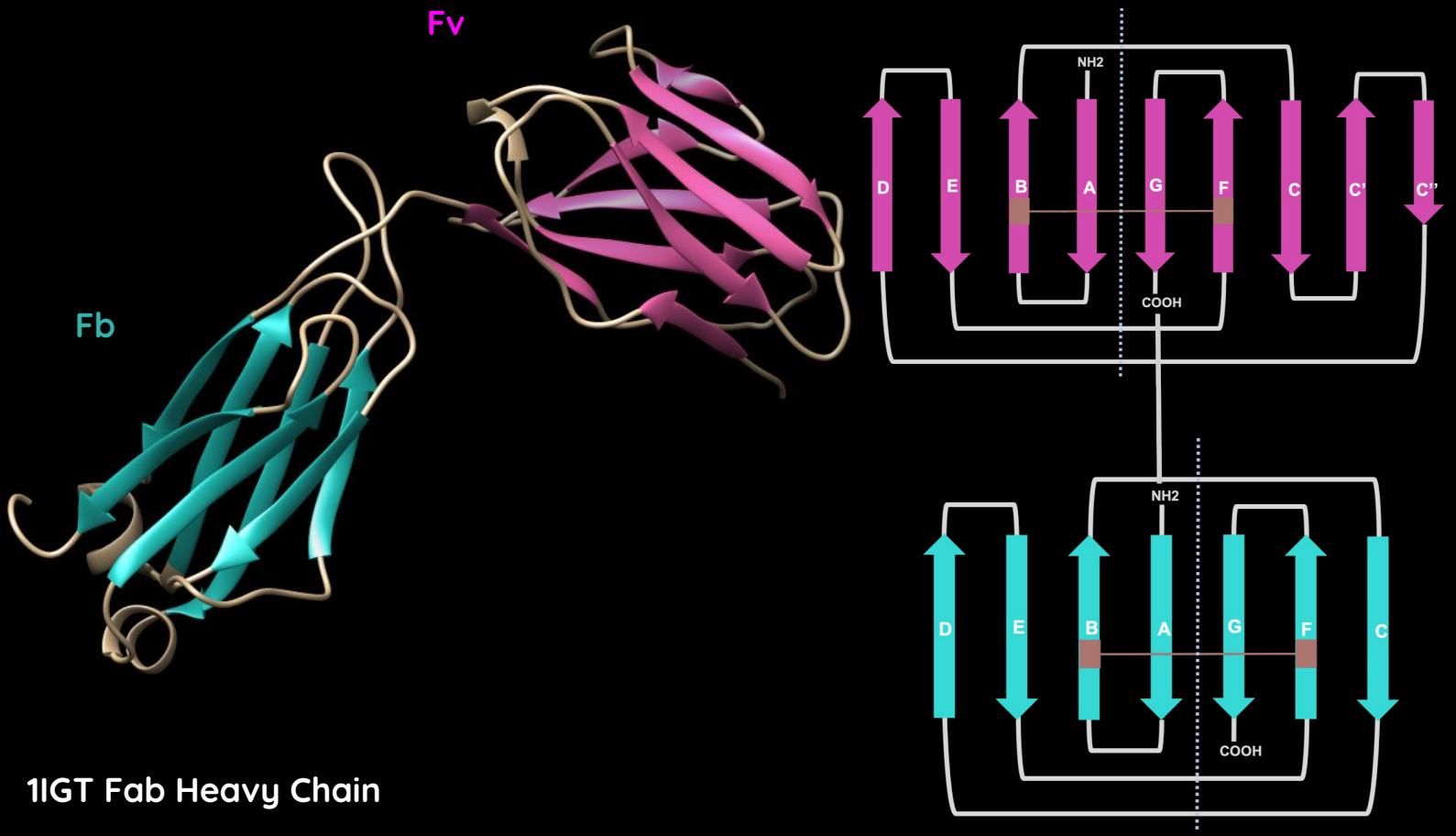
Immunoglobulin Structure: Class - *Hydrophobicity*



- Hydrophobic
- Hydrophilic
- Contralateral CH3

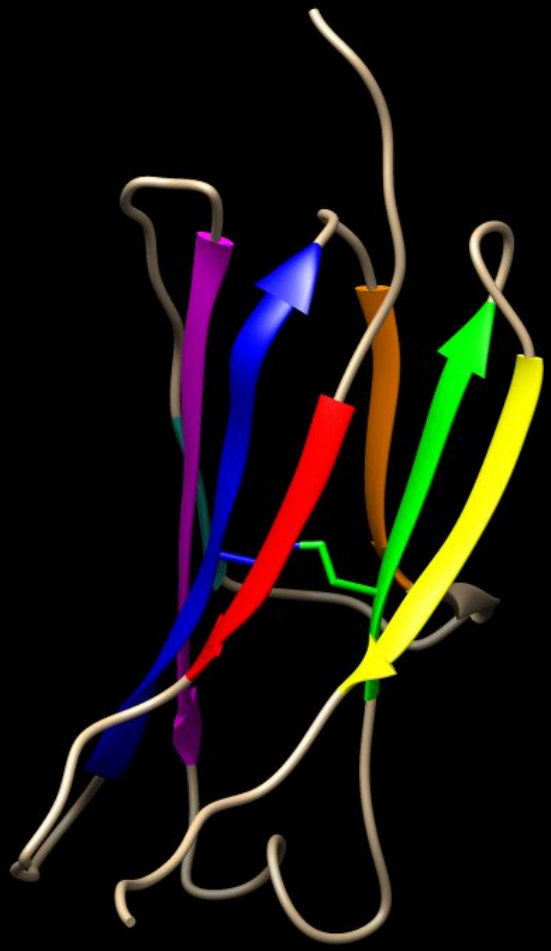
1IGT CH3

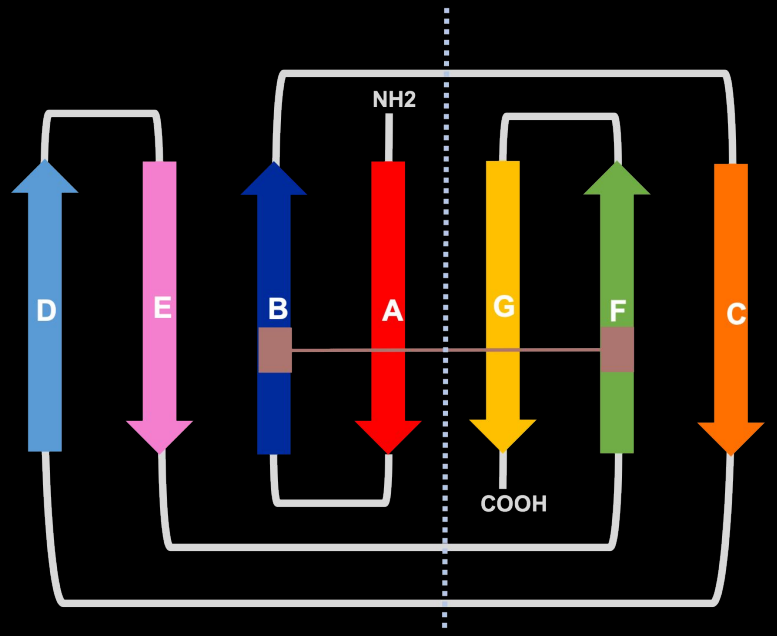
Immunoglobulin Structure: Fold - *Ig like beta-sandwich*



1IGT Fab Heavy Chain

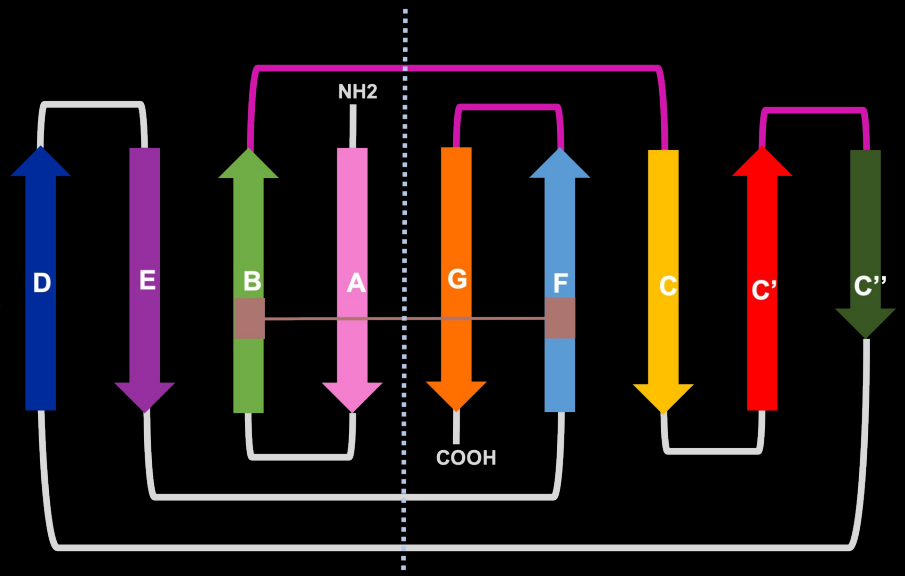
Immunoglobulin Structure: Fold - *Ig like beta-sandwich*





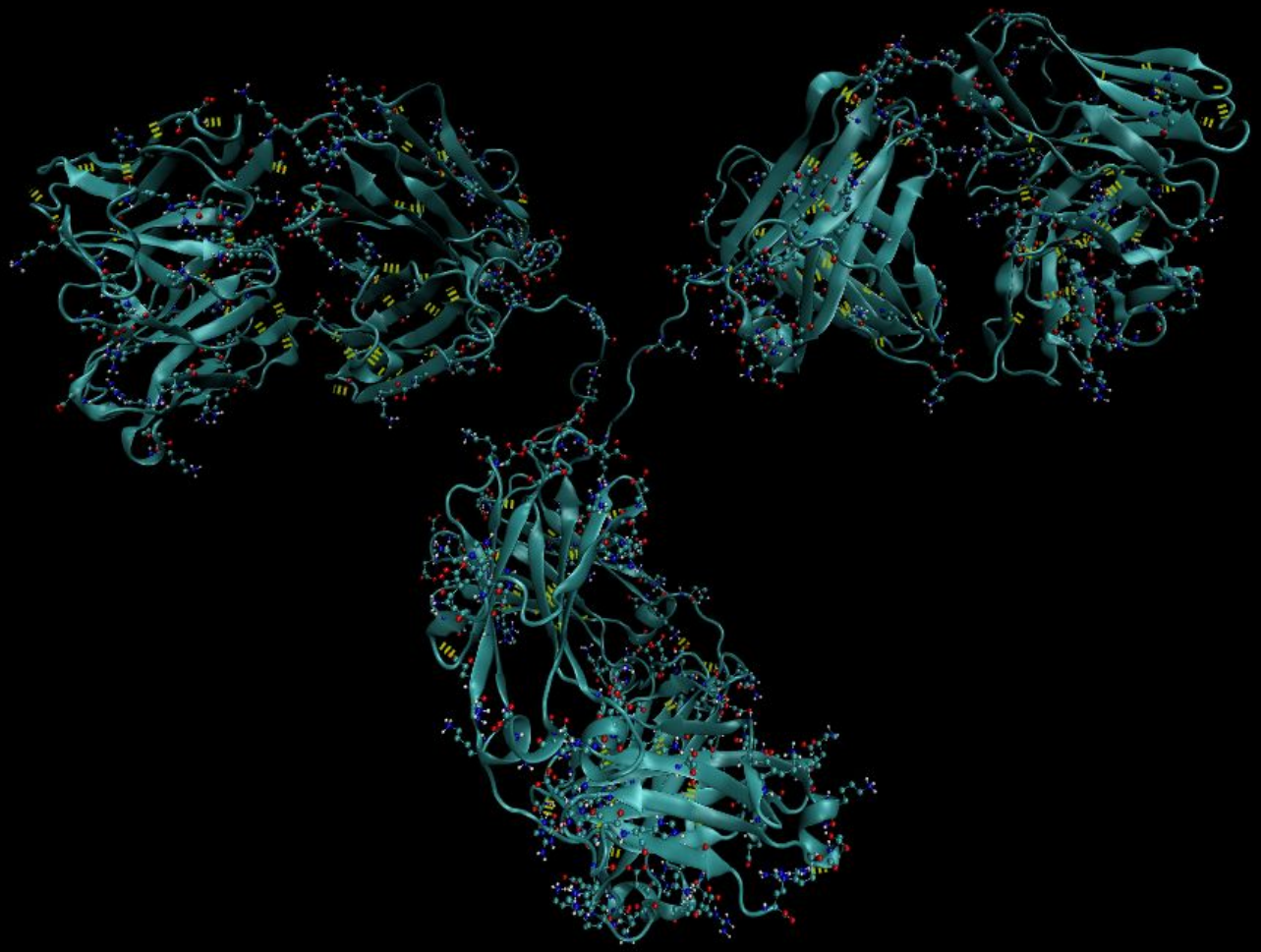
1IGT CL

Immunoglobulin Structure: Fold - *Ig like beta-sandwich*



1IGT VL

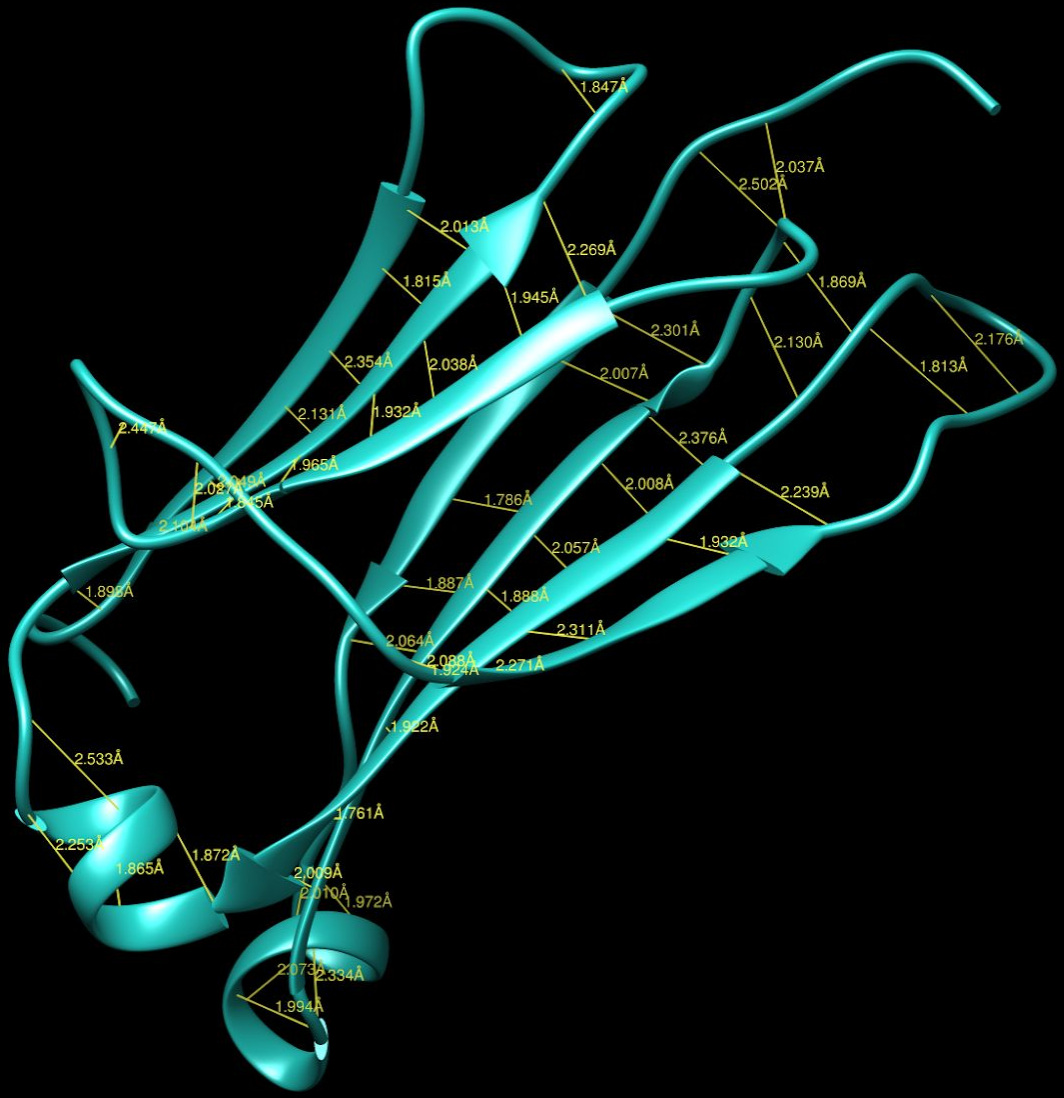
Immunoglobulin Structure: Hydrogen Bonds



1IGT

Immunoglobulin Structure: Hydrogen Bonds

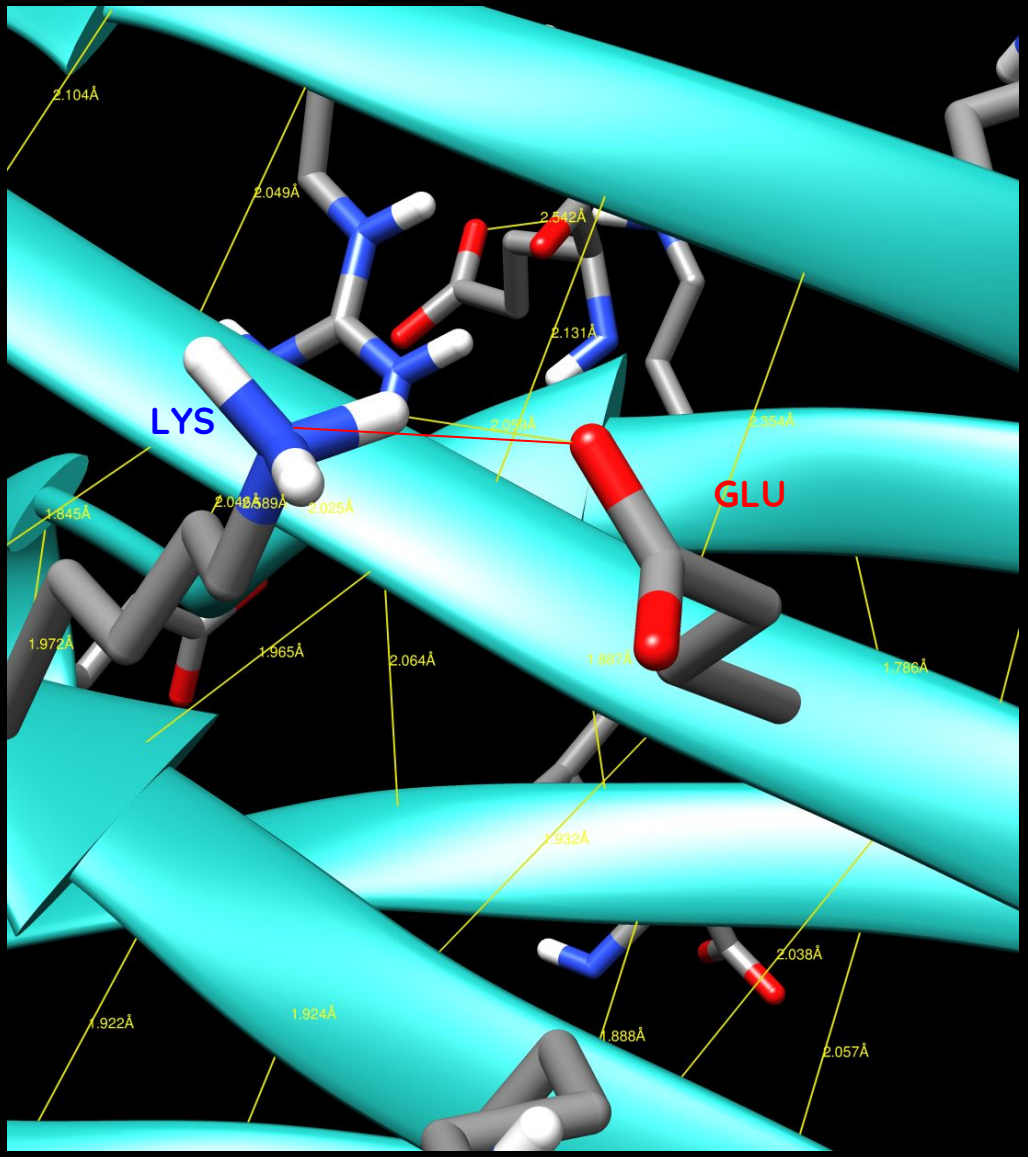
Ig like beta-sandwich



1IGT

Immunoglobulin Structure: Hydrogen Bonds

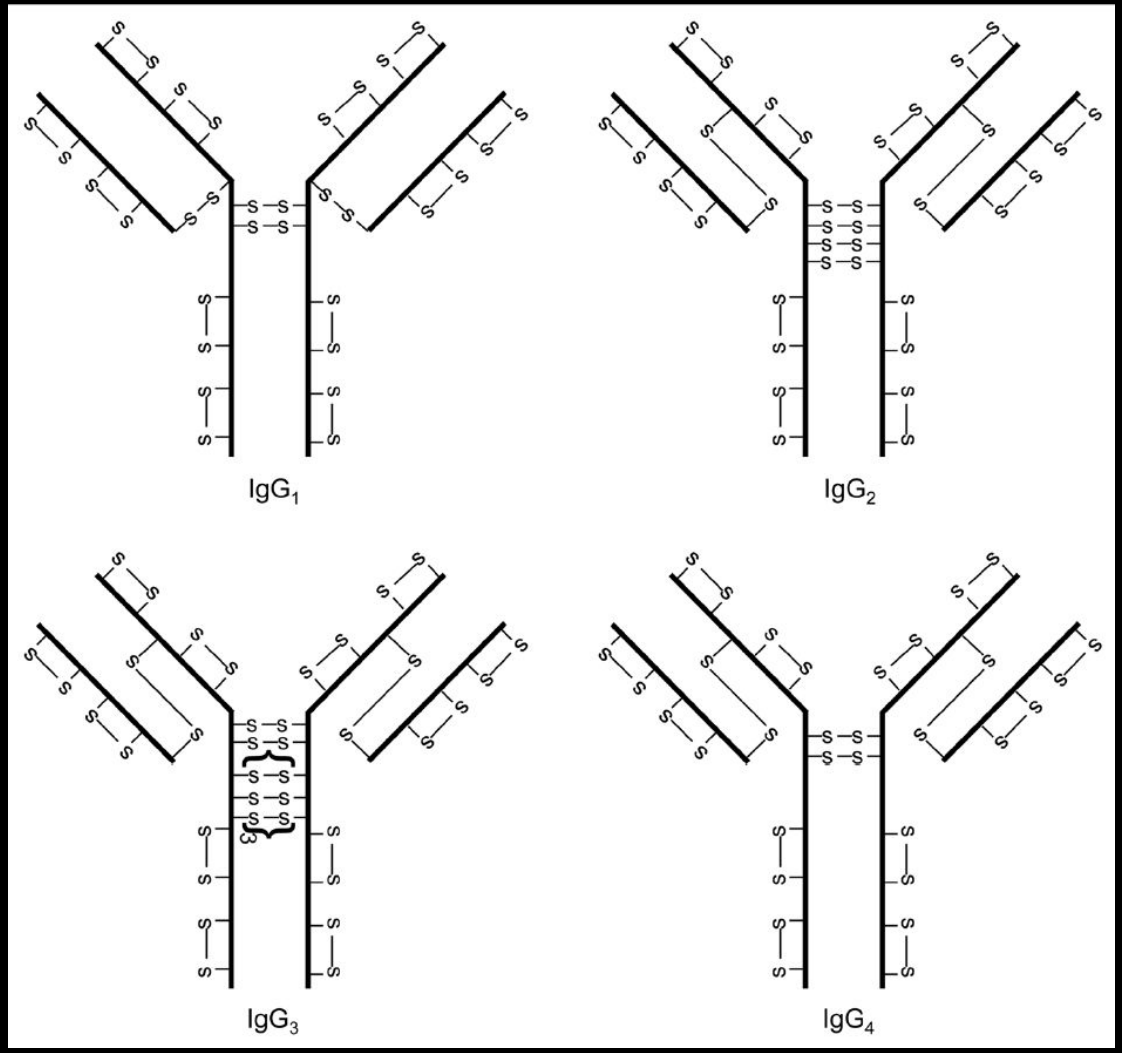
Ig like beta-sandwich



- Oxigen
- Nitrogen
- Carbon and Backbone
- Hidrogen
- Sidechain

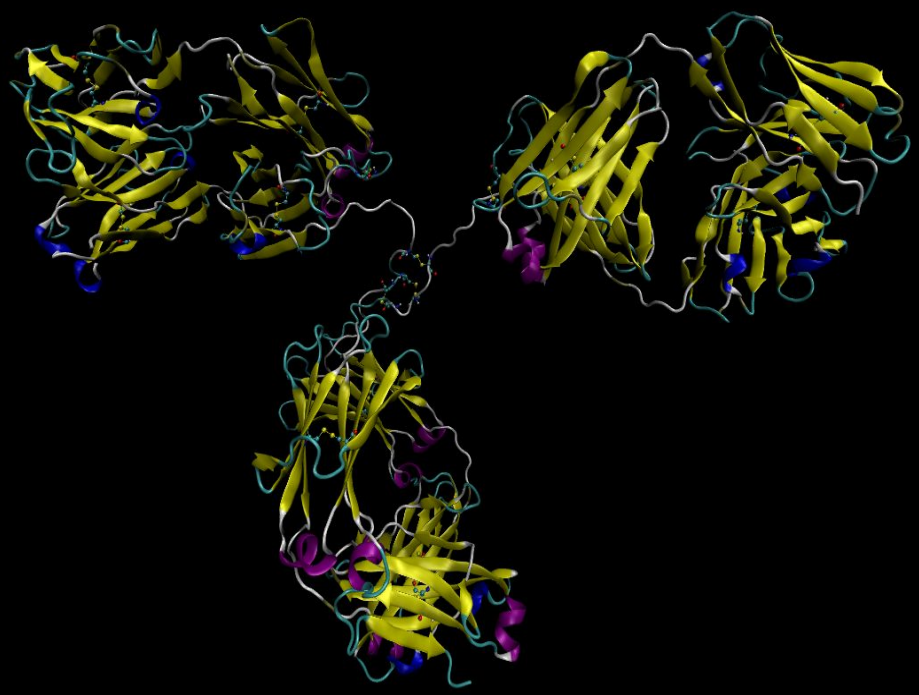
1IGT

Immunoglobulin Structure: Classical IgG disulfide bond structures

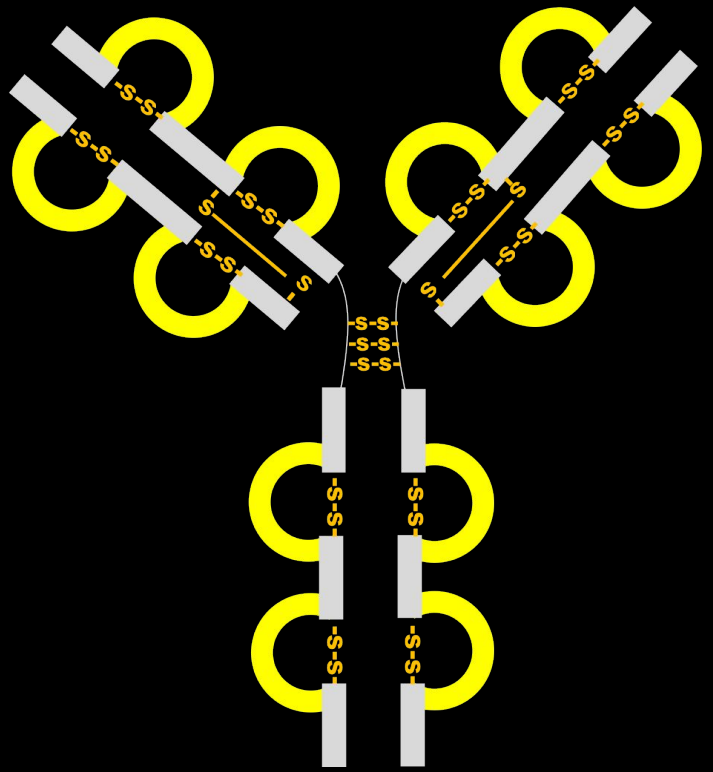


Hongcheng Liu and Kimberly May, 2012.

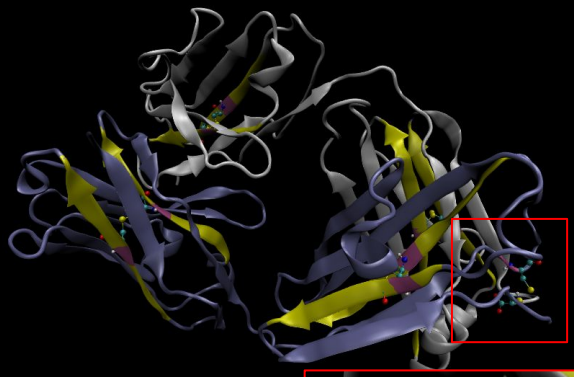
Immunoglobulin Structure: Disulfide bond structures



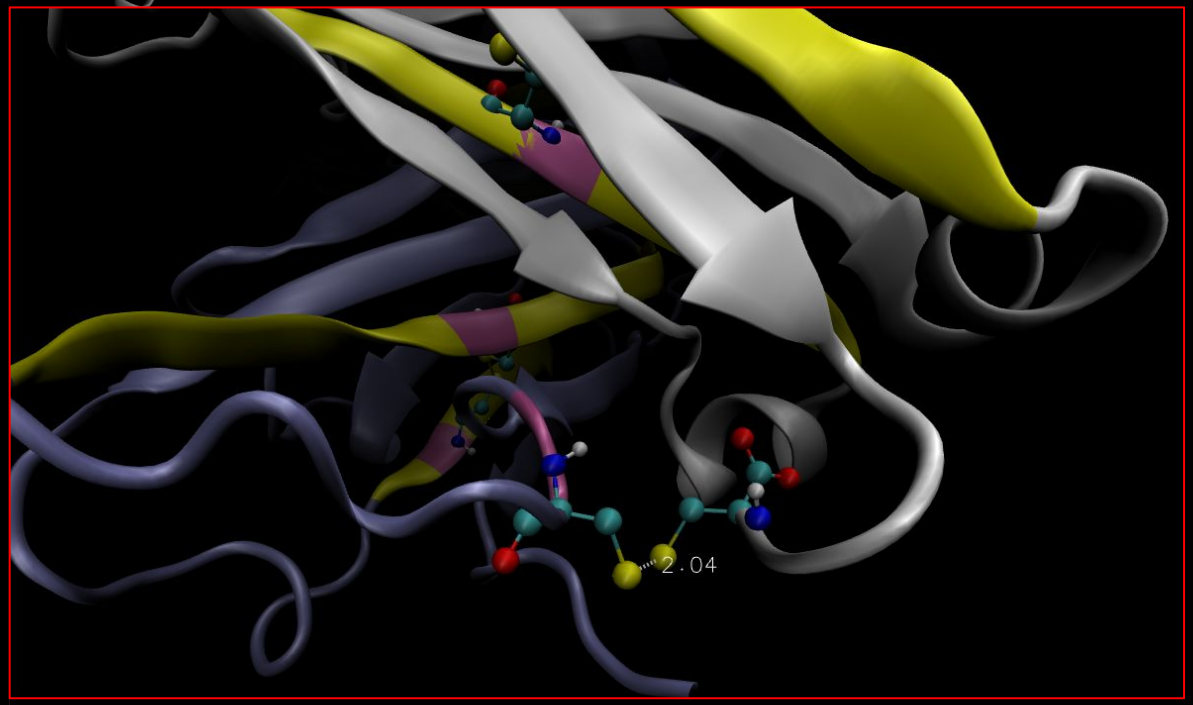
1IGT



Immunoglobulin Structure: Disulfide bond structures

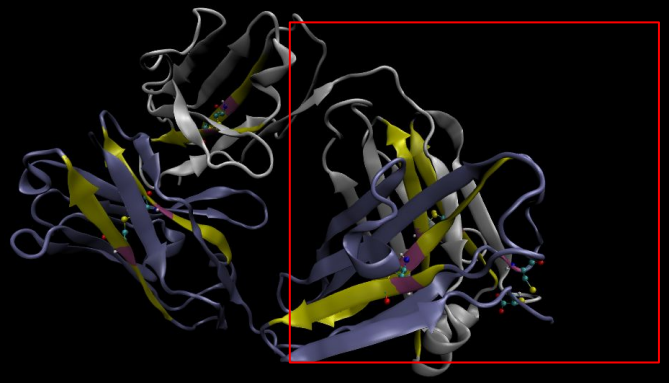


- Sulfur
- Oxigen
- Nitrogen
- Carbon and Backbone
- Hidrogen



1IGT Fab

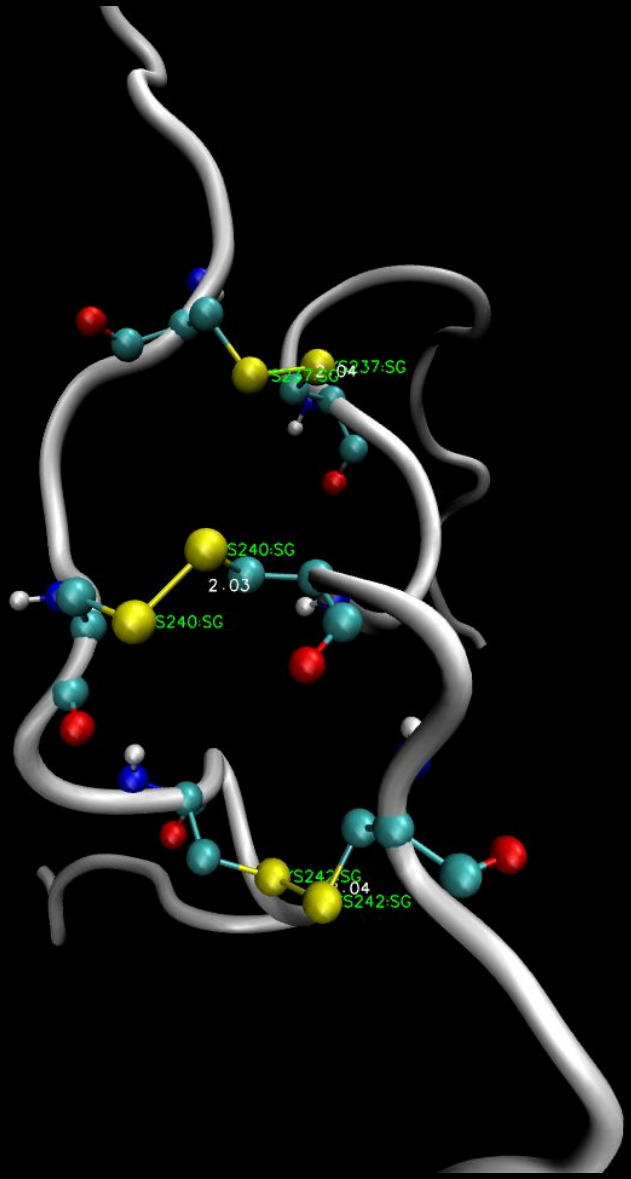
Immunoglobulin Structure: Disulfide bond structures



- Sulfur
- Oxigen
- Nitrogen
- Carbon and Backbone
- Hidrogen

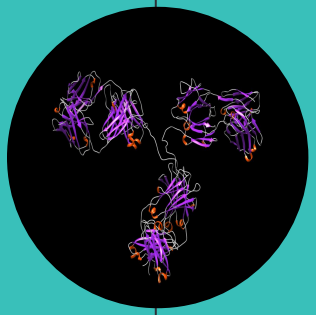
1IGT Fab

Immunoglobulin Structure: Disulfide bond structures



- Sulfur
- Oxigen
- Nitrogen
- Carbon and Backbone
- Hidrogen

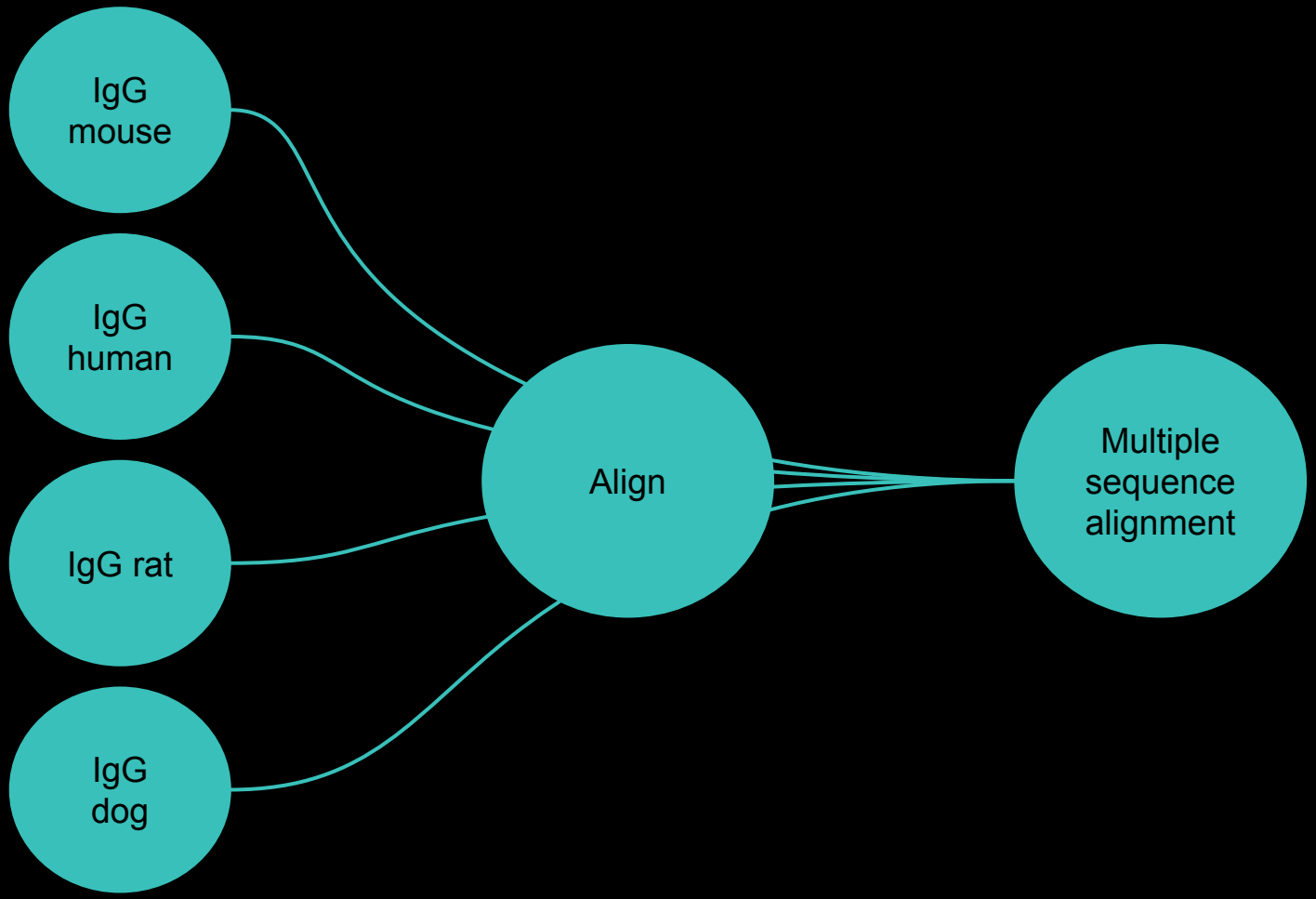
1IGT hinge



Evolution of IgG chains

Methodology

Objective of the evolutive study



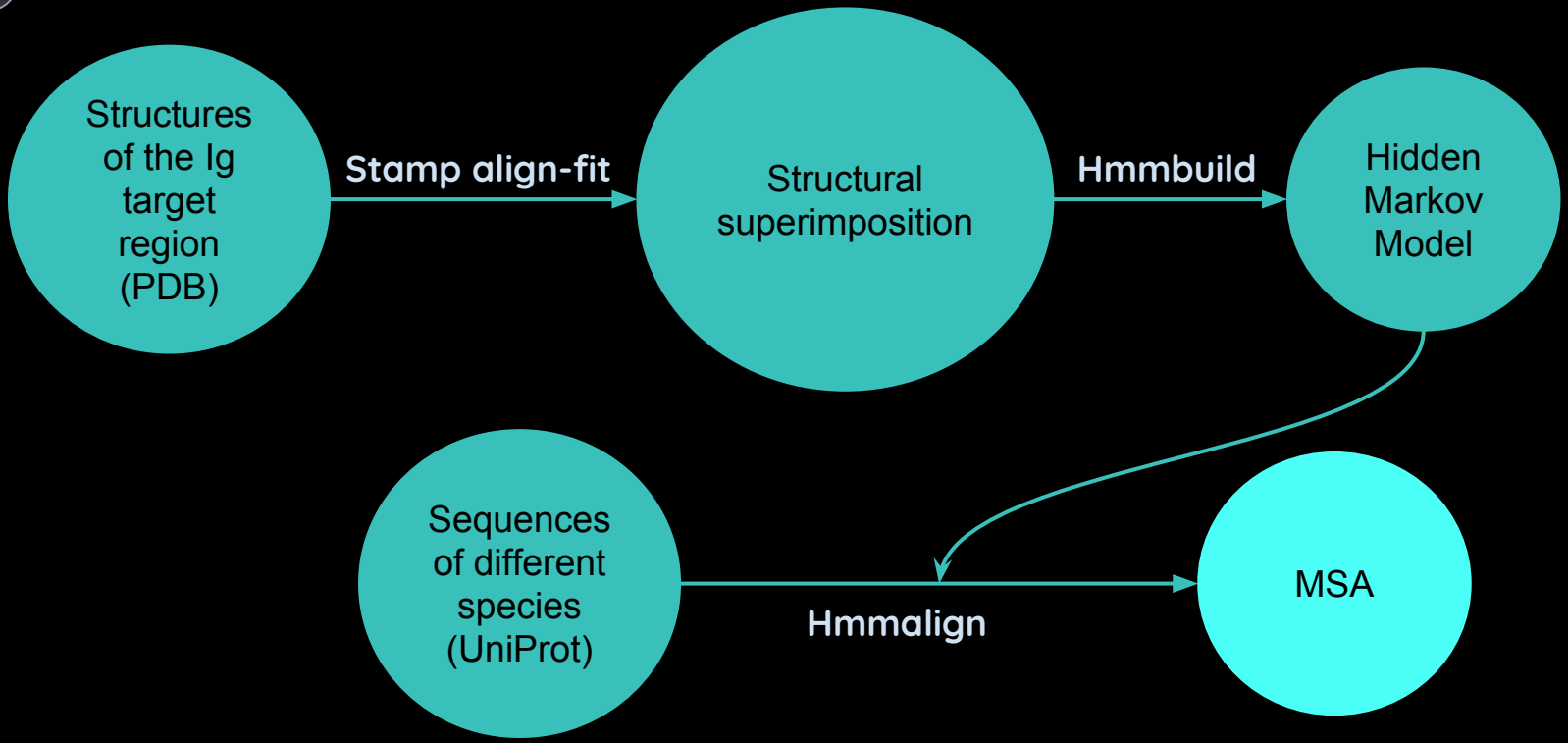
Objective of the evolutive study

Very few different species with Ig structure in PDB

Solution: using UniProt sequences

However... UniProt is not awesome

Objective of the evolutive study



Methods of the evolutive study

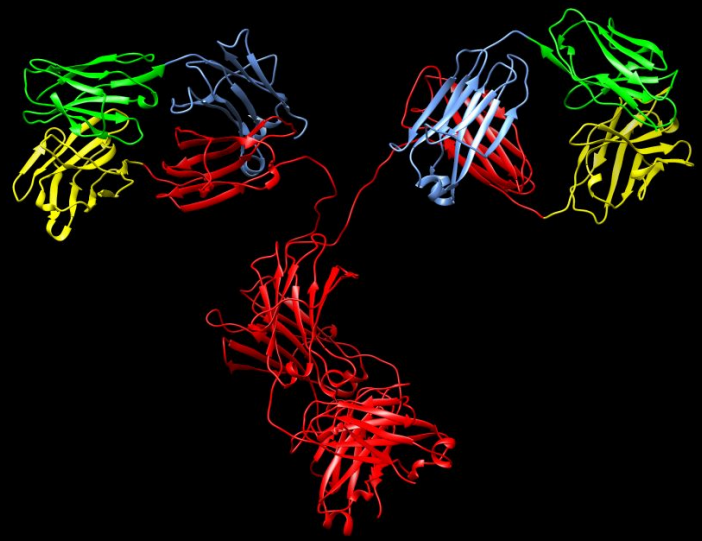
Discovering database problems

PDB

UniProt

- Fab
- Fc

1IGT

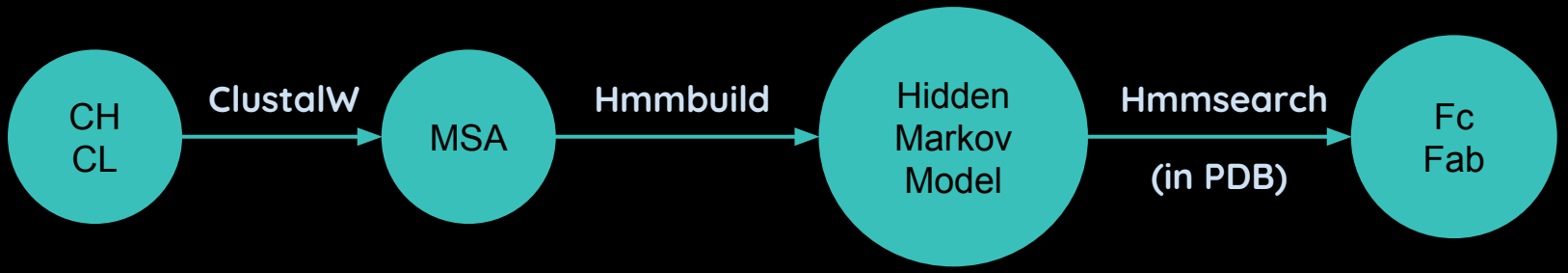


- CH
- VH
- CL
- VL

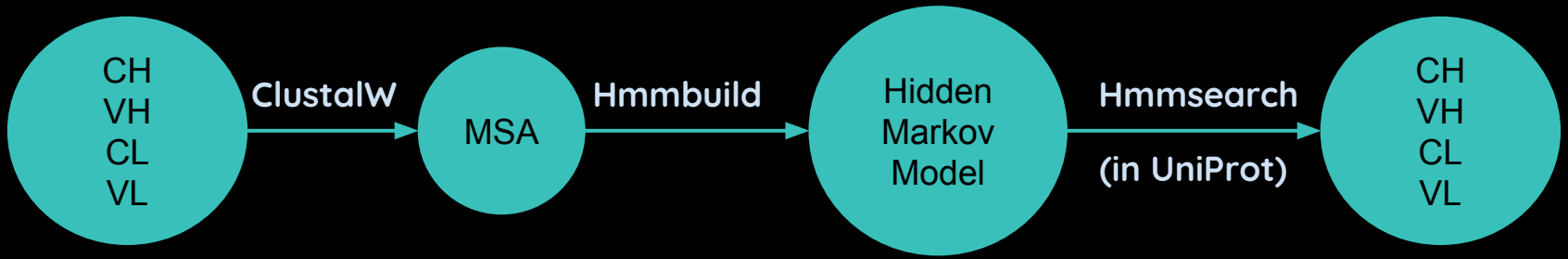
Methods of the evolutive study

Obtaining the data

Structures



Sequences



Methods of the evolutive study

Solving database problems: obtained HMMs

PDB

1IGT

- LC
- HC (Fab)
- HC (Fc)

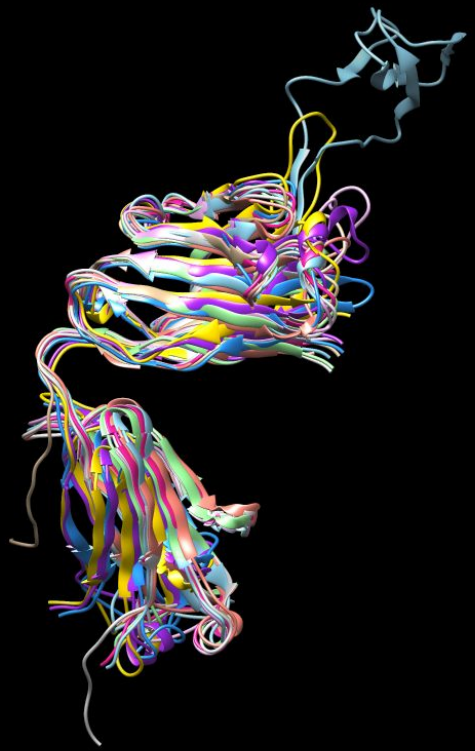
Methods of the evolutive study

Solving database problems: obtained superimpositions

HC (Fc)

n=11

HC (Fab)



n=10

LC (Fab)

n=10

Methods of the evolutive study

Solving database problems: obtained MSA

Fab (HC)

- VH
- CH1

Fab (LC)

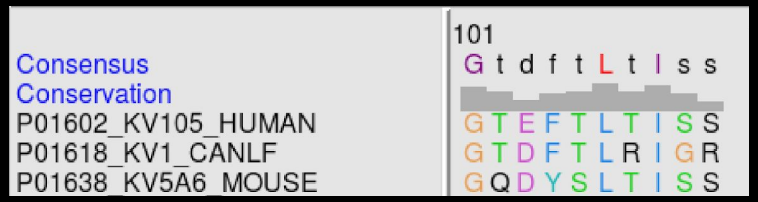
- VL
- CL

Fc

- CH2-3

1IGT

Visualization of the results

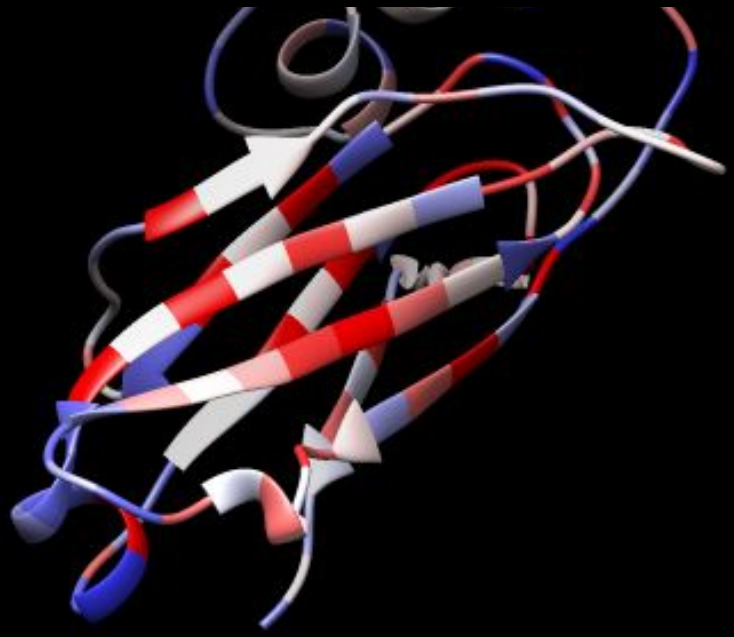


Clustal X Default Colouring

Category	Colour	Residue at position	{ Threshold, Residue group }
Hydrophobic	BLUE	A,I,L,M,F,W,V	{>60%, WLVIMAFCHP}
		C	{>60%, WLVIMAFCHP}
Positive charge	RED	K,R	{>60%,KR},{>80%, K,R,Q}
Negative charge	MAGENTA	E	{>60%,KR},{>50%,QE},{>85%,E,Q,D}
		D	{>60%,KR}, {>85%, K,R,Q}, {>50%,ED}
Polar	GREEN	N	{>50%, N}, {>85%, N,Y}
		Q	{>60%,KR},{>50%,QE},{>85%,Q,E,K,R}
		S,T	{>60%, WLVIMAFCHP}, {>50%, TS}, {>85%,S,T}
Cysteines	PINK	C	{>85%, C}
Glycines	ORANGE	G	{>0%, G}
Prolines	YELLOW	P	{>0%, P}
Aromatic	CYAN	H,Y	{>60%, WLVIMAFCHP}, {>85%, W,Y,A,C,P,Q,F,H,I,L,M,V}
Unconserved	WHITE	any / gap	If none of the above criteria are met

ClustalX coloring method

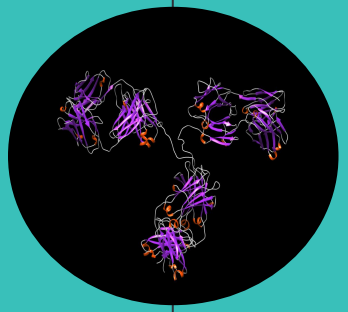
Visualization of the results



Highly variable

Highly conserved

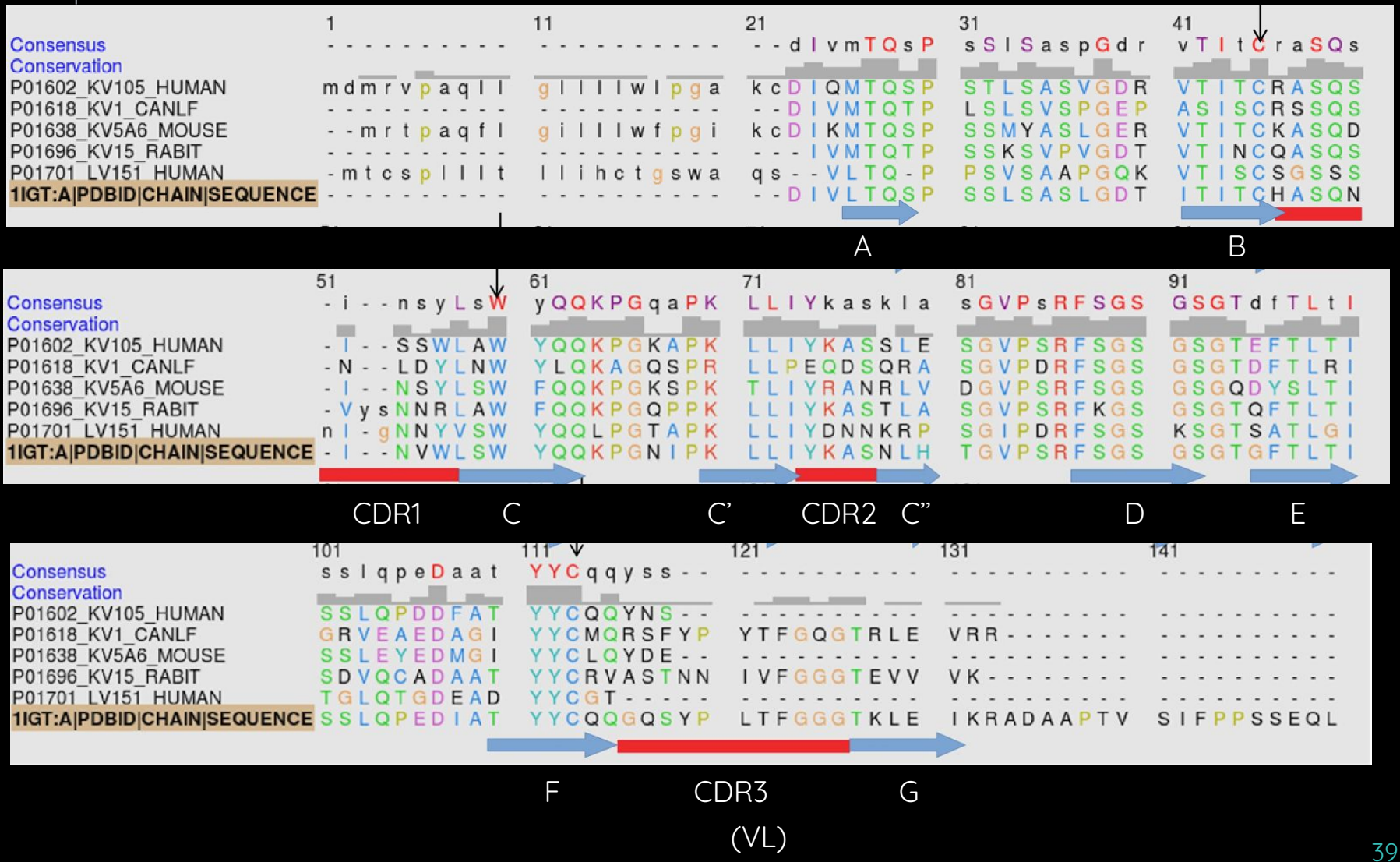
1IGT



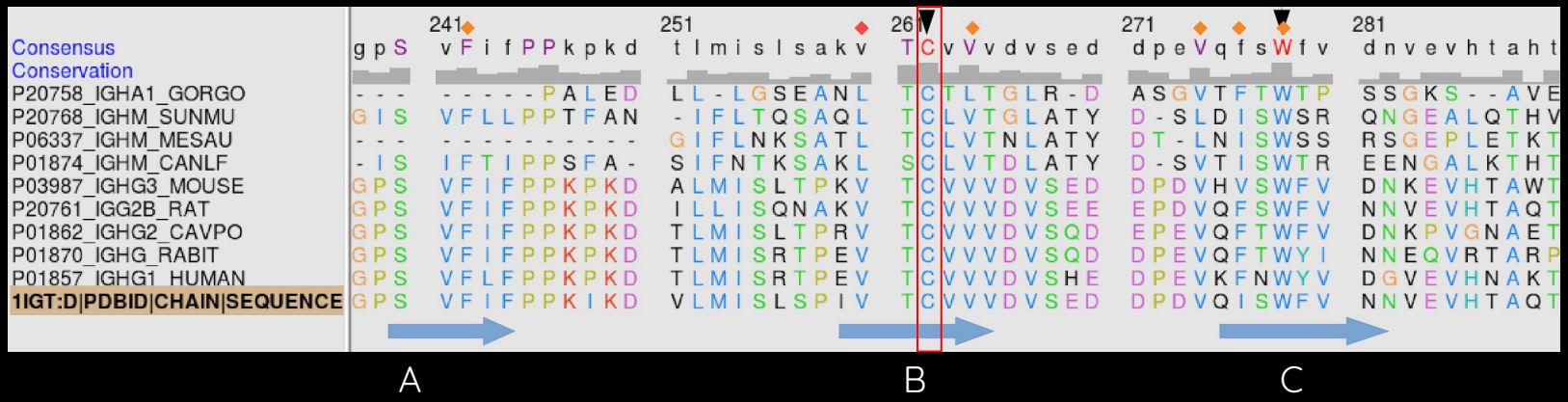
Evolution of IgG chains

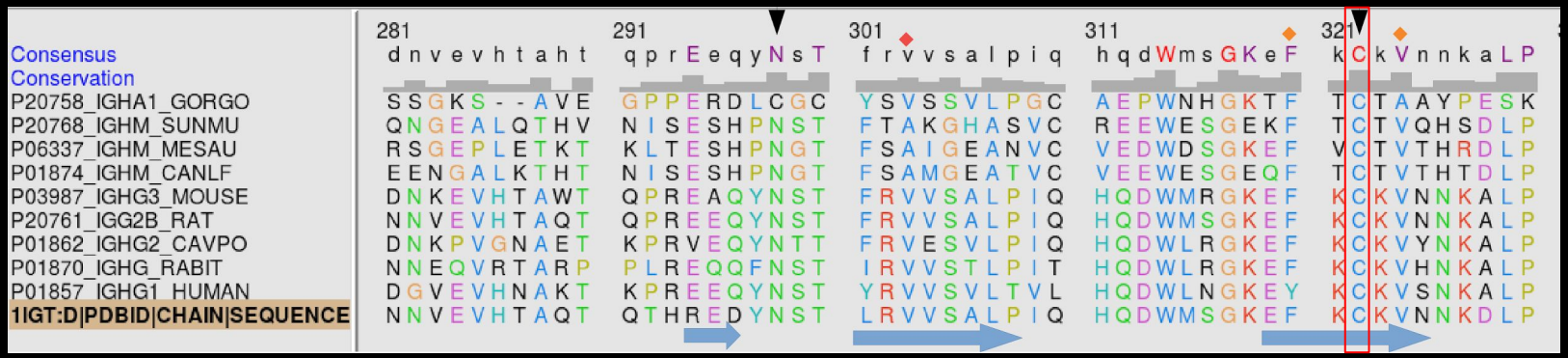
Results

CDR are highly variable regions



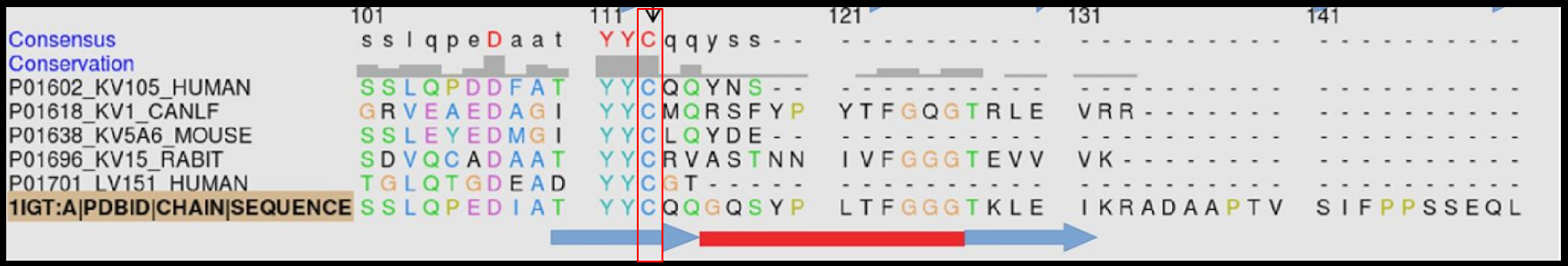
Disulphide bridge-forming Cys are totally conserved in constant and variable Ig domains





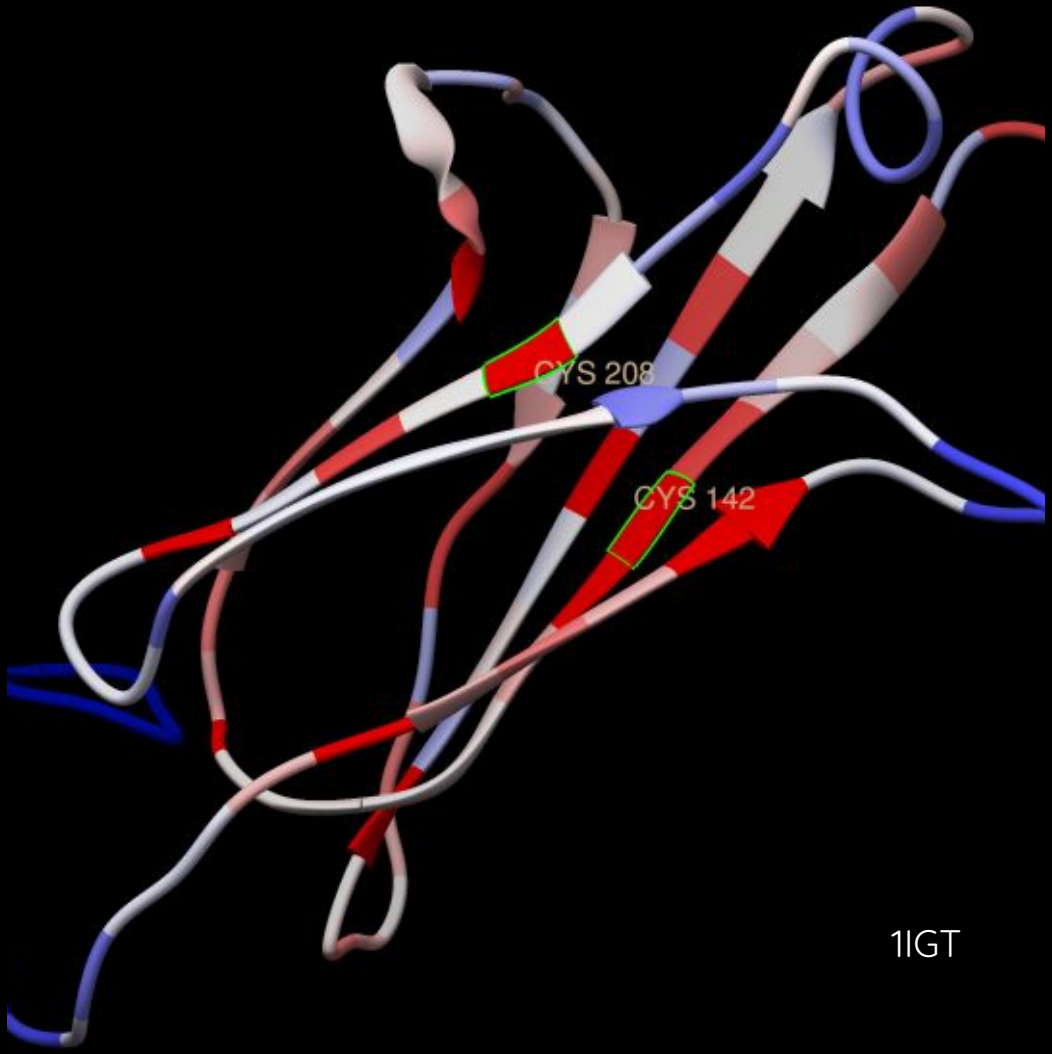
(CH2)

Disulphide bridge-forming Cys are totally conserved in constant and variable Ig domains



(VL)

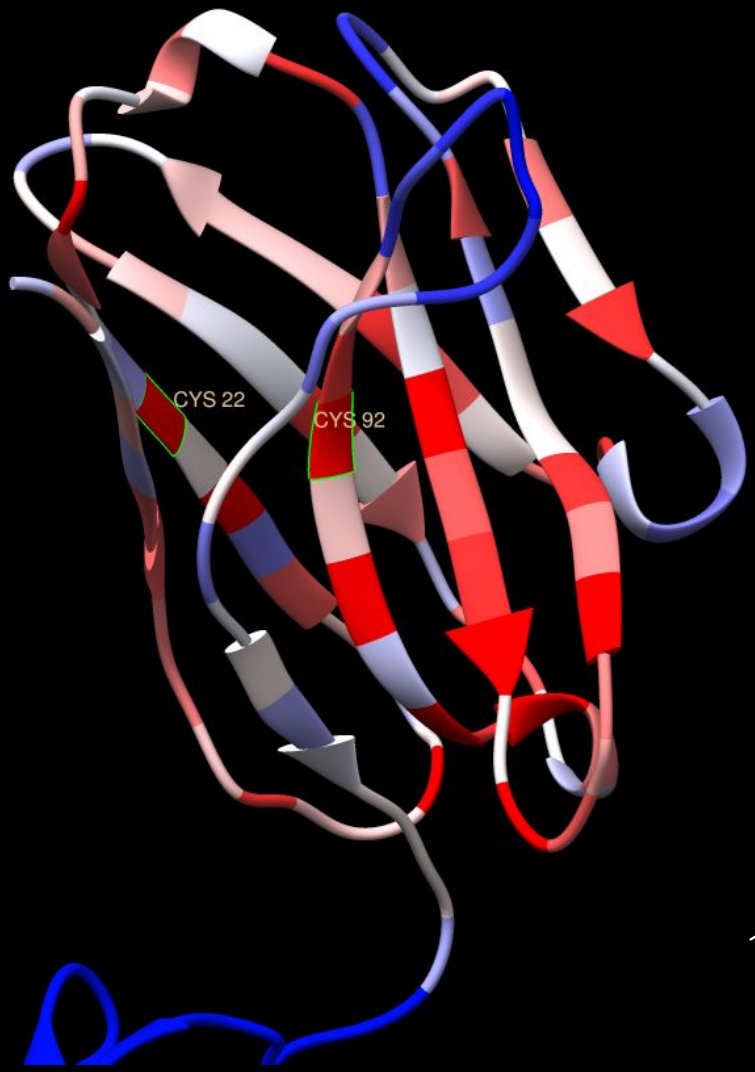
Cys residues are greatly conserved in constant and variable Ig domains



1IGT

(CH1)

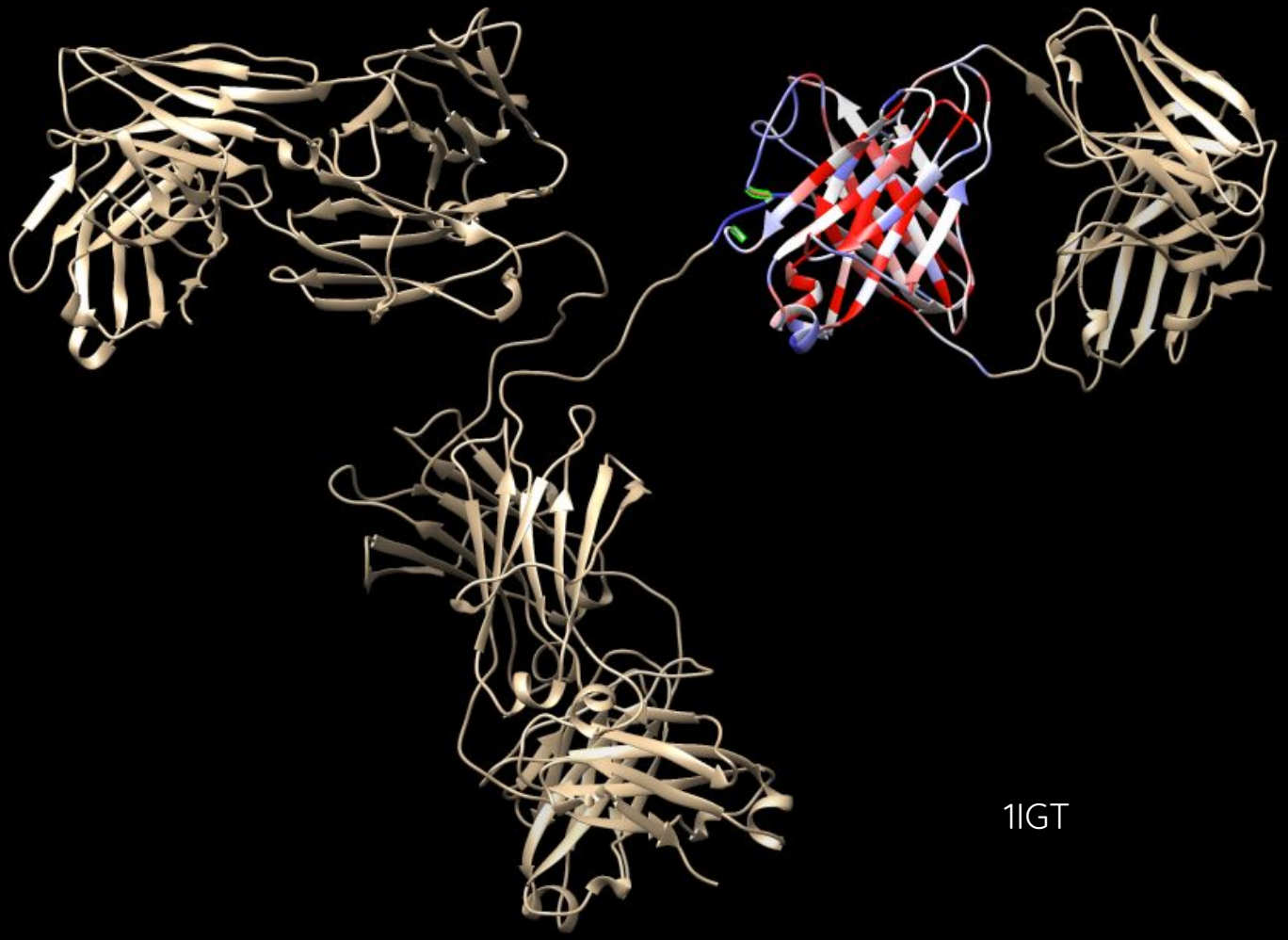
Cys residues are greatly conserved in constant and variable Ig domains



1IGT

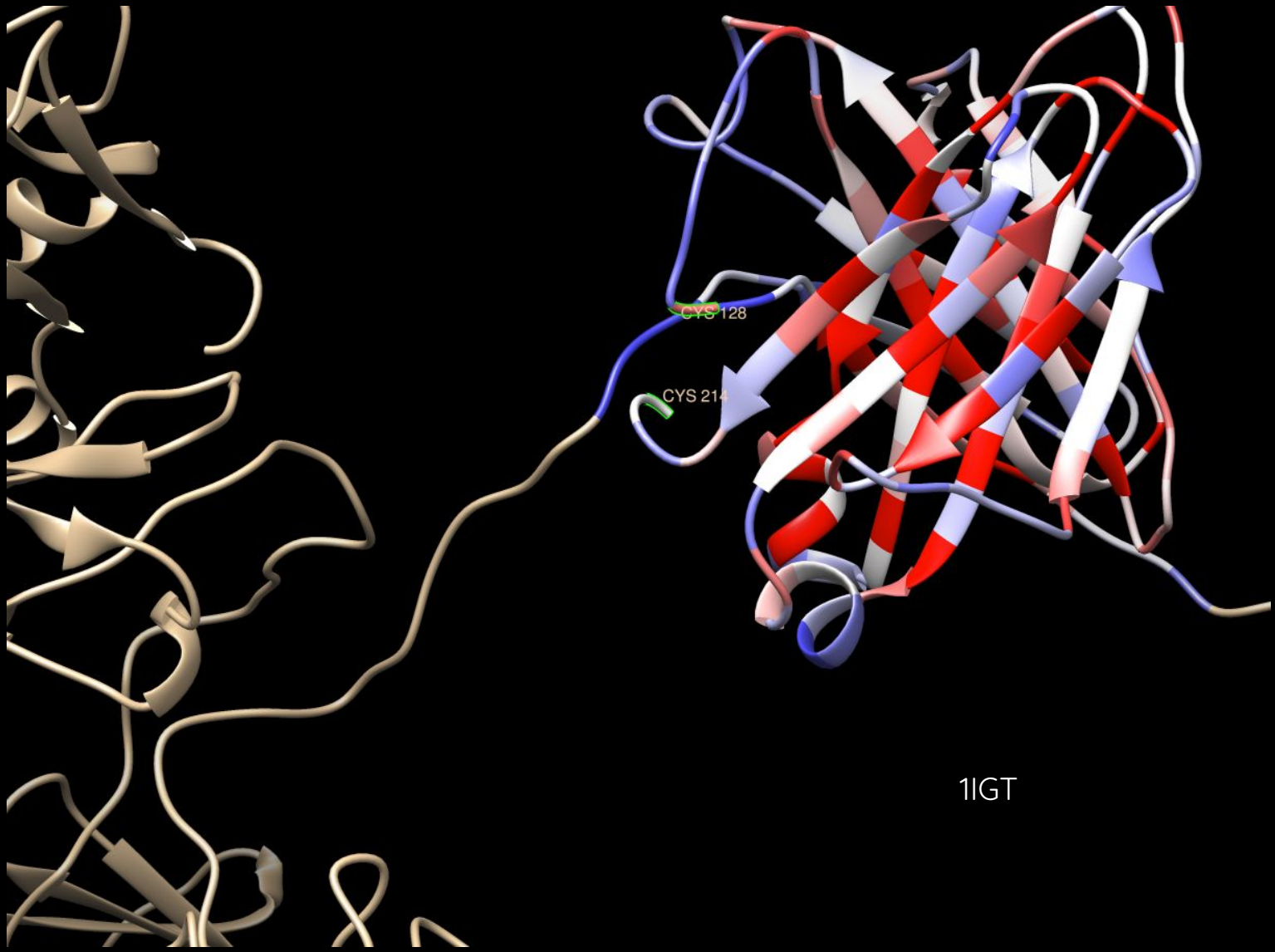
(VH)

Cys joining HC and LC are greatly conserved



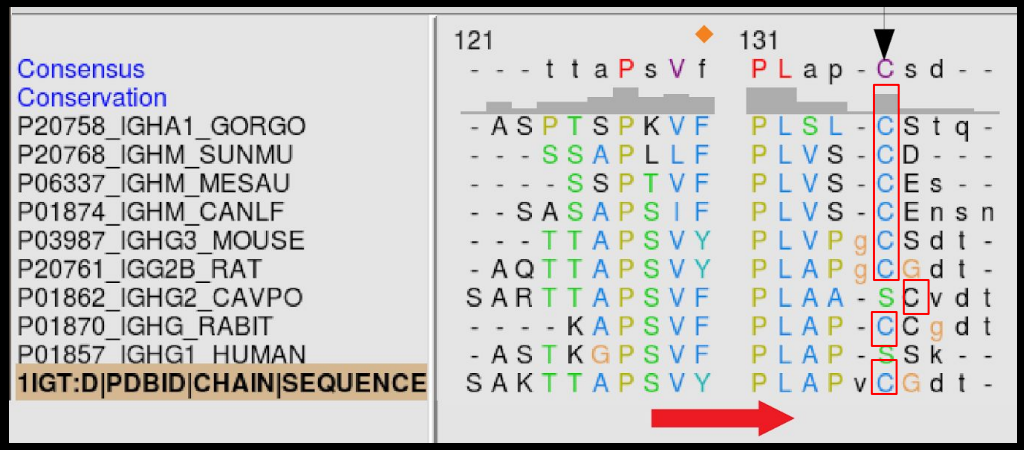
1IGT

Cys joining HC and LC are greatly conserved



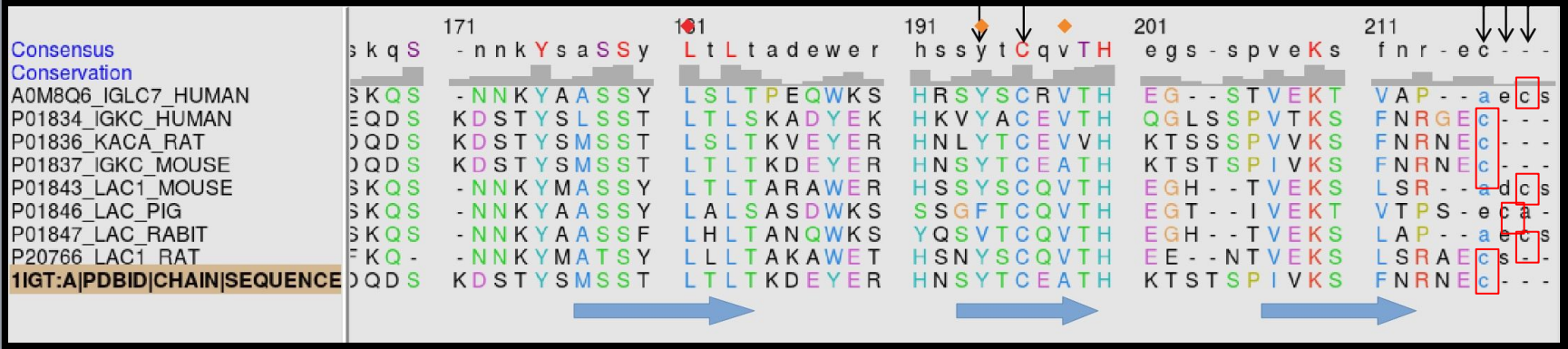
1IGT

Cys joining HC and LC are greatly conserved



A

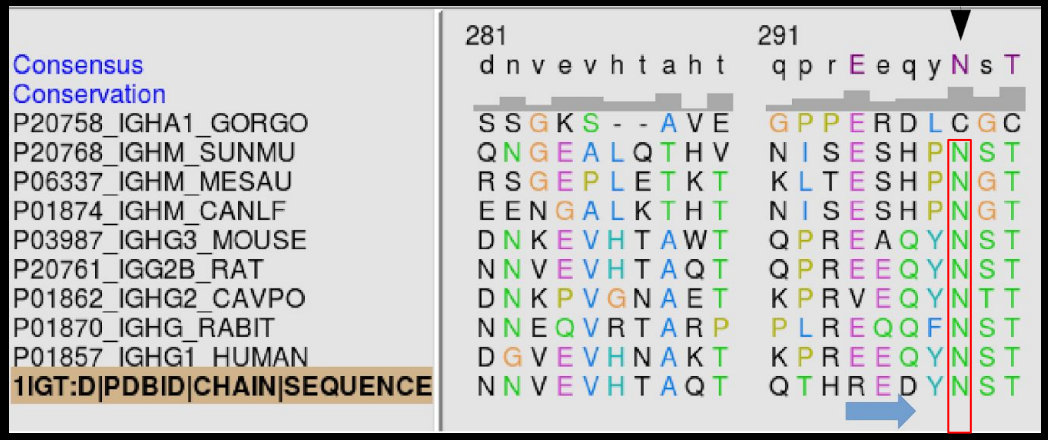
(CH1)



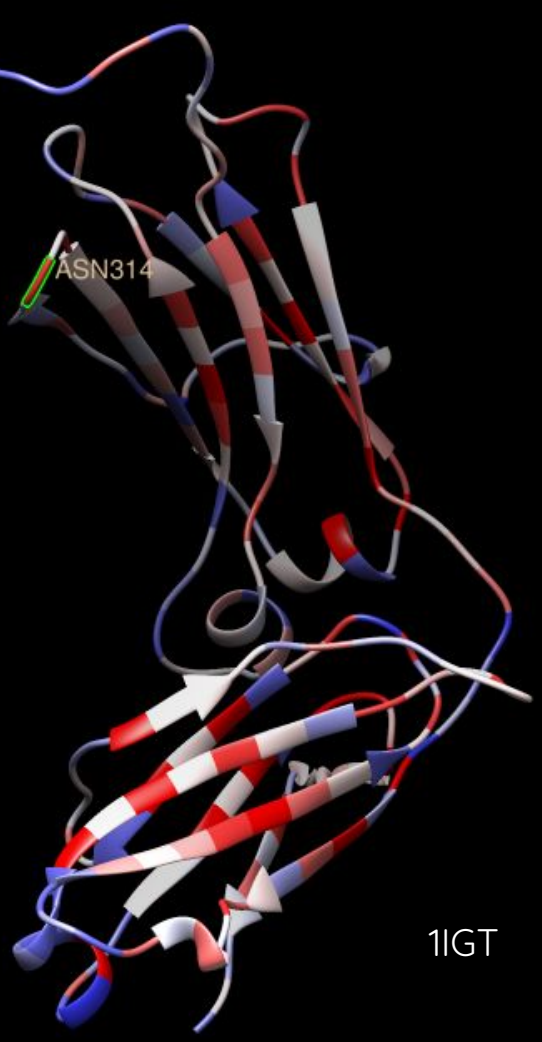
E F G

(CL)

Asn314 is necessary for glycosylation

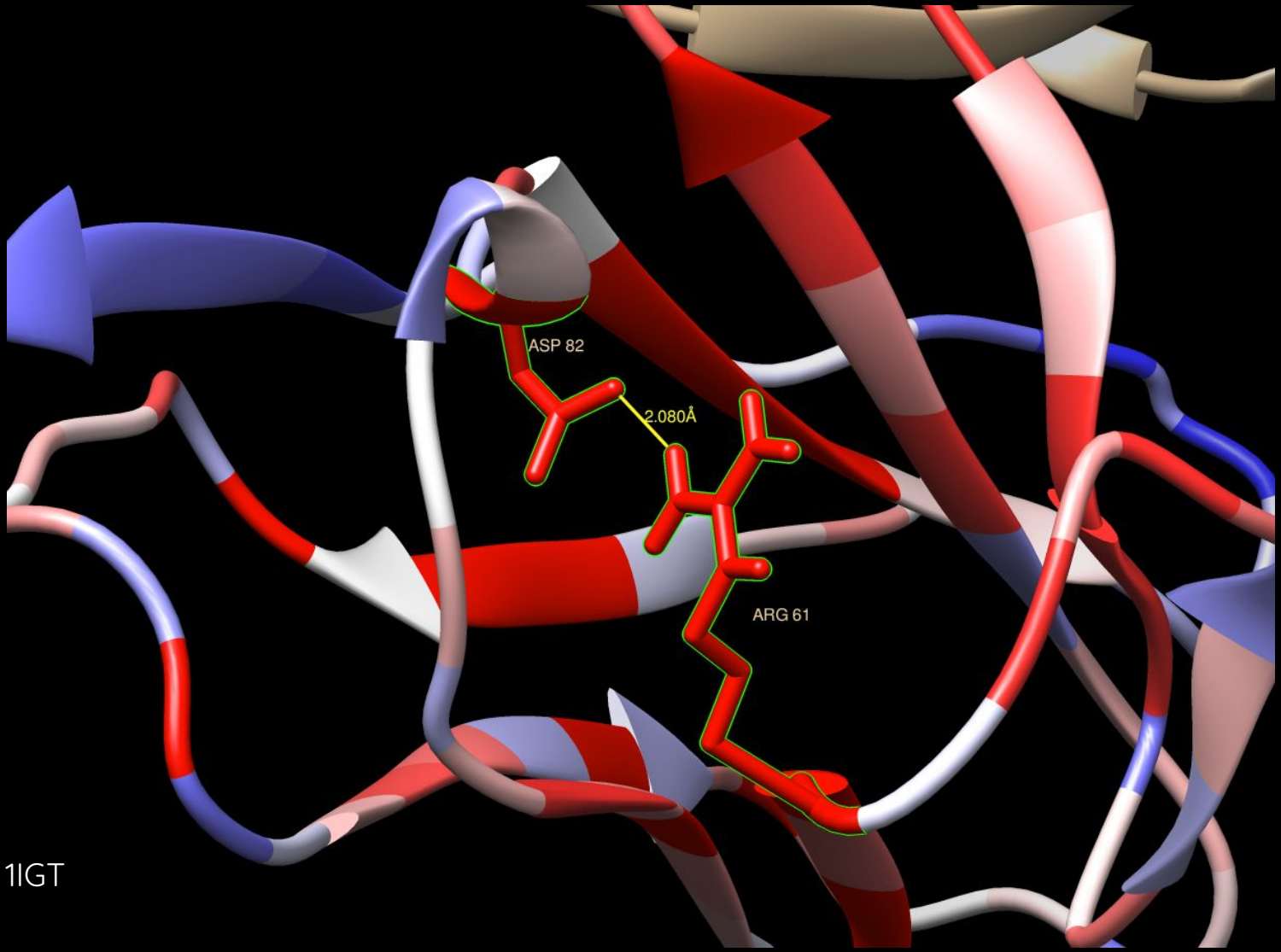


(CH2)



1IGT

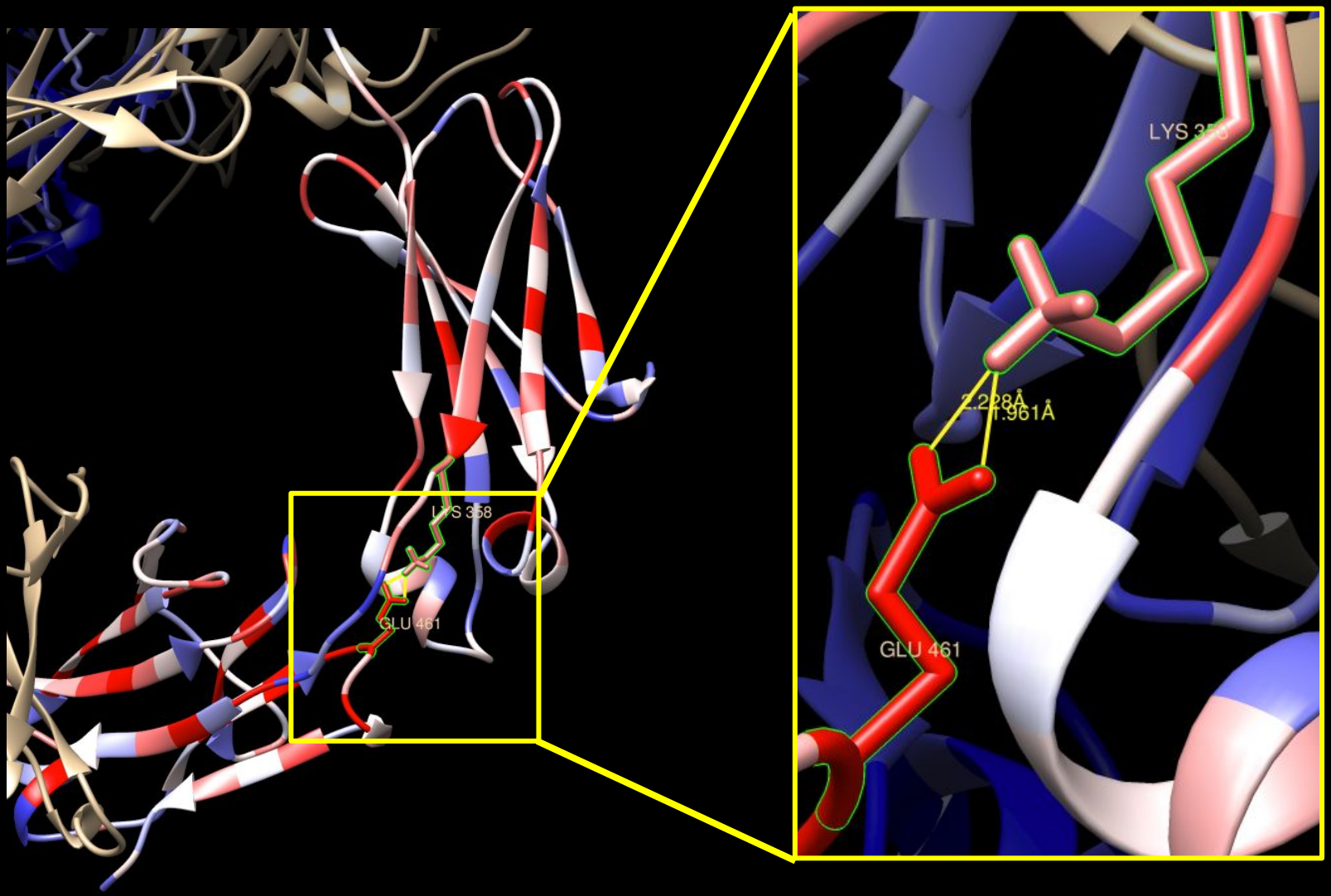
Conservation of charged residues: Within the same region



1IGT

(VL)

Conservation of charged residues: Between two regions



1IGT

(CH2 and CH3)

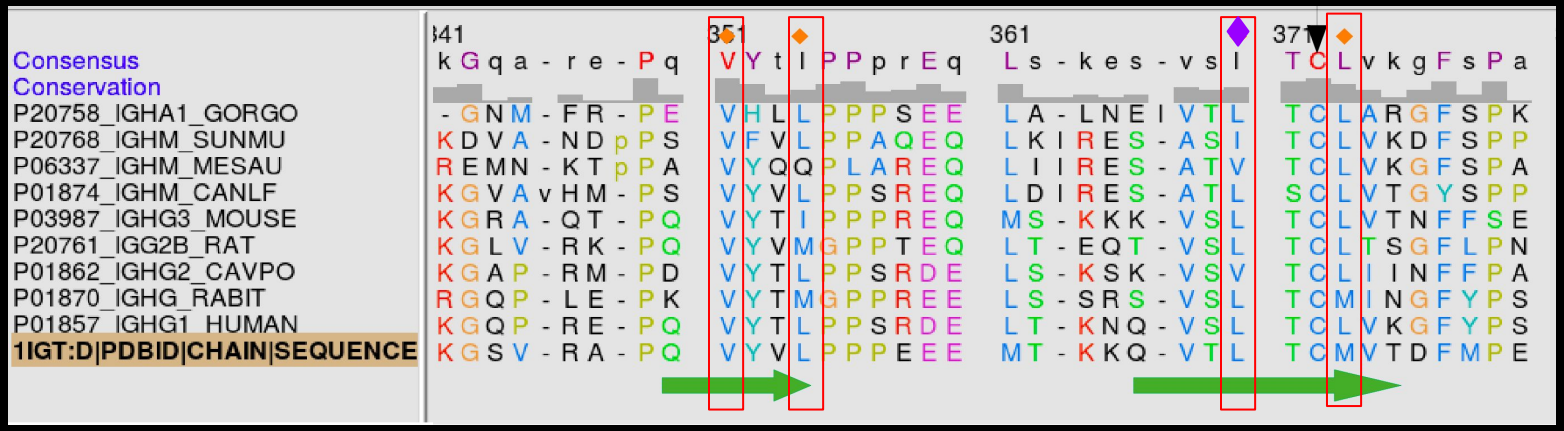
Hydrophobic side-chains are faced inside of the beta chains

1IGT

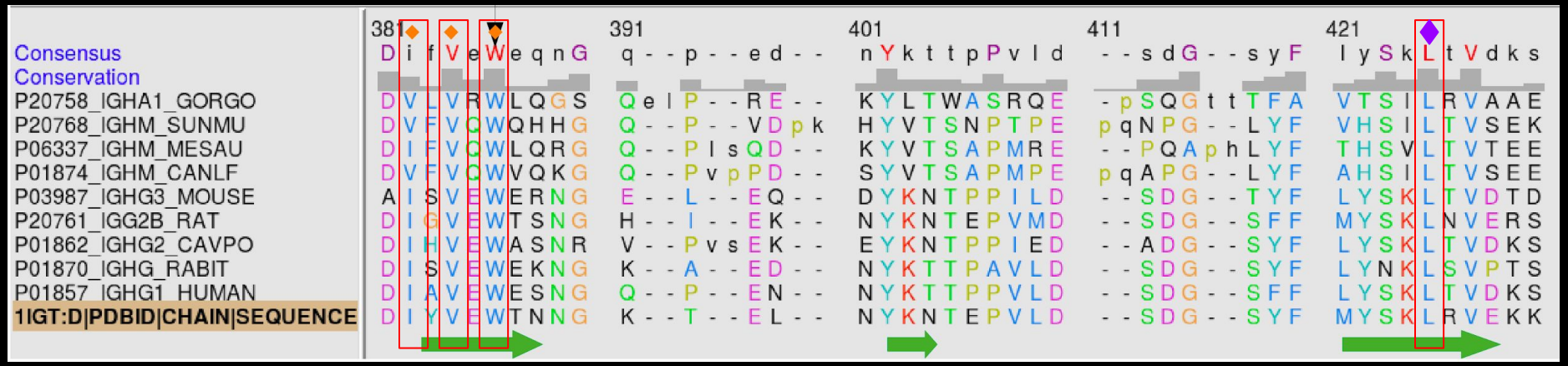
Hydrophobic
Polar
Cys

(CH2 and CH3)

There are many conserved topohydrophobic amino acids



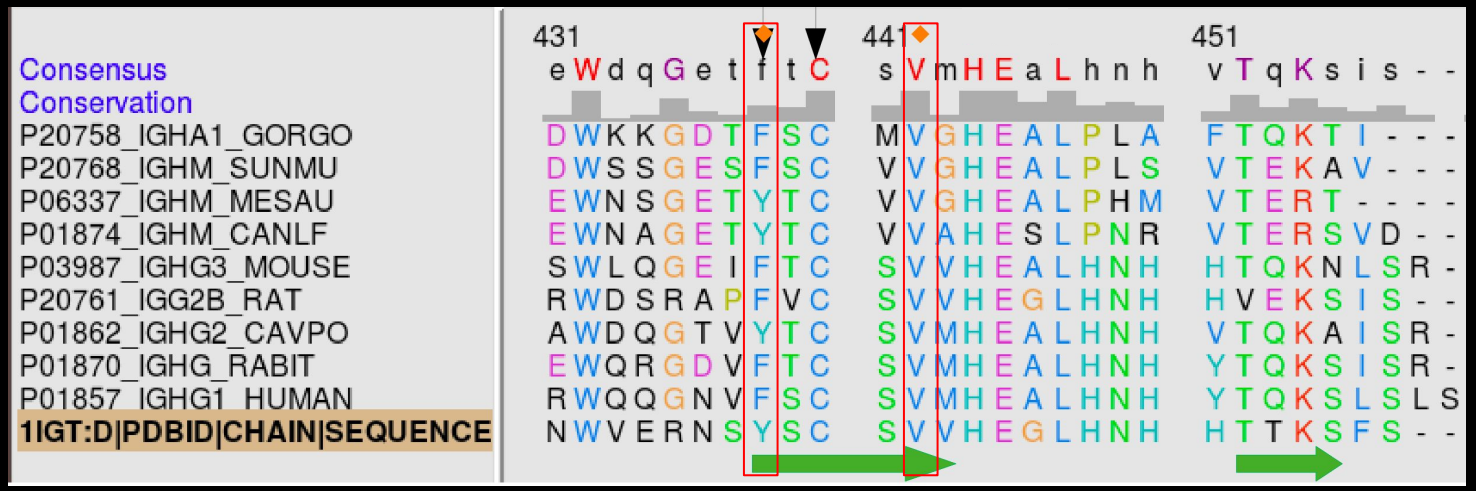
A B



C D E

(CH3)

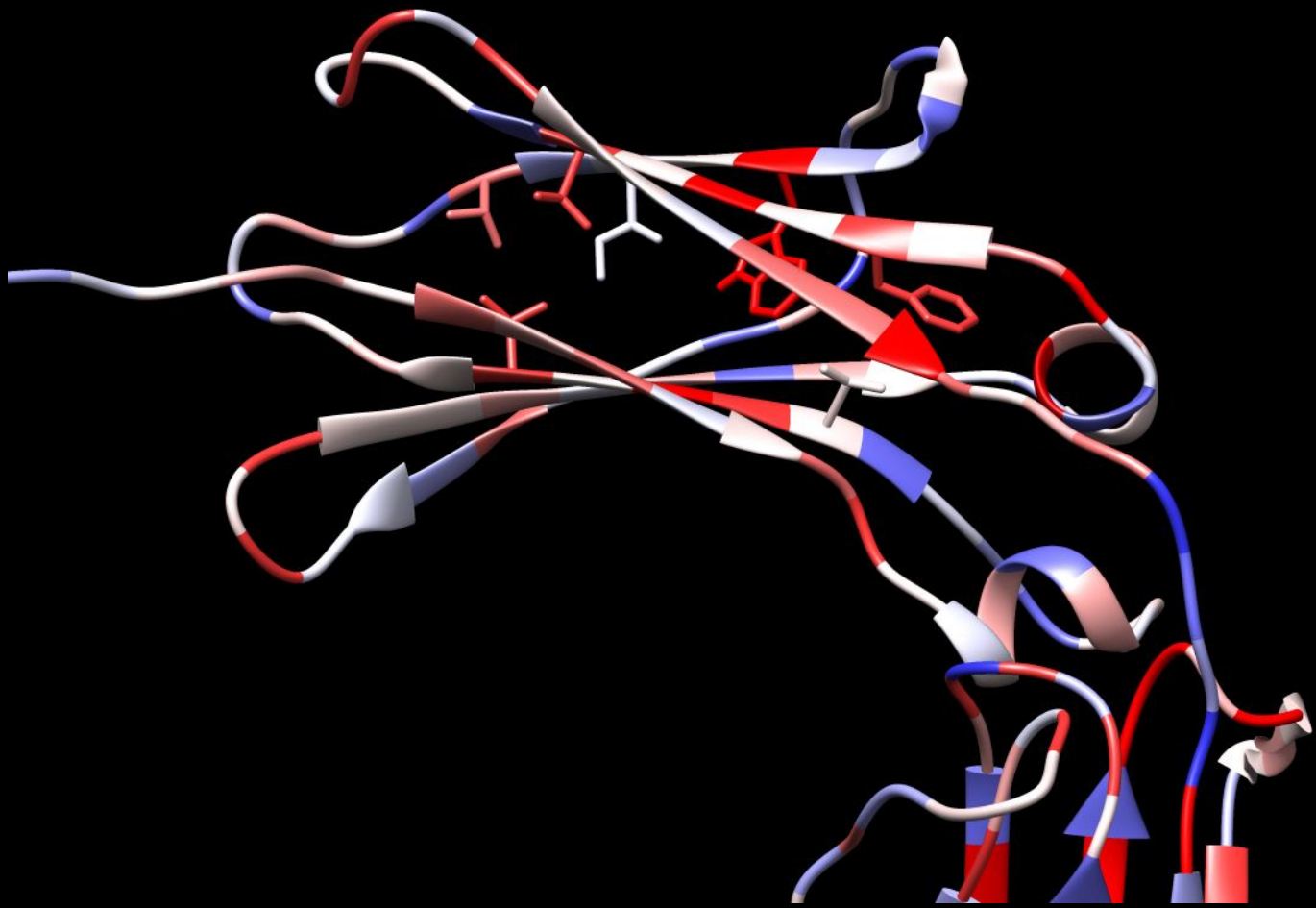
There are many conserved topohydrophobic amino acids



Type of amino acid	Position	Symbol
Topohydrophobic	A3, A7, B4, C1, C3, C5, F1, F5	◆
Aliphatic	B1, E5	◆

(CH3)

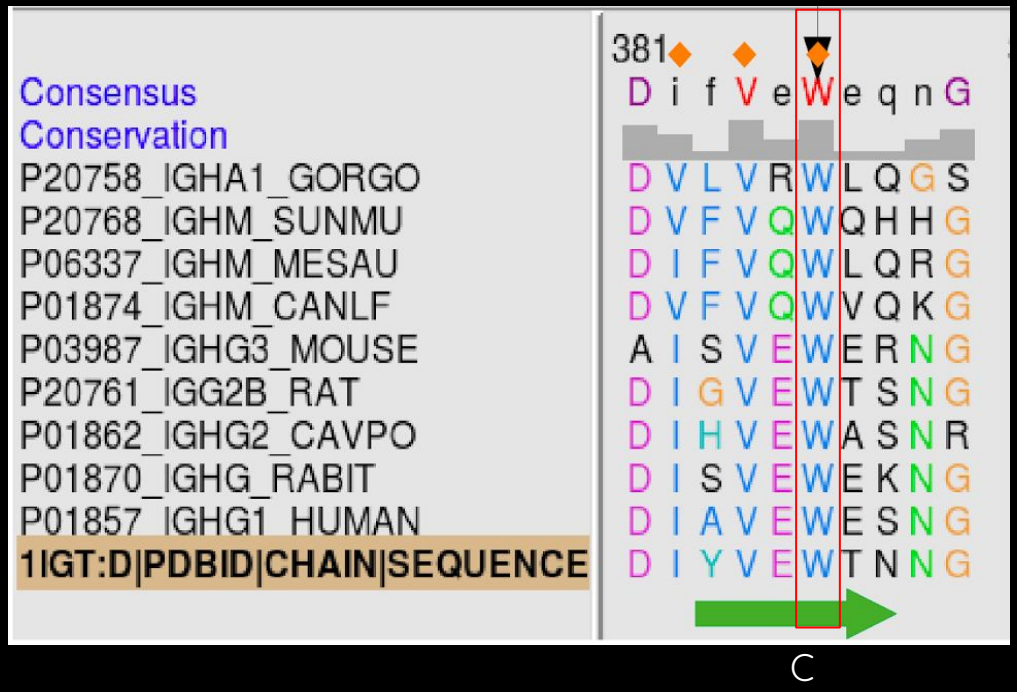
Topohydrophobic side-chains are faced inside of the beta chains



1IGT

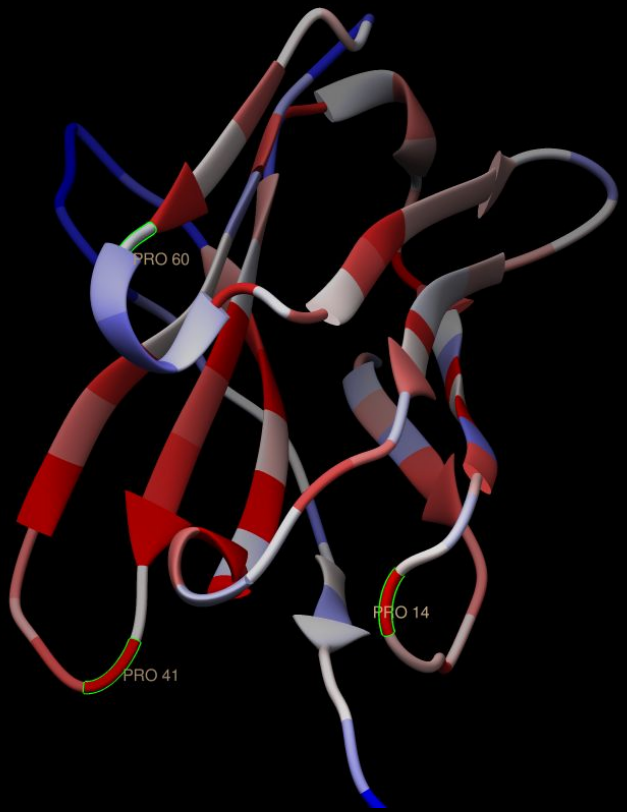
(CH2)

Trp (in C5) is highly conserved



(CH3)

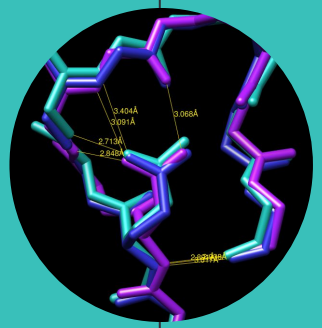
The role of proline



(VH)

1IGT

(hinge, CH2 and CH3)



CDRs: Canonical Structures

Canonical structures for the hypervariable regions of immunoglobulins

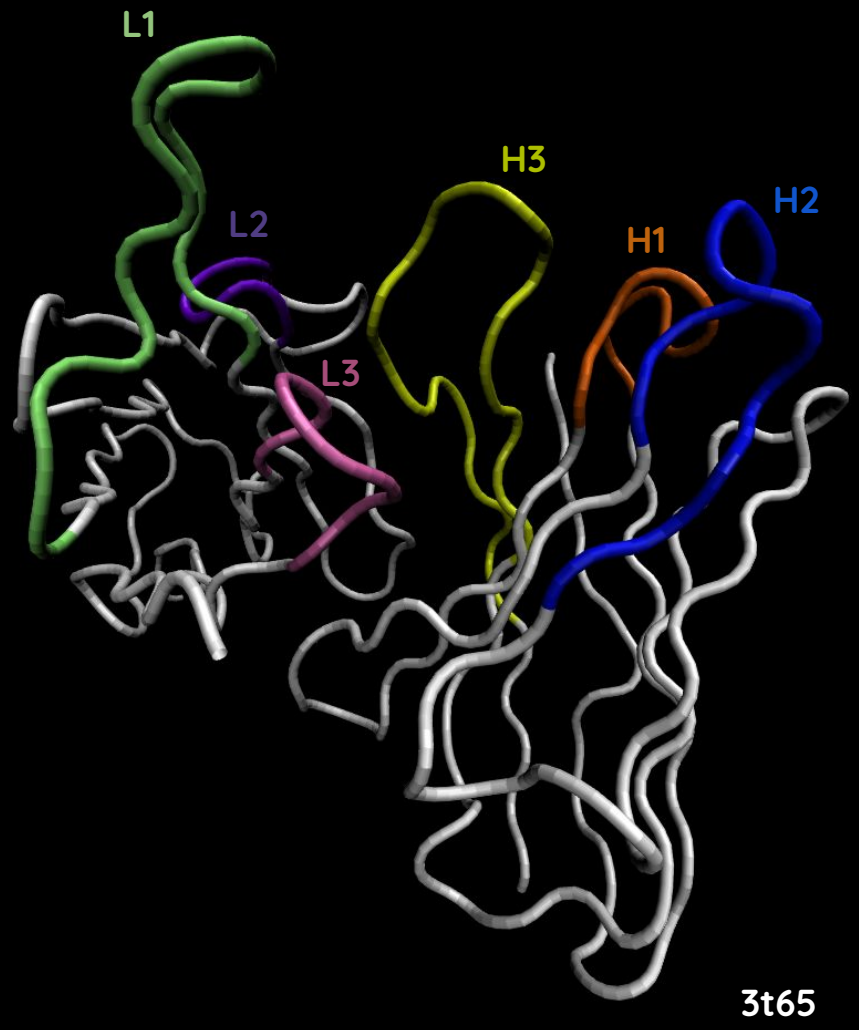
Light chain

L1	**κ→** 1, **2a, 2b**, 3, 4, 5, 6 **λ→** 1, 2, **3a**, 3b, 4
L2	**1**
L3	**κ→ 1,** 2, 3, 4, 5 **λ→ 1a, 1b, 1c,** 2

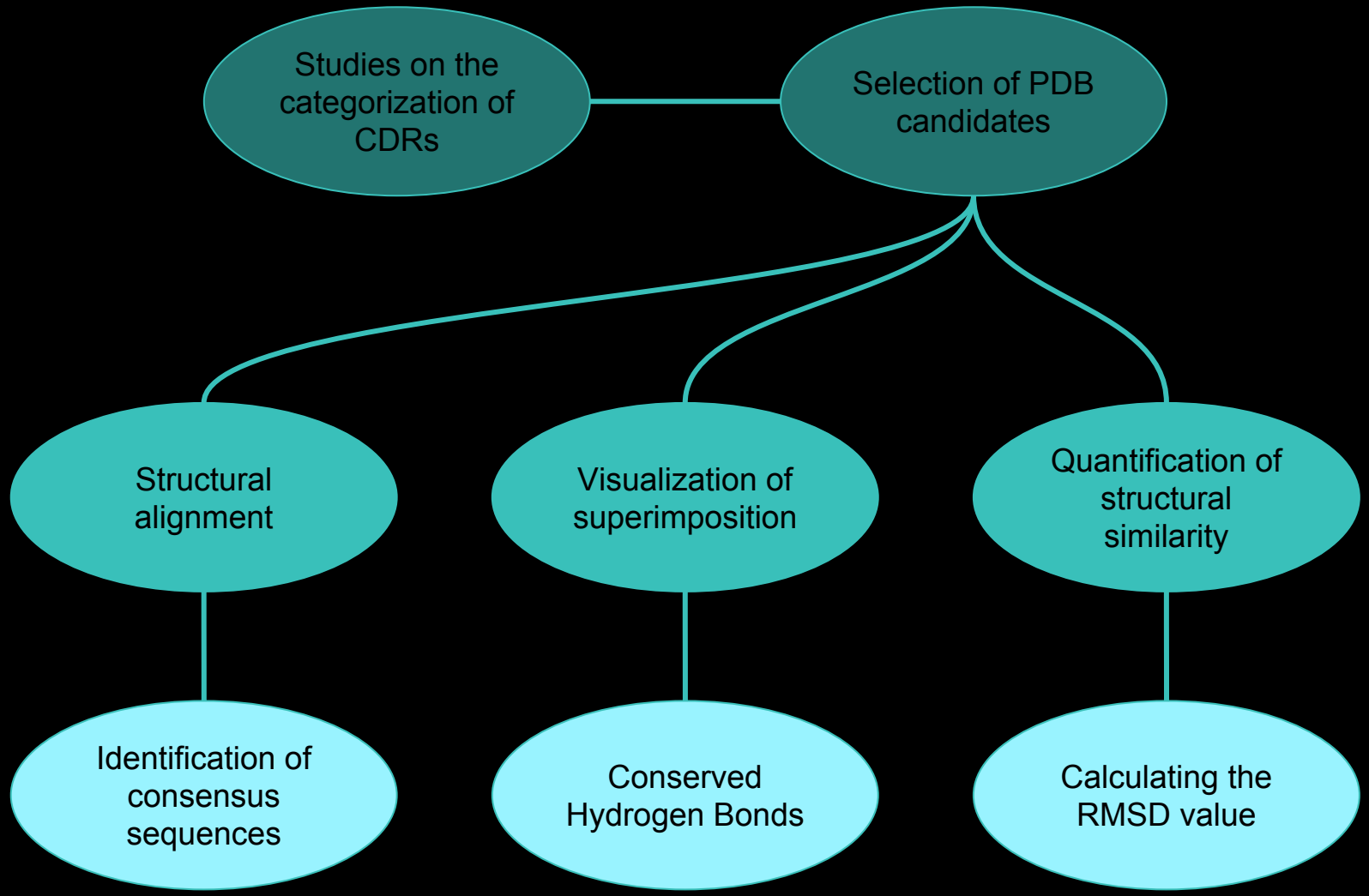
Heavy chain

H1	**1**, 2, 3
H2	1, **2a,** 2b, 2c, 3a, **3b,** 3c, 4
H3	No canonical structures

Chothia *et al.* classification



Methodology



L1-κ2

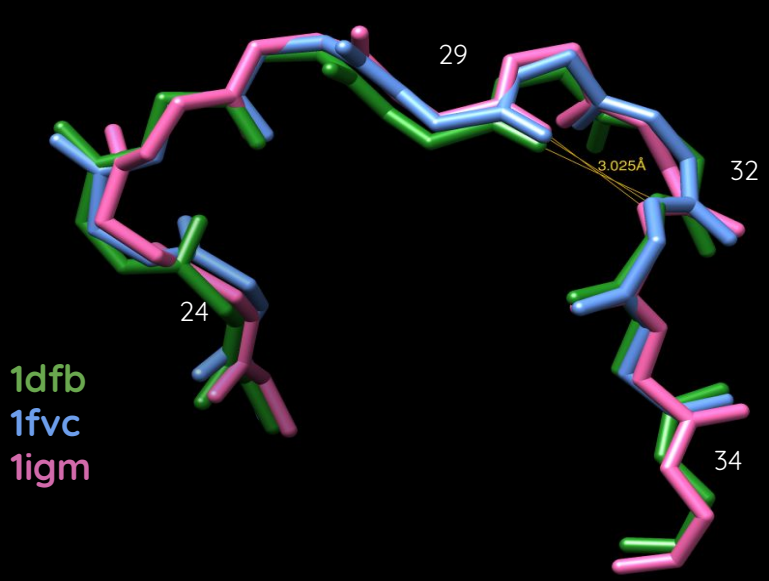
L1-κ2a

HBond: 29 (O) - 32 (N)

Consensus: rASQdisnyla

```
1dfb  GDRVTITCRASQSISRWLAWYQQKP
1fvc  GDRVTITCRASQDVNTAVAWYQQKP
1igm  GDRVTITCQASQDISNYLAWYQQKP
```

RMSD = 0,721



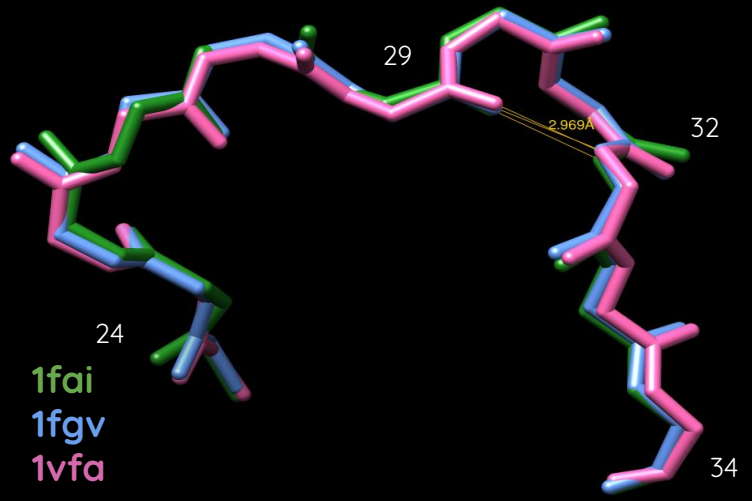
L1-κ2b

HBond: 29 (O) - 32 (N)

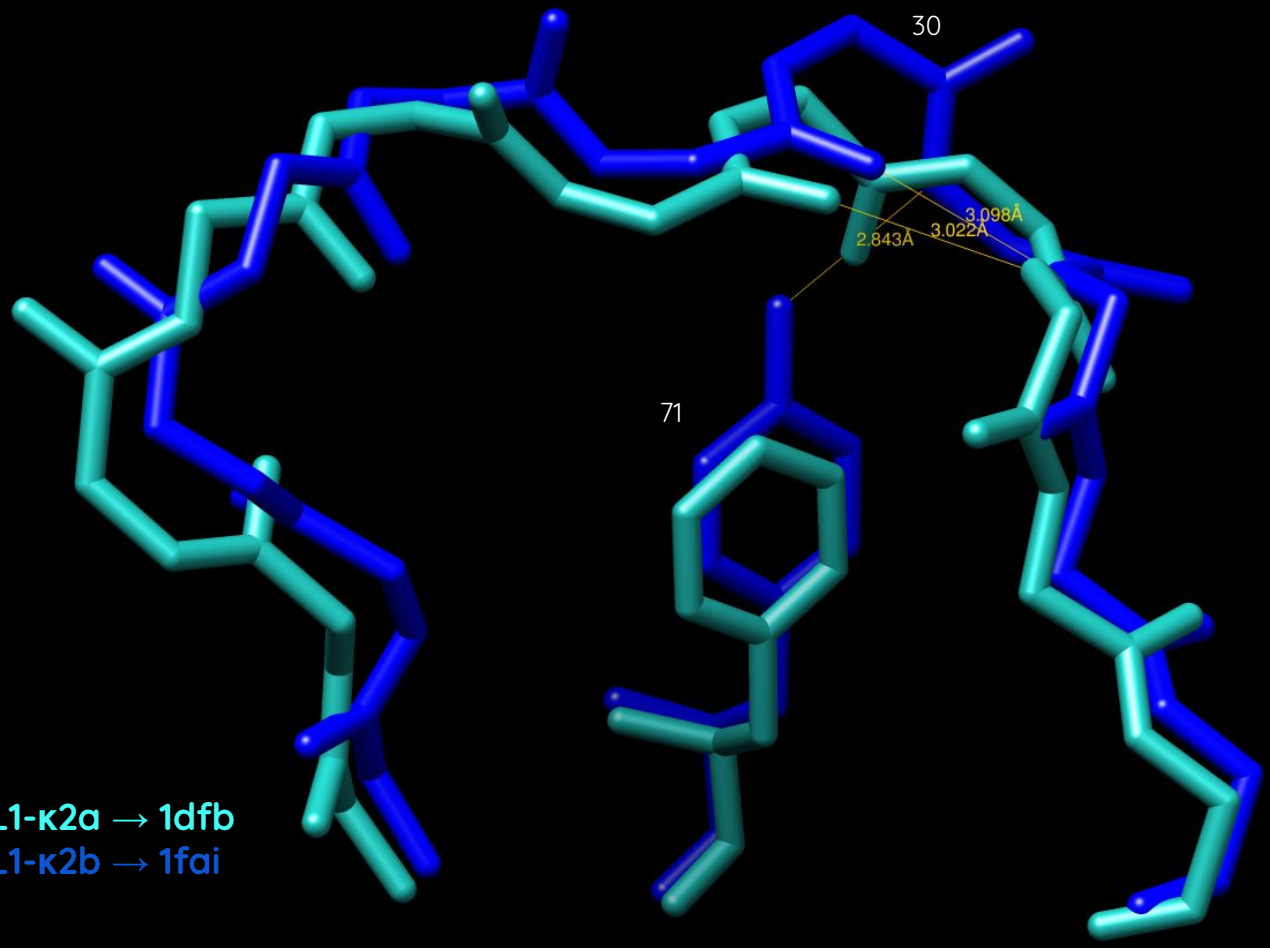
Consensus: rASqdIsnyLn

```
1fai  ISCRASQDISNYLNWYQQKPDGTVK
1fgv  ITCRASQDINNYLNWYQQKPGKAPK
1vfa  ITCRASGNIHNYLAWYQQKQGKSPQ
```

RMSD = 0,807



L1-κ2



L1-κ2a → 1dfb
L1-κ2b → 1fai

L1-κ2a	**Phe71**
L1-κ2b	**Tyr71**

HBond: 71 (O) --- 31 (N)

L1-λ3a

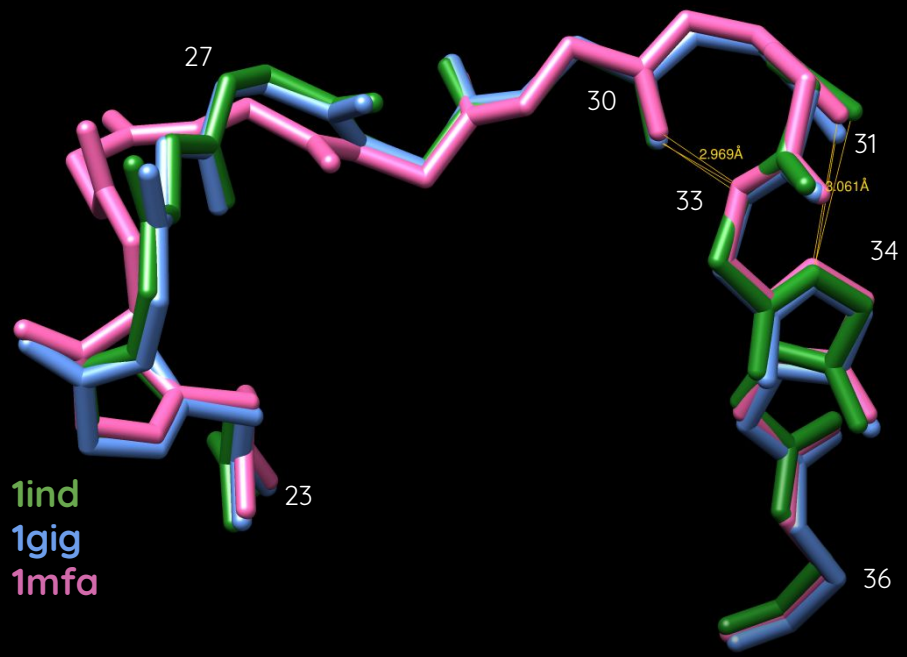
HBond:

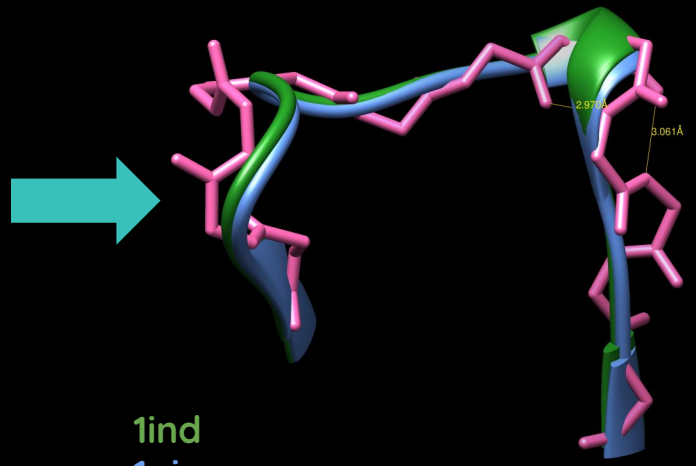
30 (O) - 33 (N)
31 (O) - 34 (N)

Consensus: RSStGavTtsNYAn

```
1ind  VTLTCRSSTGAVTTSNYANWVQEKPD
1gig  VTLTCRSSTGAVTTSNYANWVQEKPD
1mfa  VTLTCRSSTGTVTSGNHANWVQEKPD
```

RMSD = 0,890





1mfa
1gig and 1ind

Tyr28
Ala28

L2-1

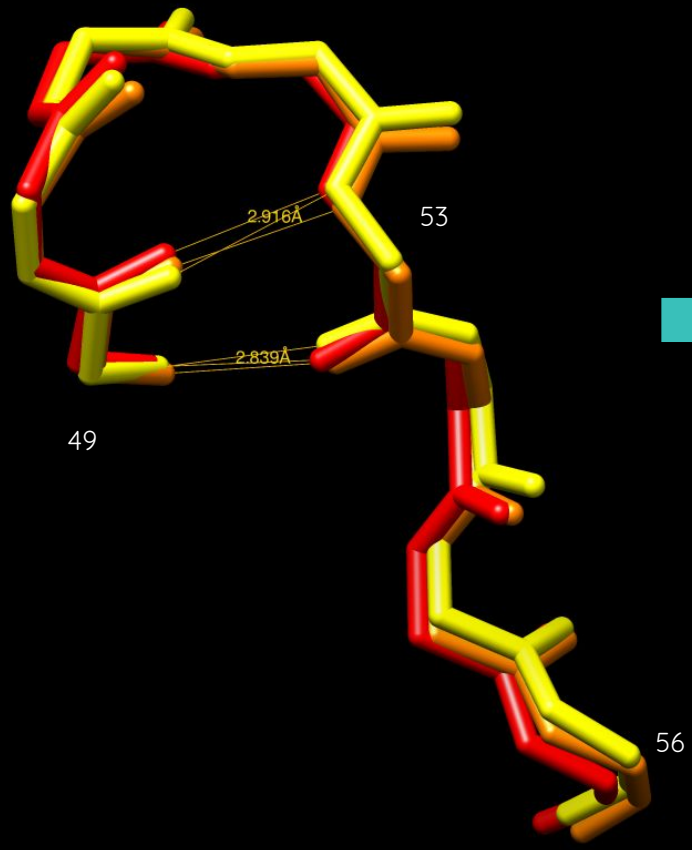
HBond:

49 (N) - 53 (O)
49 (O) - 53 (N)

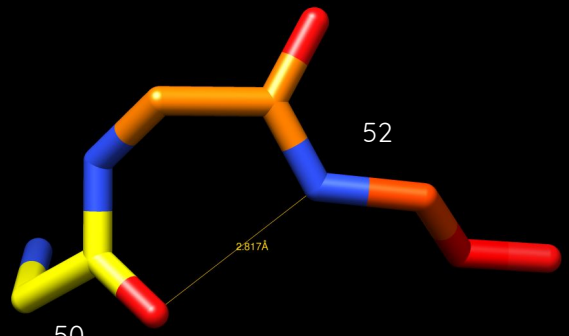
Consensus: Y-asnlas

```
1fgv KLLIYYTSTLESGVPSRFSGSGS
1flr KVLIYKVSNRFSGVPDRFSGSGS
1hil KVLIYWASTRESGVPDRFTGSGS
```

RMSD = 0,597



1fgv
1flr
1hil



HBond: 50 (O) - 52 (N)

L3-κ1

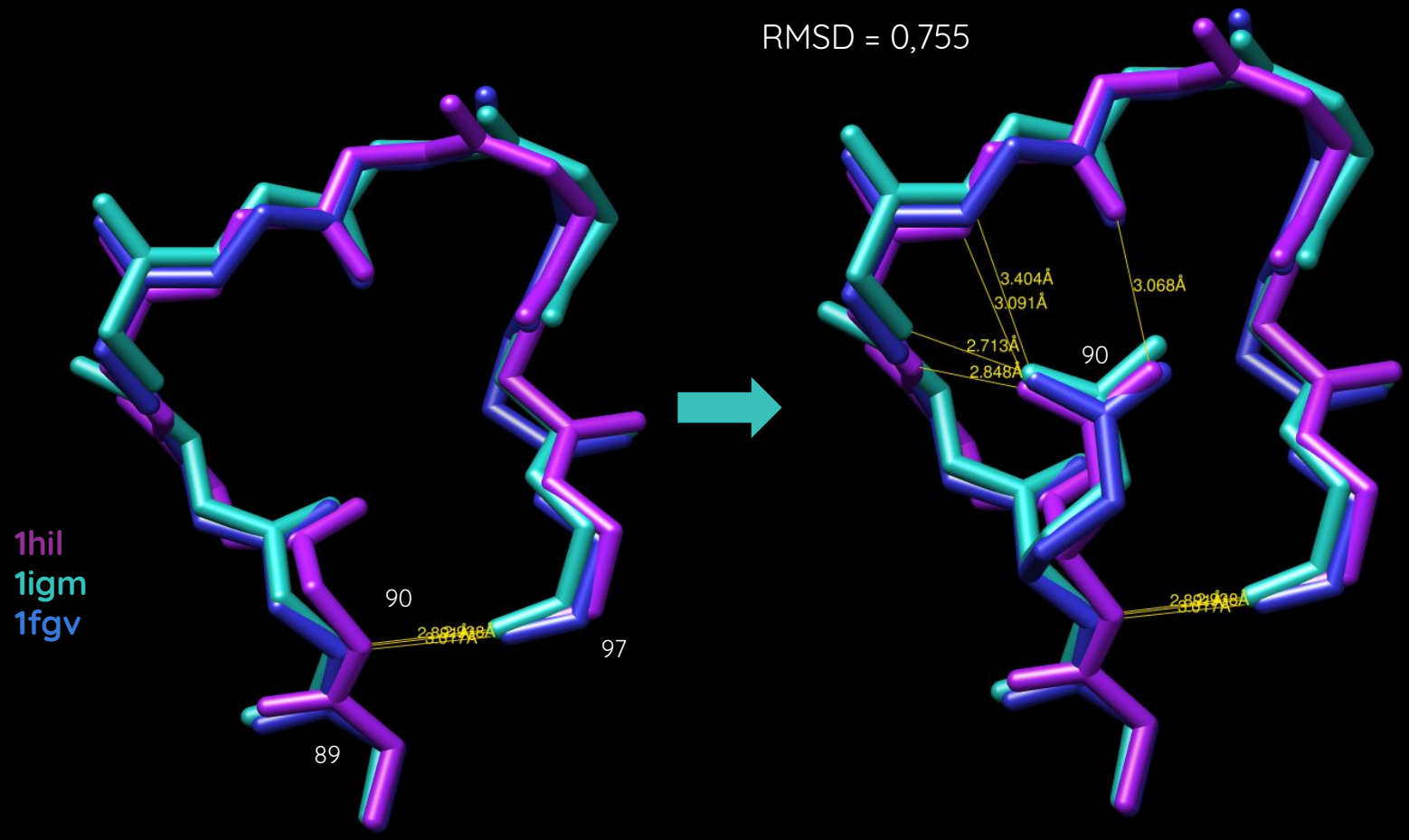
HBond:

90 (N) - 97 (O)

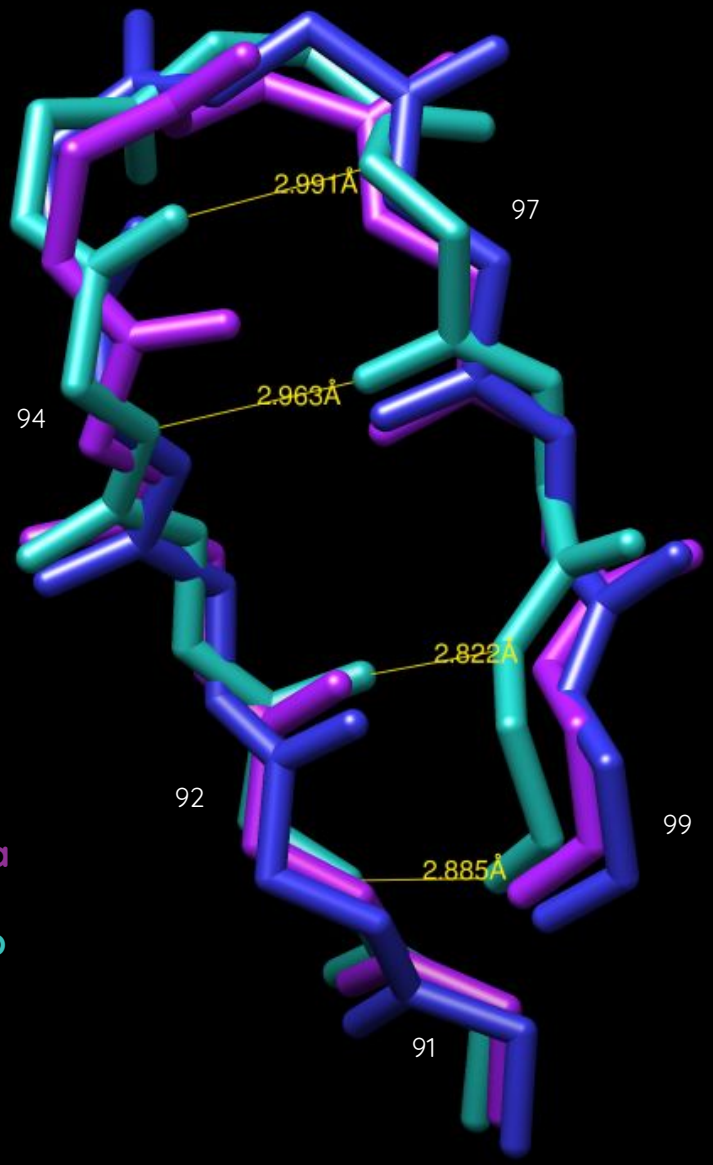
Consensus: qQgss-PIT

```
1hil  /YYCQNDYSNPLTFGGGTKLELK
1fgv  TYYCQQGNTLPPTFGAGTKVEIK
1igm  TYYCQQYQNLPLTFGPGTKVDIK
```

RMSD = 0,755



L3-λ1



HBond:
92 (N) - 99 (O)
92 (O) - 99 (N)
94 (N) - 97 (O)
94 (O) - 97 (N)

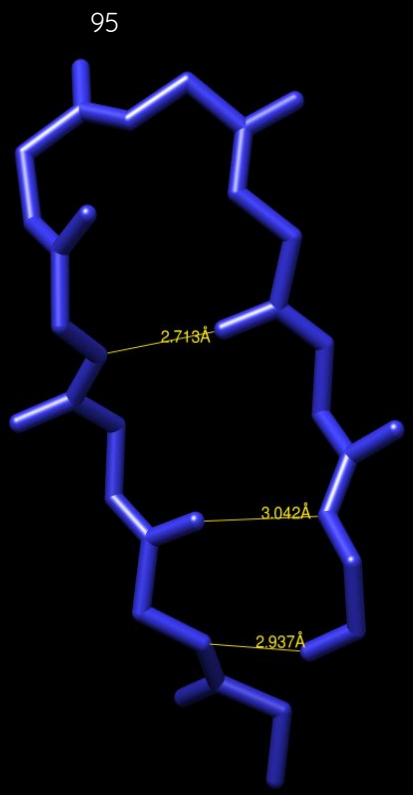
Consensus: alw-snhwv

```
1mfa YFCALWSNNHWIFGGGT
1gig YFCALWYSNHWVFGGGT
7fab YYCQSYDRSLRVFGGGT
```

RMSD = 1,045

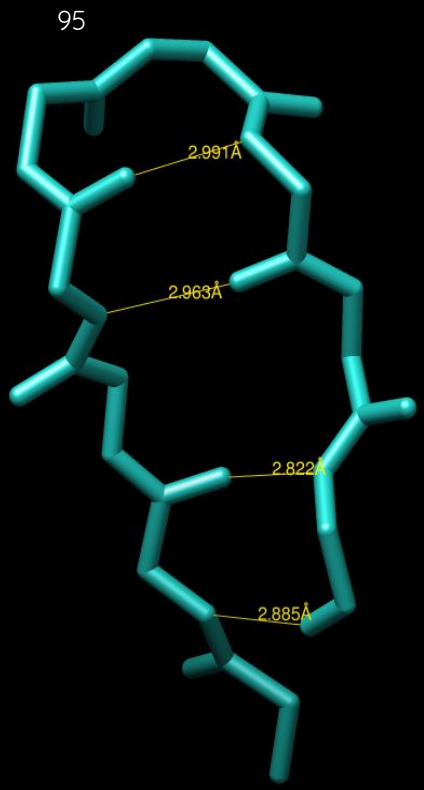
L3-λ1

L3-λ1a



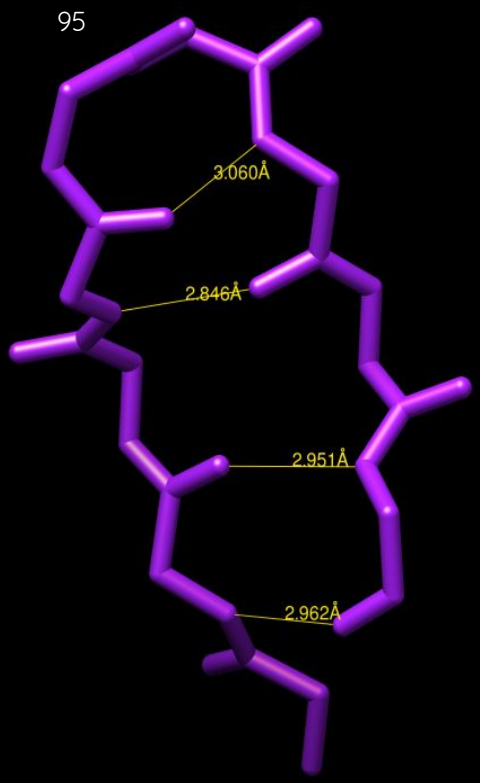
1gig

L3-λ1b



7fab

L3-λ1c



1mfa

H1-1

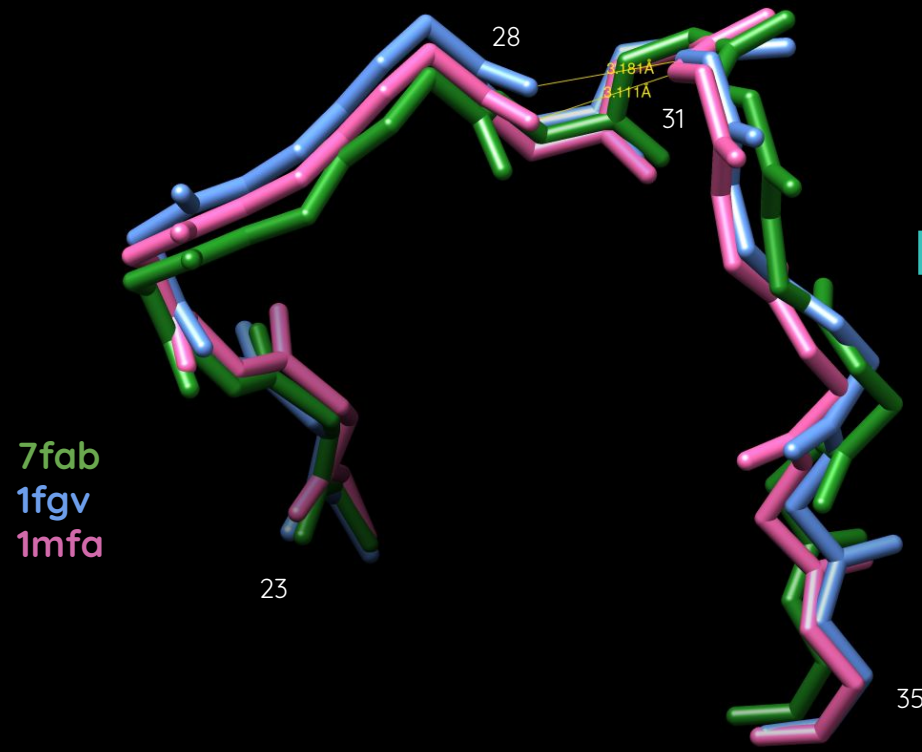
HBond:

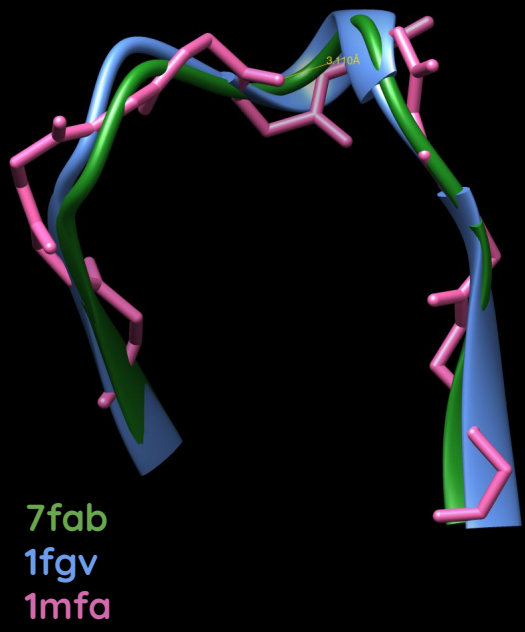
28 (O) - 31 (N)

Consensus: kaSGftftdyymh

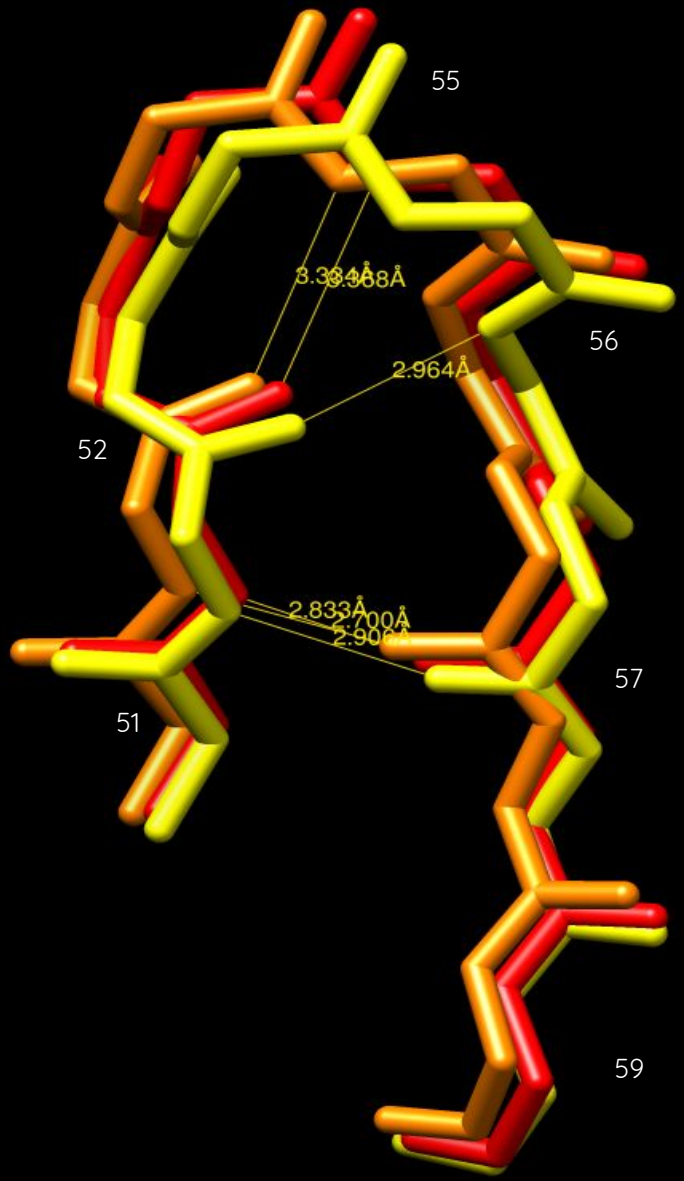
```
7fab LTCTVSGTSFDDYYWTWVRQP
1fgv LSCATSGYTFTEYTMHWMRQA
1mfa MSCKASGYTFTNYWMHWIKQR
```

RMSD = 2,008





H2-2a



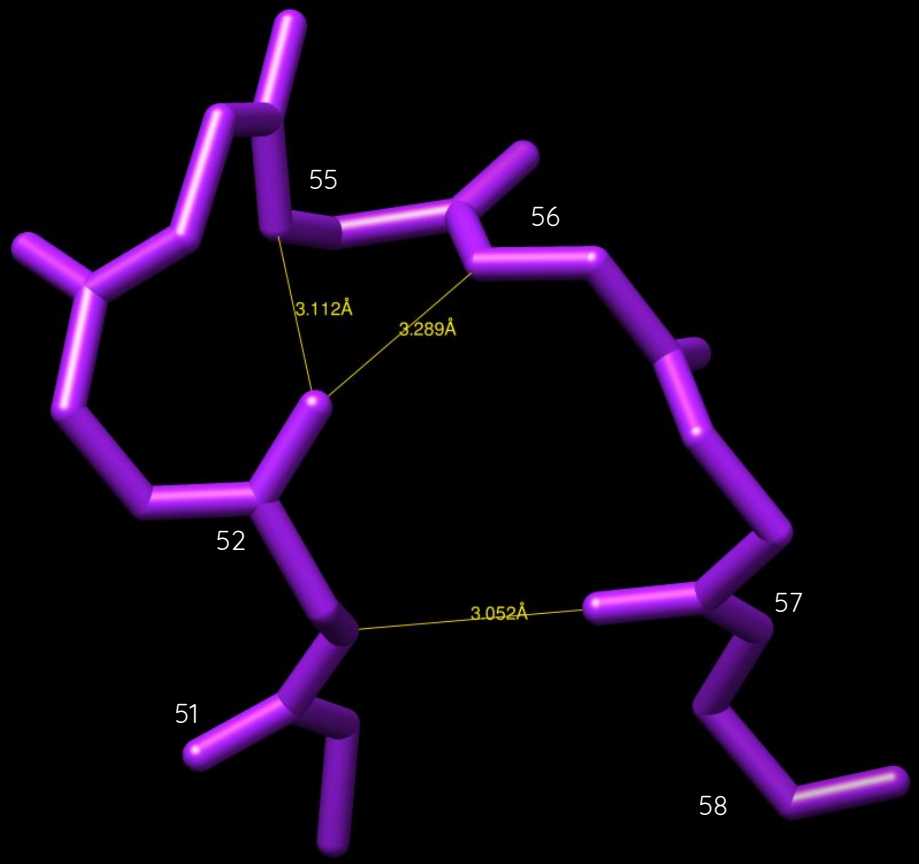
HBond:
52 (N) - 57 (O)
52 (O) - 55 (N)
52 (O) - 56 (N)

Consensus: -lypgng-t-

```
1mfa  GLEWIGAIYPGNSATFY
1fgv  GLEWVAGINPKNGGTSY
1fvc  GLEWVARIYPTNGYTRY
```

RMSD = 0,931

H2-3b



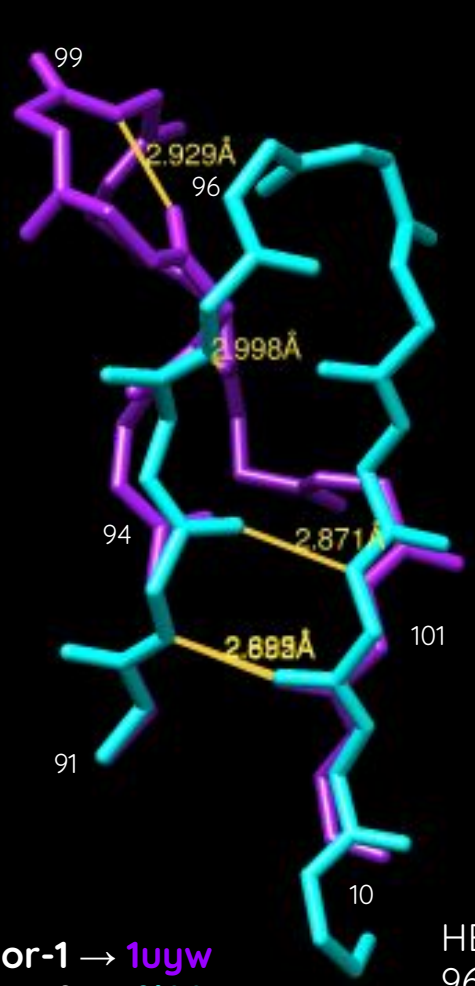
HBond:
52 (N) - 57 (O)
52 (O) - 55 (N)
52 (O) - 56 (N)

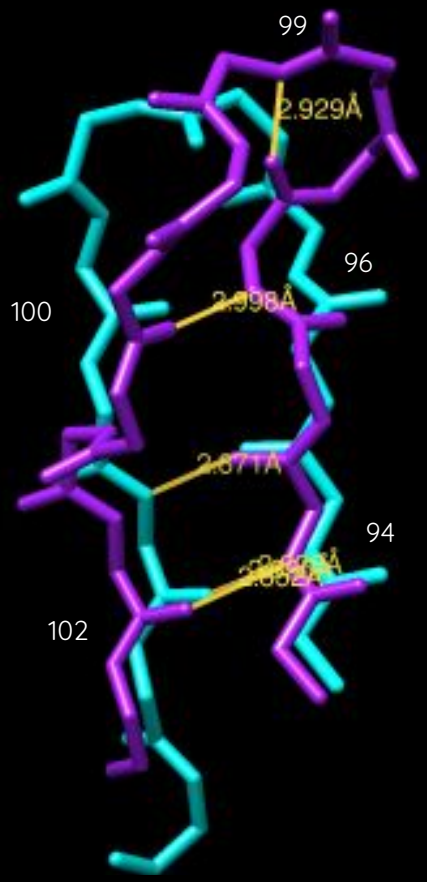
Consensus: -lssgggnty

1igm

Gly53
Gly55
Gly56

H3





H3-anchor-1 → 1uyw
H3-anchor-2 → 2j88

HBond **1uyw**:
96 (O) - 99 (N)
96 (O) - 100 (N)
94 (N) - 102 (O)

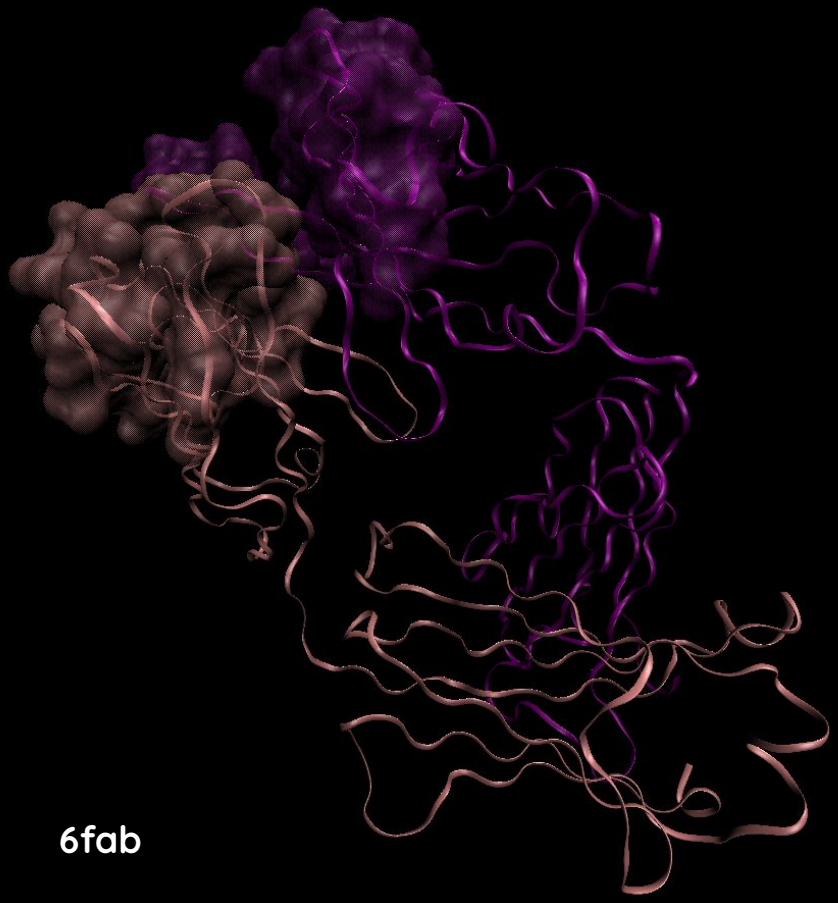
HBond **2j88**:
94 (N) - 101 (O)
94 (O) - 101 (N)

Strong Geometrical Restrictions Associated to the Mechanism of Immune Recognition

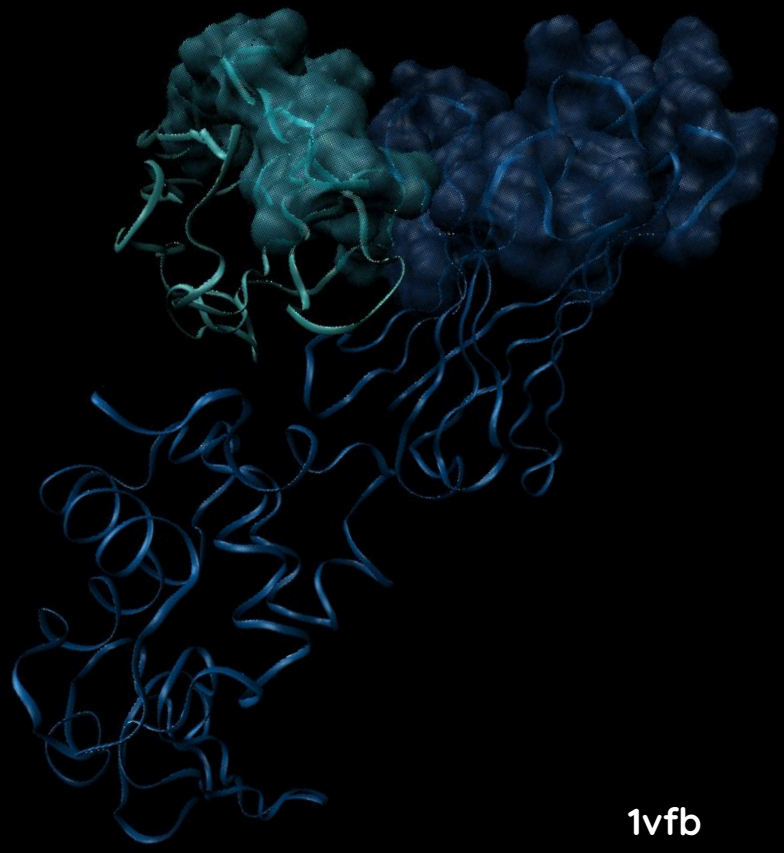
Nomenclature: class H1-H2-L1-L2-L3
H1, L2 & L3 are usually type 1, resulting in 1-X-Y-1-1

Class 1-4-4-1-1
→ Long loops
→ Small molecules (haptens)
→ Cleft at the antigen-binding sites

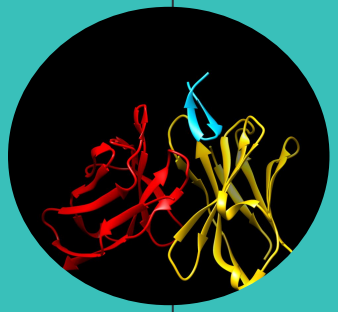
Class 1-1-2-1-1
→ Short loops
→ Large antigens (proteins)
→ Flat antigen-binding sites



6fab



1vfb



Characterization of an antigen-antibody interaction: antibody AP33 and E2 antigen from HCV

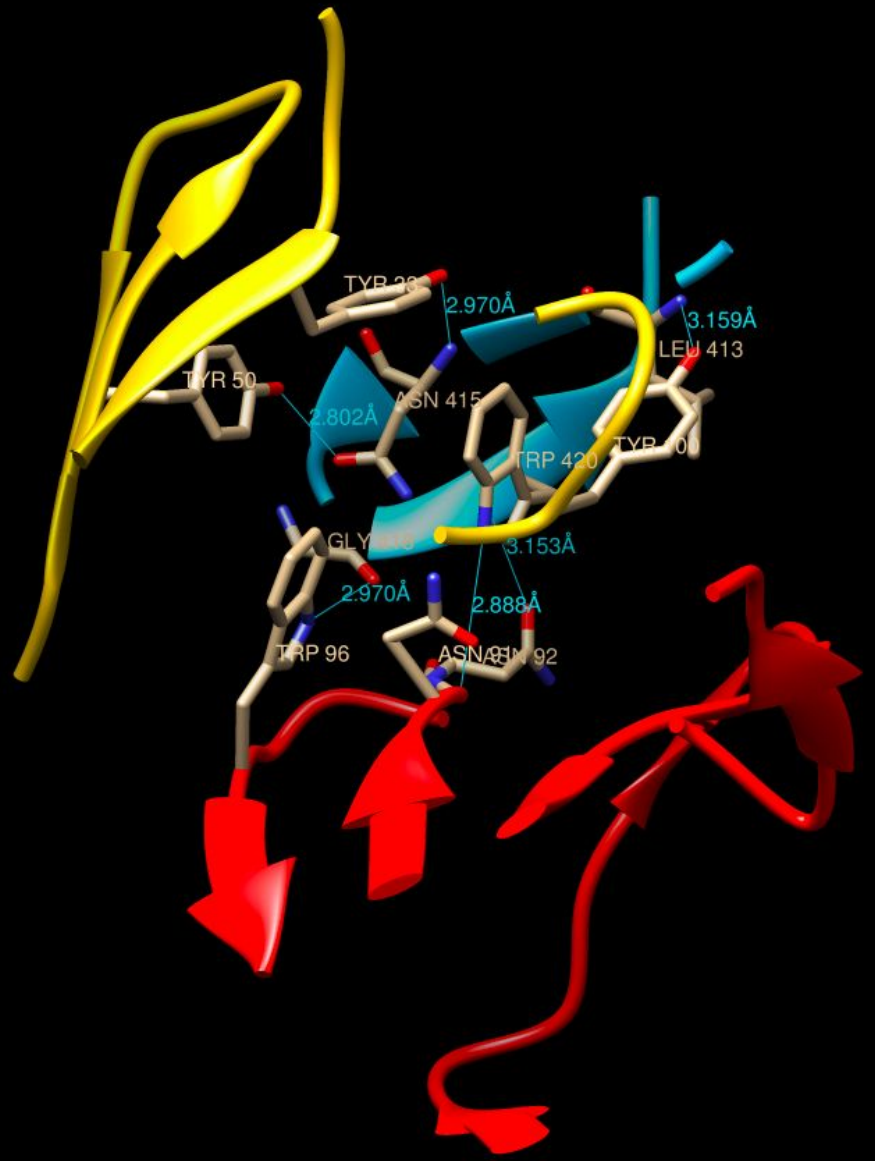
The crystallographic structure

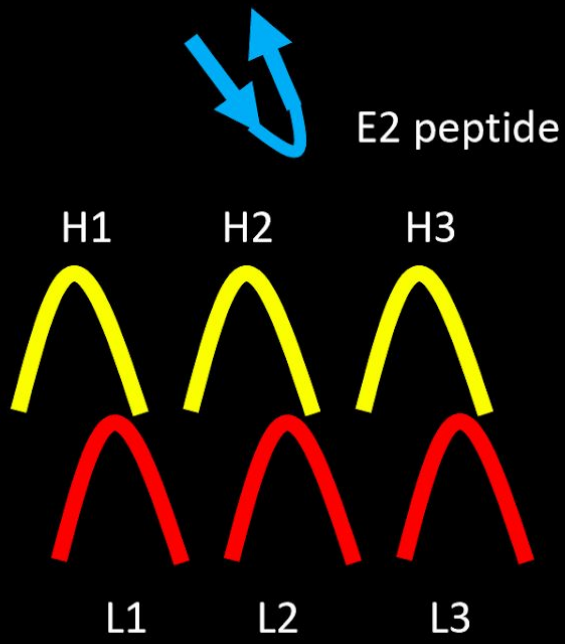
LC
HC
Antigen

(Fab)

4G6A

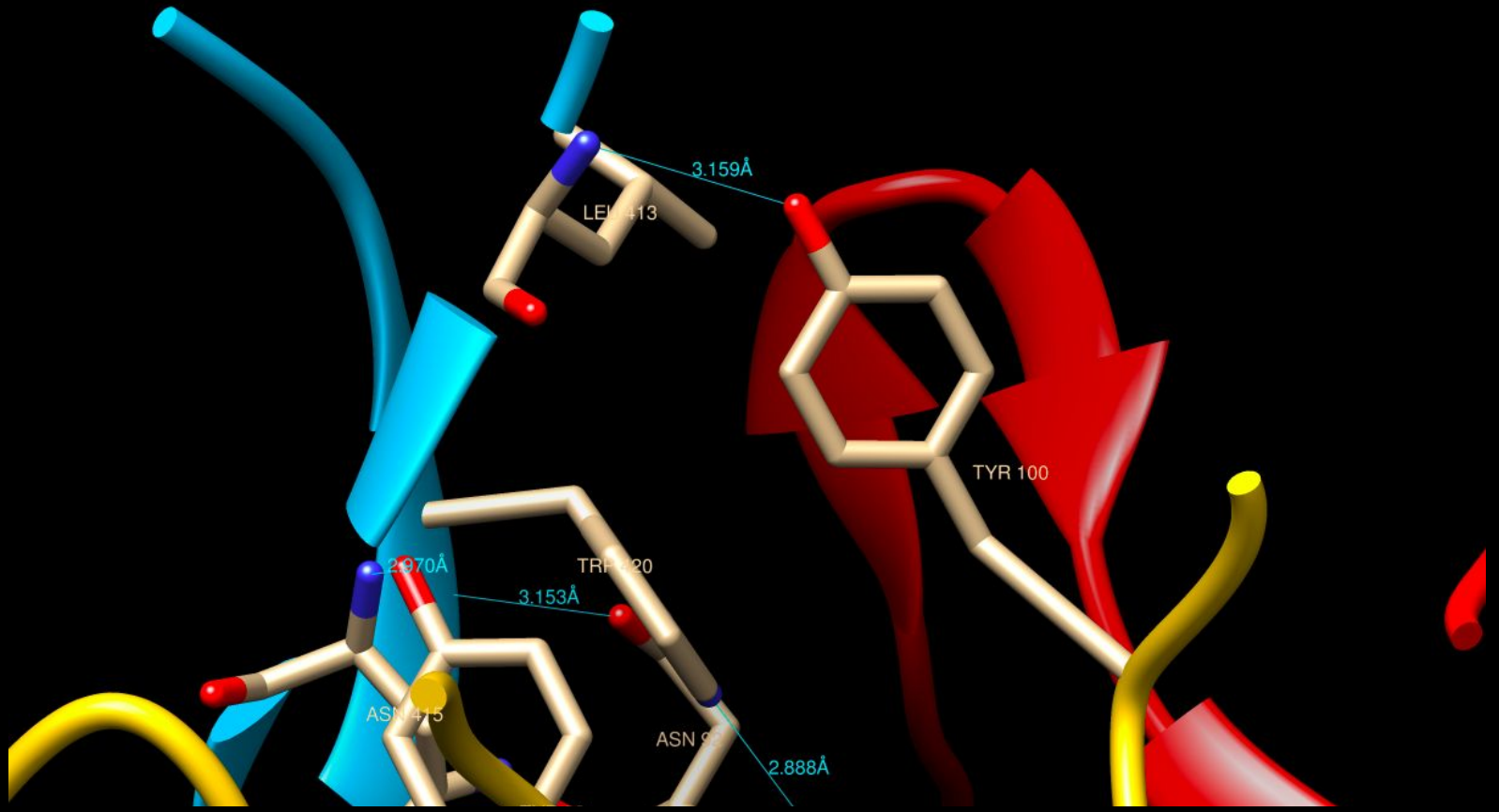
Hydrogen bonds





4G6A

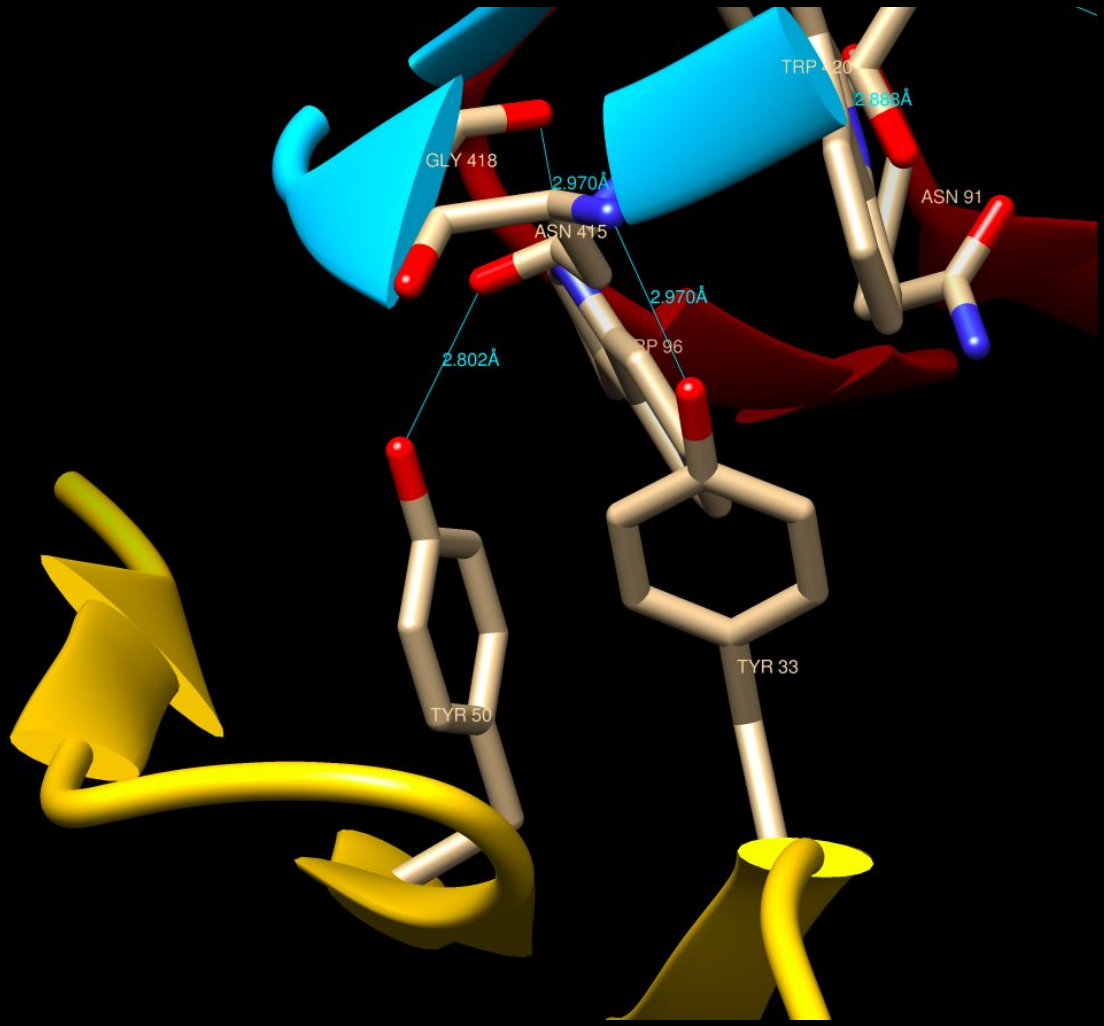
Hydrogen bonds



LEU 413 - TYR 100

4G6A

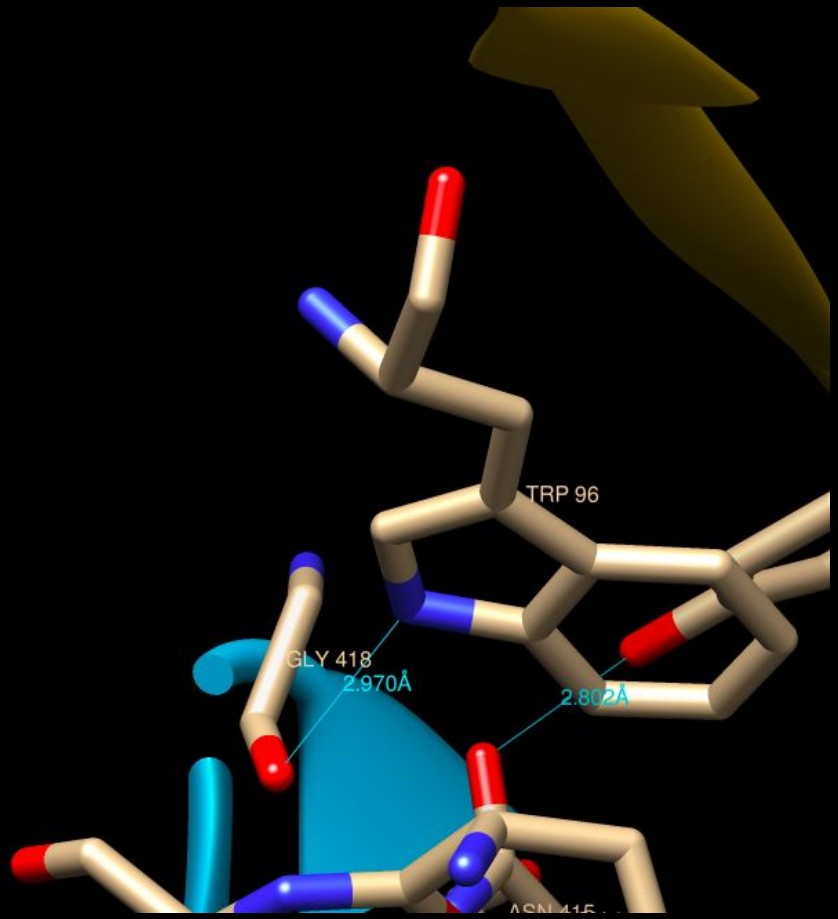
Hydrogen bonds



ASN 415 - TYR 50
ASN 415 - TYR 33

4G6A

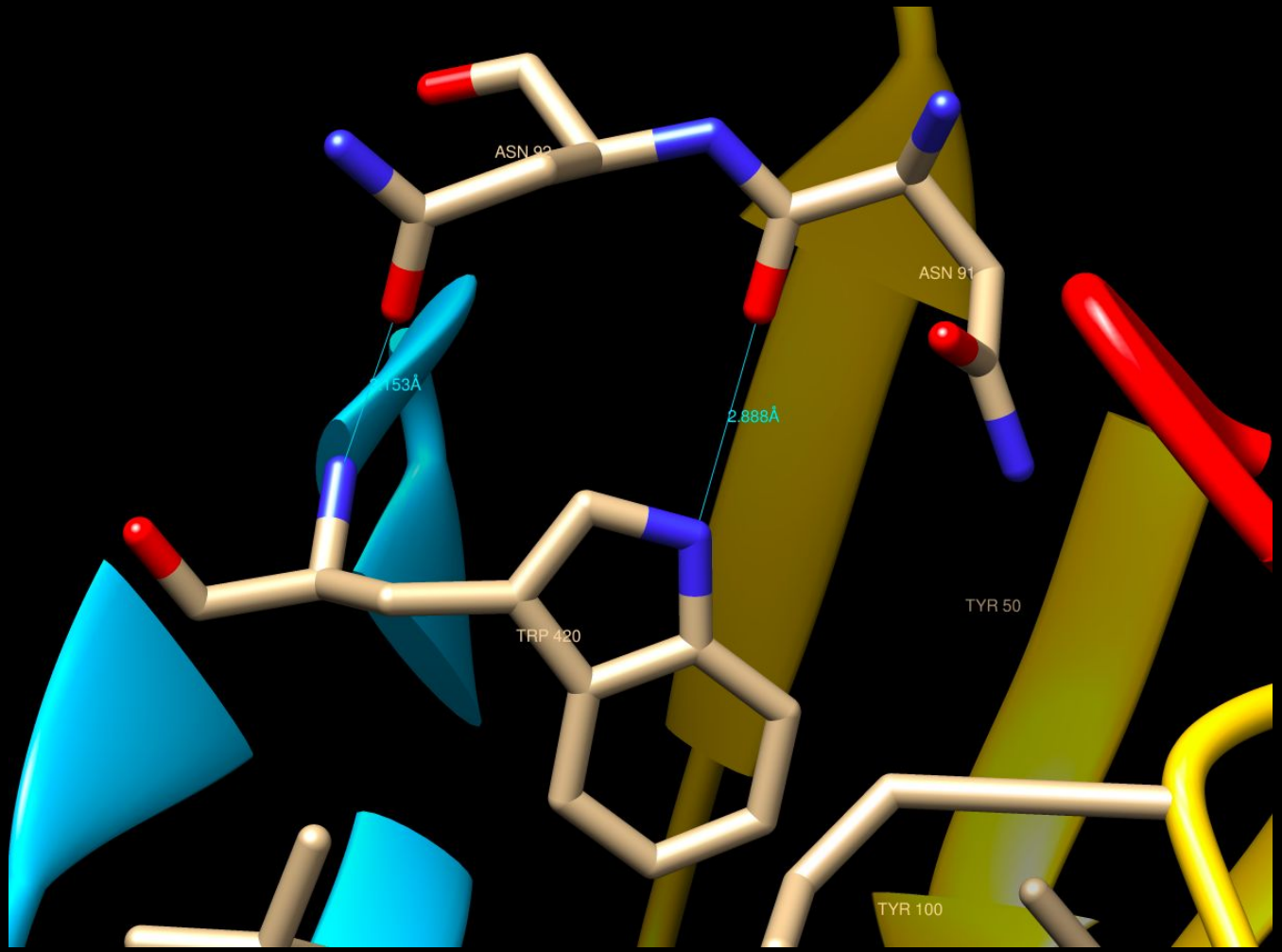
Hydrogen bonds



GLY 418 - TRP 96

4G6A

Hydrogen bonds

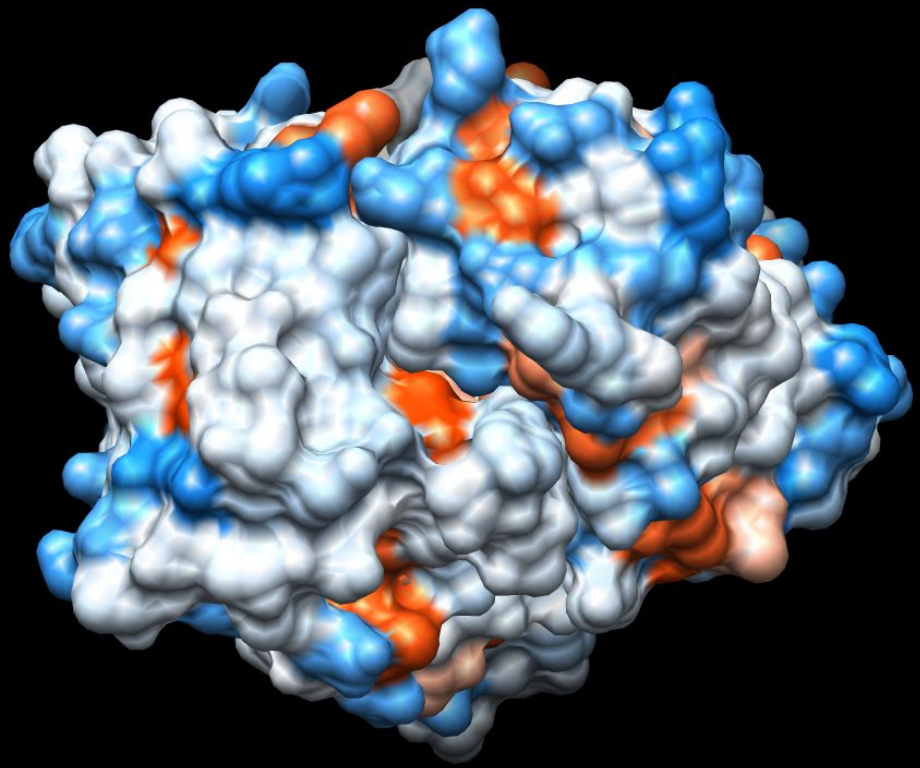


TRP 420 - ASN 91
TRP 420 - ASN 92

4G6A

Hydrophobicity surface

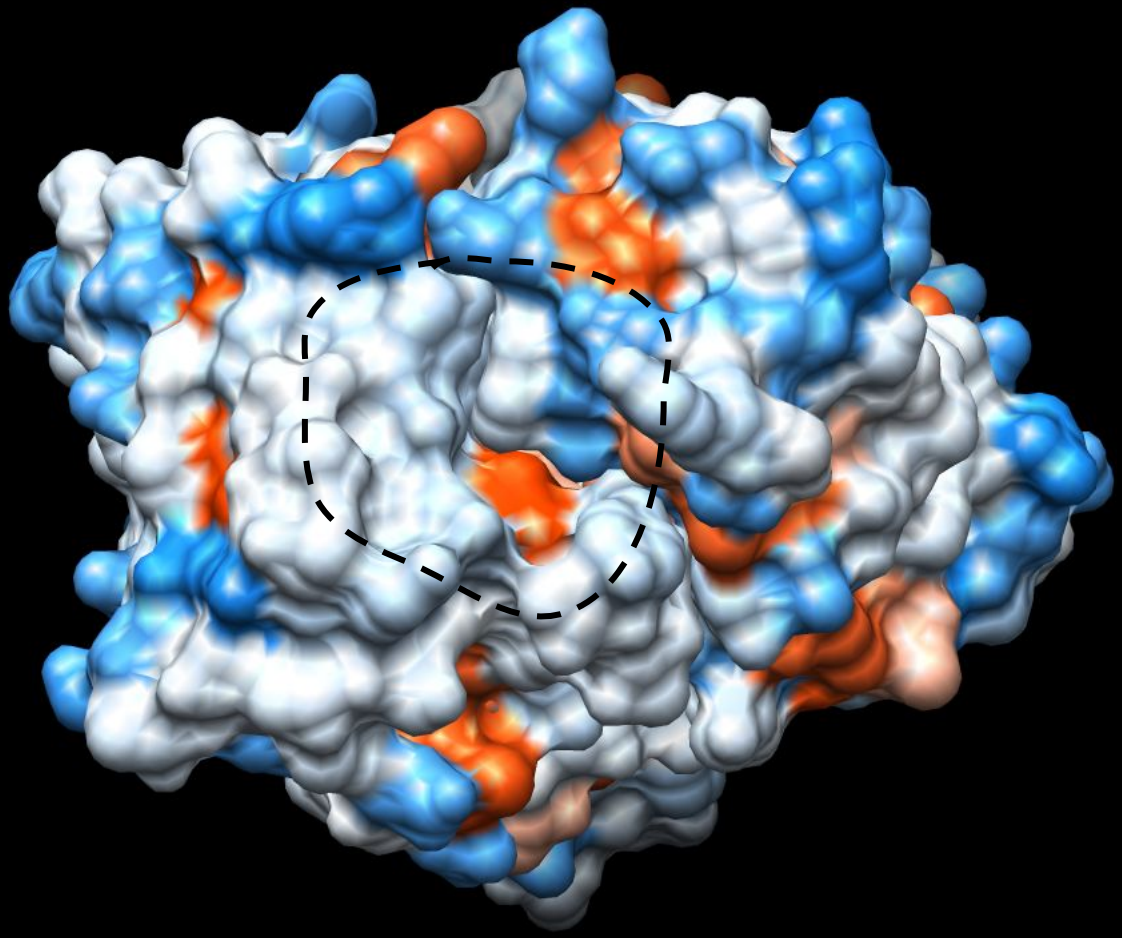
Hydrophilic Hydrophobic



Fab (top)

4G6A

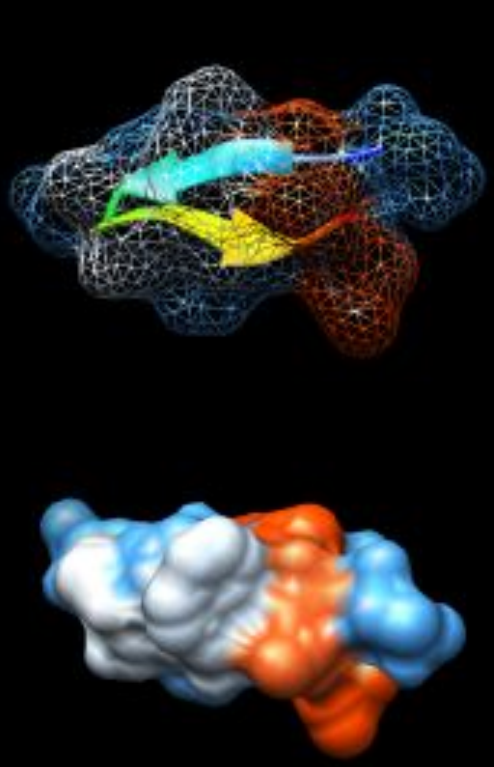
Hydrophobicity surface

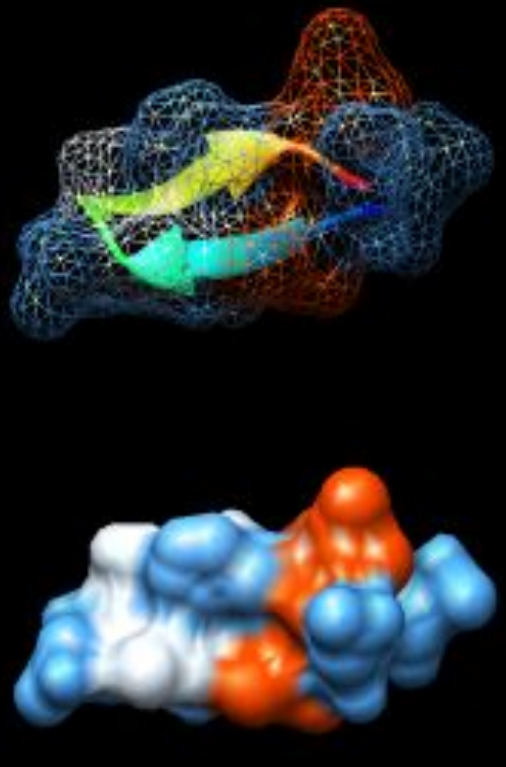


Fab (top)

4G6A

Hydrophobicity surface

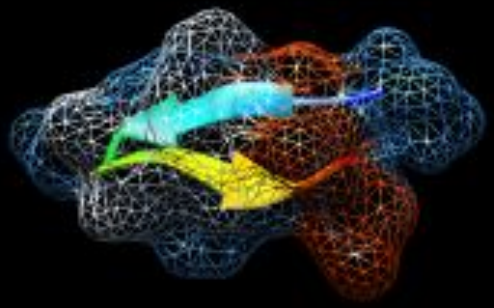


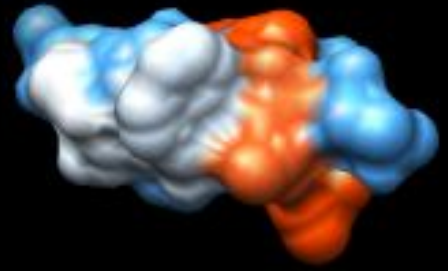


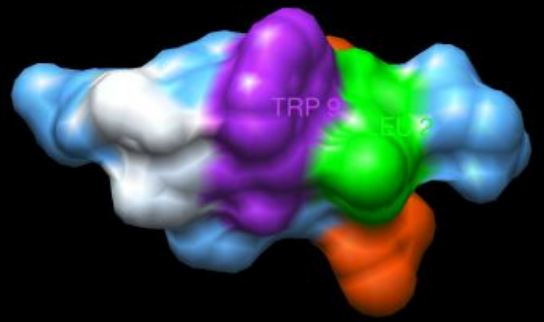
Ligand (bottom)

Ligand (top)

Hydrophobicity surface



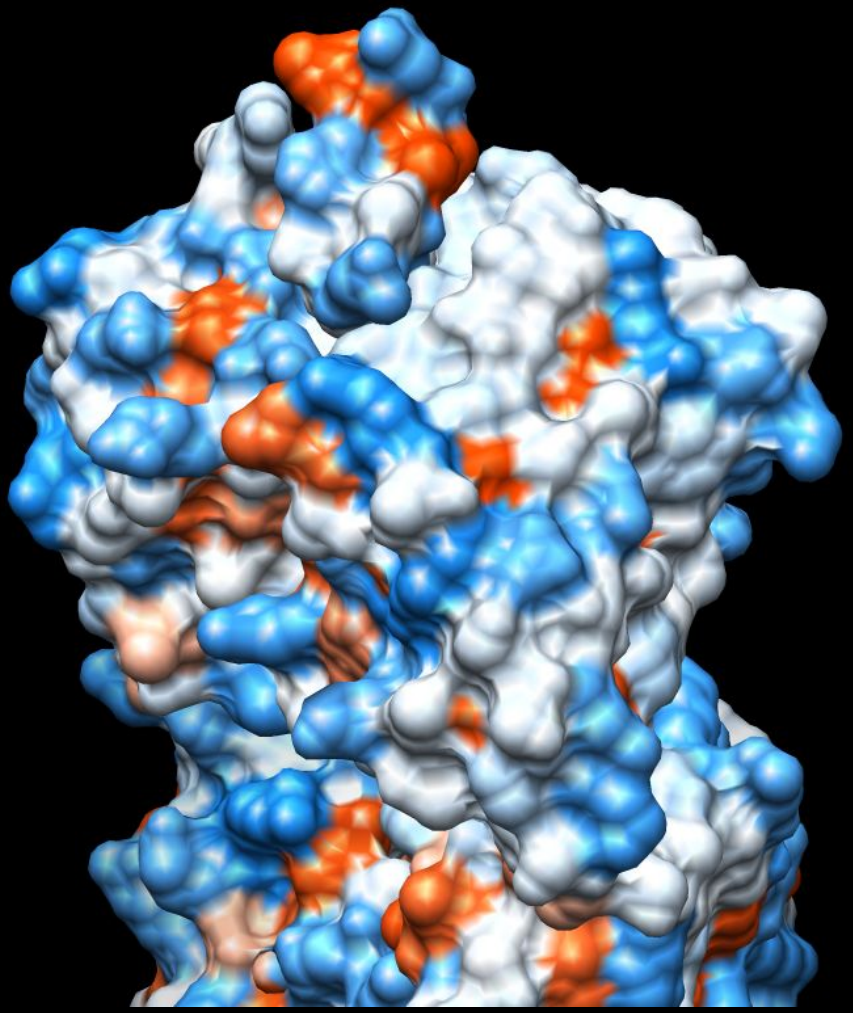




Trp426
Leu414

Ligand (bottom)

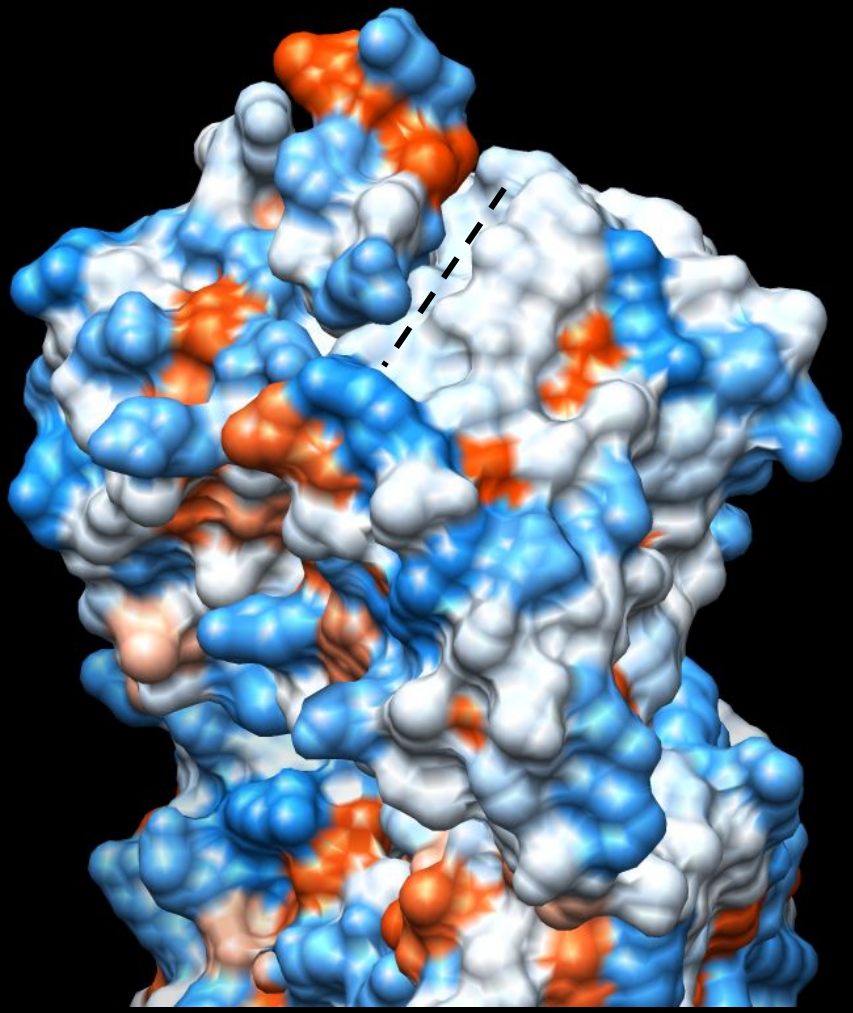
Hydrophobicity surface



Fab + ligand

4G6A

Hydrophobicity surface

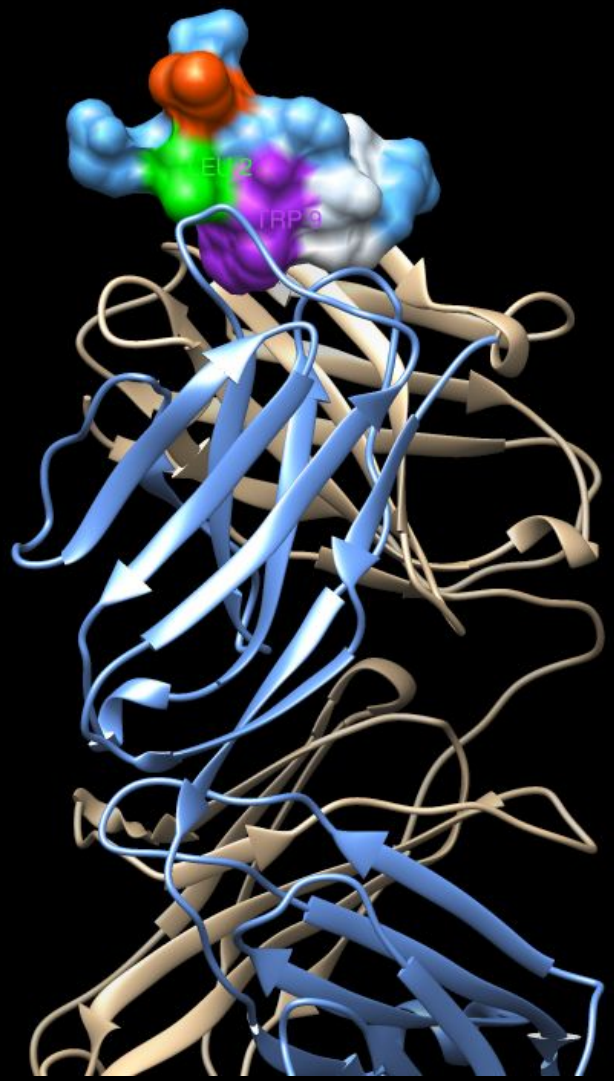


Fab + ligand

4G6A

Hydrophobicity surface

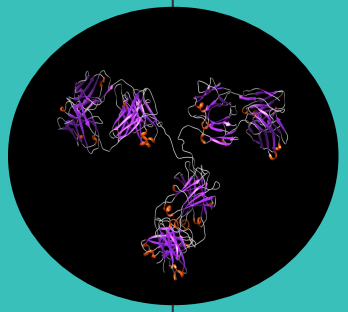
Ligand



Heavy chain

Light chain

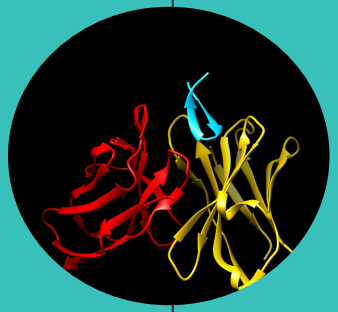
4G6A



Conclusions

Conclusions

- Immunoglobulins are heterodimers whose main motif is a greek-key, which commonly consists of seven beta strands conforming 2 beta-sheets.
- Hydrogen bonds, disulfide bonds and hydrophobic bonds are the main responsible of the maintenance of the fold, the secondary structure and the tertiary and quaternary structure, respectively.
- Besides Igs being very variable proteins, there are some highly conserved residues: Cys-forming disulfide bonds, topohydrophobic positions, and residues with special roles (Asn, Pro, charged residues...).
- Despite CDRs being hypervariable regions, there are some chain conformations that are more frequently found, defining canonical structures.
- CDRs are a clear example of the fact that structure is generally more conserved than sequence.
- Correlation between the architecture of the antigen-binding site and their recognised antigen provides insight into the general mechanism of the molecular recognition process mediated by Igs.
- In the antigen-antibody binding, both hydrophobic interactions and hydrogen bonds are formed between the CDRs of the Fab and the epitope.



Multiple Choice Questions

1. About the topohydrophobic amino acids of the immunoglobulins:

a) They create hydrophobic interactions that permit the fold of the two beta-sheet domains
b) They are located on the surface of Ig's, solubilizing them
c) The two of answers above are correct
d) They are not conserved along evolution
e) All of the above are correct

2. About the interaction between the antigen E2 and the antibody AP33:

a) The antigen interacts with the Fc region of the antibody
b) Hydrogen bonds are not involved in this interaction, only hydrophobic interactions and Van der Waals forces.
c) When both proteins interact, hydrophobic surfaces are joined and only the polar parts remain exposed.
d) All of the CDR's of AP33 are involved in the interaction with E2.
e) All of the above are correct

3. Choose the correct option regarding cysteine residues in immunoglobulins:

a) Cysteine residues are not conserved through evolution.
b) Cysteine residues do not form hydrogen bonds.
c) Cysteine residues are located in a particular position.
d) There is not any Cys joining the heavy chain with the light chain.
e) Each immunoglobulin fold consist of just 1 Cys residue.

4. About the role of proline in immunoglobulins:

a) Proline is not favored neither in beta-sheets nor alpha-helix
b) Proline is the amino acids that forms most hydrogen bonds.
c) Both are correct.
d) Proline is not important for the structure of a protein, and thus is not conserved at all.
e) All are correct.

5. About canonical structures in CDRs, find the correct answer:

a) Because of its variability, canonical structures are hard to establish in H3 CDR.
b) The first classification was done by Chothia et al in 1989.
c) The two of the above are correct.
d) Canonical structures only exist in the light chain CDRs.
e) All kind of CDRs share the same consensus sequence.

6. Regarding CDR H3 loop, find the INCORRECT answer:

a) Cluster H3–anchor-1 covers about two-thirds of the H3 loop structures.
b) CDR H3 loop is known to have a substantial impact on the antigen binding.
c) According to North et al, H3 can be split in an anchor region and a “head” or apex region.
d) CDR H3 loop structures are very easy to predict.
e) The main reason of its variability is the full loop structure.

7. Regarding the Immunoglobulin-like beta-sandwich fold:

a) It always consists of sandwich which contains 7 strands in 2 sheets: greek-key.
b) Some members of the fold have additional strands.
c) It always consists of sandwich which contains 9 strands in 2 sheets: greek-key.
d) Only V-set domains are considered to have immunoglobulin-like beta-sandwich fold.
e) This fold is exclusive of Deuterostomes.

8. Which of the following statements about immunoglobulins are FALSE:

1. Are all beta proteins, which means that the structure is composed mainly of beta-sheets, although the stabilization of the structure is possible thanks to other structures as alpha-helices.
2. The stabilization of beta strands forming one sheet (greek-key) is obtained thanks to the hydrogen bonds between them.
3. CH3 has only hydrophobic residues in the surface.
4. Disulfide bonds distribution may change according to the Immunoglobulin type and subtype.

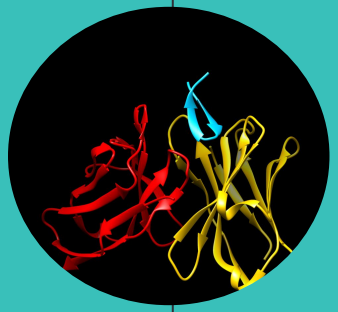
a) 1, 2, 3.
b) 1, 3.
c) 2, 4.
d) 4.
e) 1, 2, 3, 4.

9. Identify the FALSE sentence about canonical structures:

a) Refer to similar chain conformations.
b) Can be found in CDRs.
c) Present a 100% conserved sequence.
d) Have consensus sequences in most of the cases.
e) Are involved in the binding of antigens

10. Which is the most variable CDR?

a) L1
b) L3
c) K2
d) H1
e) H3



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Thank you for your attention

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