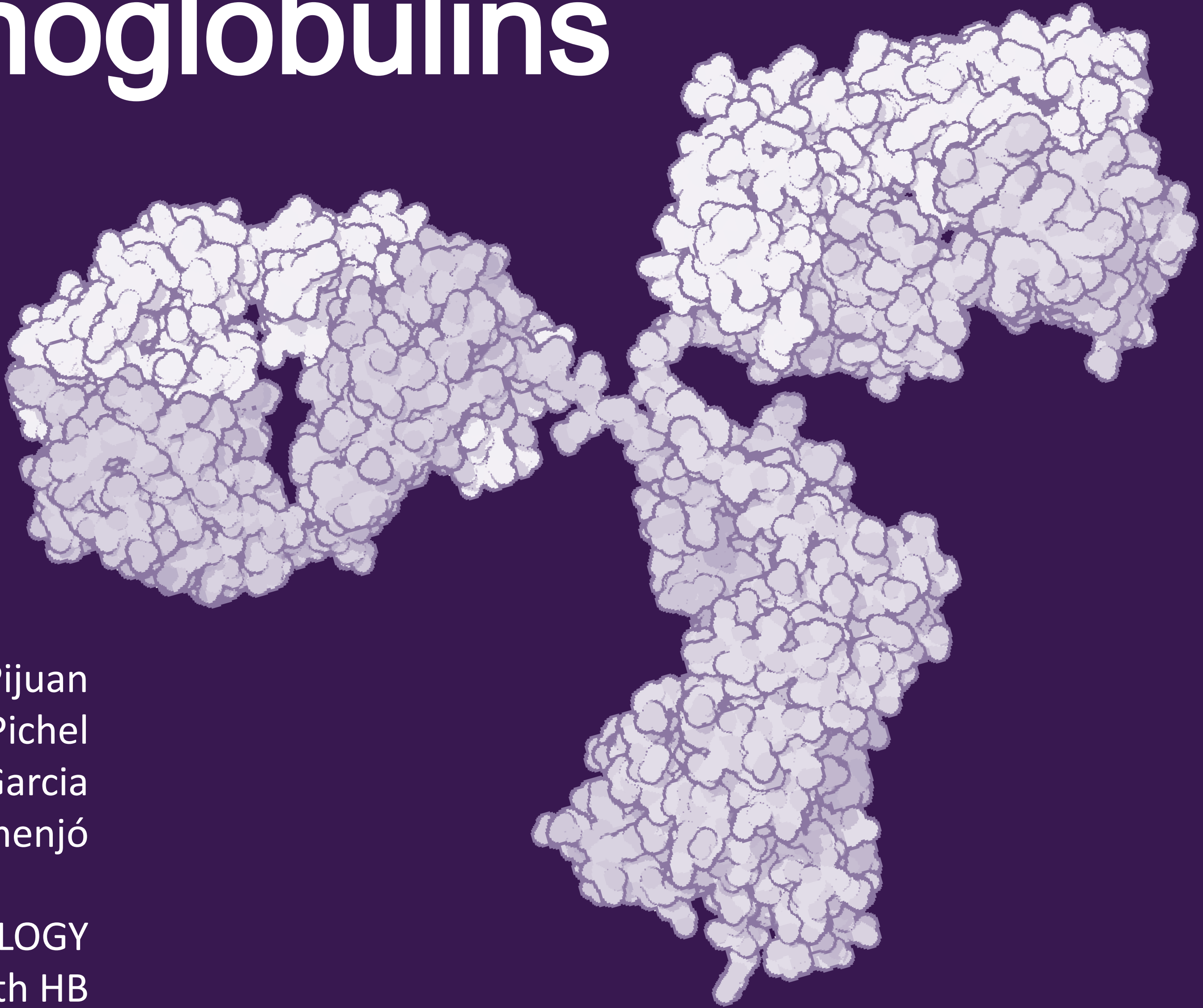
Proteins of the Immune System:

Immunoglobulins



Anna Pijuan
Núria Pichel
Marta Garcia
Eva Domenjó

STRUCTURAL BIOLOGY
4th HB

Immunoglobulins

- General structure
- Classes
- Diversity

Immunoglobulin fold

- Immunoglobulin fold
- Strand delimitation
- Constant domain
- Variable domain
- Hydrophobic core

Chain association

- Constant domains
- Variable domains
- Hinge and flexibility
- Disulfide bridges

Hypervariable regions

- Canonical structures
  - L1, L2, L3, H1, H2
- Prediction of Immunoglobulin Structure (PIGS)

IgA – Fcα Receptor

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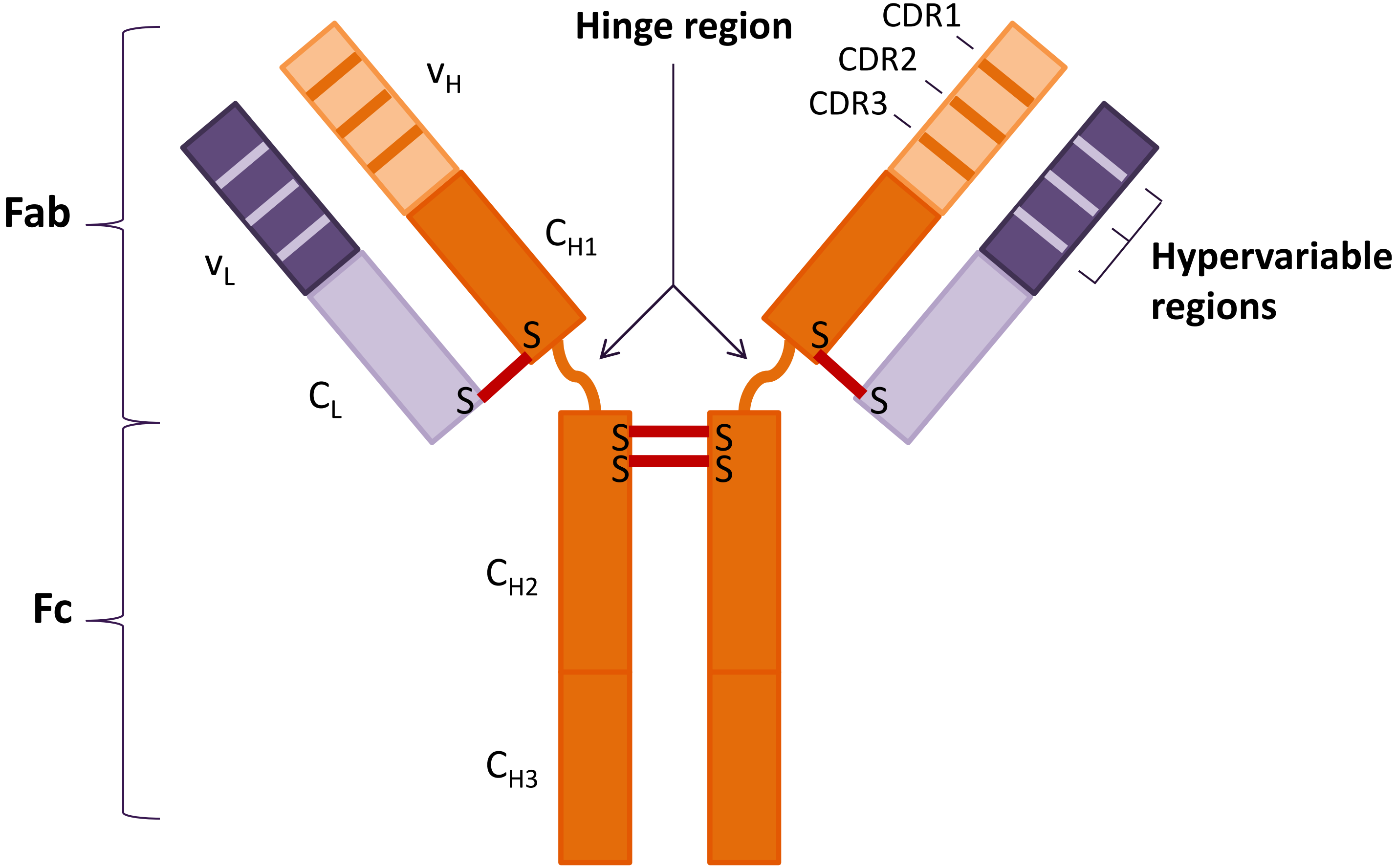
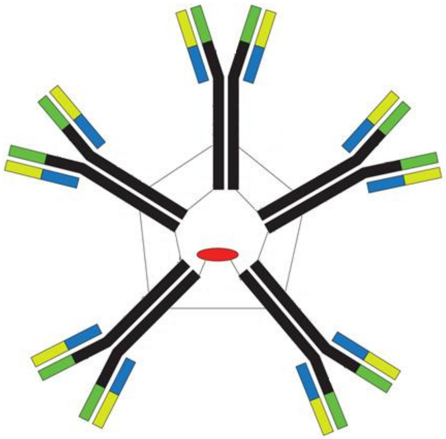
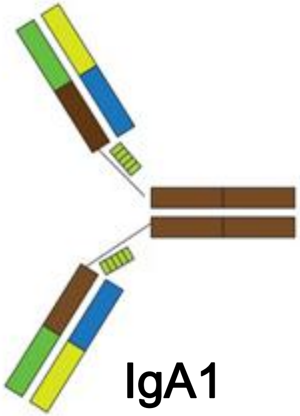


Classes

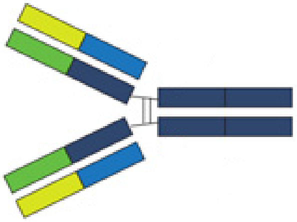


IgM

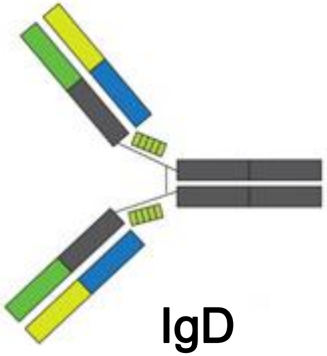


IgA1

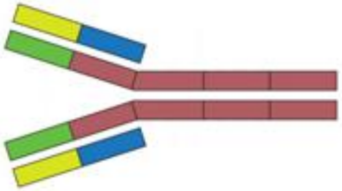
	H chain	FcR
IgM	μ	
IgA		FcαR
IgA1	α1	
IgA2	α2	
sIgA	α1 / α2	
IgG		FcγR
IgG1	γ1	I, II, III
IgG2	γ2	II
IgG3	γ3	I, II, III
IgG4	γ4	I, II
IgD	δ	FcδR
IgE	ε	FcεR I, II



IgG1

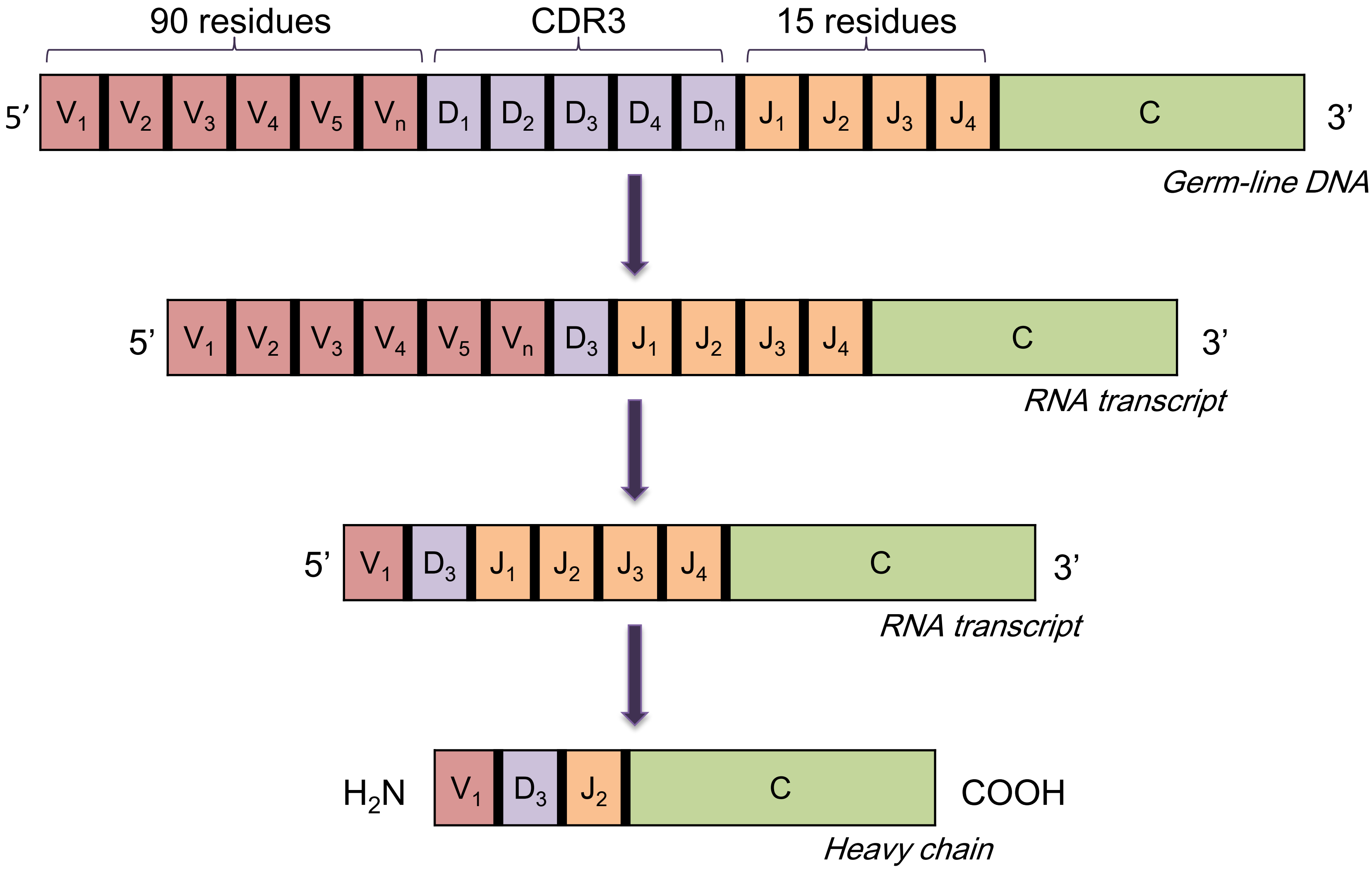


IgD



IgE

Diversity



90 residues CDR3 15 residues

5’ V_1 V_2 V_3 V_4 V_5 V_n D_1 D_2 D_3 D_4 D_n J_1 J_2 J_3 J_4 C 3’

Germ-line DNA

5’ V_1 V_2 V_3 V_4 V_5 V_n D_3 J_1 J_2 J_3 J_4 C 3’

RNA transcript

5’ V_1 D_3 J_1 J_2 J_3 J_4 C 3’

RNA transcript

H_2N V_1 D_3 J_2 C COOH

Heavy chain

Combinatorial Joining

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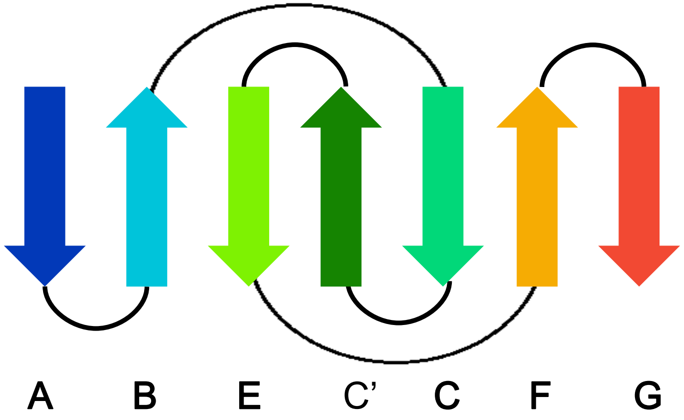
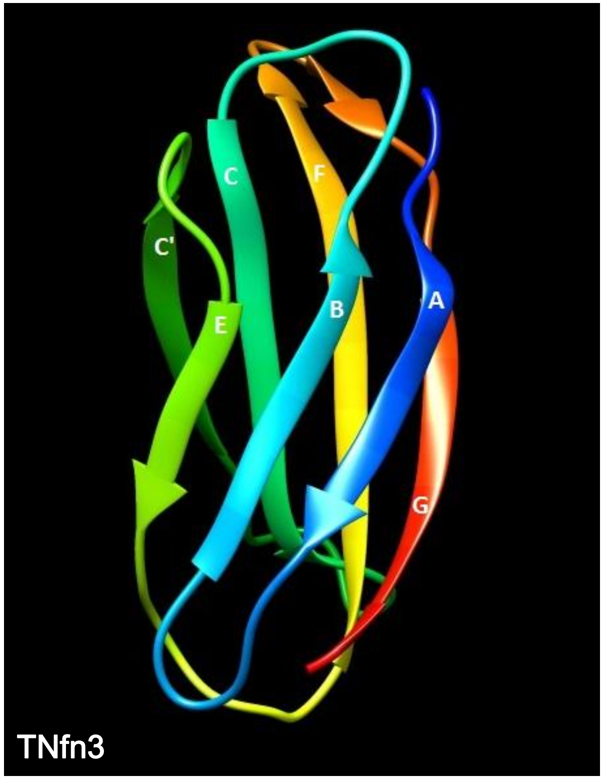
Immunoglobulin fold

Two antiparallel β sheets packed tightly against each other

- Invariably contains six strands: A, B, C, E, F and G
- B,C,E and F constitute hydrophobic core and A, C', C'', D and G are the external strands





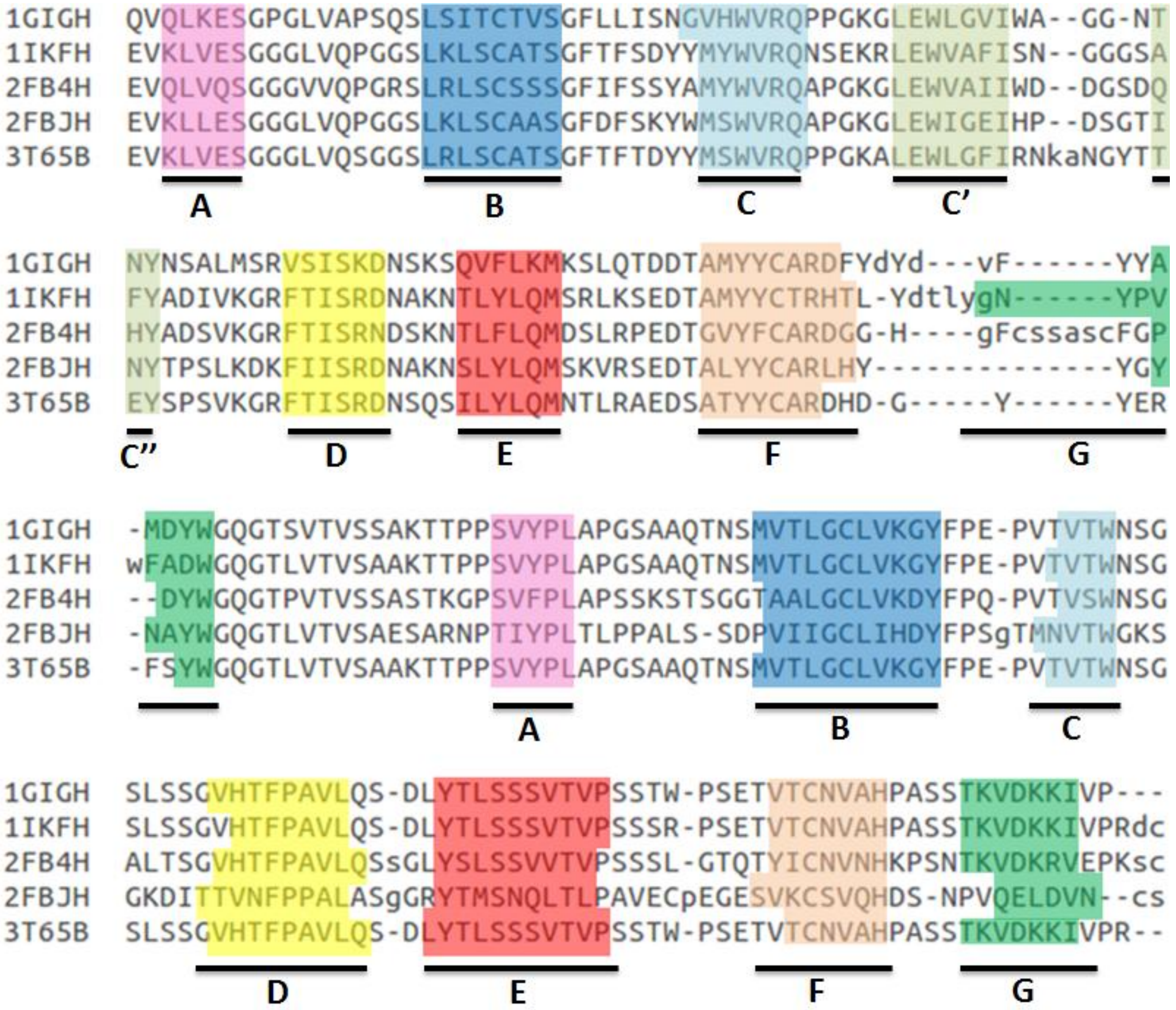
Immunoglobulin Superfamily

Lineage:

- Root: scop
- Class: All beta proteins
- Fold: Immunoglobulin-like beta-Sandwich
- Superfamily: Immunoglobulin

Family	Sheet 1	Sheet2
V-set	A,B,E,**D**	C,E,G,**C',C"**
C1-set	A,B,E,**D**	C,E,G
C2-set	A,B,E	C,E,G,**C'**
I	A,B,E,**D**	C,E,G,**C'**

Strand delimitation

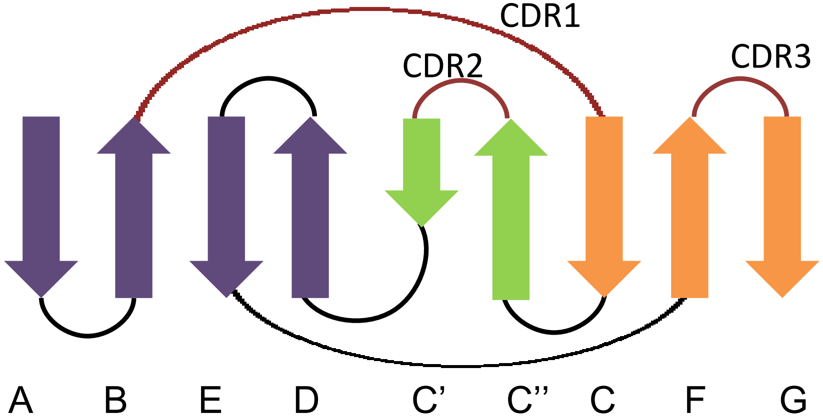


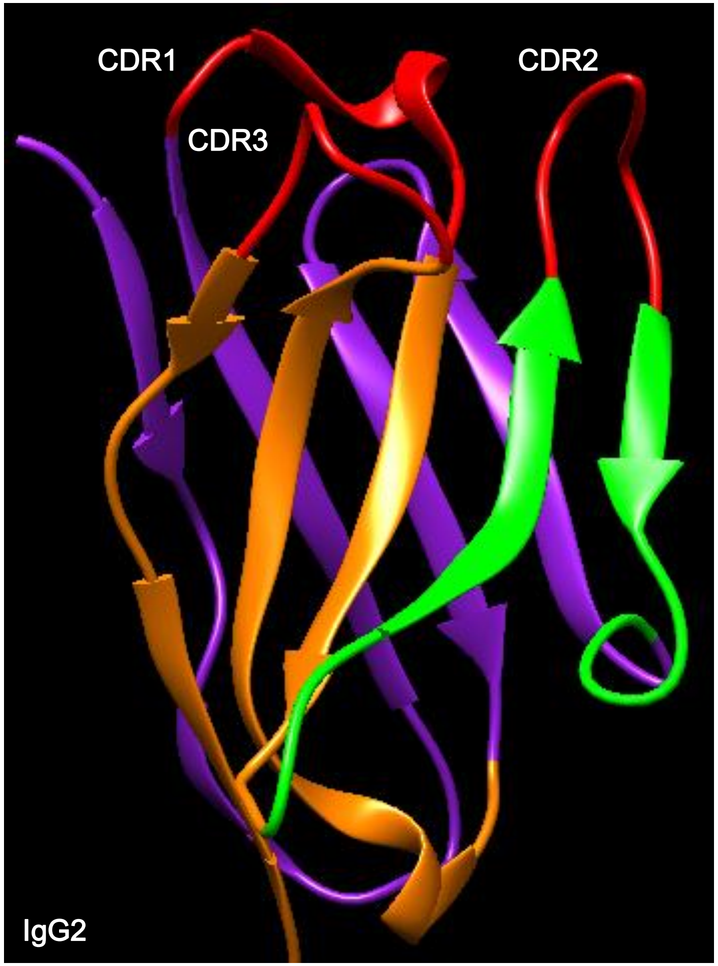
```
1GIGH   QVQLKESGPGLVAPSQSLSITCTVSGFLLISNGVHWVRQPPGKGLEWLGVIWA--GG-NT
1IKFH   EVKLVESGGGLVQPGGSLKLSCATSGFTFSDYYMYWVRQNSEKRLEWVAFISN--GGGSA
2FB4H   EVQLVQSGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGKGLEWVAIIWD--DGSDQ
2FBJH   EVKLLESGGGLVQPGGSLKLSCAASGFDFSKYWMSWVRQAPGKGLEWIGEIHP--DSGTI
3T65B   EVKLVESGGGLVQSGGSLRLSCATSGFTFTDYYMSWVRQPPGKALEWLGFIRNkaNGYTT
          A            B             C          C'

1GIGH   NYNSALMSRVSISKDNSKSQVFLKMKSLQTDDTAMYYCARDFYdYd---vF------YYA
1IKFH   FYADIVKGRFTISRDNAKNTLYLQMSRLKSEDTAMYYCTRHTL-YdtlygN------YPV
2FB4H   HYADSVKGRFTISRNDSKNTLFLQMDSLRPEDTGVYFCARDGG-H----gFcssascFGP
2FBJH   NYTPSLKDKFIISRDNAKNSLYLQMSKVRSEDTALYYCARLHY-------------YGY
3T65B   EYSPSVKGRFTISRDNSQSILYLQMNTLRAEDSATYYCARDHD-G-----Y------YER
        C''        D        E              F                  G

1GIGH   -MDYWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE-PVTVTWNSG
1IKFH   wFADWGQGTLVTVSAAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE-PVTVTWNSG
2FB4H   --DYWGQGTPVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPQ-PVTVSWNSG
2FBJH   -NAYWGQGTLVTVSAESARNPTIYPLTLPPALS-SDPVIIGCLIHDYFPSgTMNVTWGKS
3T65B   -FSYWGQGTLVTVSAAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE-PVTVTWNSG
                             A                B            C

1GIGH   SLSSGVHTFPAVLQS-DLYTLSSSVTVPSSTW-PSETVTCNVAHPASSTKVDKKIVP---
1IKFH   SLSSGVHTFPAVLQS-DLYTLSSSVTVPSSSR-PSETVTCNVAHPASSTKVDKKIVPRdc
2FB4H   ALTSGVHTFPAVLQSsGLYSLSSVVTVPSSSL-GTQTYICNVNHKPSNTKVDKRVEPKsc
2FBJH   GKDITTVNFPPALASgGRYTMSNQLTLPAVECpEGESVKCSVQHDS-NPVQELDVN--cs
3T65B   SLSSGVHTFPAVLQS-DLYTLSSSVTVPSSTW-PSETVTCNVAHPASSTKVDKKIVPR--
              D               E                F          G
```

Constant domains

7 β antiparallel strands
2 β sheets

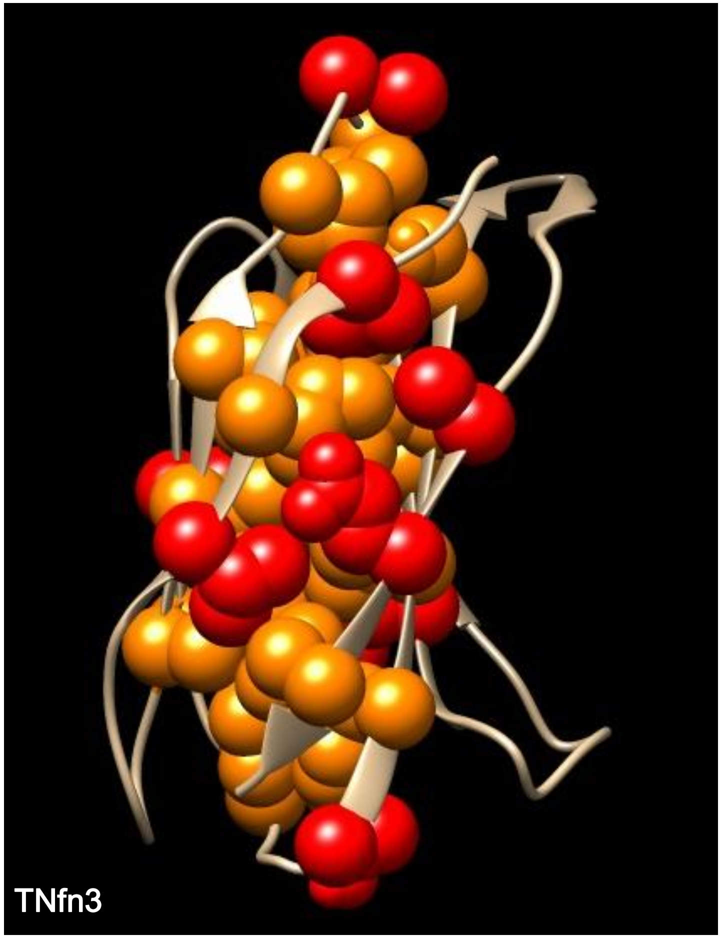
Variable domains

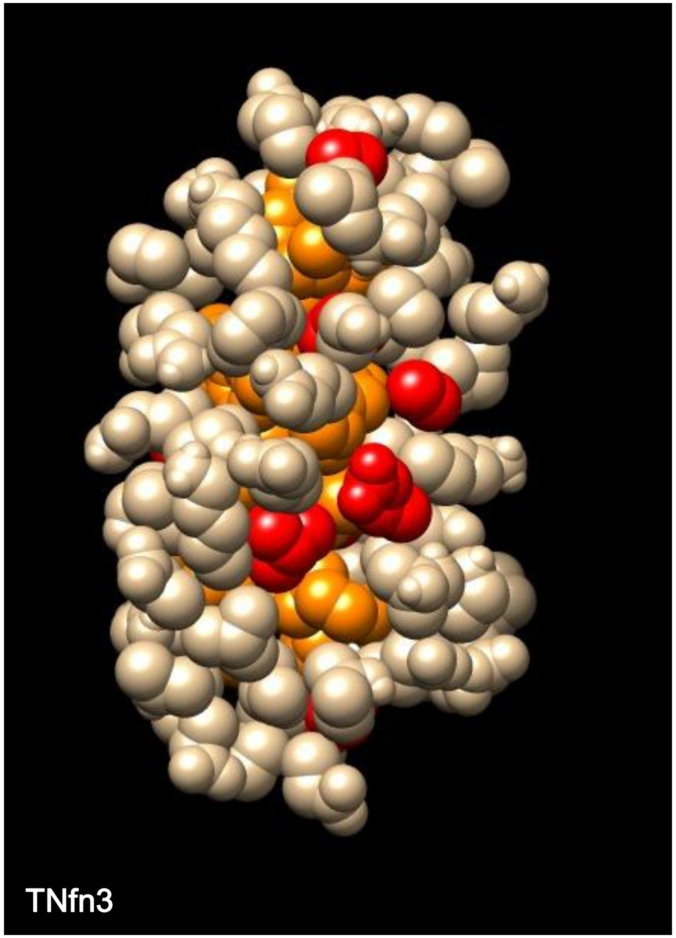




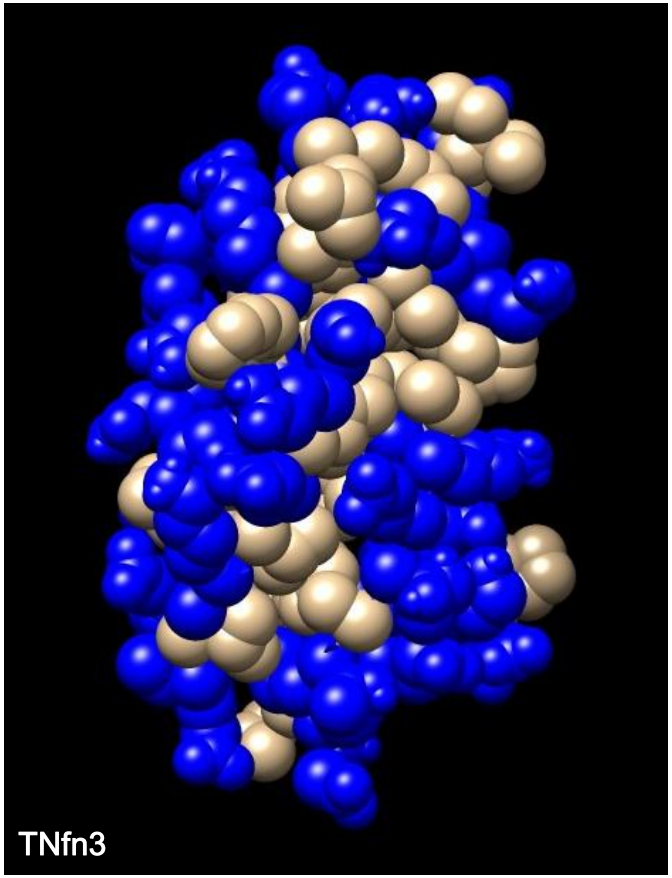
9 β antiparallel strands
2 β sheets

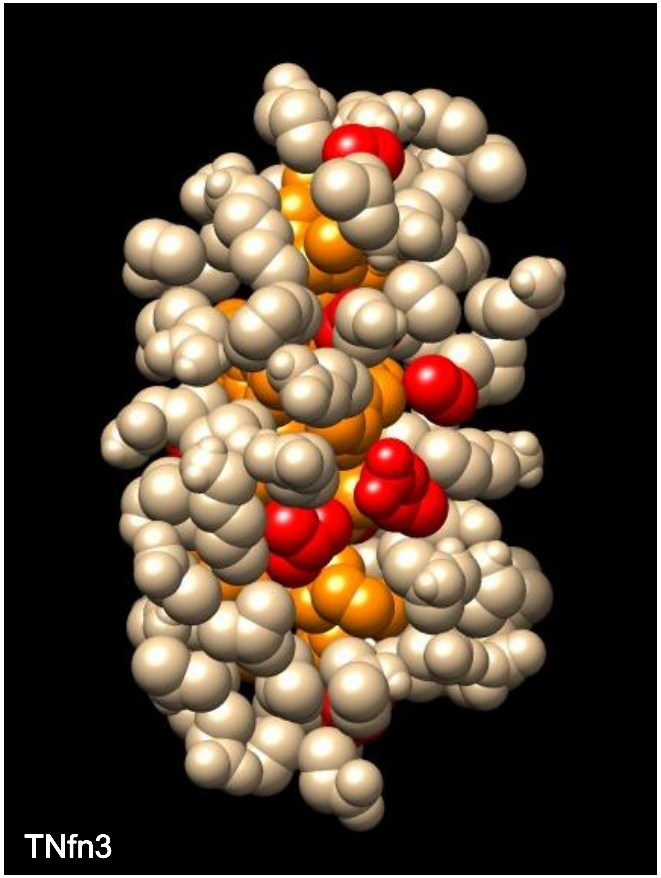
Hydrophobic core





Hydrophobic core





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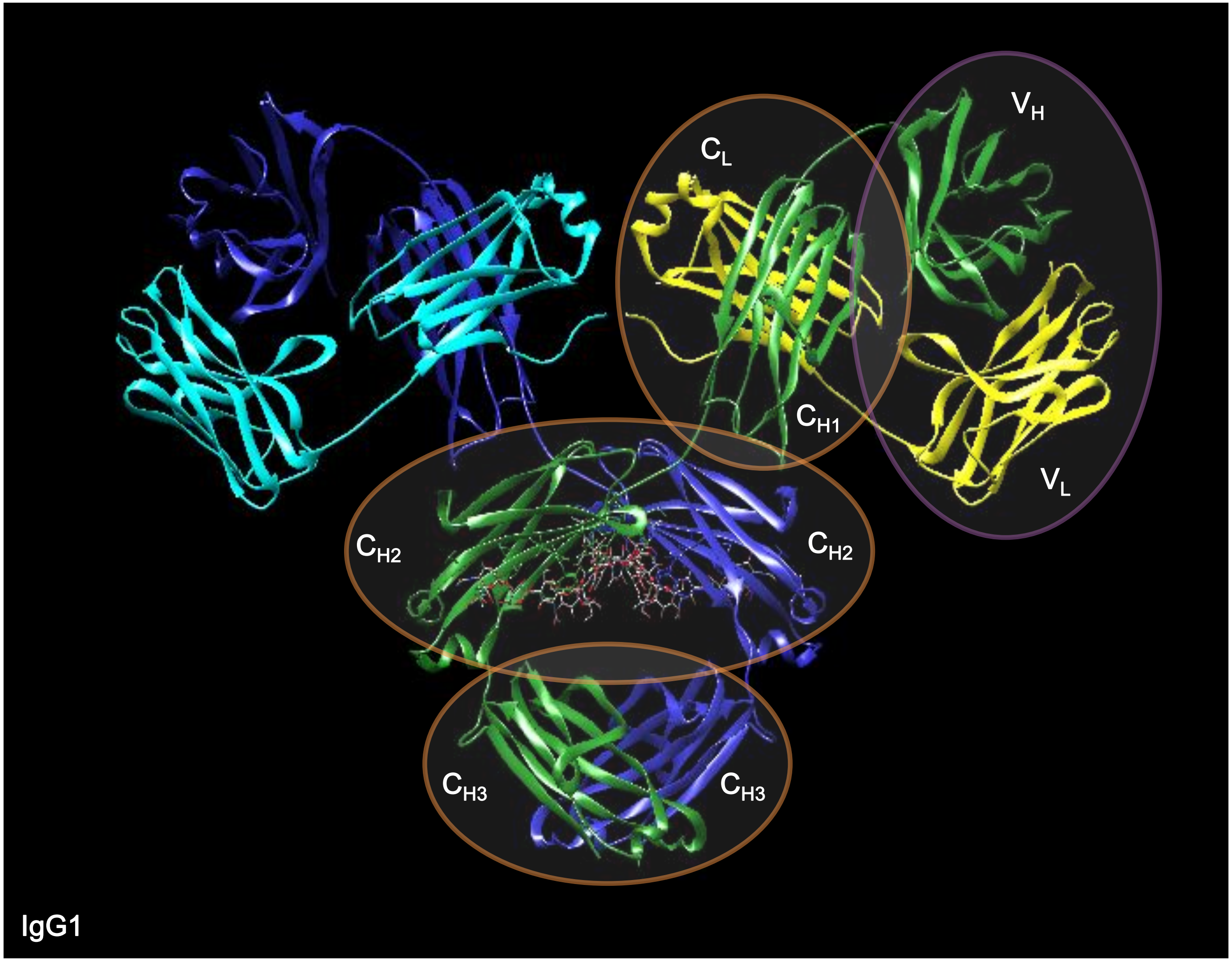
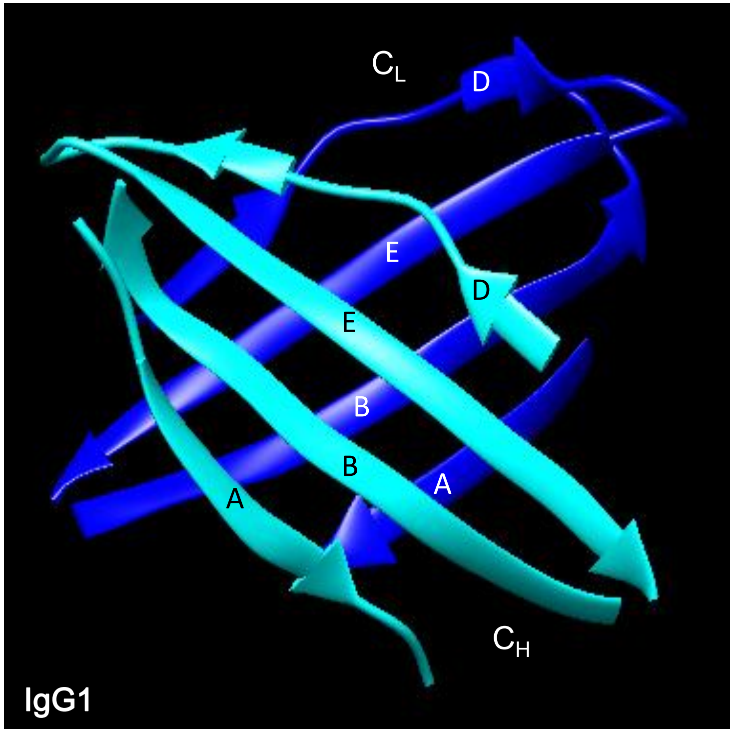


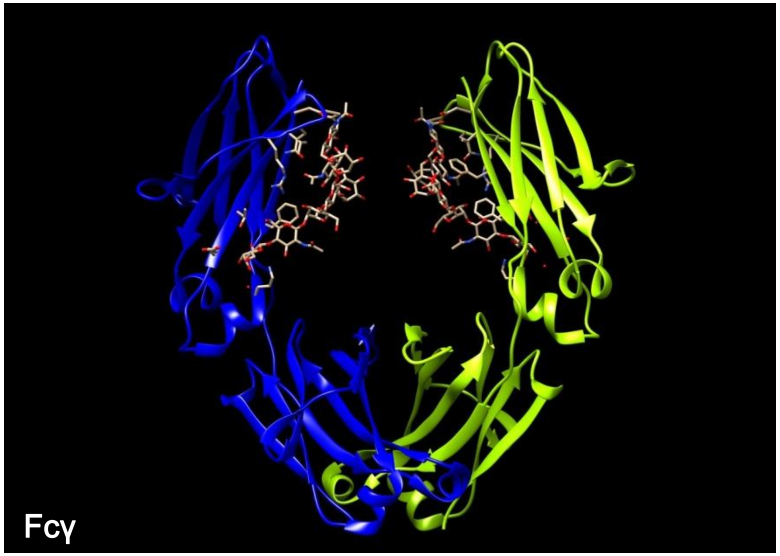
Chain association

Constant domains

Interaction between the four-stranded β sheets disposed almost at right angles to each other

There is a carbohydrate attached to each C_{H2} domain in the interface region and forms a weak bridge between them

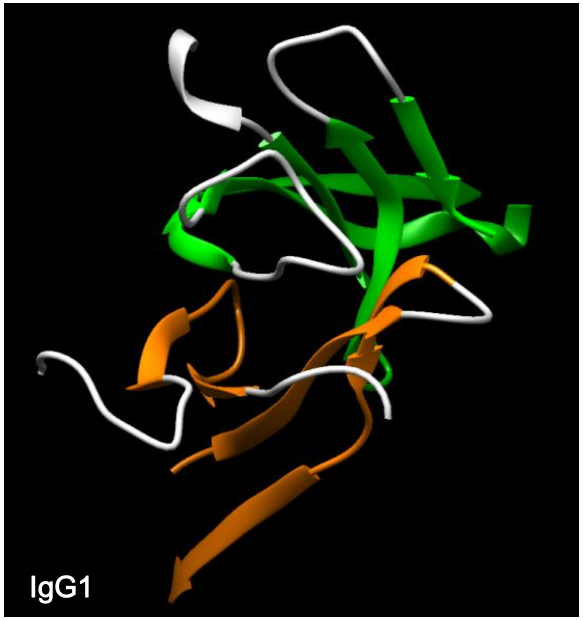


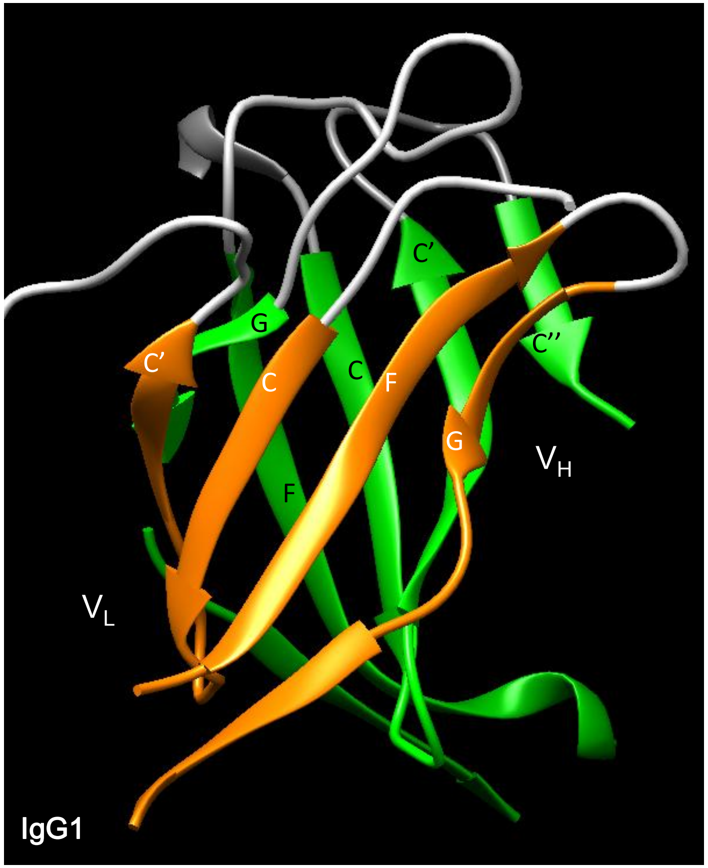


Chain association

Variable domains

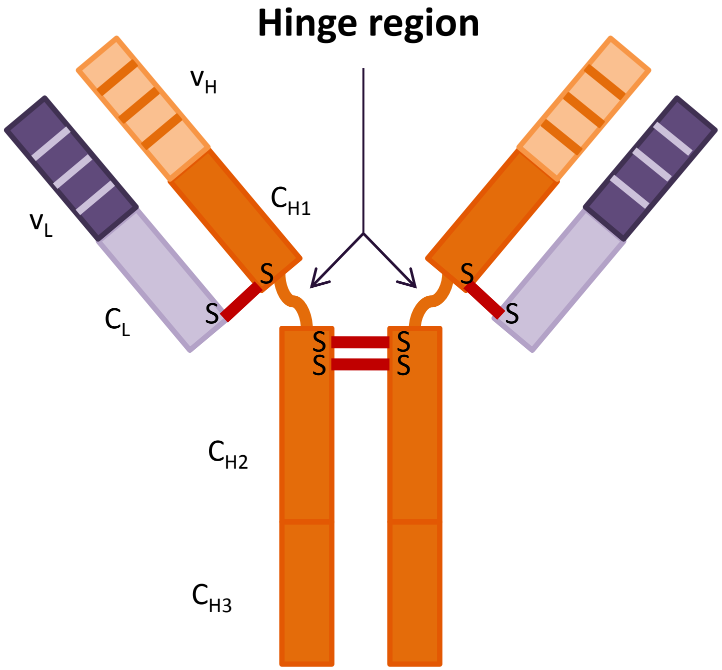
Interaction between the five-stranded β sheets orientated closer to parallel and forming a half barrel

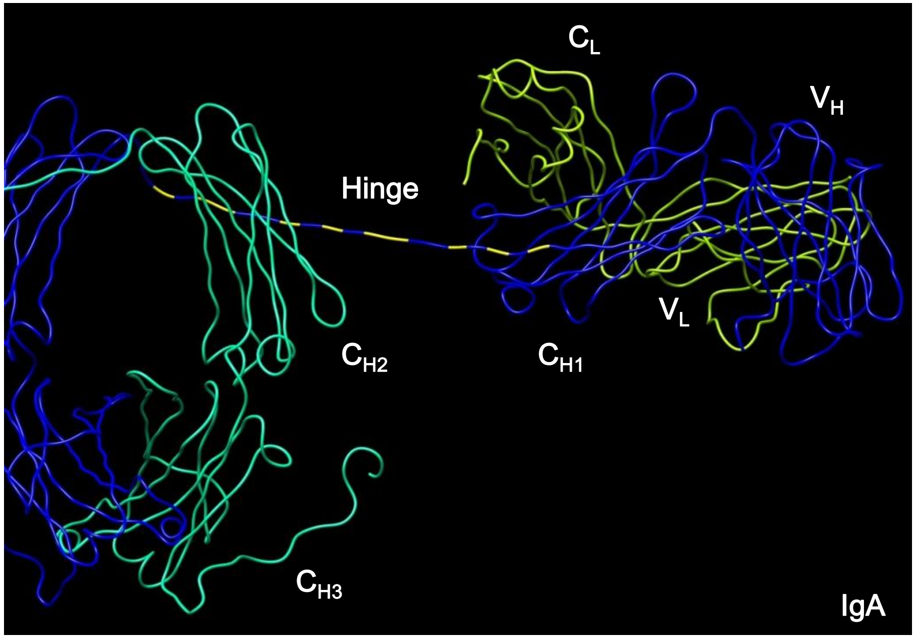




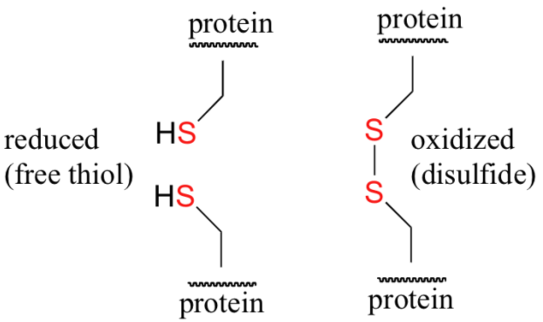
Hinge region and flexibility

- Connects C_{H1} and C_{H2}
- Proline rich domain

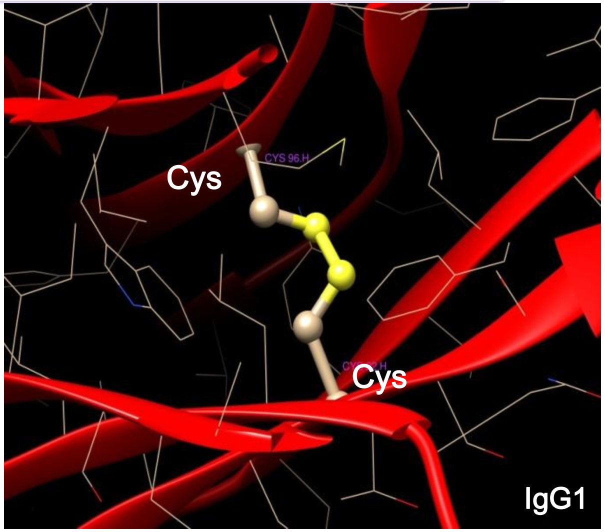


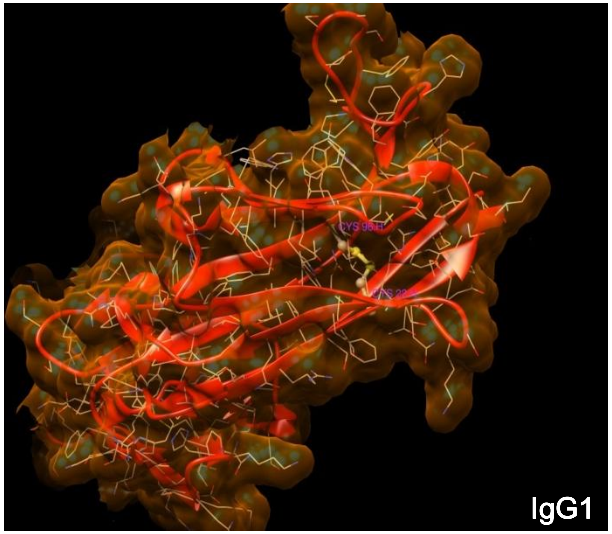


Disulfide bridges

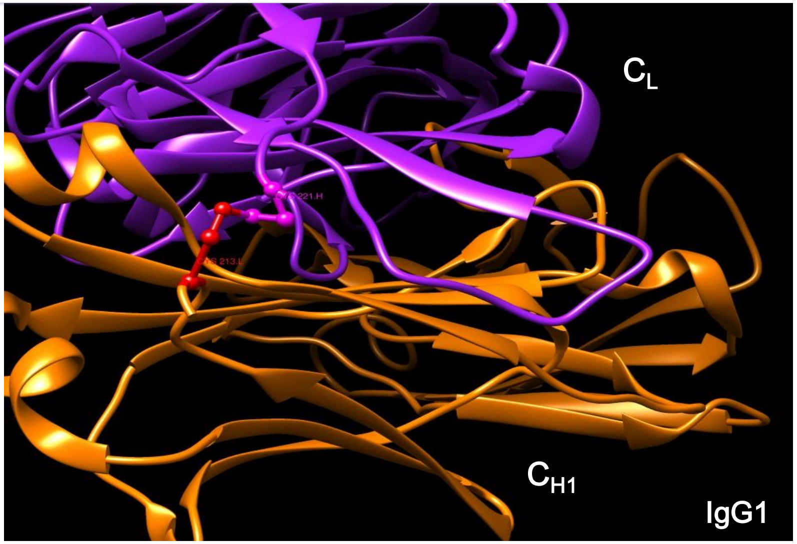


Intra-chain bridges





Inter-chain bridges



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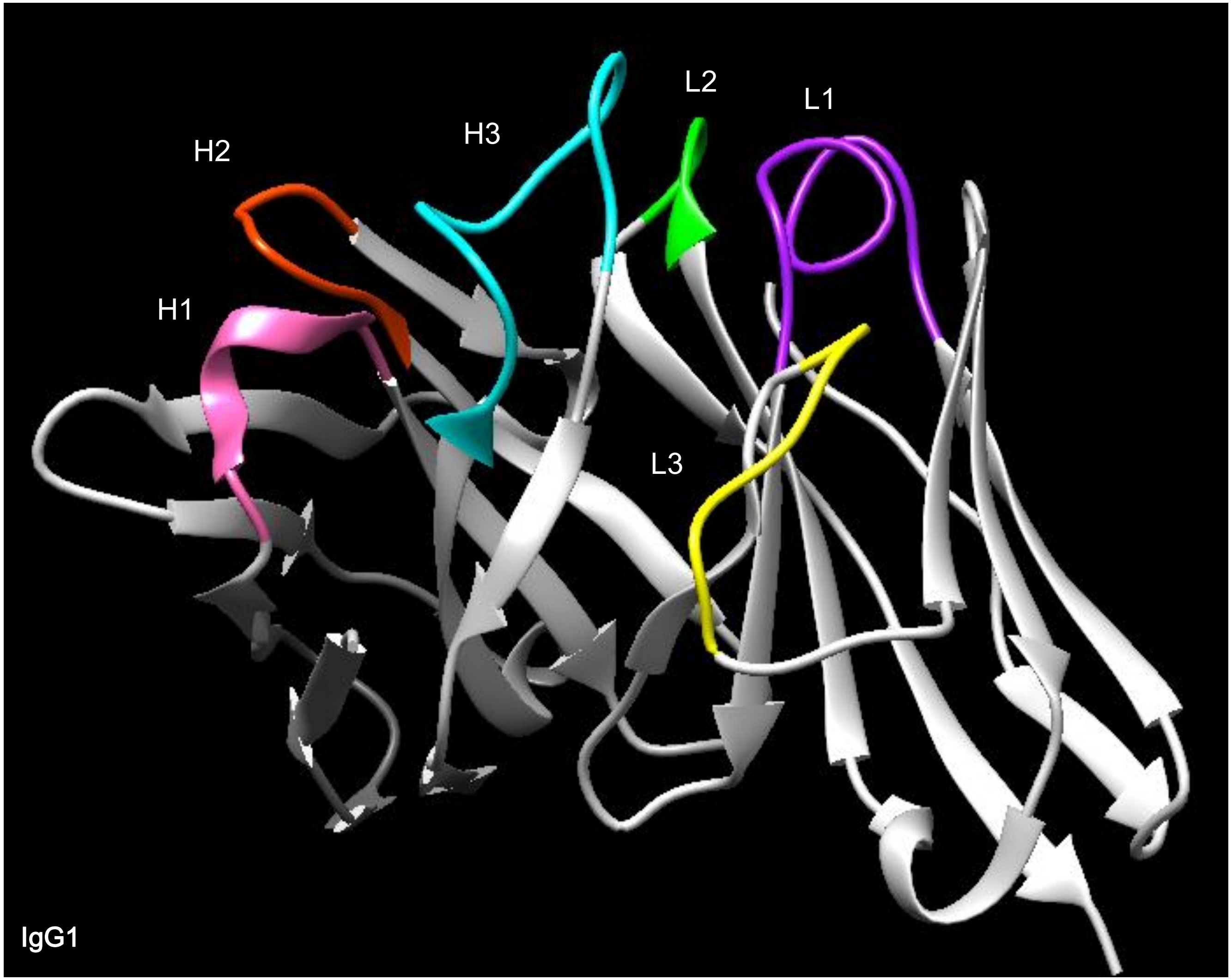


Canonical structures

5 of the 6 hipervariable regions have main chain conformations

L1	κ: 6 λ: 4
L2	1
L3	κ: 6 λ: 2
H1	3
H2	4

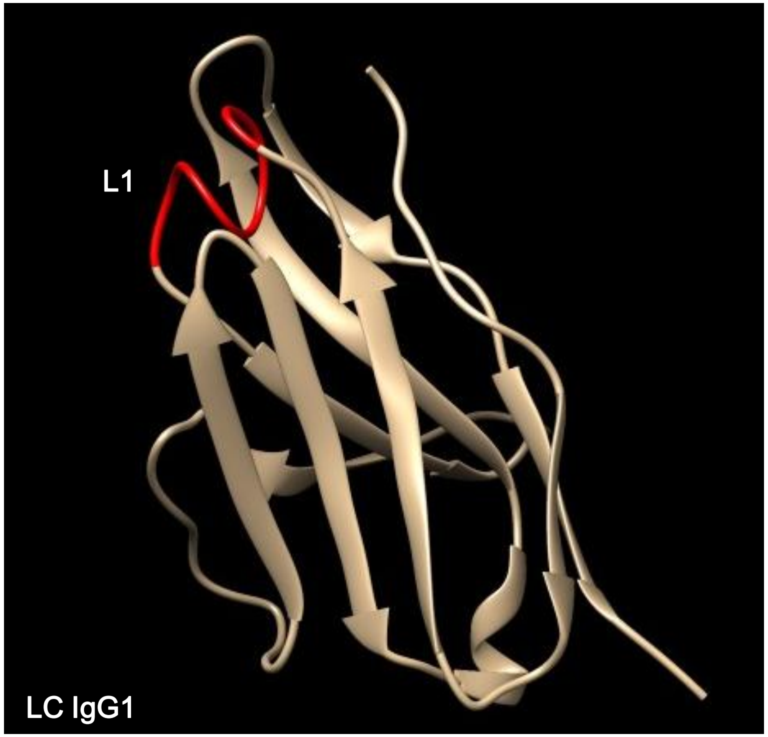
Conformation of a particular canonical structure is determined by:

- ✓ the length of the loop
- ✓ residues at key sites

L1 κ

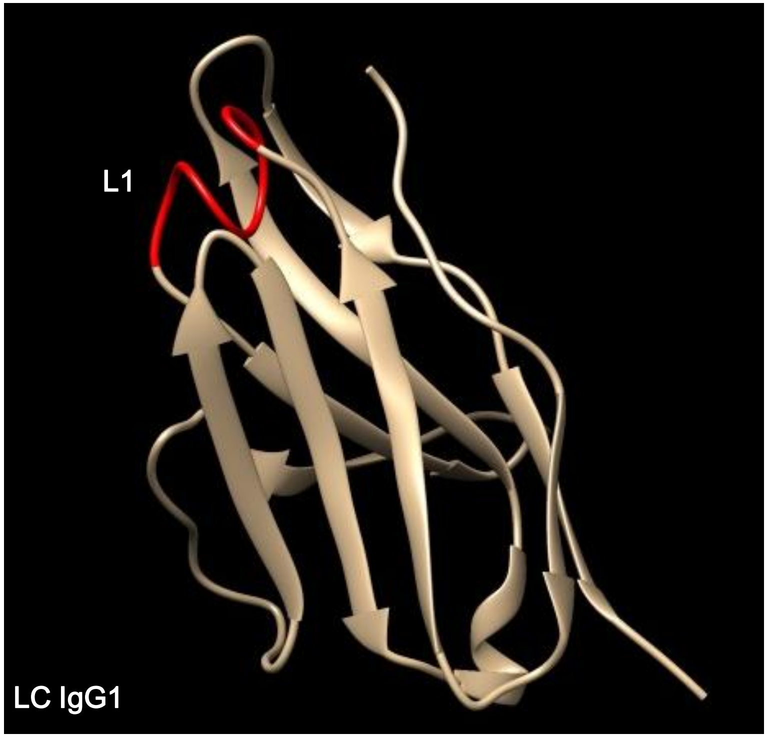
Canonical structure	1	2	3	4	5	6
N. of residues	0	1	7	6	5	2

Conformation of CDR1 which packs across the top of Vk domain, bridging the two β sheets

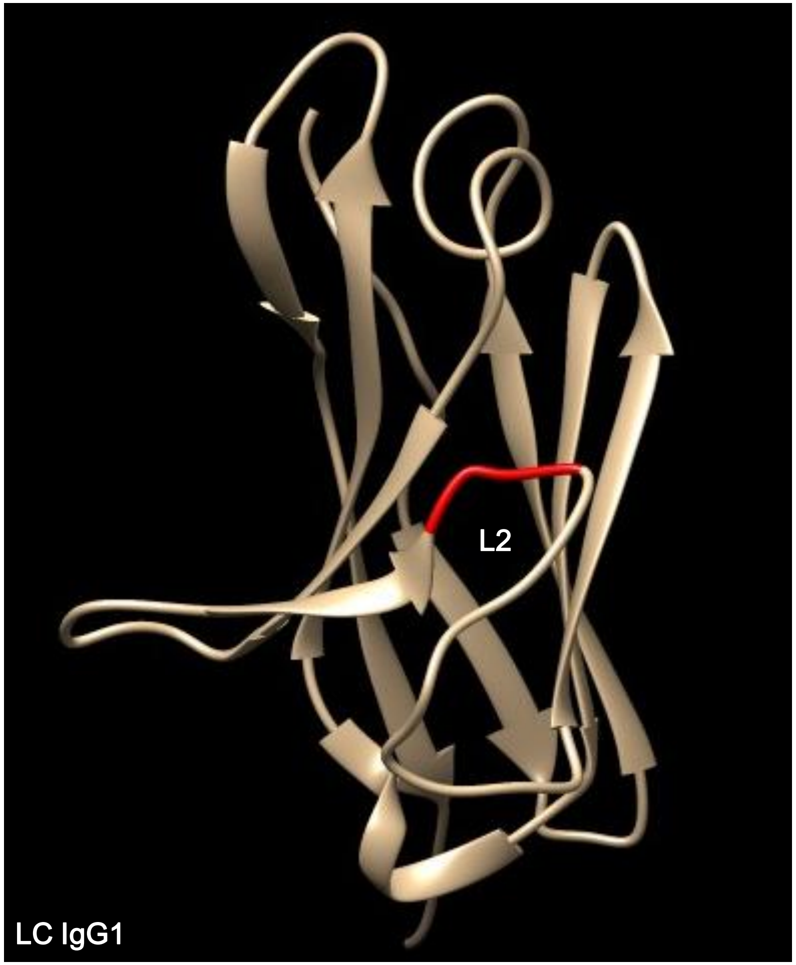


L1 λ

Canonical structure	1	2	3	4
N. of residues	10	11	11	9



L2



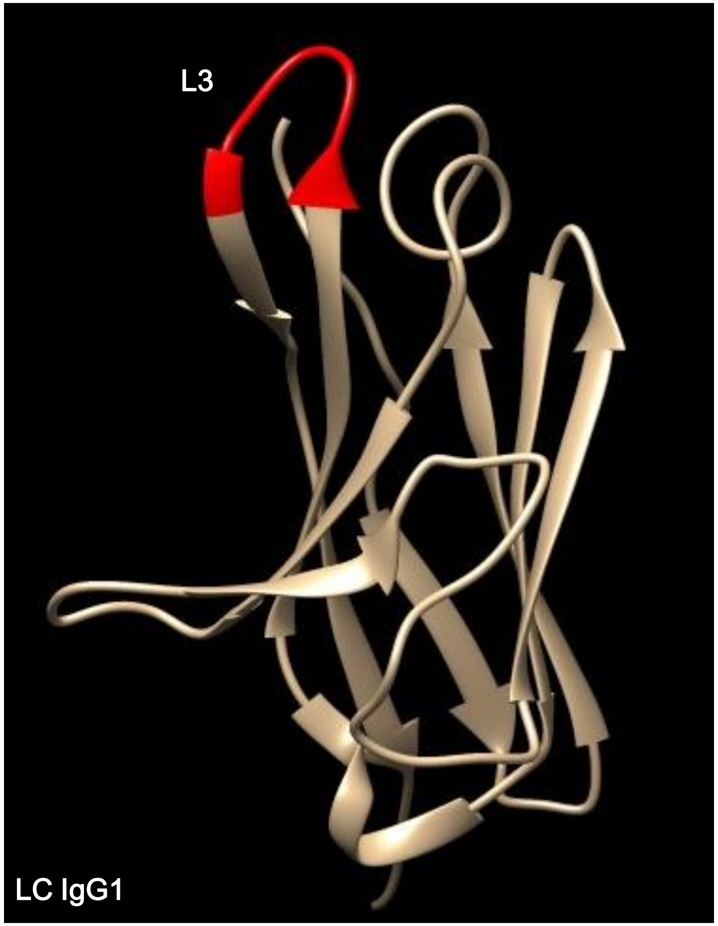
L2 hypervariable region is in the hairpin loop linking C’ and C’’ strands

Residues 50 to 56

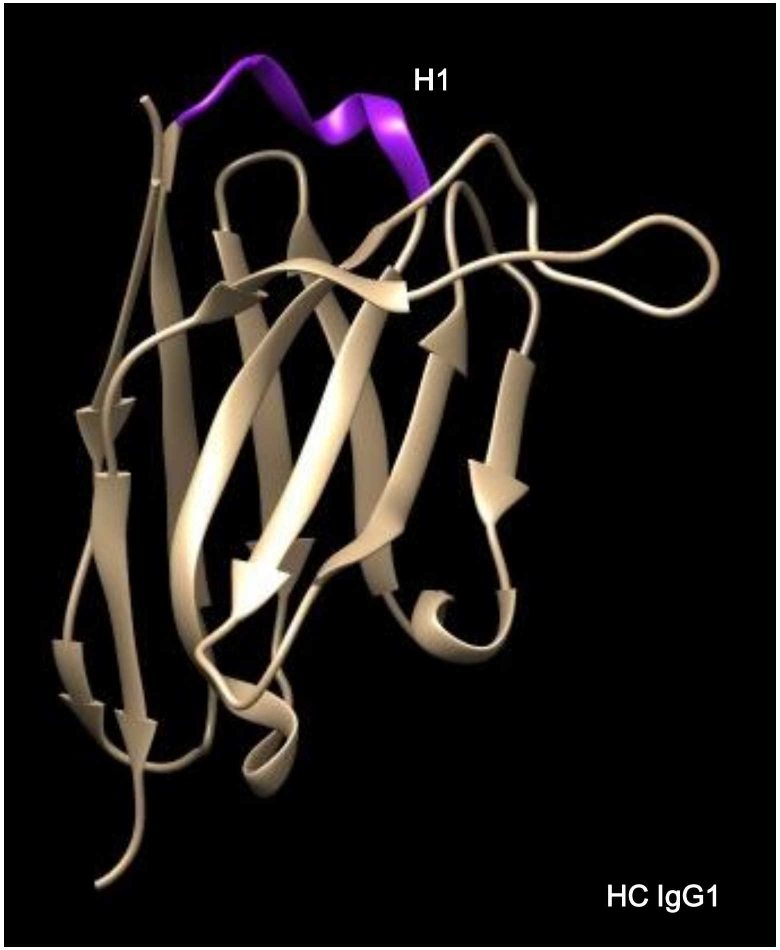
1 canonical structure

L3 κ and λ

L3 hypervariable region is in the hairpin loop linking the F and G strands
Residues 91 to 96
k: 6 canonical structures
λ: 2 canonical structures



H1



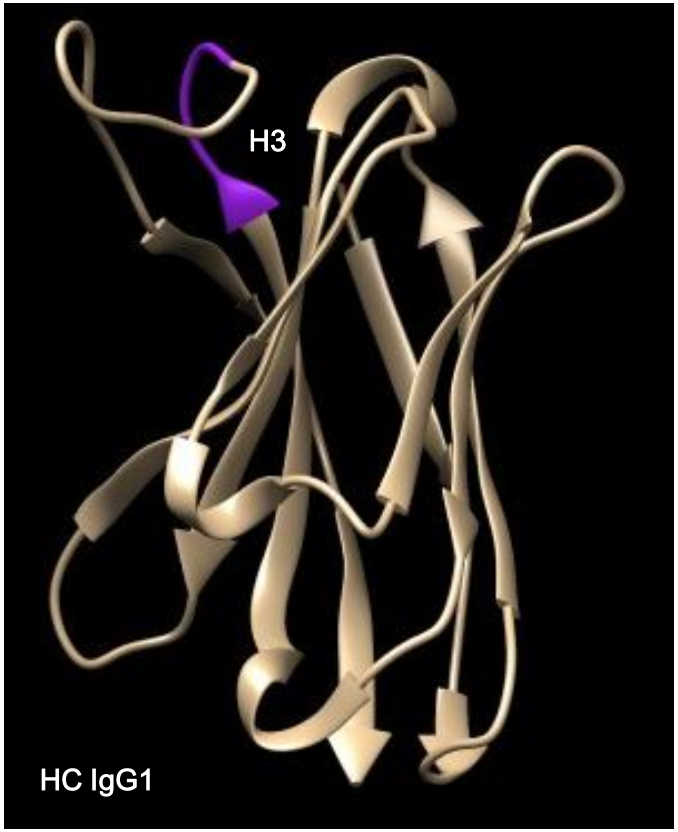
H1 region packs across the top of the V_H domain, bridging the two β-sheets
Residues 26 to 32
3 canonical structures

H2

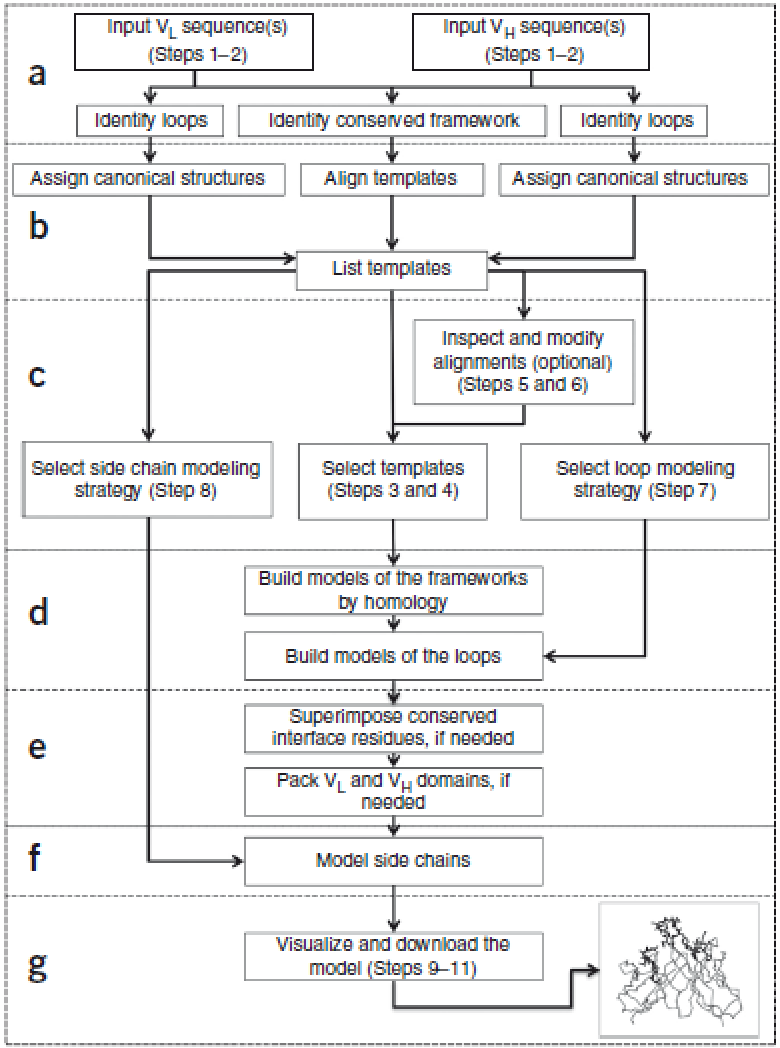
H2 region allows the binding between C' and C'' strands
Residues 52 to 56
4 canonical structures

H3

H3 is in the hairpin loop linking the F and G strands. Residues 95 to 102.

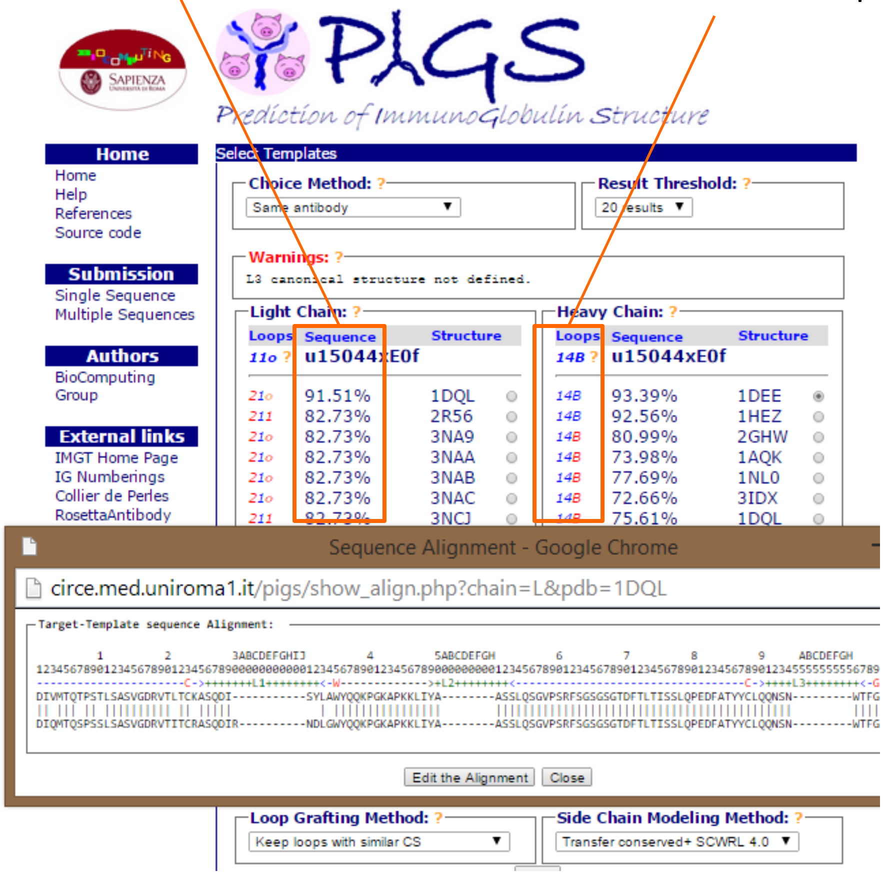


PIGS



Sequence identity

Canonical structure of the loop



Choice Method: ? Same antibody ▼

Result Threshold: ? 20 results ▼

Warnings: ?

`L3 canonical structure not defined.`

Light Chain: ?

Loops	Sequence	Structure	
11o ?	u15044xE0f		
21o	91.51%	1DQL	○
211	82.73%	2R56	○
21o	82.73%	3NA9	○
21o	82.73%	3NAA	○
21o	82.73%	3NAB	○
21o	82.73%	3NAC	○
211	82.73%	3NCJ	○

Heavy Chain: ?

Loops	Sequence	Structure	
14B ?	u15044xE0f		
14B	93.39%	1DEE	●
14B	92.56%	1HEZ	○
14B	80.99%	2GHW	○
14B	73.98%	1AQK	○
14B	77.69%	1NL0	○
14B	72.66%	3IDX	○
14B	75.61%	1DQL	○

Sequence Alignment - Google Chrome

circe.med.uniroma1.it/pigs/show_align.php?chain=L&pdb=1DQL

Target-Template sequence Alignment:

```
        1         2         3ABCDEFGHIJ         4         5ABCDEFGH         6         7         8         9     ABCDEFGH
1234567890123456789012345678900000000000012345678901234567890000000001234567890123456789012345678901234567890123455555555556789
---------------------C->+++++++L1++++++++<-W-------------->+L2++++++++<-----------------------------------C->++++L3++++++++<-G
DIVMTQTPSTLSASVGDRVTLTCKASQDI-----------SYLAWYQQKPGKAPKKLIYA--------ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQQNSN---------WTFG
|| ||| || |||||||||| || |||||            | ||||||||||||||||        |||||||||||||||||||||||||||||||||||||||||||         ||||
DIQMTQSPSSLSASVGDRVTITCRASQDIR----------NDLGWYQQKPGKAPKKLIYA--------ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQQNSN---------WTFG
```

Loop Grafting Method: ? Keep loops with similar CS ▼

Side Chain Modeling Method: ? Transfer conserved+ SCWRL 4.0 ▼

http://www.biocomputing.it/pigs

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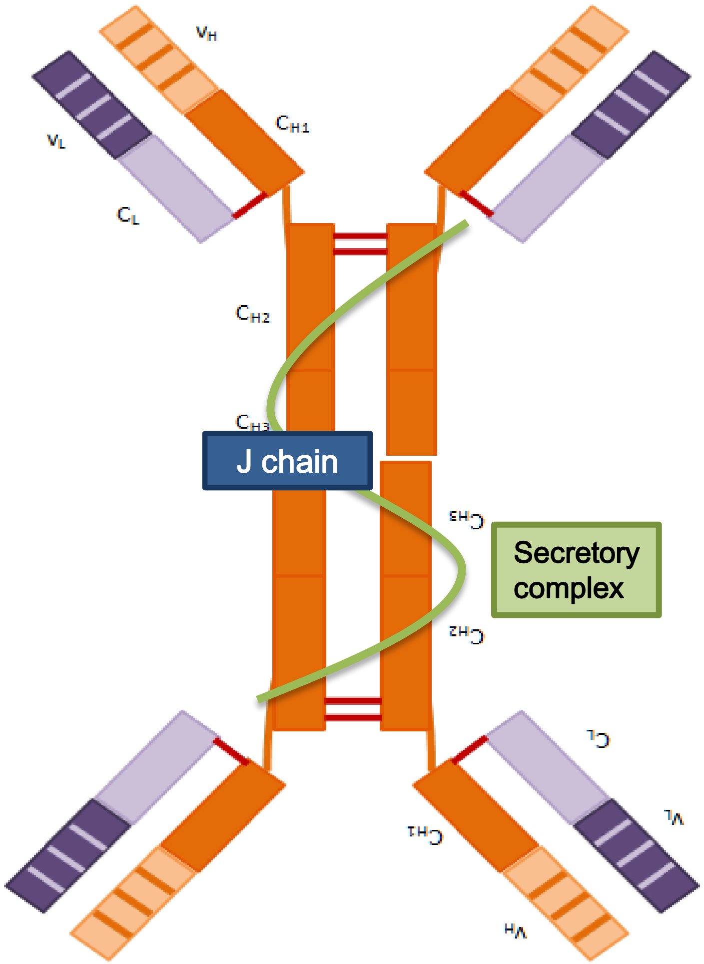
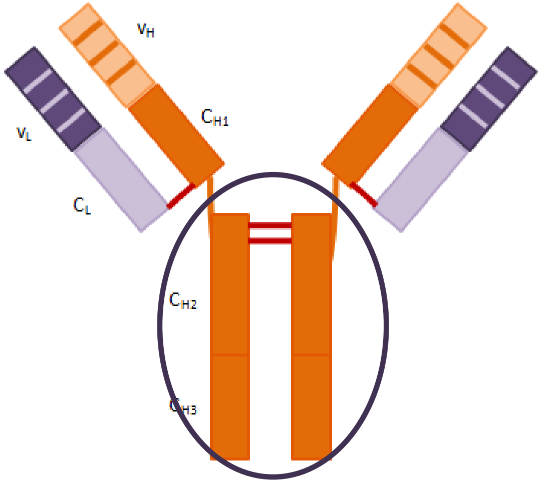
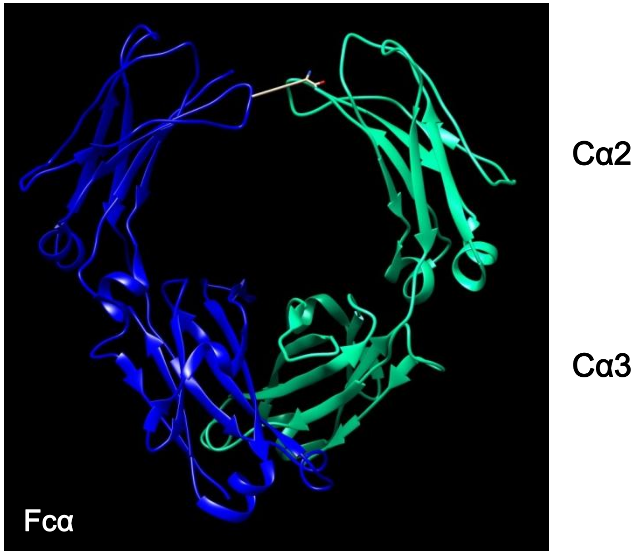
Immunoglobulin A

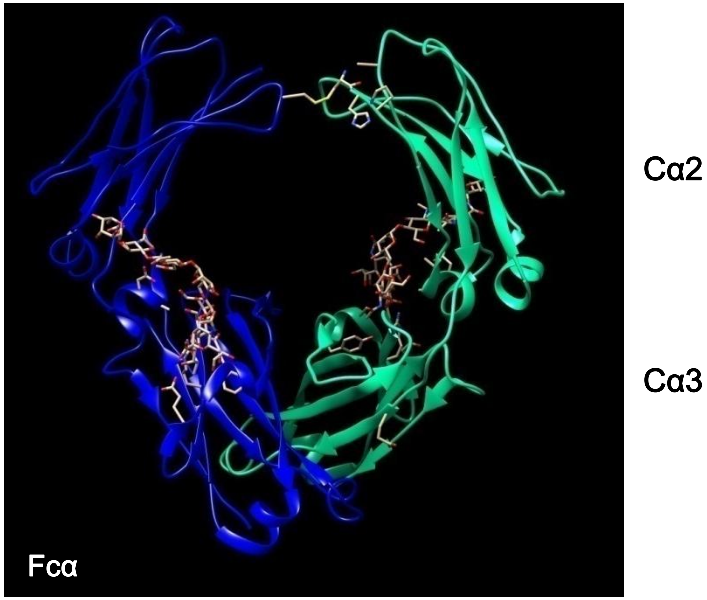
- Predominant in mucosal regions
- First barrier against pathogens
- Two forms:
  - Monomeric: similar to IgG and IgE
  - Dimeric: secretory IgA formed with J chain and SC

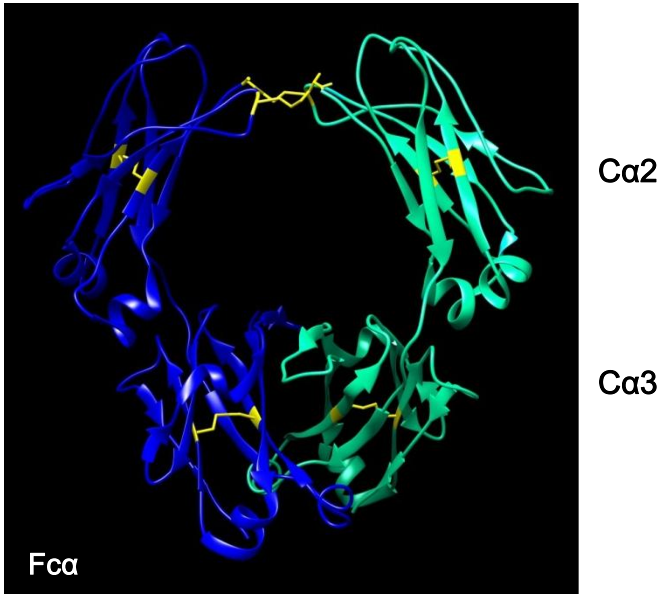


Fc alpha



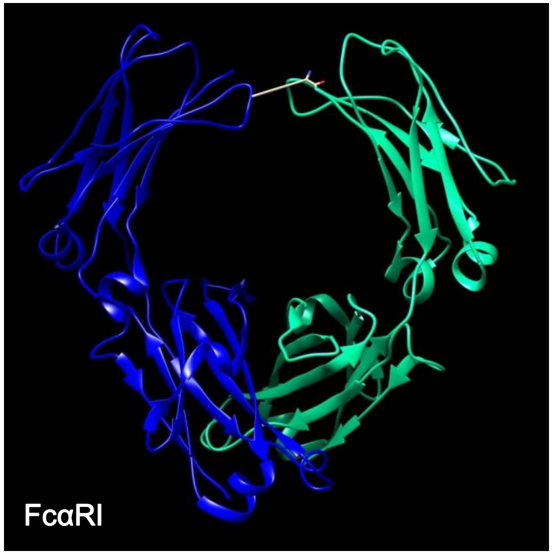


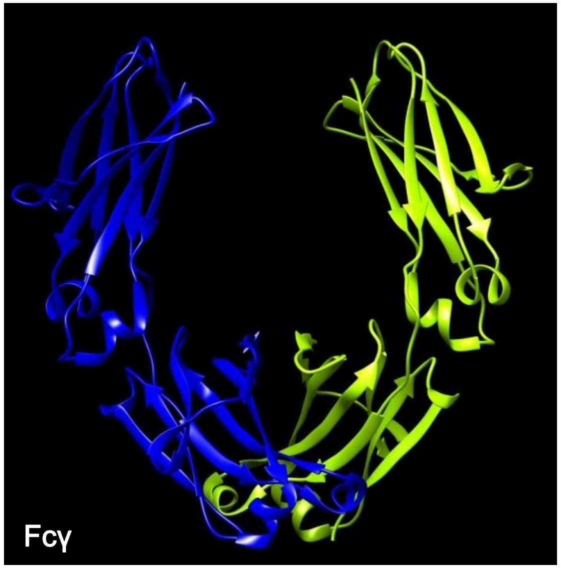


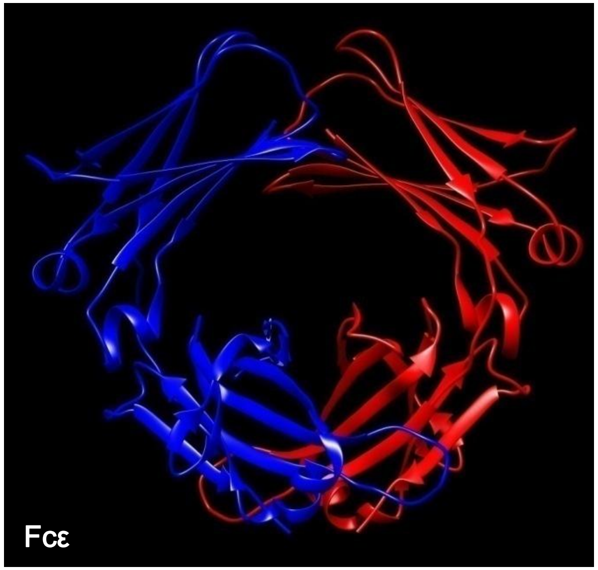


Fc alpha vs gamma/epsilon

General structure

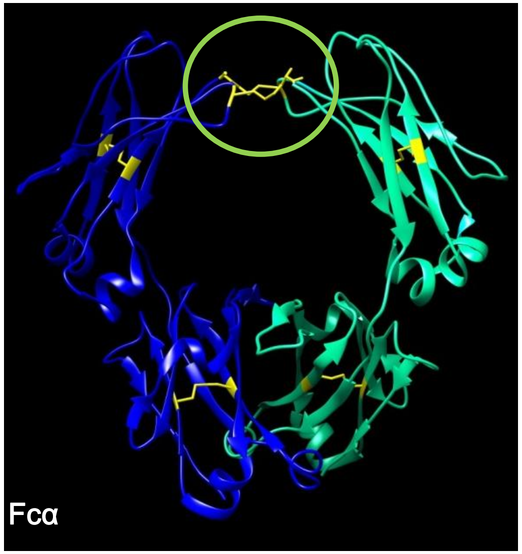


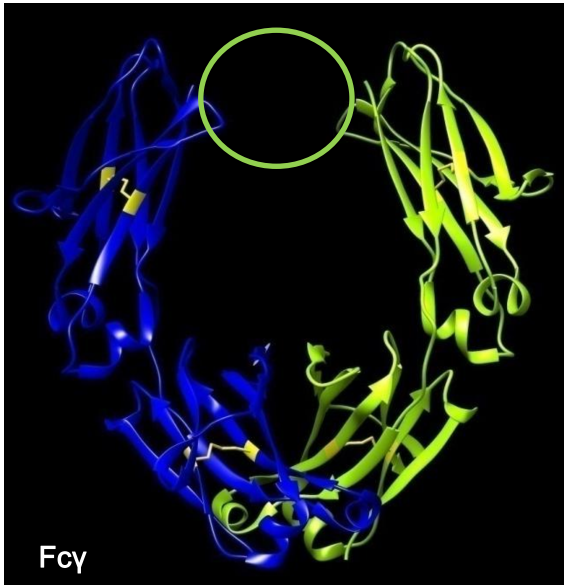


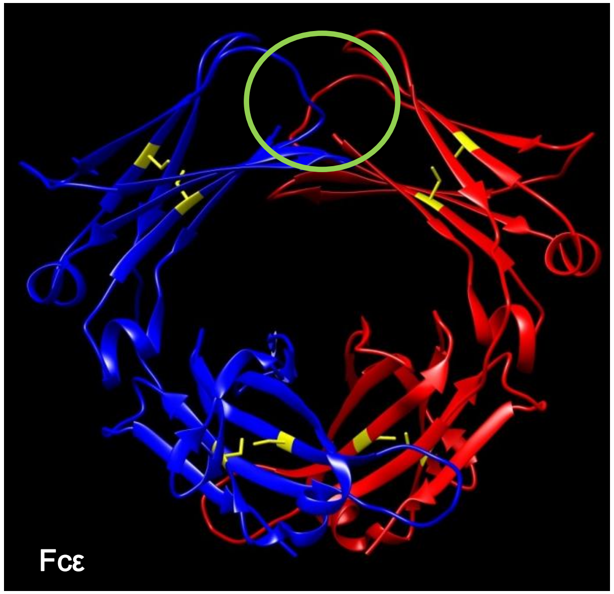


Fc alpha vs gamma/epsilon

Disulfide bonds

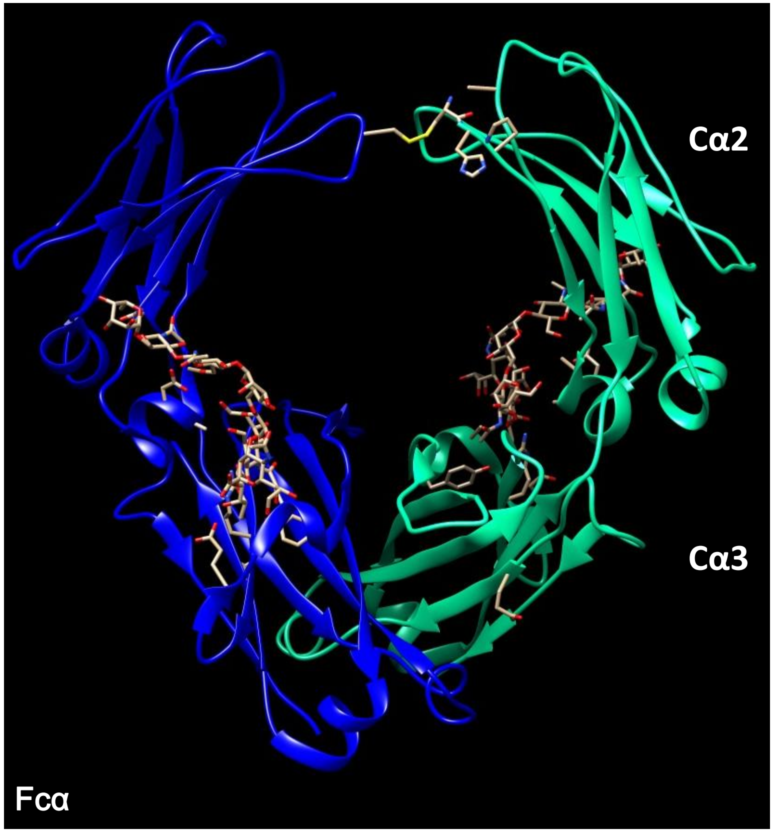


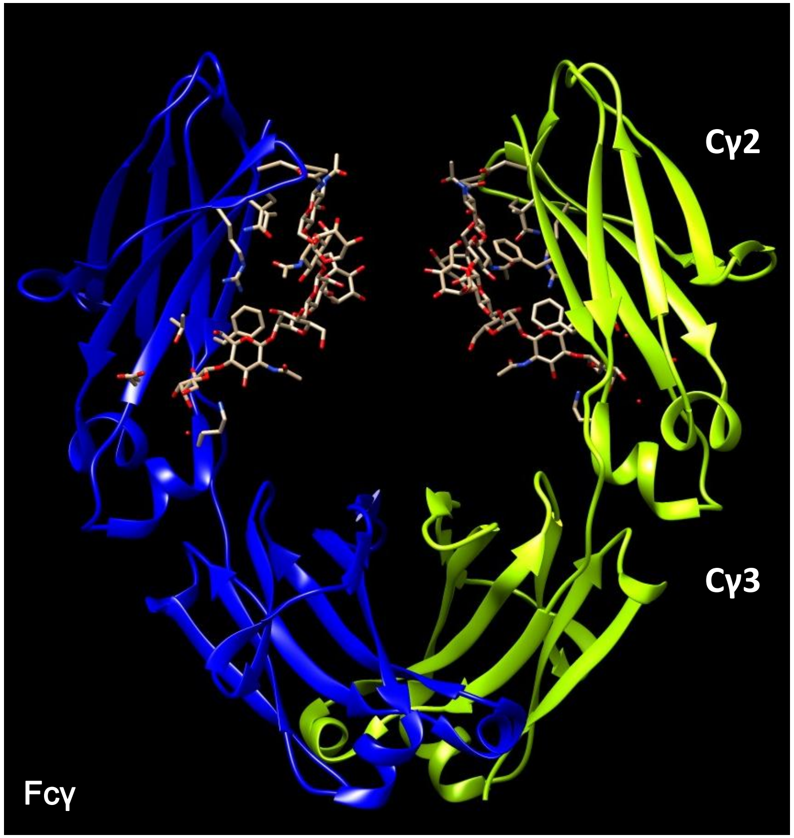




Fc alpha vs gamma/epsilon

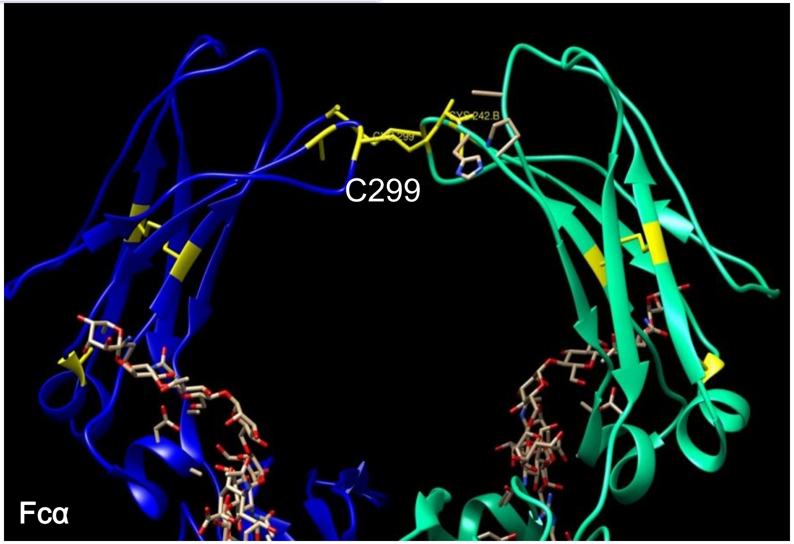
Glycosilation

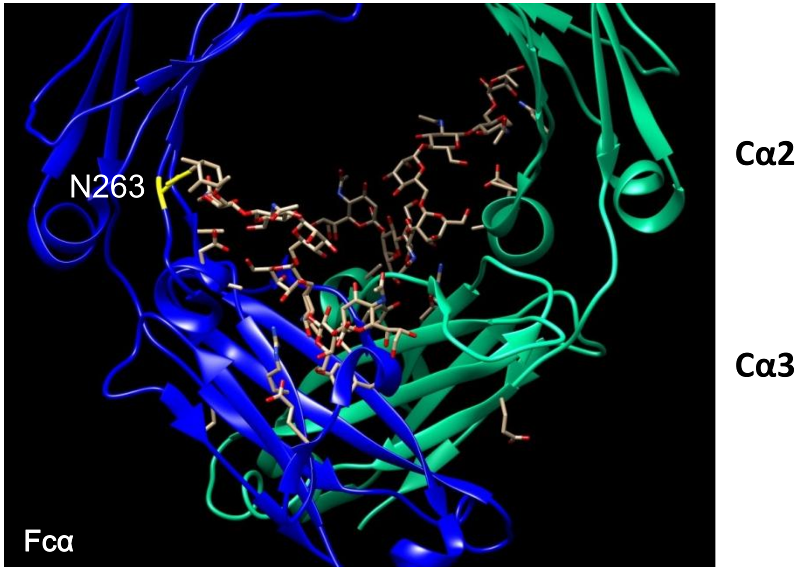


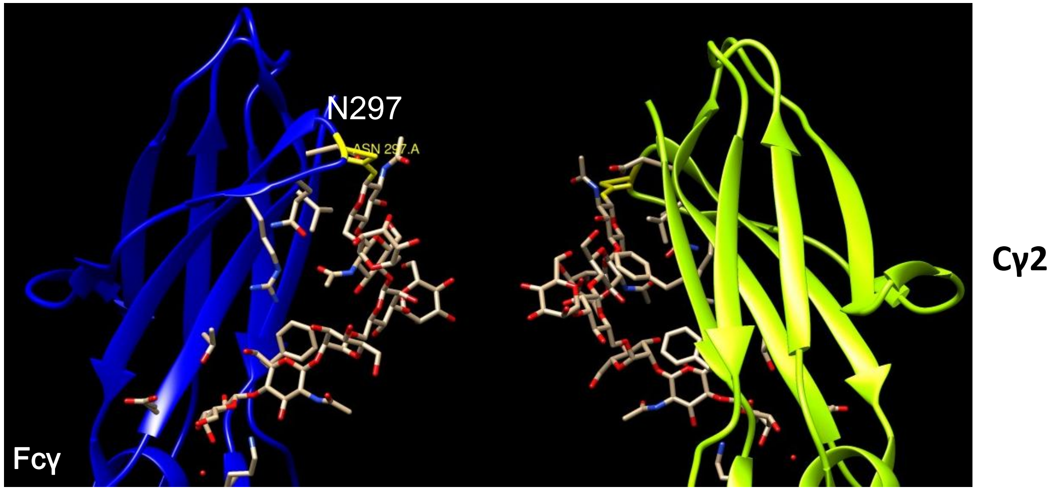


Fc alpha vs gamma/epsilon

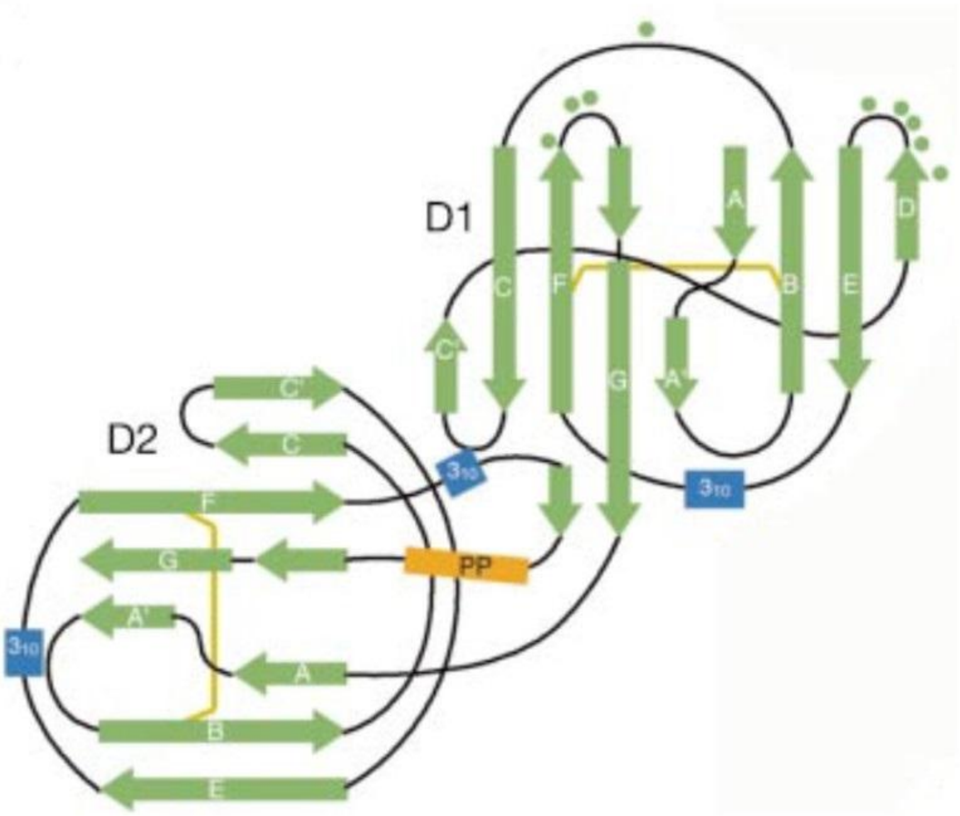
Cys - Asn change

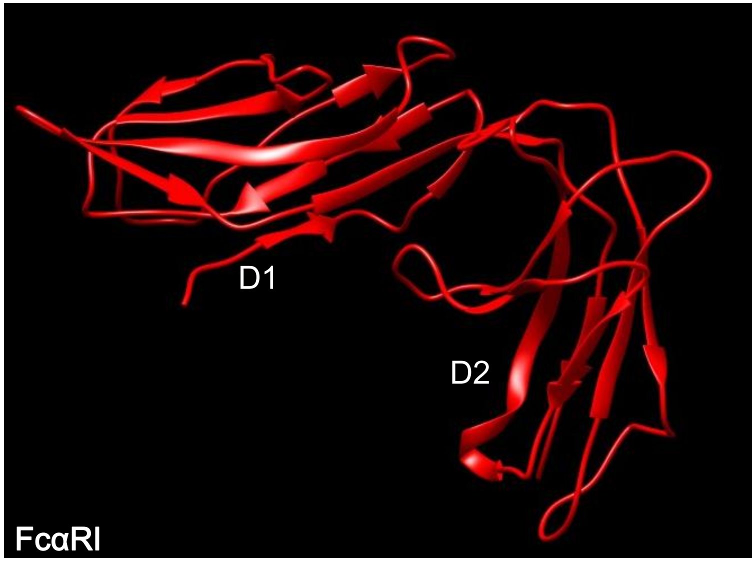




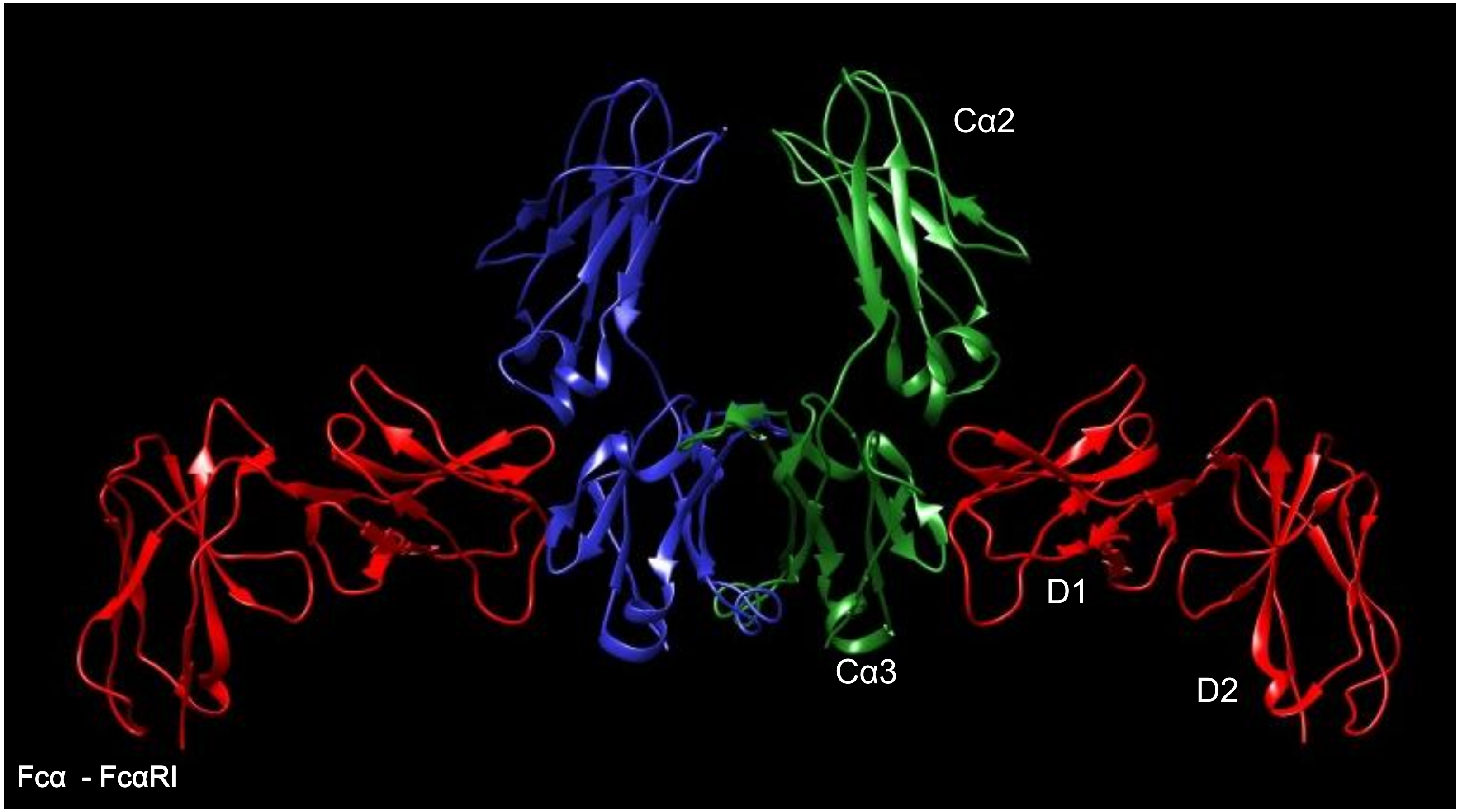


IgA and Fcα Receptor I

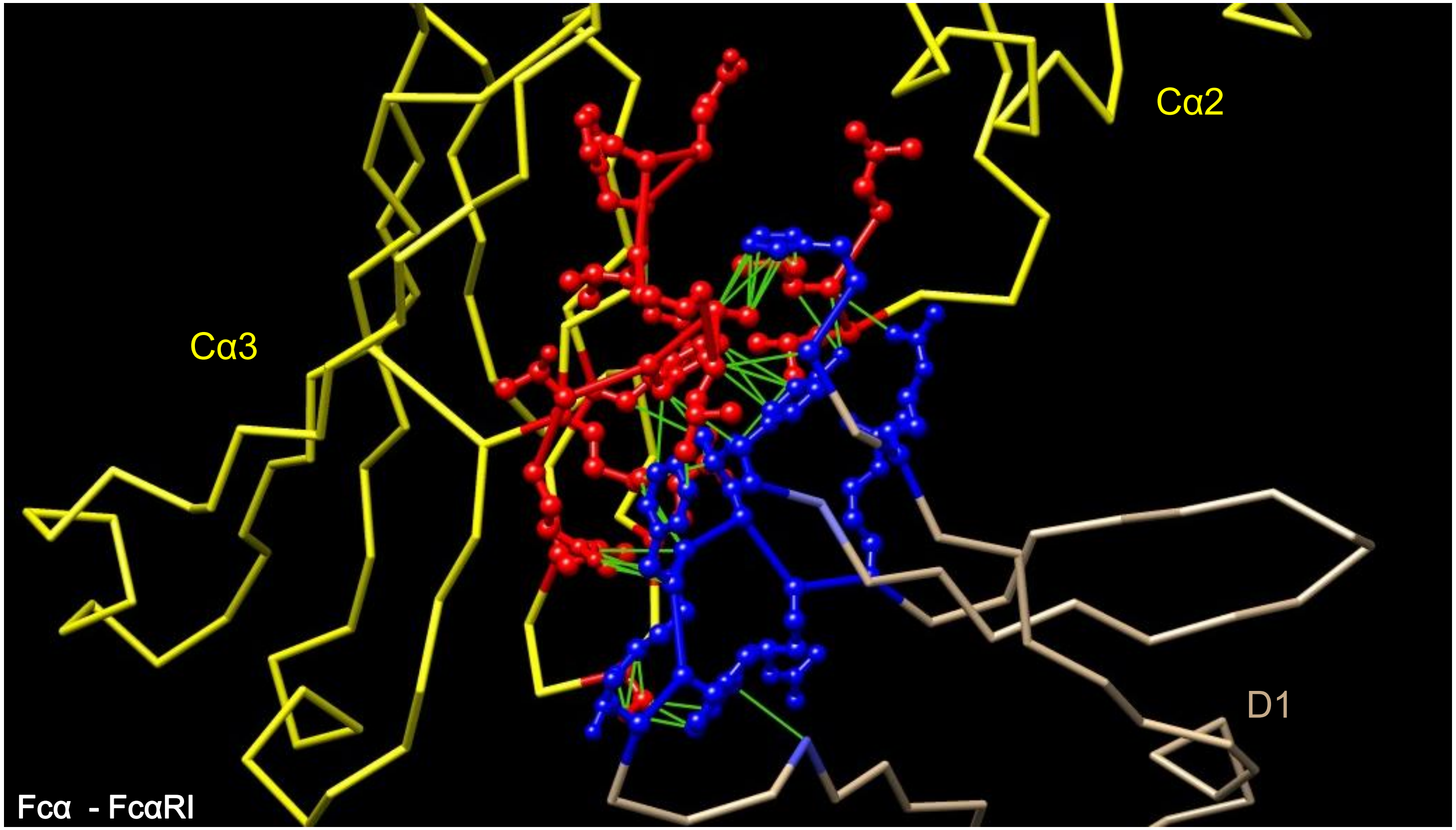




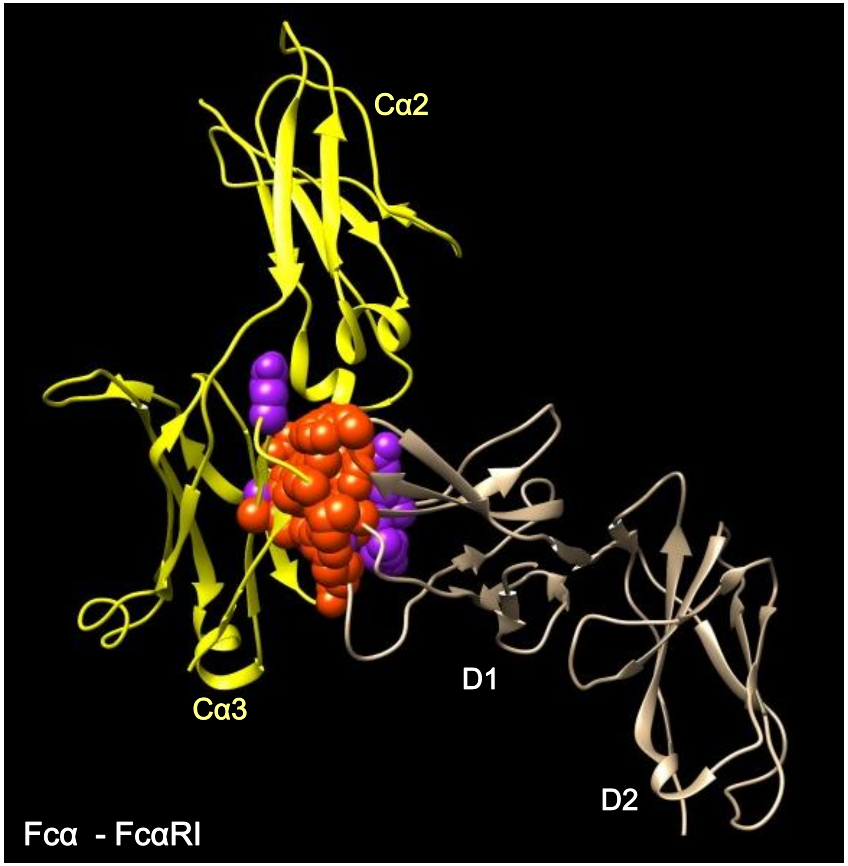
IgA – Fcα Receptor I

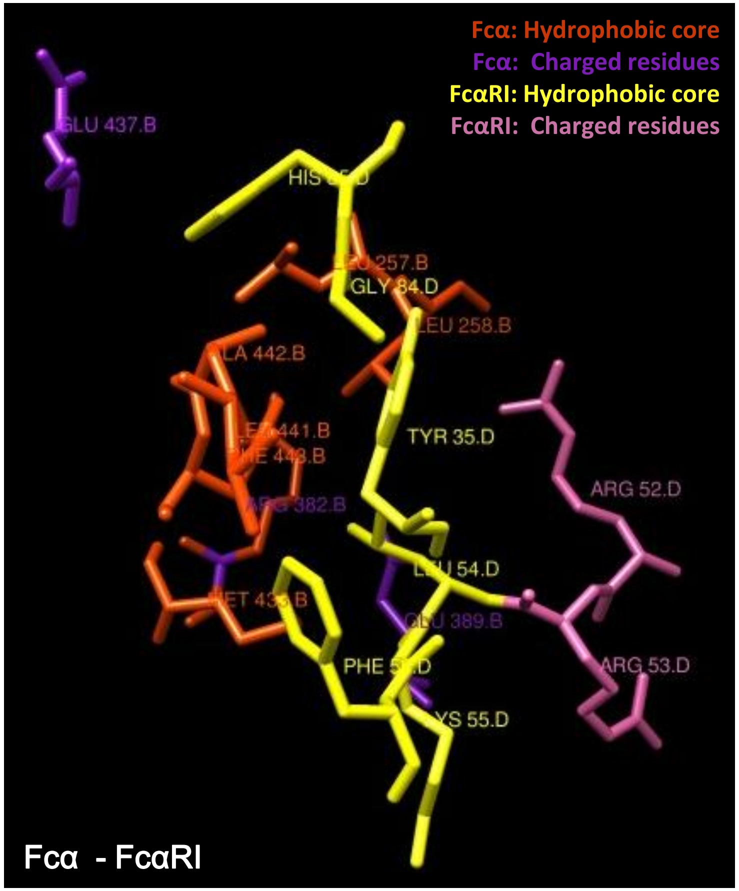


IgA – Fcα Receptor

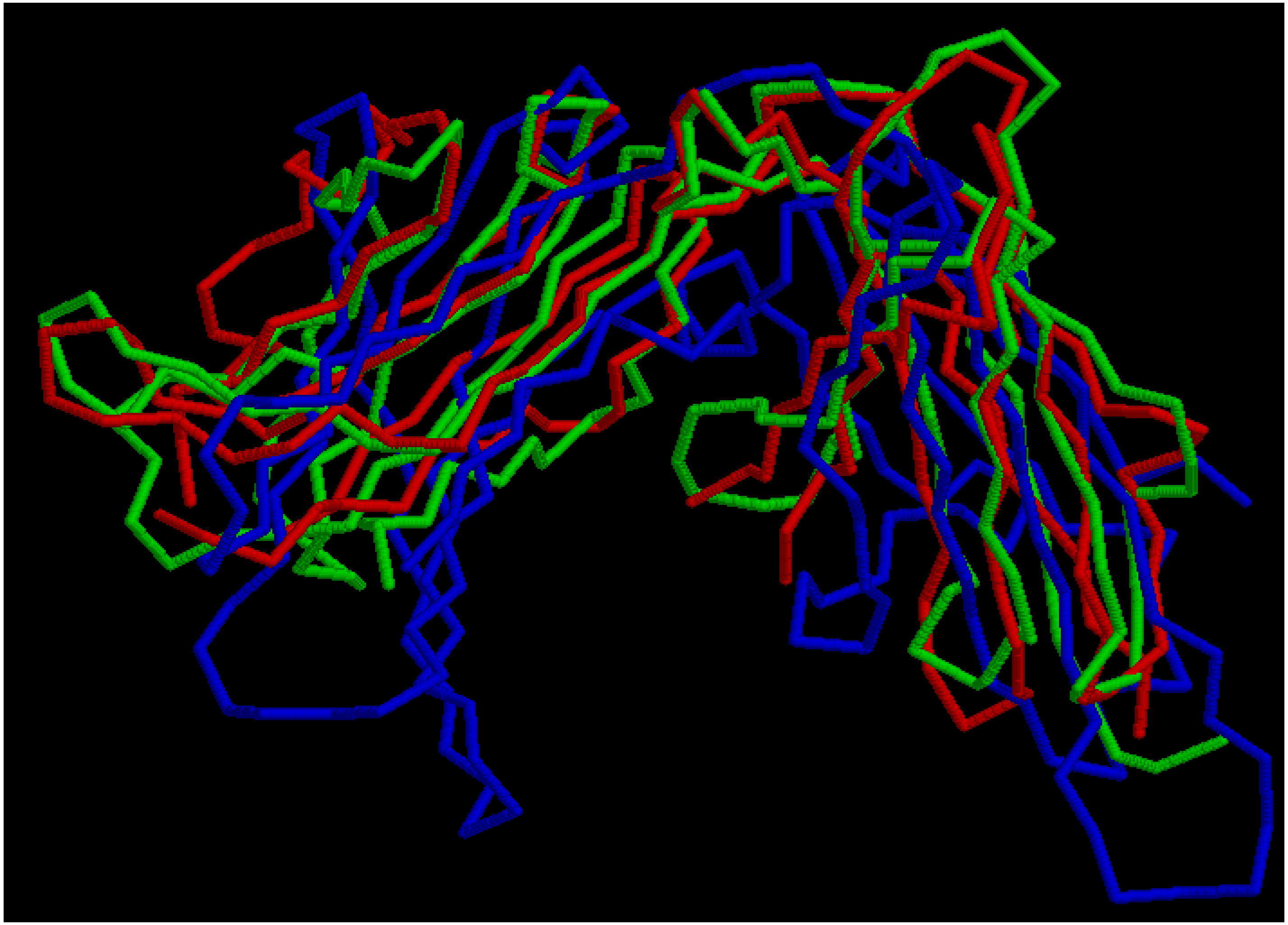


IgA – Fcα Receptor





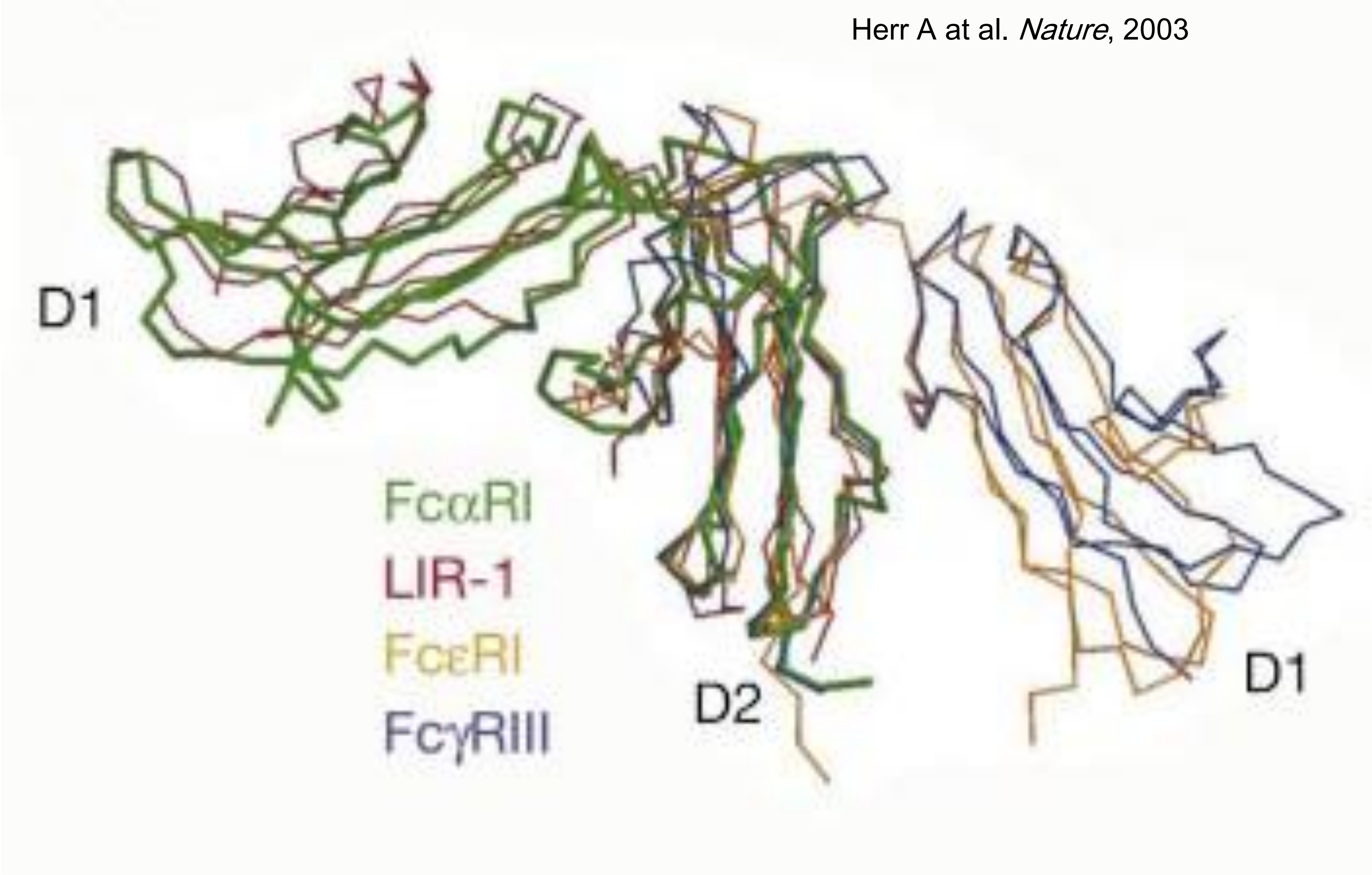
Fc Receptors superimposition



Blue: FcƐRI / Green: LIR-1 / Red: FcαRI

Fc Receptors superimposition

Herr A at al. *Nature*, 2003



Conclusions

- ✓ Immunoglobulins are composed by 2 heavy chains and 2 light chains
- ✓ Each Ig domain is about 110 residues. There are variable and constant domains
- ✓ There is a common structure in the Ig Superfamily known as the Immunoglobulin fold
- ✓ Disulfide bonds stabilize the conformation of the Ig domain
- ✓ Hypervariable regions determine the antigen binding surface and 5 of them have canonical structures

- ✓ IgA has 6 disufide bonds: 4 intra-domain and 2 inter-chain
- ✓ The primary differences between IgA and other Igs are in the position of N-linked carbohydrates and disulfide bonds
- ✓ In the FcαRI:Fcα complex the interaction occurs between D1 and Cα3 and Cα2, respectively

Thank you for your attention

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PDBs

1MAM → IgG2B-k

7FAB → IgG1-λ

2FB4 → IgG1

1MCO → IgG1

1IGA→ IgA

1OVZ → FcαRI

1OW0 → FcαRI bound to IgA1-Fc

1G0X → LIR

1DN2 →Fcγ

1O0V o 1J87 → FcεRI

3WN5 → FcγFc

2FCB → FcγRIIB ectodomain

1FP5 → Fcε (biological assembly)

2WQR → Fcε

1TEN → TNfn3

Exam questions

1. About the different isotypes of immunglobulins:
    a. IgM is the predominant isotype of immunoglobulins
    b. IgA is found as a pentamer in serum
    c. IgD induces the major response to protein antigens
    d. IgG is found in mucosal surfaces and in secretions
    e. IgE is associated to hypersensitivity reactions

2. The immunoglobulin fold:
    a. Is an all alfa structure
    b. Is composed of 4 α-helices arranged in 20º angles forming a bundle
    c. Consists in two antiparallel β sheets packed tightly against each other
    d. Can only be found in antibodies
    e. Forms an up and down β barrel

3. About disulfide bonds:
    a. There are only intra-chain disulfide bonds in all immunoglobulins
    b. There are only inter-chain disulfide bonds in all immunoglobulins
    c. There aren’t inter-chain disulfide bonds in immunoglobulins
    d. There aren’t inter-chain disulfide bonds in immunoglobulins
    e. There are both inter-chain and intra-chain disulphide bonds in immunoglobulins

Exam questions

4. Choose the correct sentence about the immunoglobulins:
   a. The constant fragment of an immunoglobulin is the part that binds the antigen
   b. H3 has canonical structures IgD induces the major response to protein antigens
   c. CDRs are the regions that give variability to the immunoglobulin
   d. Hypervariable regions are located in the hydrophobic core of an immunoglobulin
   e. All of them are correct

5. About CDRs and canonical structures:
   a. Canonical structures are determined by the length and the residues in key sites of the loop
   b. L1 canonical structure links the two beta sheets of the immunoglobulin domain
   c. A and B are correct
   d. The framework region does not take part in the conformation of the loops
   e. All of them are false

Exam questions

6. Choose the correct answer:
    a. **Variable domains have two extra β strands inserted between C and D strands**
    b. Different strands cannot be delimitated in the sequence of immunoglobulins
    c. Loops have exactly the same sequence in every Ig chain
    d. Constant domains have 4 β strands arranged in a single β sheet
    e. No aminoacid conservation occurs within immunoglobulin sequences

7. The FcαRI:
    a. **Has two immunoglobulin-like ectodomains (D1 and D2)**
    b. Has a similar fold as LIR-a receptor
    c. Forms a 1:1 complex with IgA Fcα regions.
    d. Interaction with Fcα has only charged residues
    e. Is more similar to FcεRI than to LIR-1

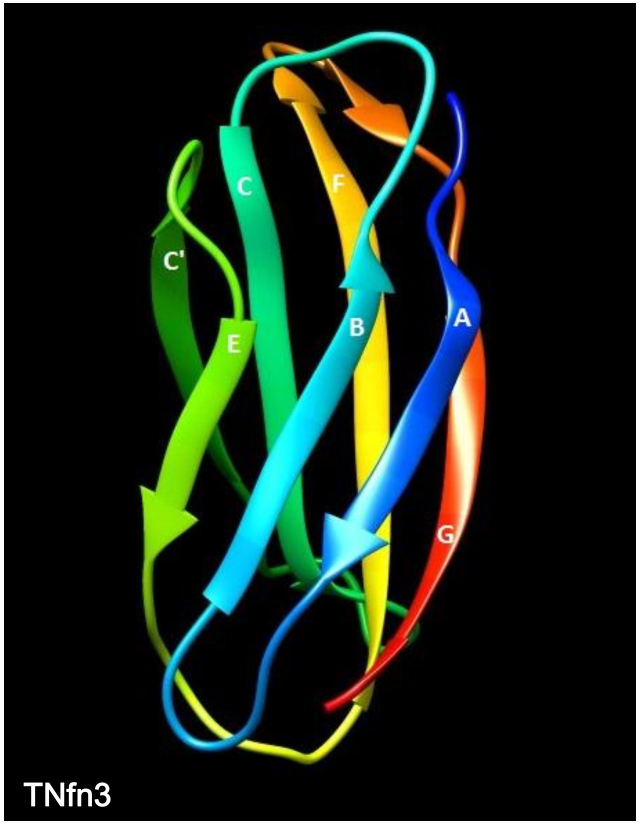
8. Select the correct answer:
    a. It is easy to crystallize an IgA alone
    b. Fcα is a two-fold asymmetric dimer of IgA heavy chains
    c. N-linked carbohydrates are attached on the center of the molecule in IgE
    d. There are many variations in the loop regions between different Ig isotypes
    e. **There are 6 disulfide bonds in IgA: 4 intra-domain and 2 inter-chain**

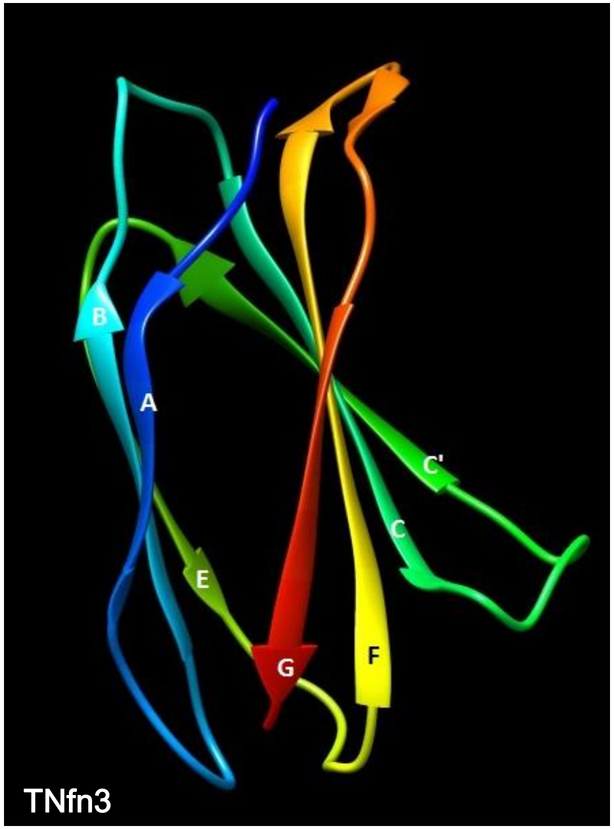
Exam questions

9. About chain association:
   a. The directions of the β strands in the two constant domains are almost parallel
   b. Chain association of constant domains is the same between C_{H2}-C_{H2} than between C_{H3}-C_{H3}
   c. C_{H2}-C_{H2} are associated by glycans that are attached to each C_{H2} domain
   d. Chain association between immunoglobulin domains is equal for constant and variable domains
   e. Constant domains are linked mainly to the five-stranded β-sheet completing a barrel structure of eight antiparallel β-strands

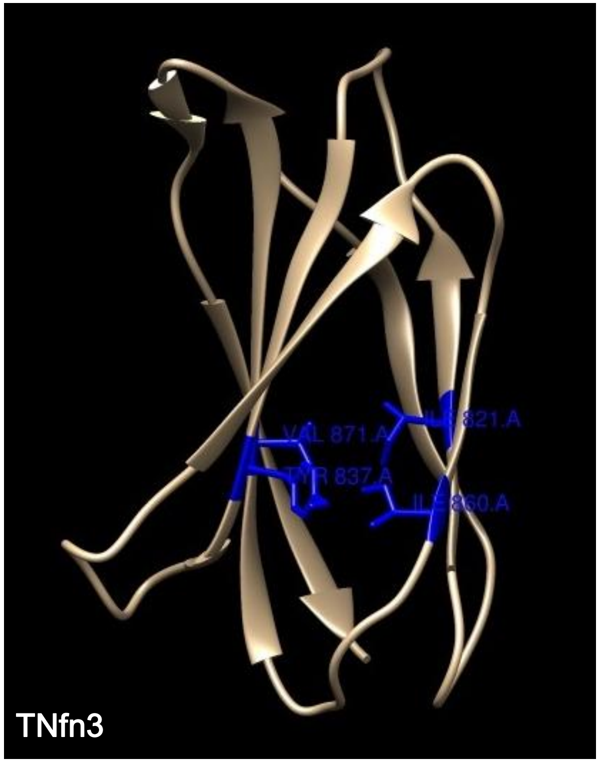
10. The hydrophobic core in Ig domains
    a. Is the main difference between Ig-like families
    b. Stabilizes the structure together with the disulfide bonds
    c. Involves the external strands A, C', C'' and D.
    d. Is constituted by charged residues
    e. All of them are correct

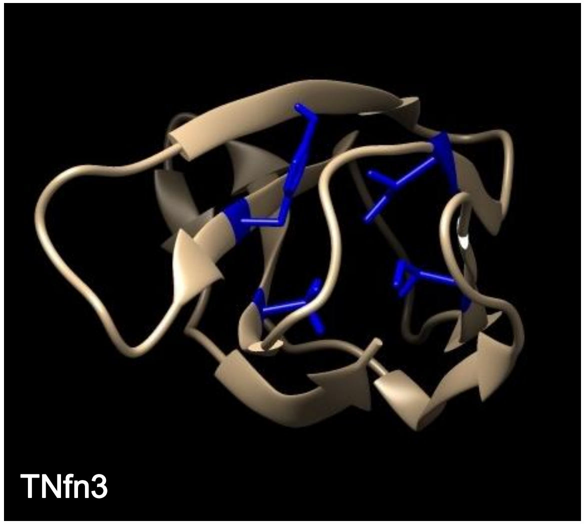
Hydrophobic core (extra)

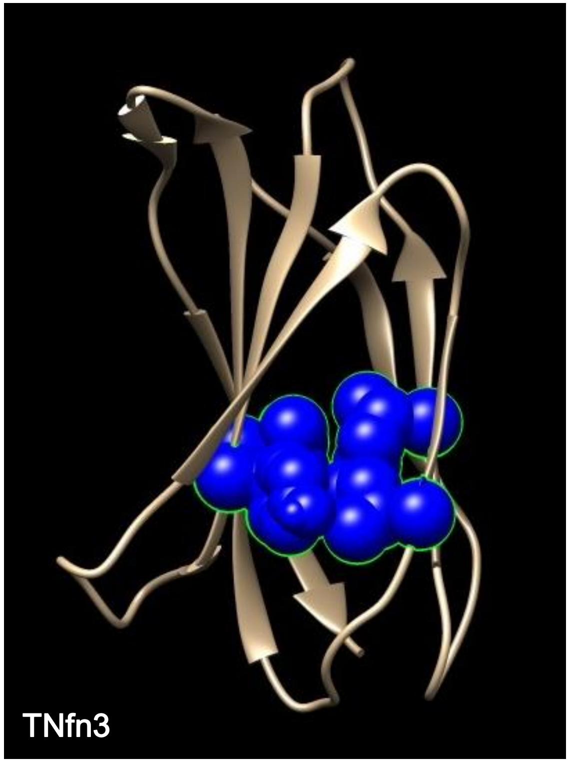




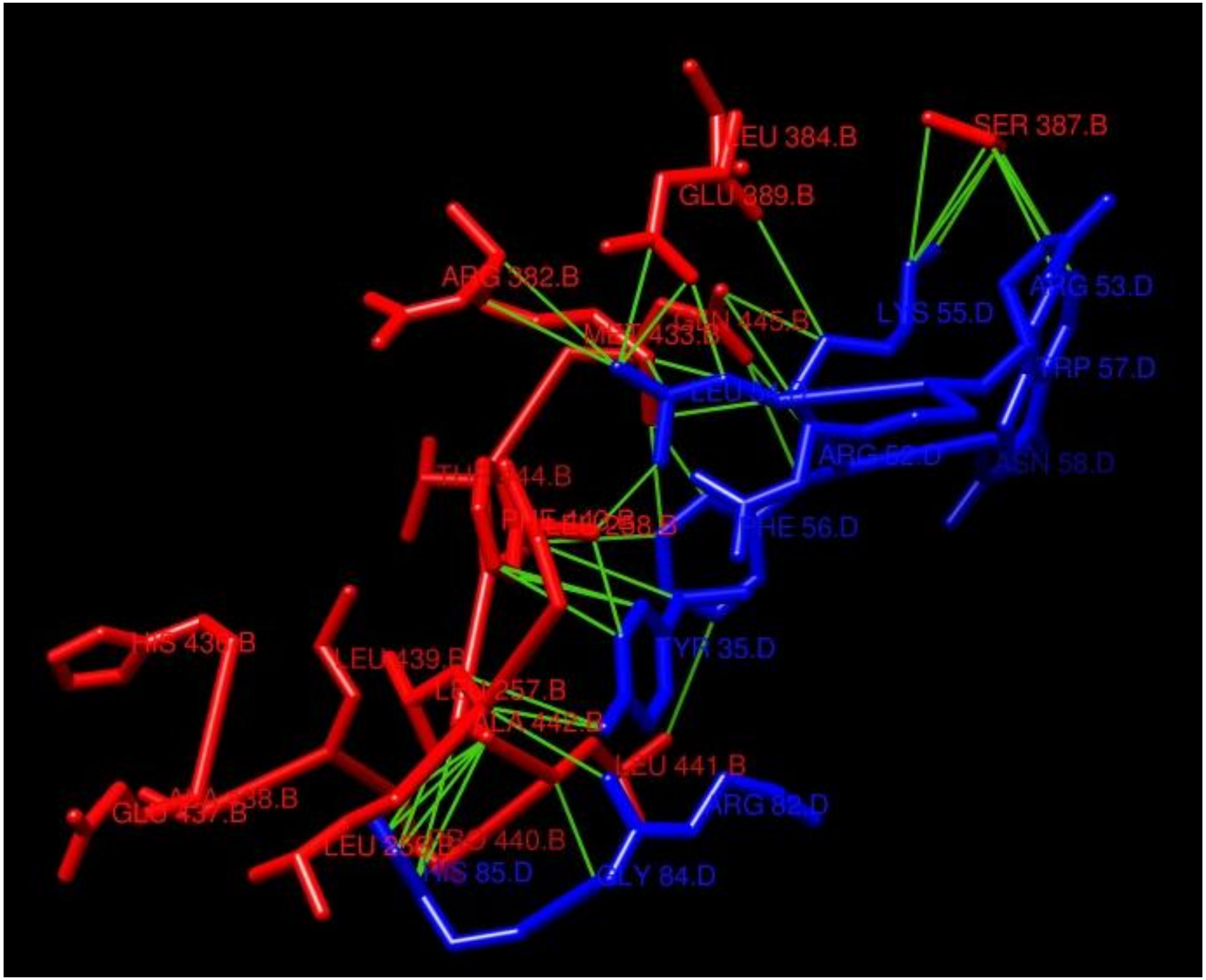
TNfn3 ring (extra)



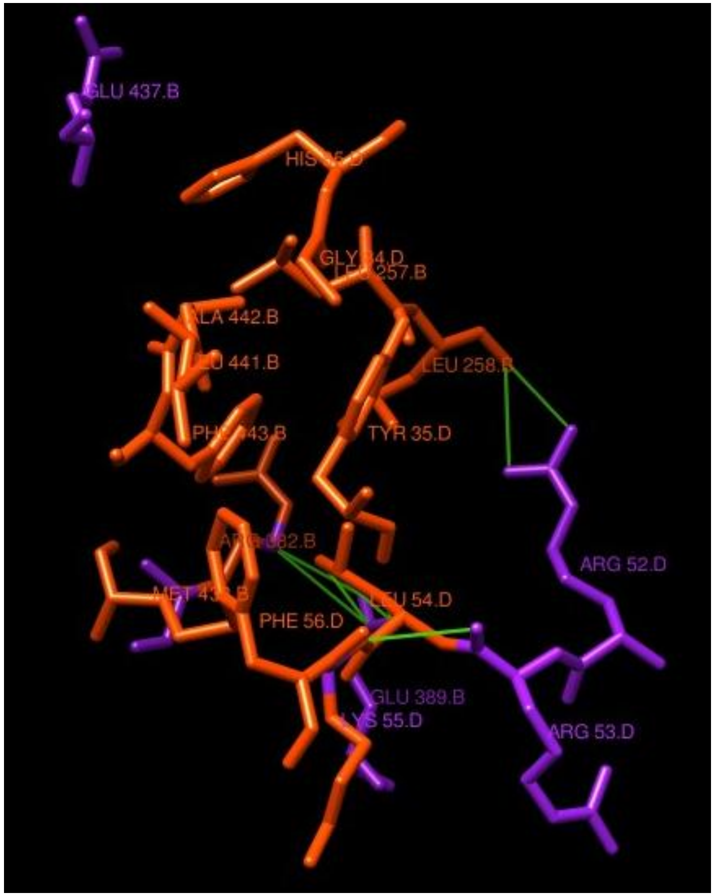


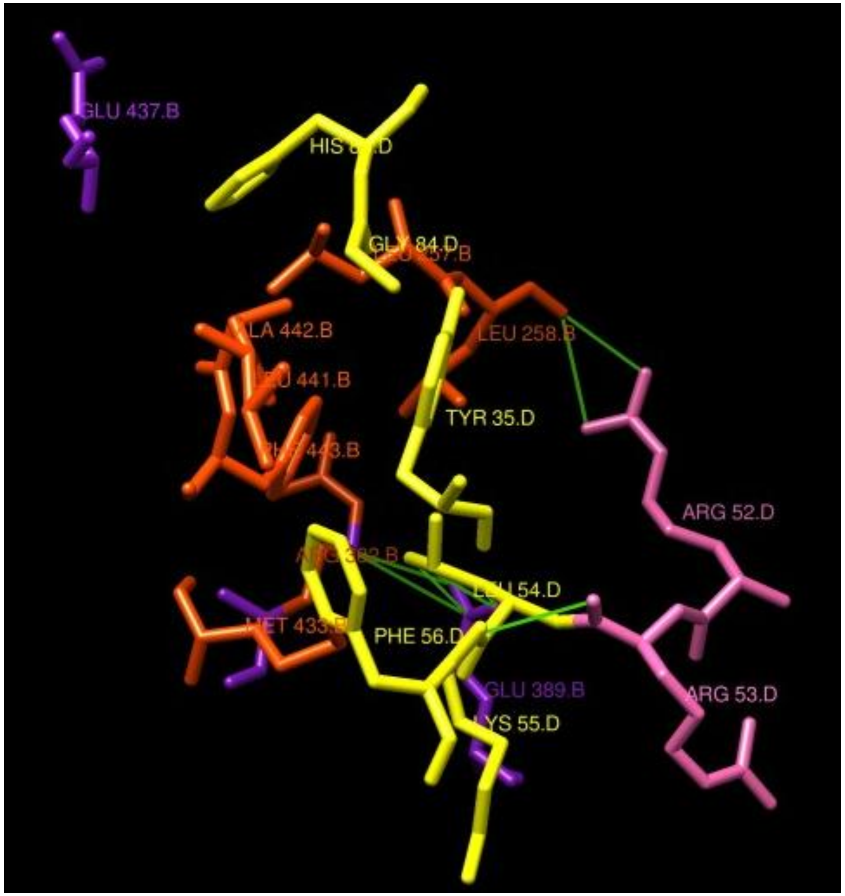


IgA – Fcα Receptor (extra)

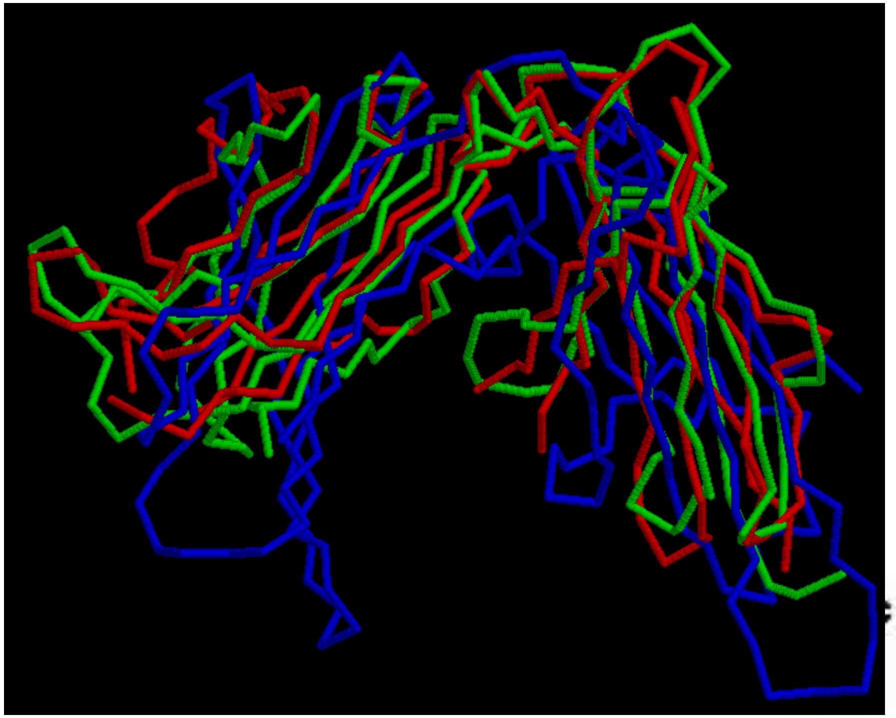


IgA – Fcα Receptor





Fc Receptors superimposition



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Herr A et al. *Nature*, 2003