

IMMUNOGLOBULIN G

Ona Lupiañez, Martina Montané, Lídia Moreno and Txell Puig

Outline

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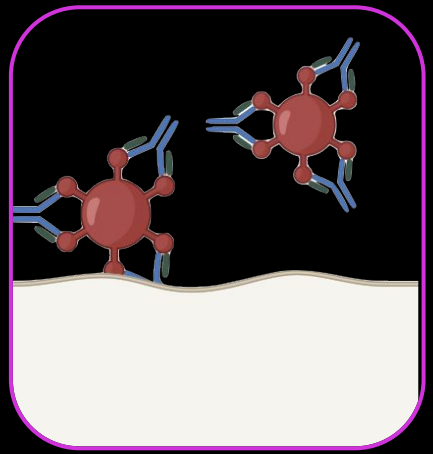
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6. **QUESTIONS**
7. **REFERENCES**

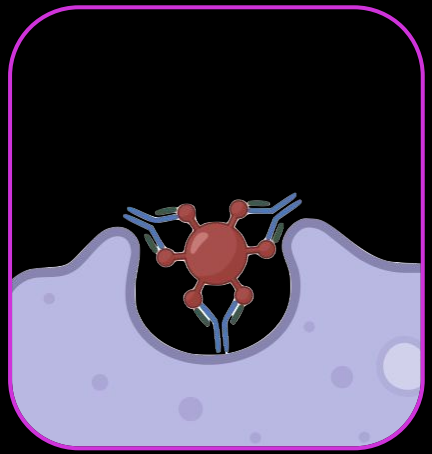
INTRODUCTION

Function

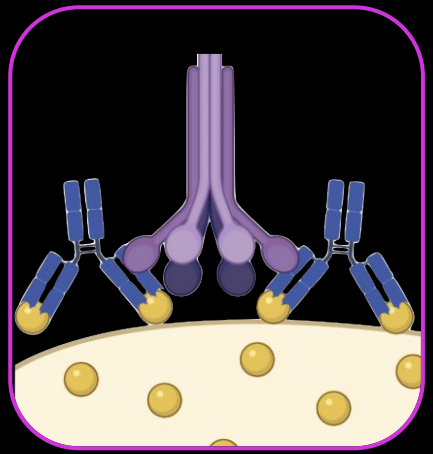
Neutralization



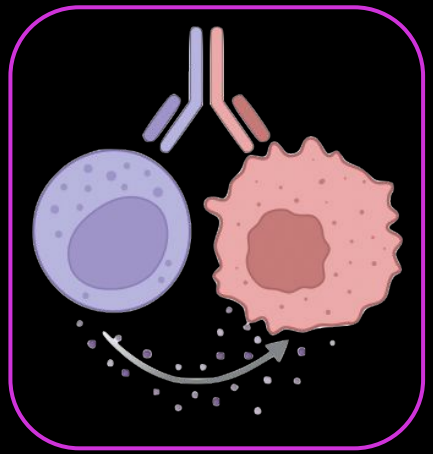
Phagocytosis



Complement activation

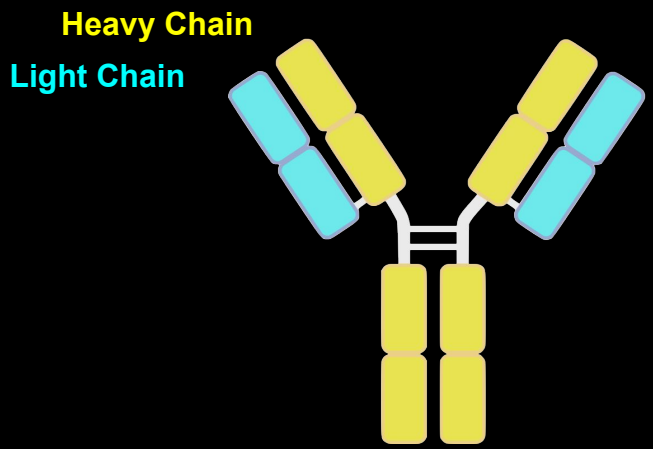


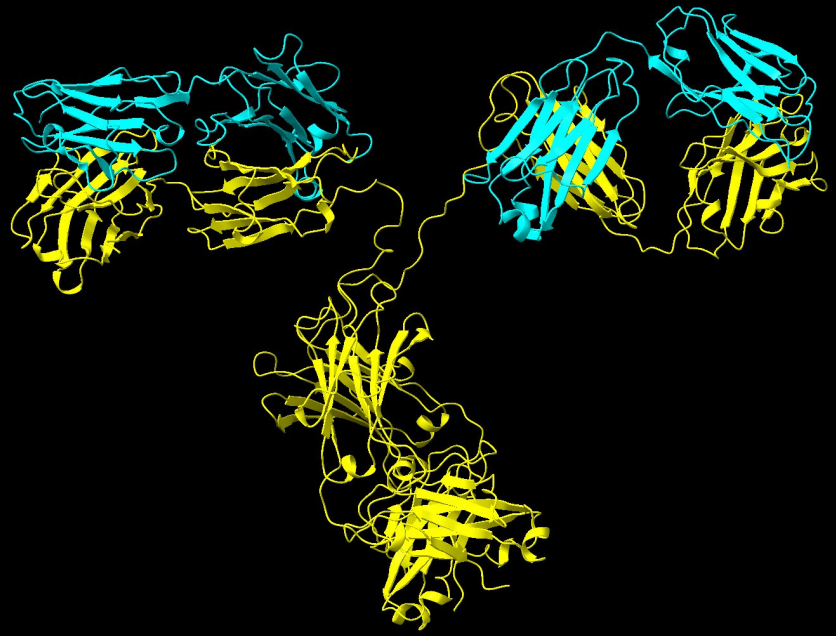
ADCC



Immunoglobulins provide specific and effective protection against a wide range of infections or alterations.

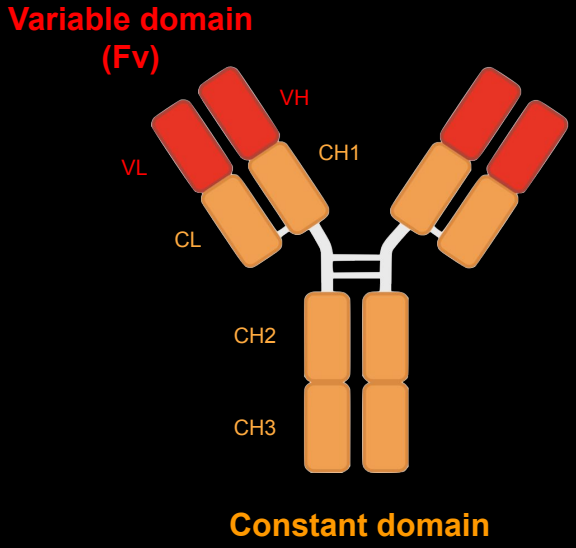
Ig structure

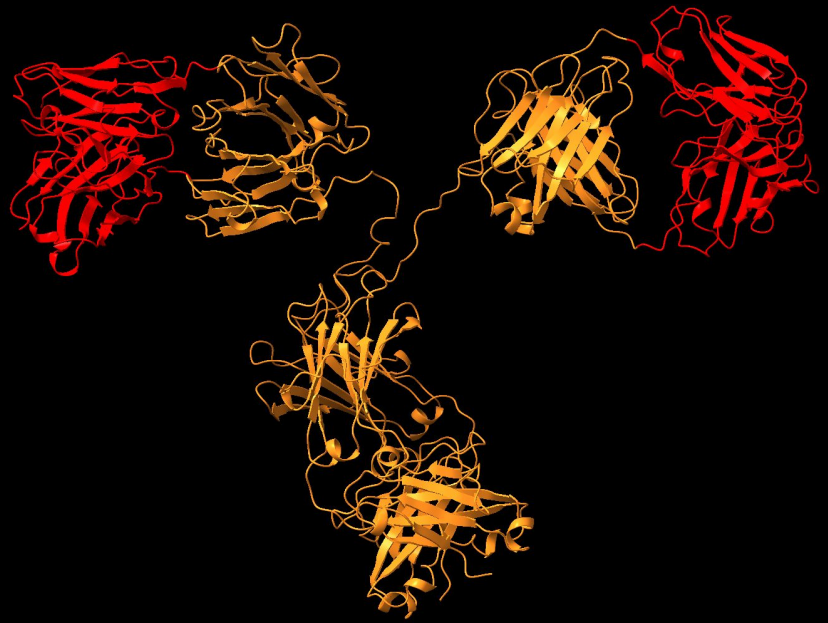




1IGT
2.80 Å, *Mus musculus*

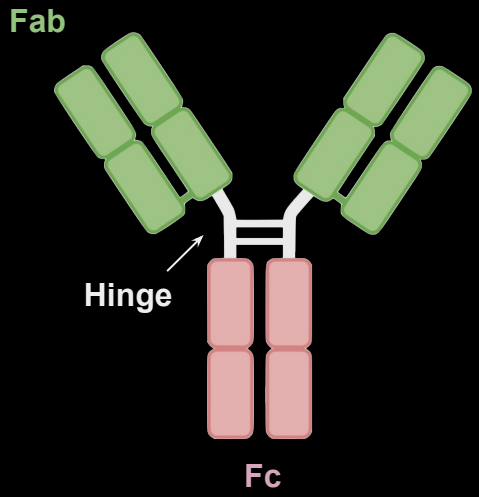
Ig structure

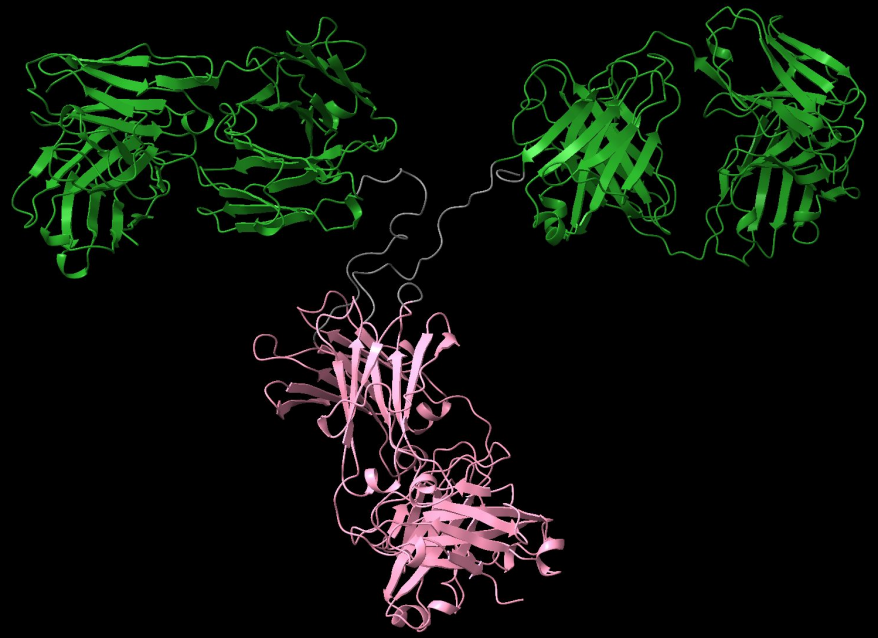




1IGT
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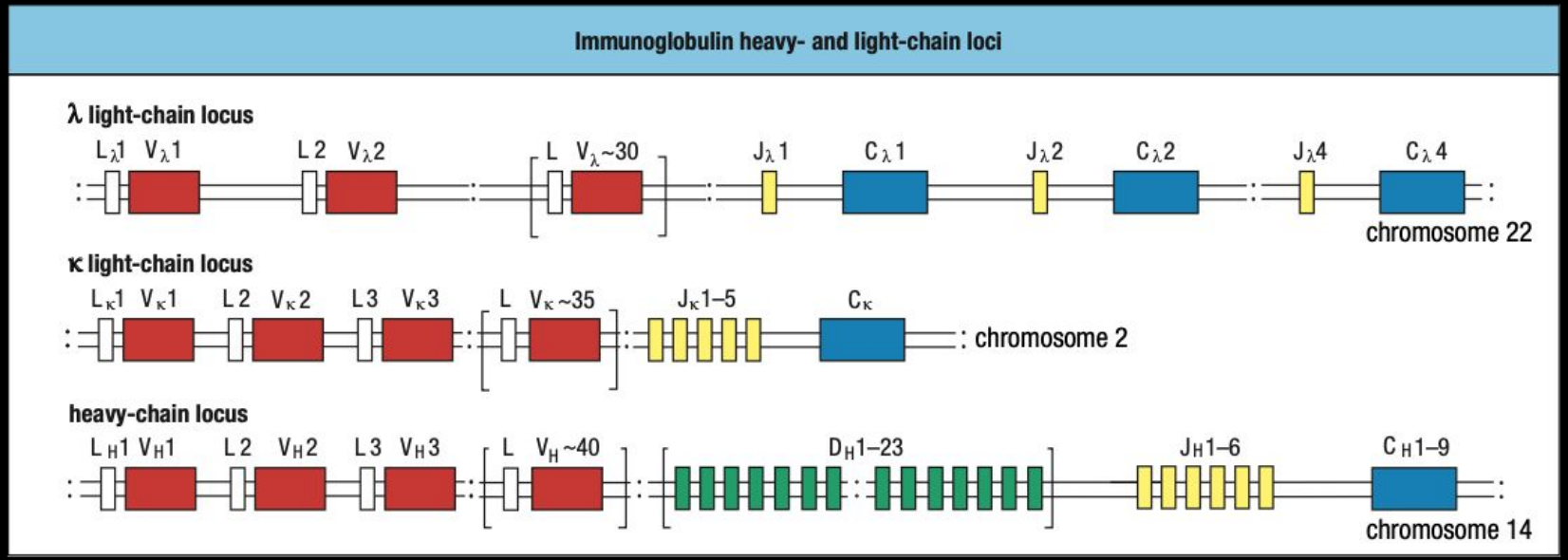
Ig structure





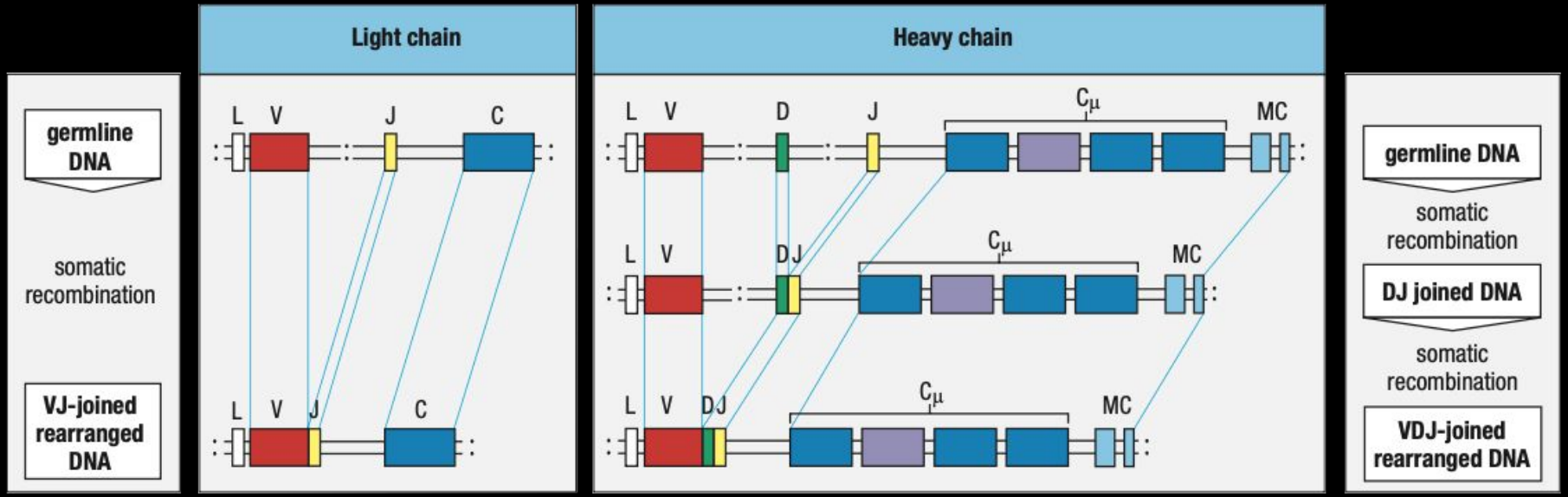
1IGT
2.80 Å, *Mus musculus*

Ig HC and LC loci germline organization



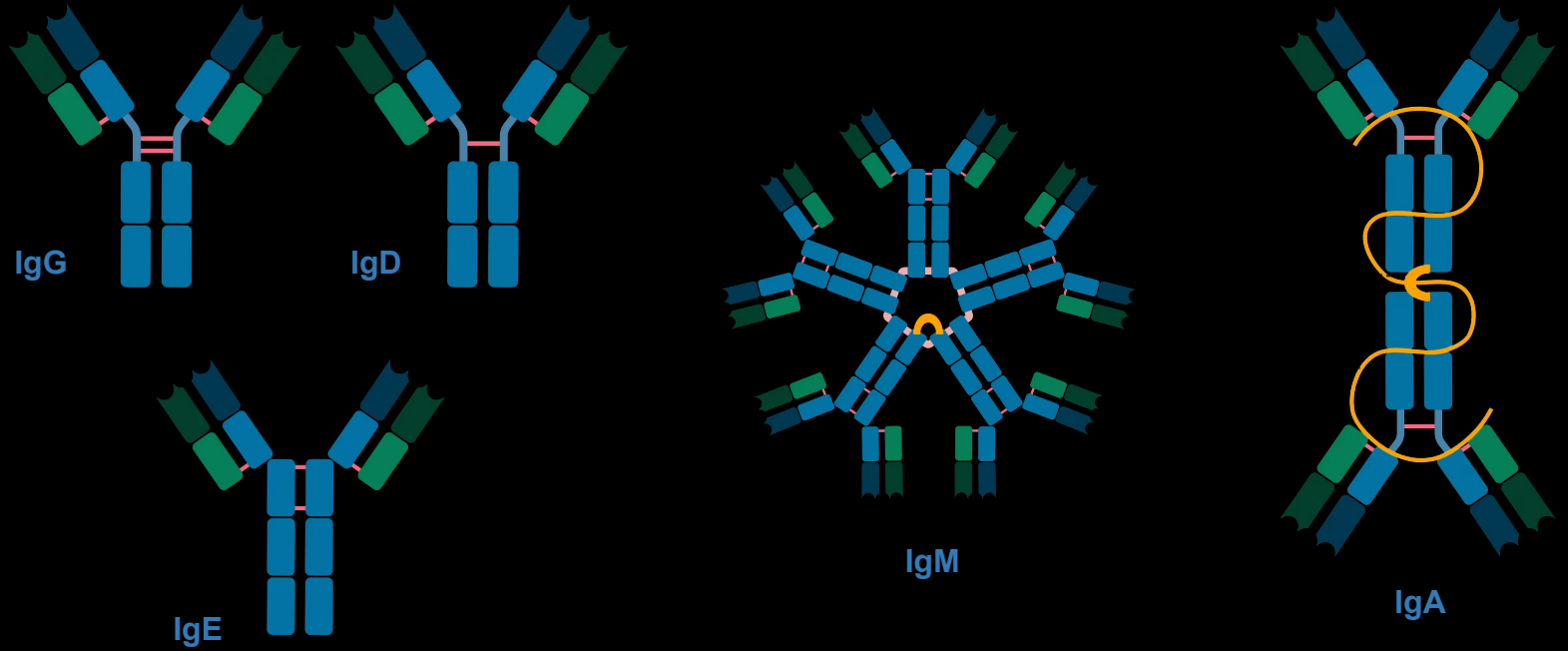
Reprinted from *The Immune System* (4th ed., p. 92), by P.Parham, 2015, Garland Science.

Antigen binding sites diversity - Somatic recombination



Reprinted from *The Immune System* (4th ed., p. 93), by P.Parham, 2015, Garland Science.

Ig isotypes



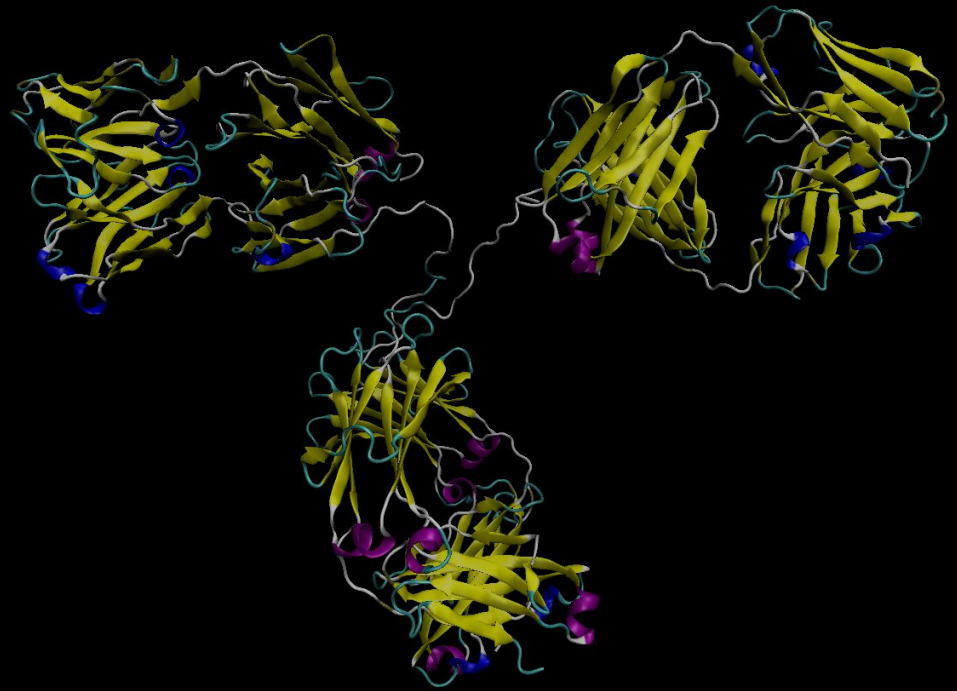
IMMUNOGLOBULIN G

SCOP classification

Class	All β proteins
Fold	Immunoglobulin-like β-sandwich
Superfamily	Immunoglobulin (Ig) domain-like
Family	V set domains-like C1 set domains C2 set domains

IgG structure

- β-sheet
- Helix
- α-helix
- Loops
- Coil

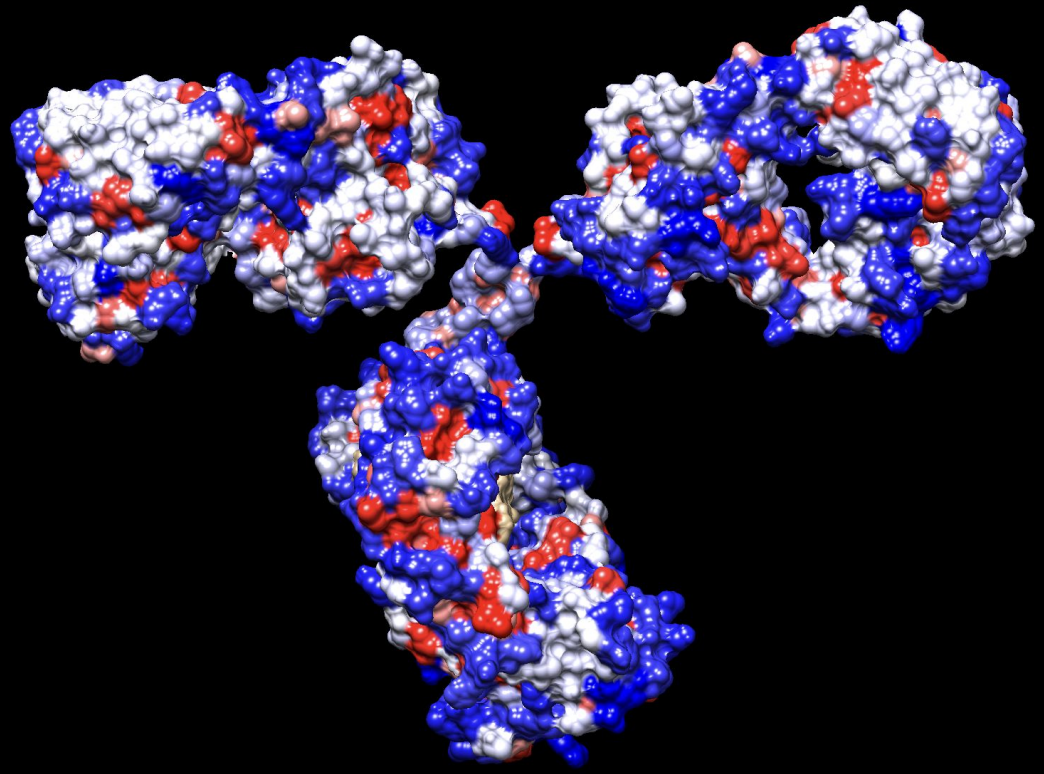


1IGT
2.80 Å, *Mus musculus*

IgG architecture

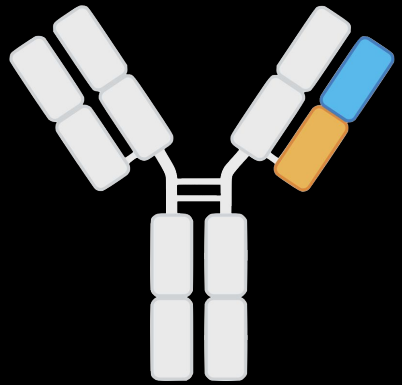


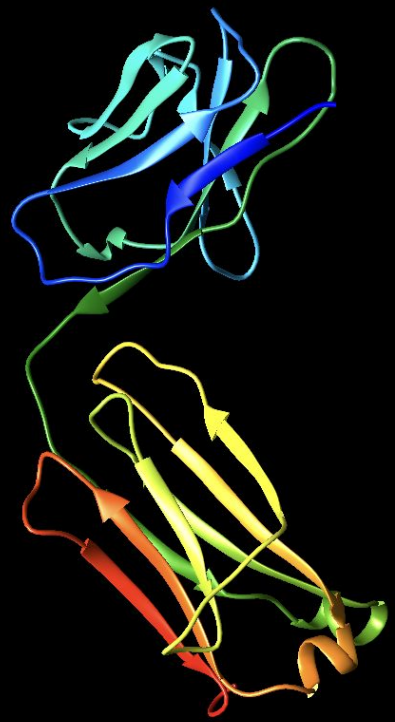
Hydrophobic Hydrophilic

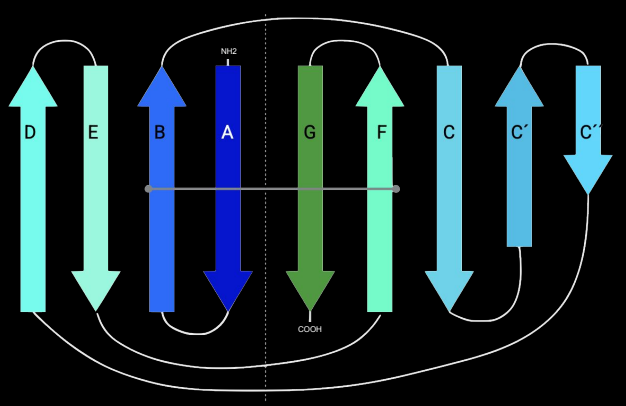


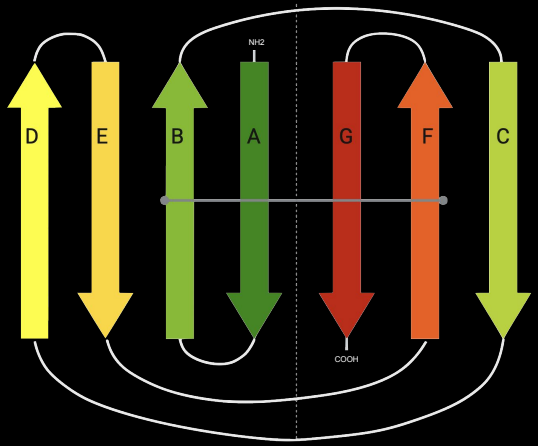
1IGT
2.80 Å, *Mus musculus*

Fold: β-sandwich



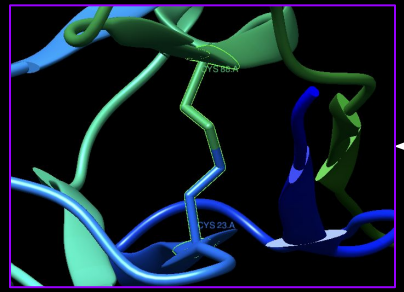


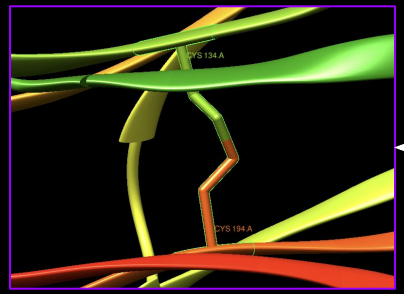




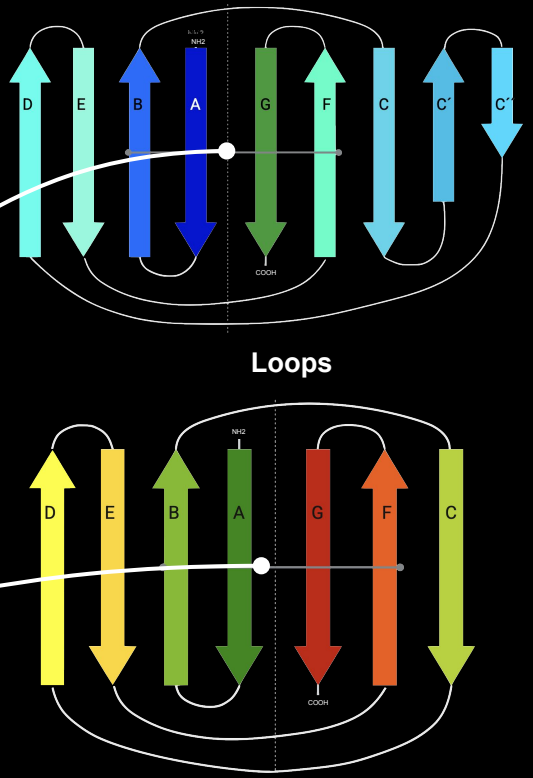
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Fold: β-sandwich





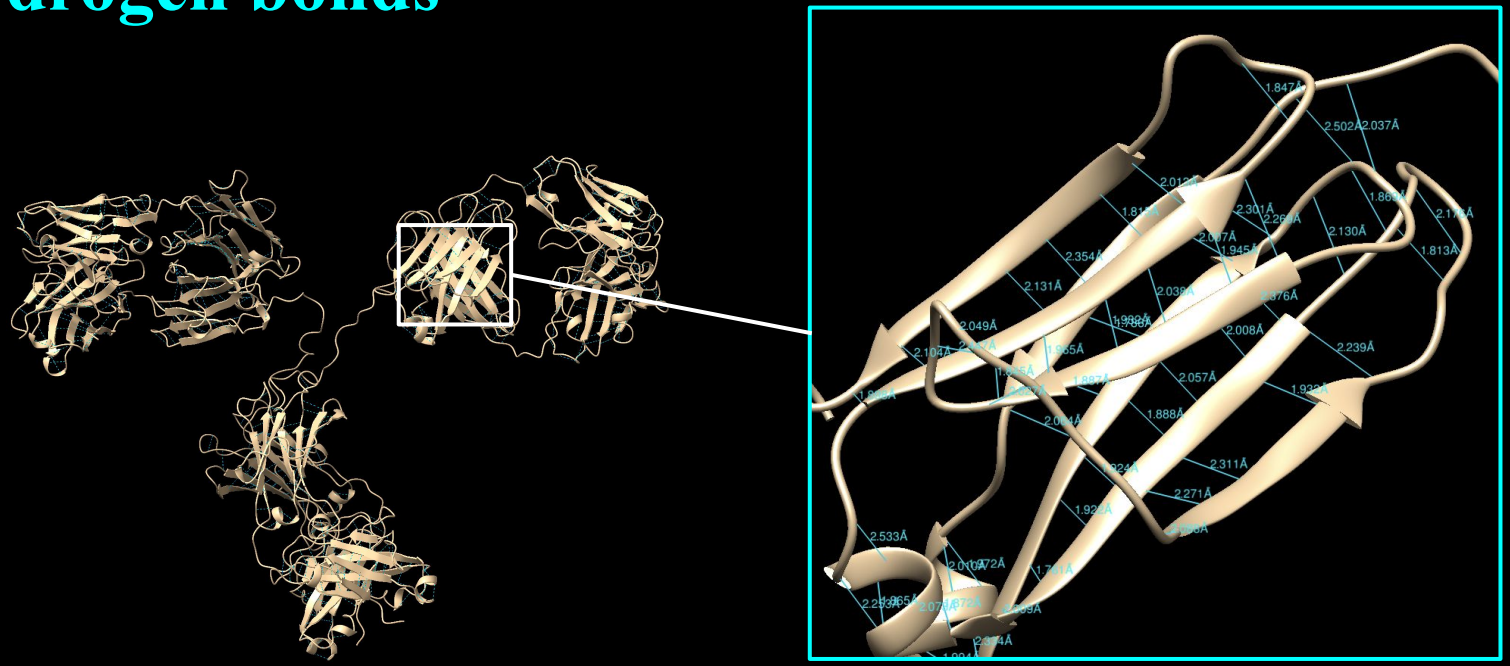
Disulfide bridges



1IGT
2.80 Å, *Mus musculus*

Stable structural framework for all Ig domains

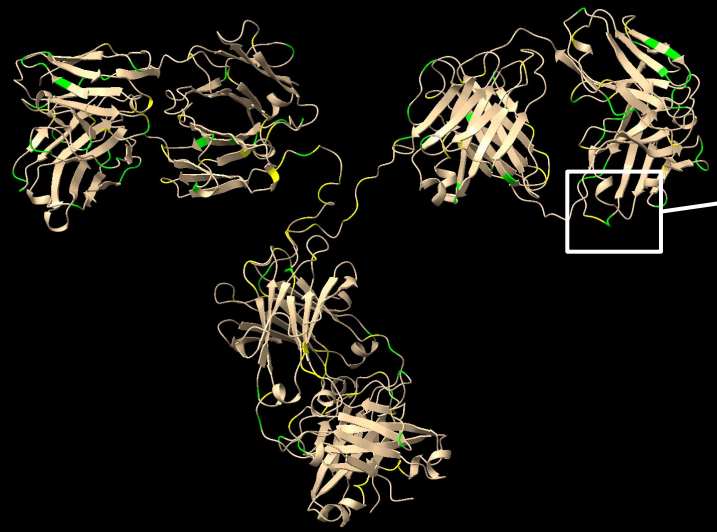
Hydrogen bonds

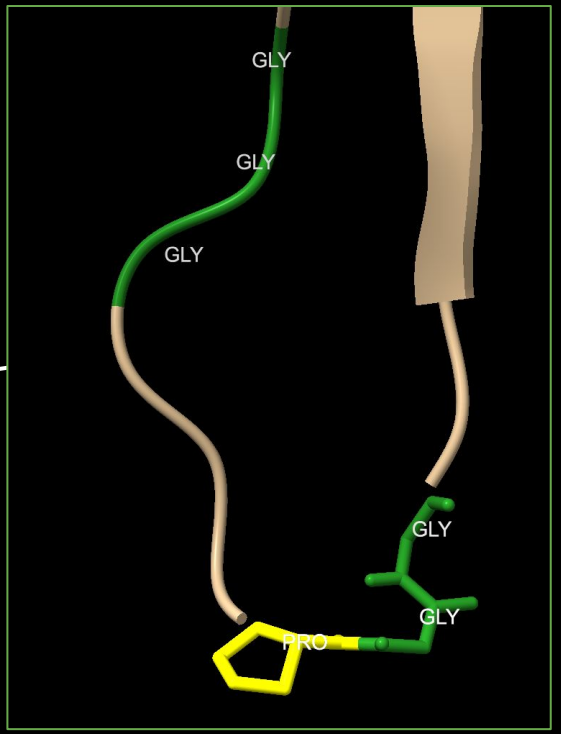


Stabilization of the different chains

1IGT
2.80 Å, *Mus musculus*

Glycine and Proline

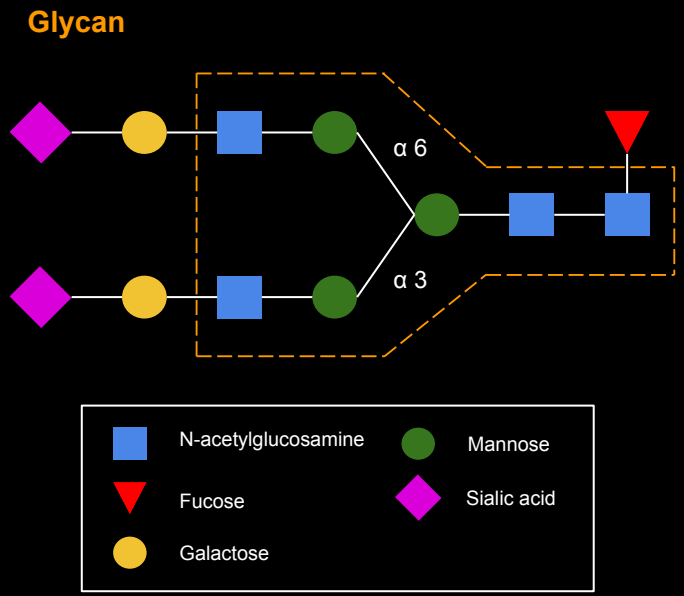


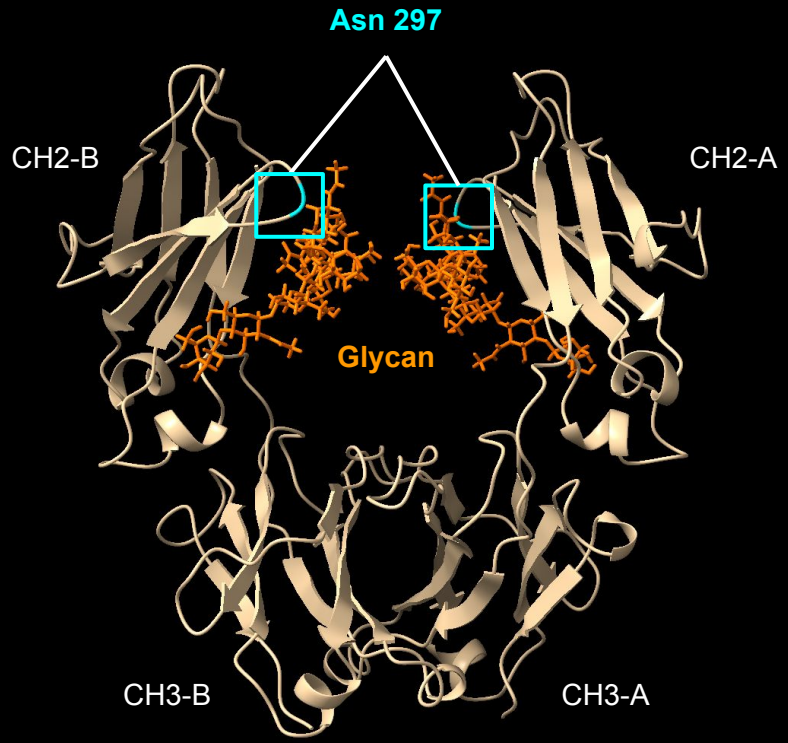


Favor sharp turns and loops

1IGT
2.80 Å, *Mus musculus*

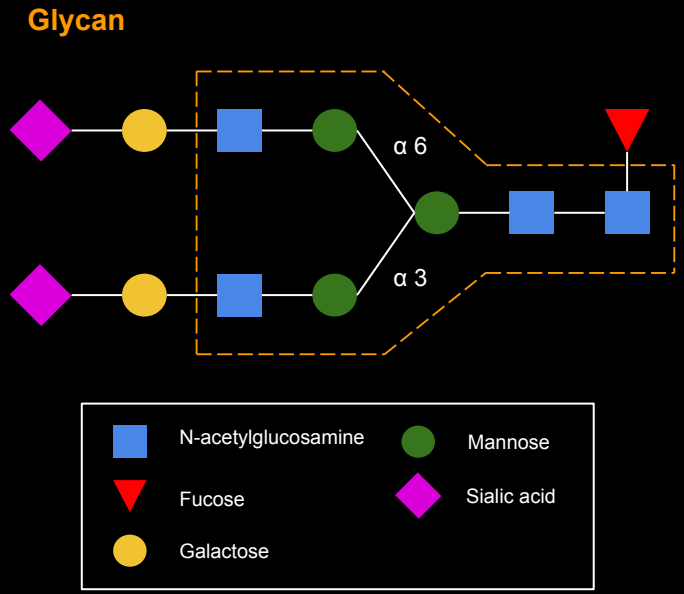
Fc glycosylation





3 AVE
2.00 Å, *Homo sapiens*

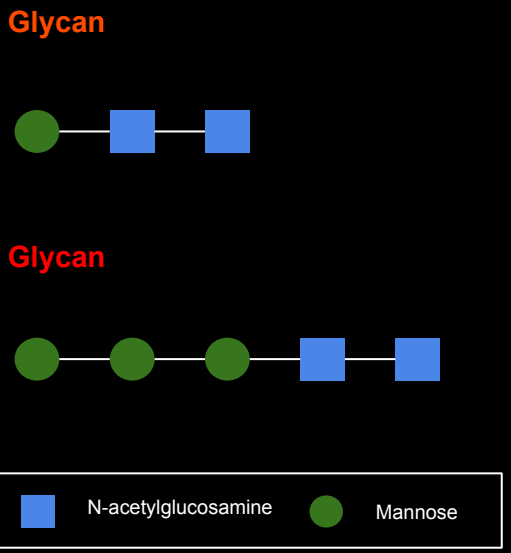
Fc glycosylation

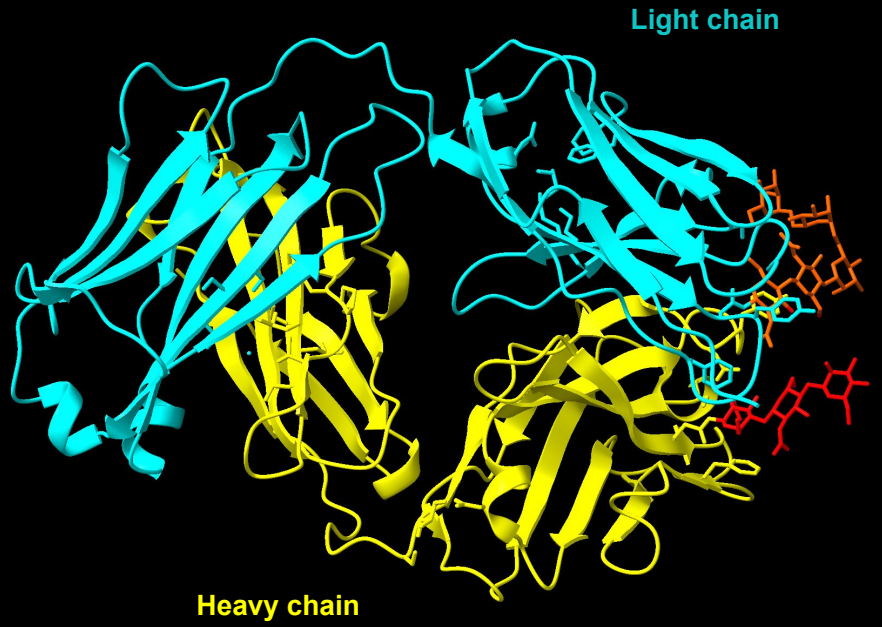


- Stabilizes IgG quaternary structure
- Critical for engagement with **FcγR**:
  - Antibody-dependent cellular cytotoxicity (**ADCC**)
  - Complement dependent cytotoxicity (**CDC**)

3 AVE
2.00 Å, *Homo sapiens*

Fab glycosylation





7ARN
1.57 Å, *Homo sapiens*

Fab glycosylation

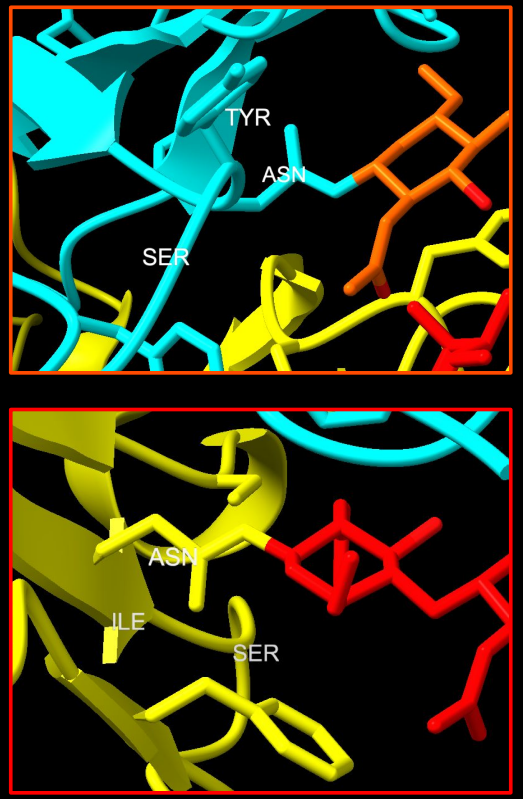
- Alter binding affinity
- Change antibody flexibility
- Improve solubility

Most commonly present in:

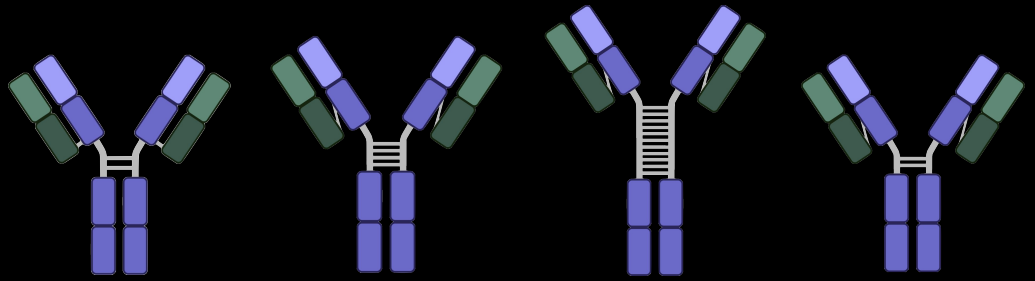
- **Autoimmune diseases**: Rheumatoid Arthritis
- **Chronic infections**: HIV-1
- Some broadly **neutralizing antibodies**: Sars-CoV-2

7ARN
1.57 Å, *Homo sapiens*

N - X - S/T motif



Disulfide bridges - subclasses

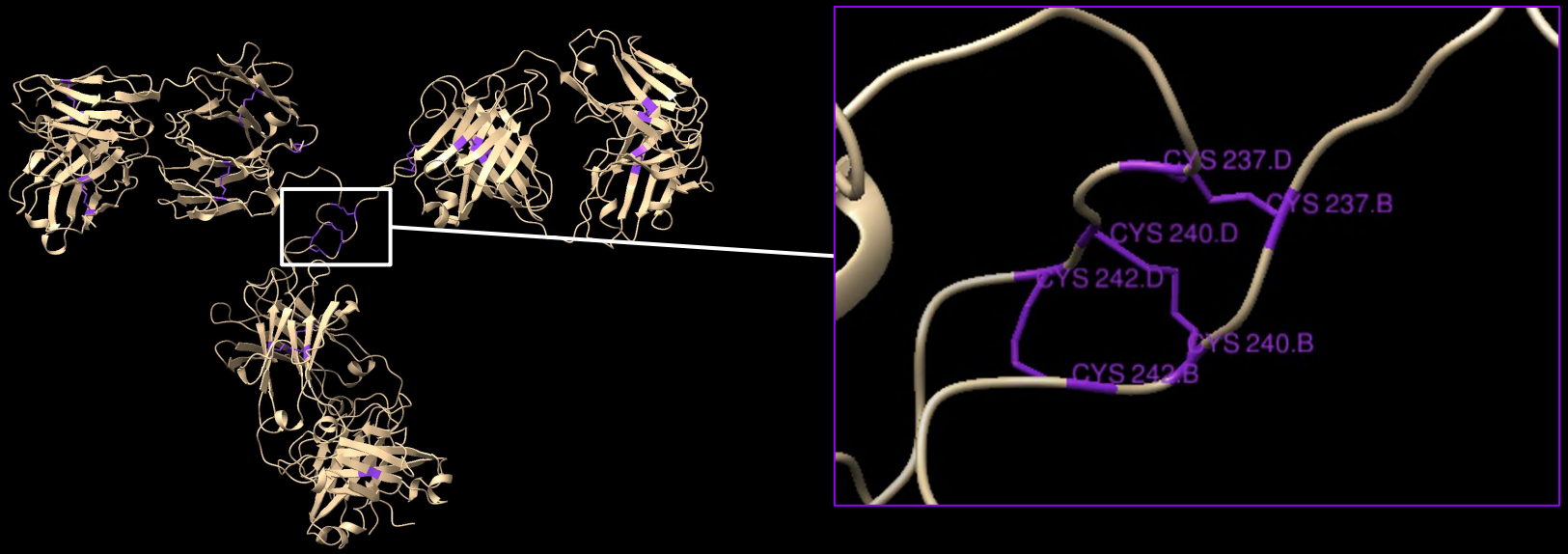


	IgG1	IgG2	IgG3	IgG4
nº hinge disulfide bonds	2	4	11	2

Similarities → more than 90% identical at amino acid level

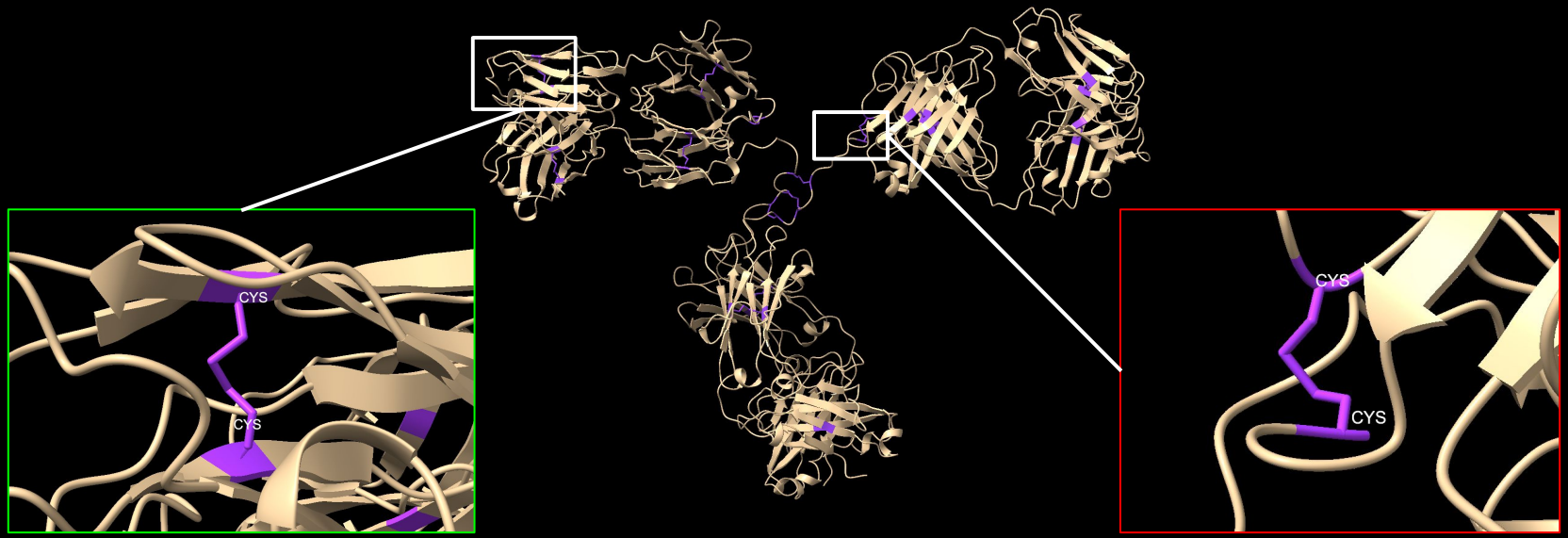
Unique profile → structure, antigen-binding, immune-complex formation, complement activation, triggering of the FcγR, half-life and placental transport.

Disulfide bridges - subclasses



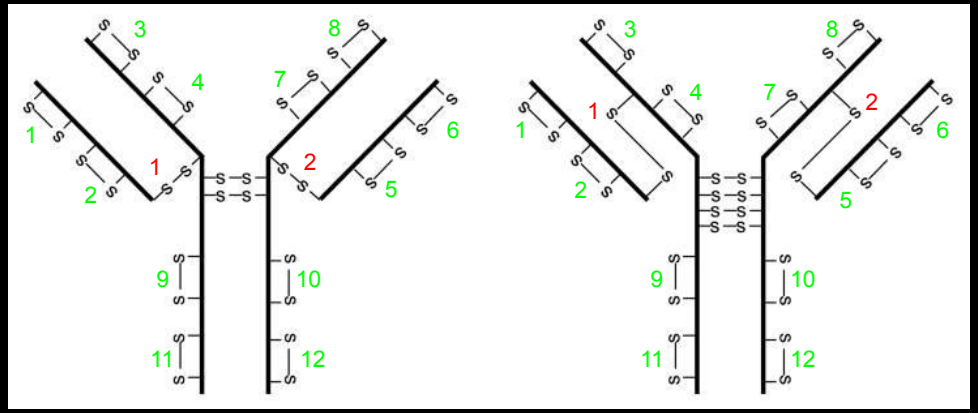
1IGT
2.80 Å, *Mus musculus*

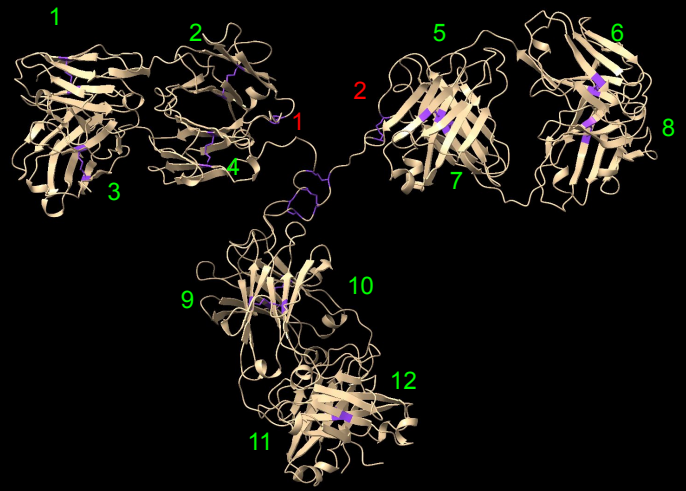
Disulfide bridges - intrachain and interchain



1IGT
2.80 Å, *Mus musculus*

Disulfide bridges - intrachain and interchain





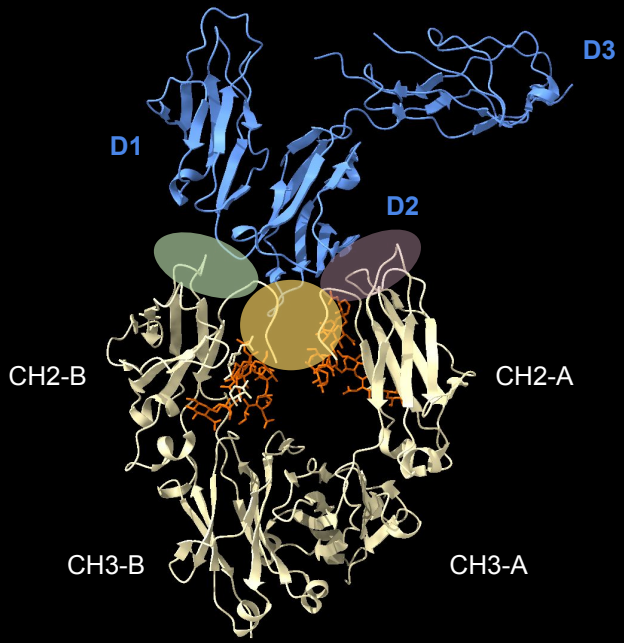
1IGT
2.80 Å, *Mus musculus*

Fc

Fcγ receptors

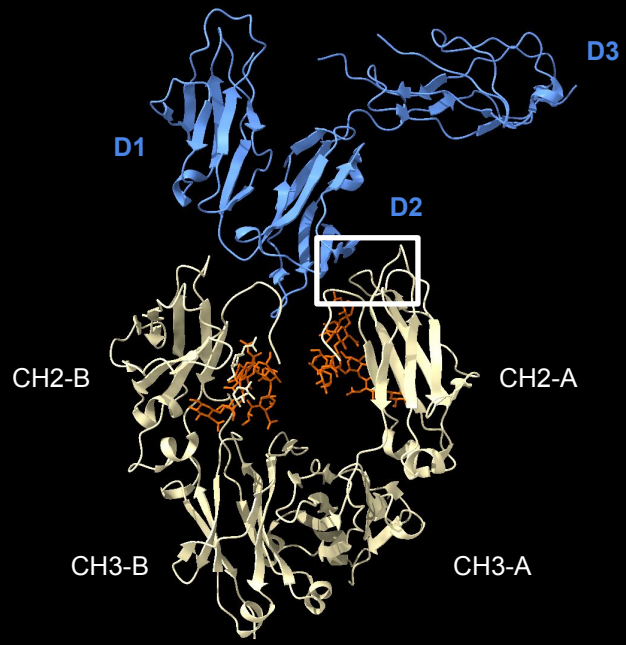
Receptor	FcγRI CD64	FcγRIIa CD32A	FcγRIIb CD32B	FcγRIIc CD32C	FcγRIIIa CD16A	FcγRIIIb CD16B
Function	Activating	Activating	Inhibitory	Activating	Activating	Activating
Affinity	High	Low	Low	Low	Low	Low

FcγRI

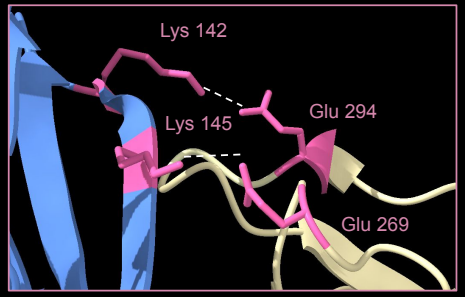


4X4M
3.48 Å, *Homo sapiens*

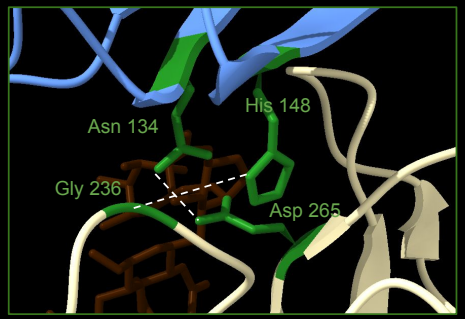
IgG Fc chain A - FcγRI



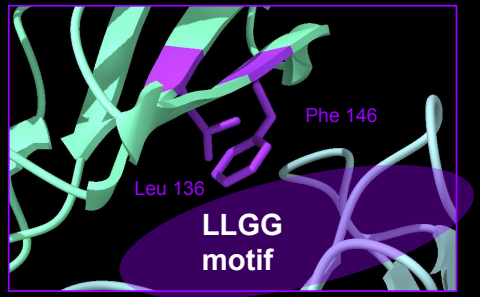
4X4M
3.48 Å, *Homo sapiens*



Salt bridges

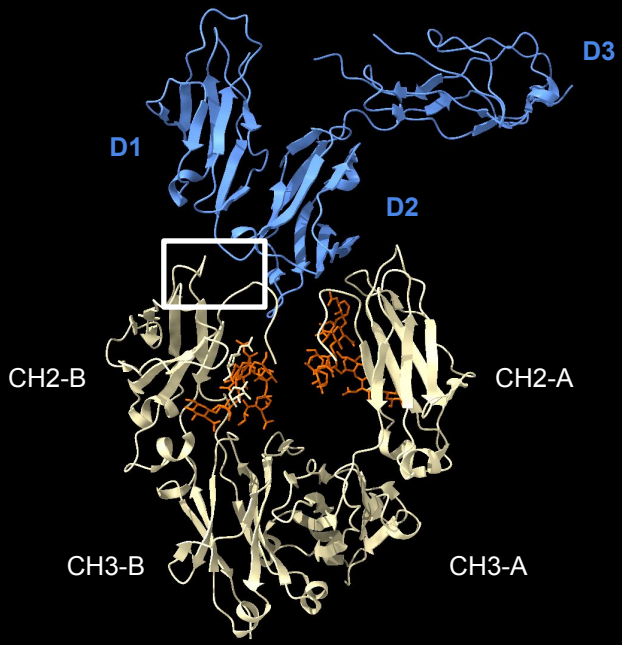


H bonds



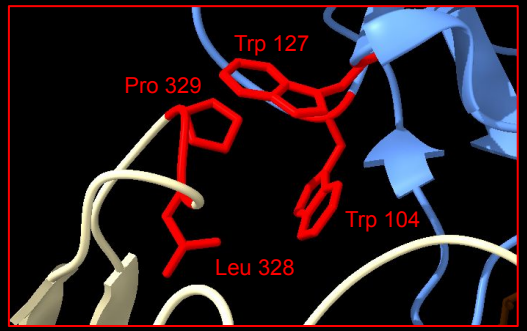
Van der Waals

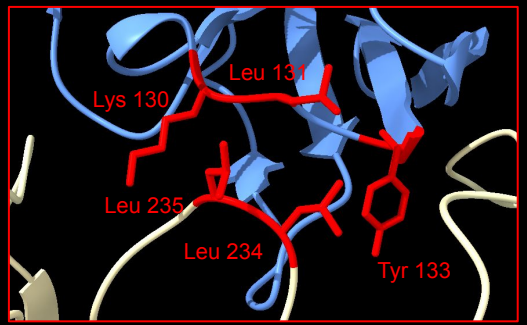
IgG Fc chain B - FcγRI



4X4M
3.48 Å, *Homo sapiens*

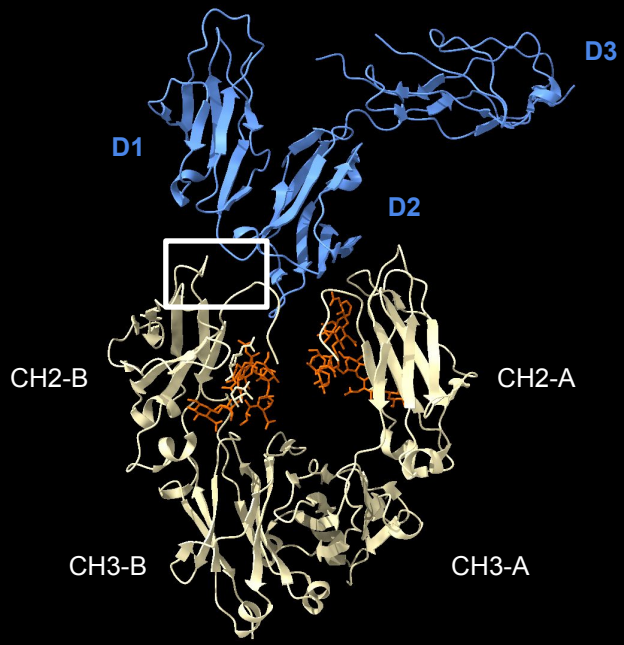
Trp-Pro-Trp sandwich



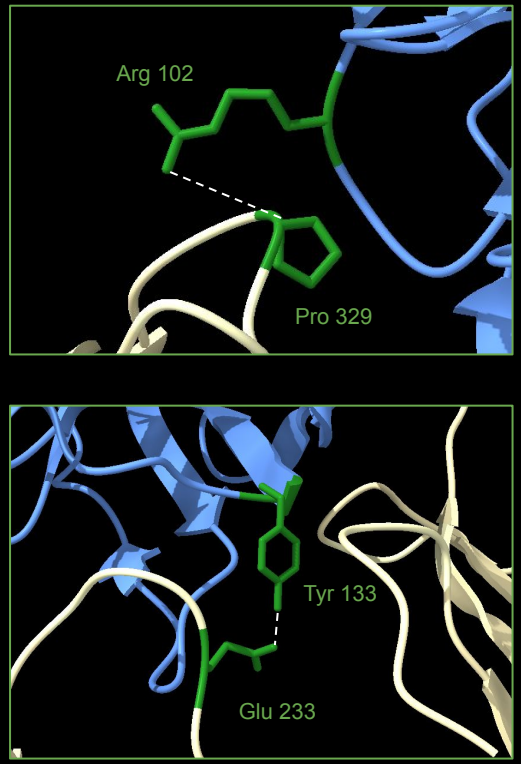


Hydrophobic clusters

IgG Fc chain B - FcγRI

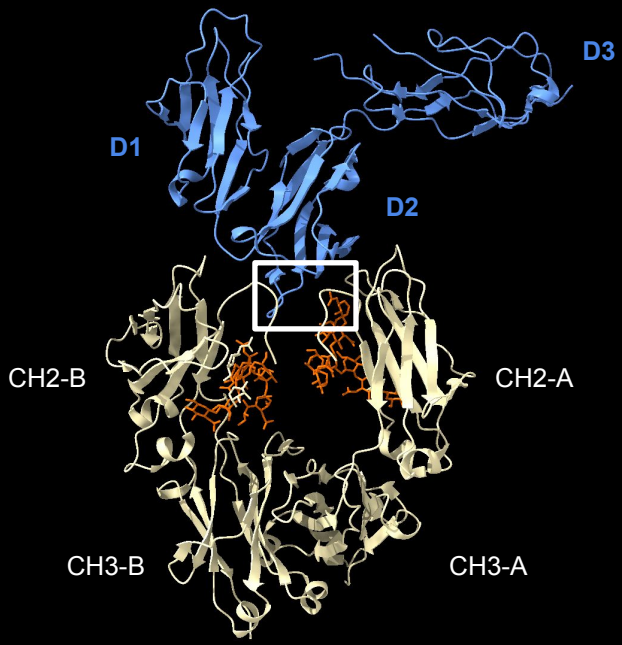


4X4M
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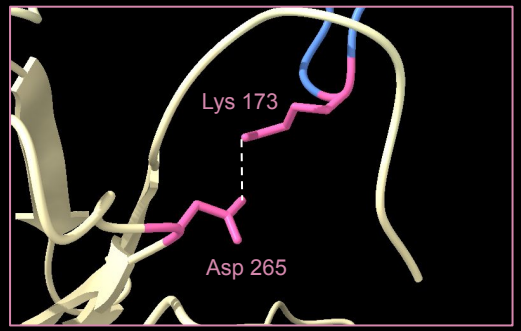


H bonds

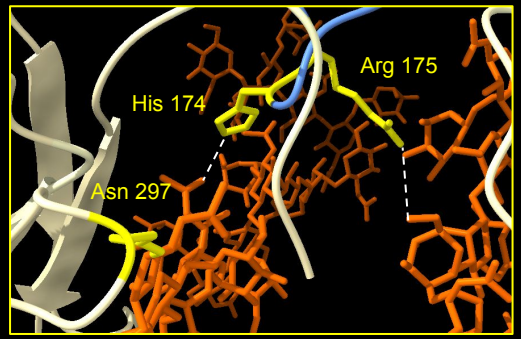
IgG Fc glycan - FcγRI FG loop (171MGKHRY176)



4X4M
3.48 Å, *Homo sapiens*



Salt bridge

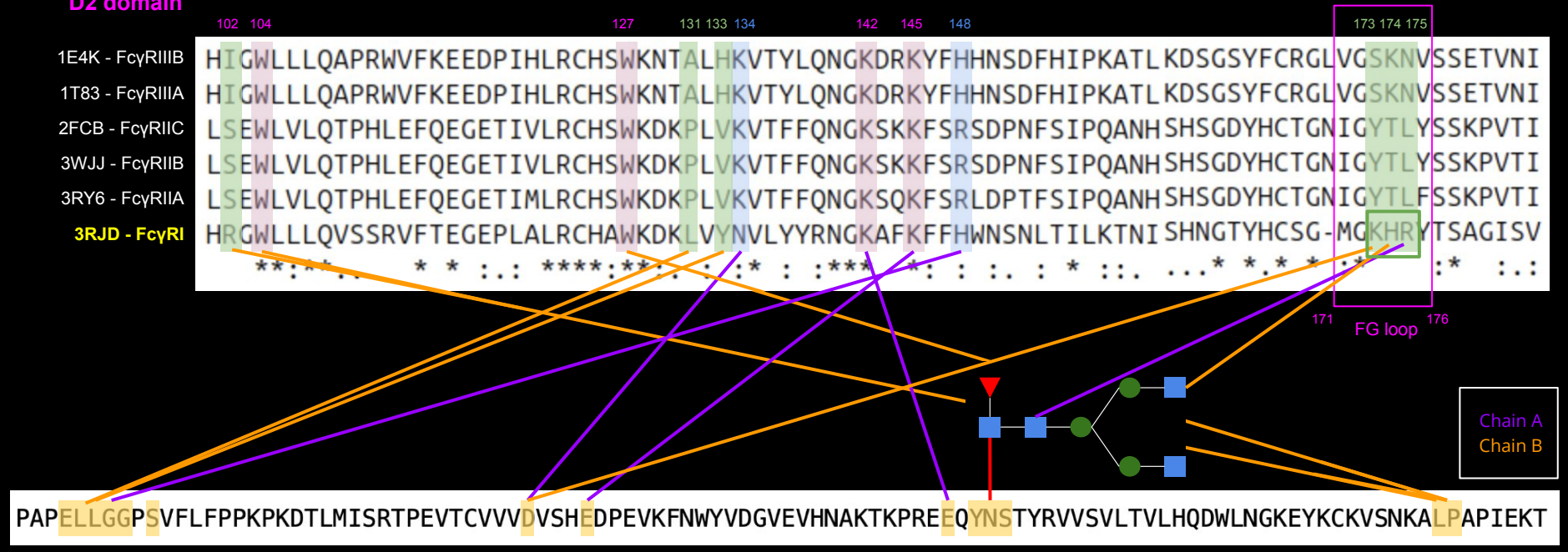


Interaction with the glycan at Asn 297 in both chains (A and B)

Sequence alignment - Fcγ R

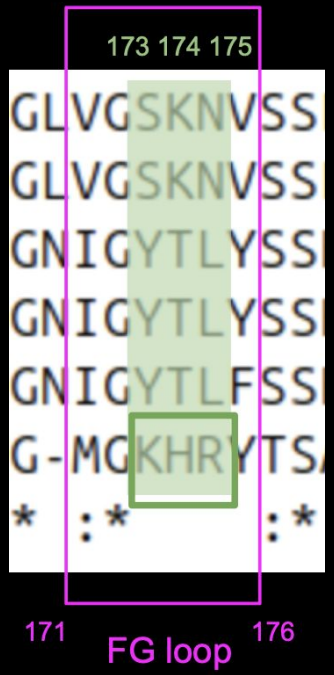
( ) → not conserved
(*) → identical residues
(:) → less conserved

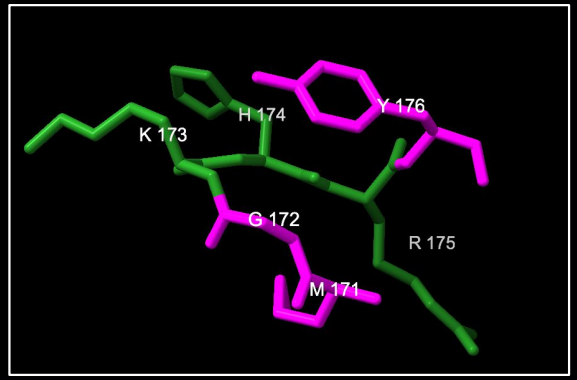
D2 domain



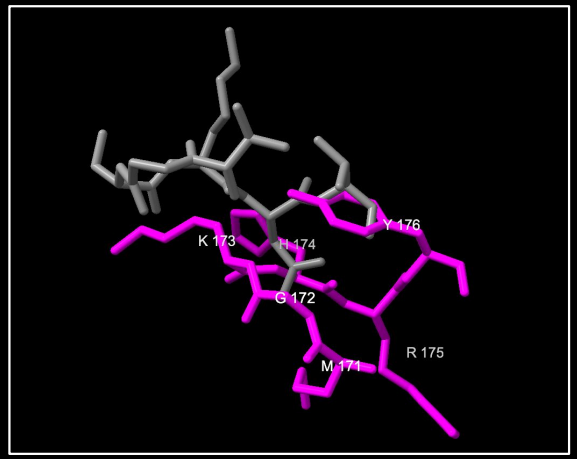
Cγ2 domain

FG loop - KHR motif





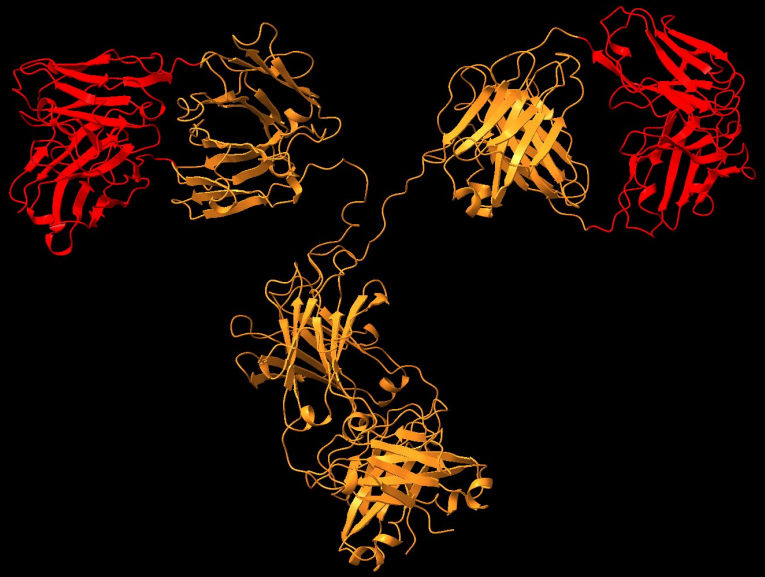
KHR motif



FcγRIII
FcγRI

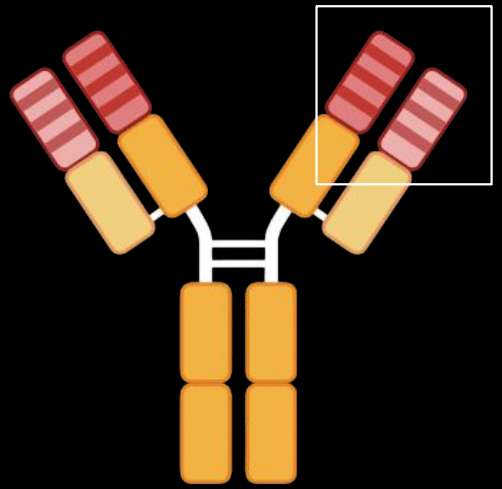
Fv

Variable domain (Fv region)

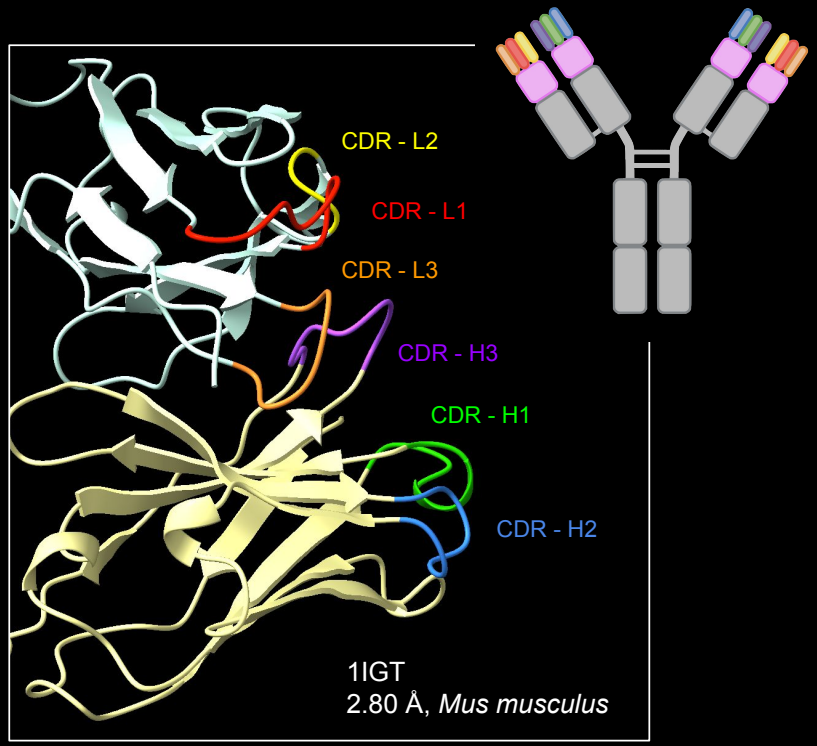


1IGT
2.80 Å, *Mus musculus*

FR: framework region
CDR: complementarity-determining region



Fv region - CDRs

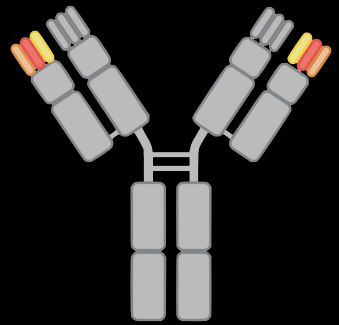


Light chain		canonical structure
L1	lambda (λ)	1, 2, 3a, 3b, 4
	kappa (k)	1, 2a, 2b, 3, 4, 5, 6
L2	lambda (λ)	1
	kappa (k)	
L3	lambda (λ)	1a, 1b, 1c, 2
	kappa (k)	1, 2, 3, 4, 5, 6
Heavy chain		
H1	1, 2, 3	
H2	1, 2a, 2b, 3a, 3b, 3c, 4	
H3	NO canonical structure	

Fv region - Light chain λ

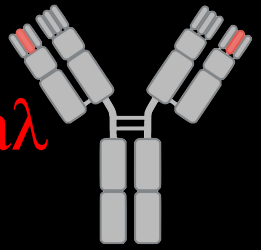
Multiple sequence alignment

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 5 2RHE          ESVLTQPPSASGTPGQRVTISCTGSATDIGS-NSVIWYQQVPGKAPKLLIYYNDLLPSGV
 6 7FAB          ASVLTQPPSVSGAPGQRVTISCTGSSSNIGAGHNVKWYQQLPGTAPKLLIFHN-------
 7 8FAB          SYELTQPPSVSVSPGQTARITCS--ANALPN-QYAYWYQQKPGRAPVMVIYKDTQRPSGI
 8 1GIG          QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPGV
 9 1MFA          QIVVTQESALTTSPGETVTLTCRSSTGTVTSGNHANWVQEKPDHLFTGLIGDTNNRAPGV
10 1IND          QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPGV
11                  :** .: : :**: . ::*   :  :     . * *: *.     :*
12
13 2FB4          PDRFSGSKSGASASLAIGGLQSEDETDYYCAAWDVSLNAYVFGTGTKVTVLGQPKANPTV
14 2RHE          SDRFSASKSGTSASLAISGLESEDEADYYCAAWNDSLDEPGFGGGTKLTVLGQPK-----
15 7FAB          NARFSVSKSGTSATLAITGLQAEDEADYYCQSYDRSLR--VFGGGTKLTVLRQPKAAPSV
16 8FAB          PQRFSSSTSGTTVTLTISGVQAEDEADYYCQAWDNSAS--IFGGGTKLTVLGQPKAAPSV
17 1GIG          PARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNHW--VFGGGTKLTVLGQPKSSPSV
18 1MFA          PARFSGSLIGDKAALTITGAQPEDEAIYFCALWSNNHW--IFGGGTKLTVLGQPKSSPSV
19 1IND          PARFSGSLIGDKAALTITGAQTEDEARYFCALWYSNLW--VFGGGTKLTVLGQPKSSPSV
20                *** *  * ..:*:* * :.***: *:*  :  .     ** ***:*** ***
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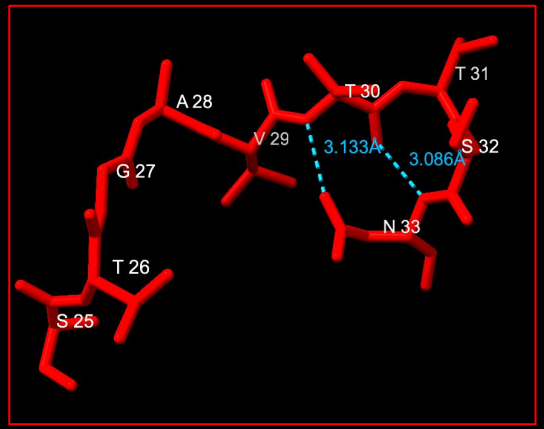


L1 λ
L2 λ
L3 λ

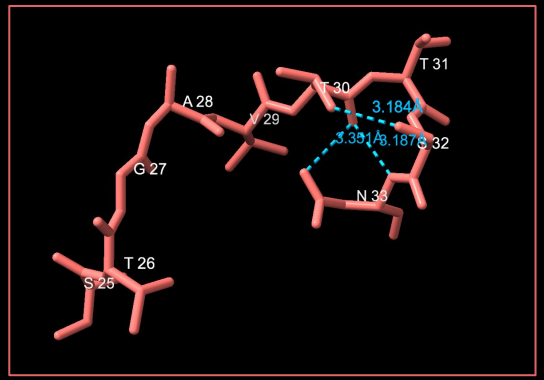
Fv region - L1-3aλ





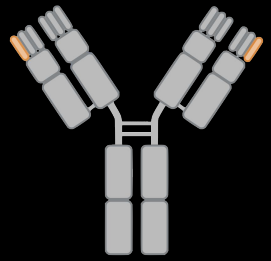


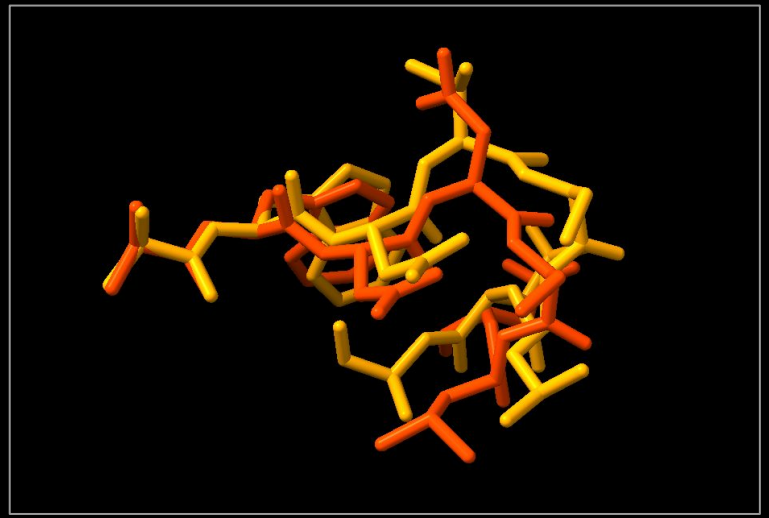
1 GIG
2.30 Å, *Mus musculus*

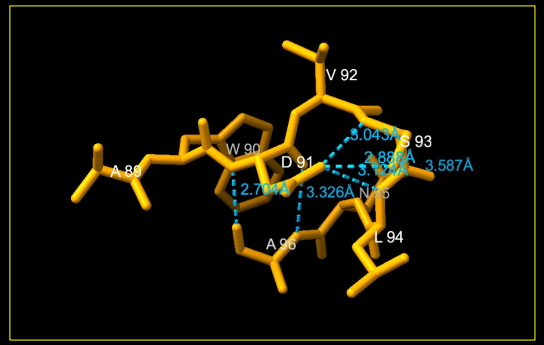


1 IND
2.20 Å, *Mus musculus*

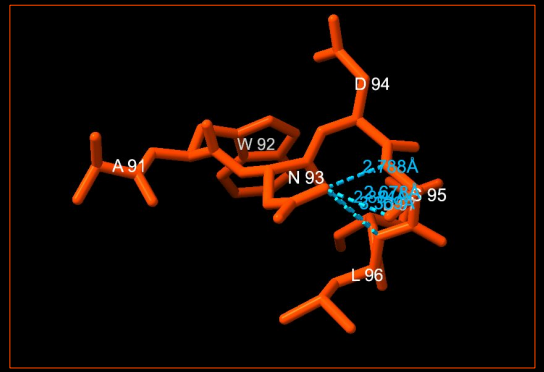
Fv region - L3-2λ







2 FB4
1.90 Å, *Homo sapiens*

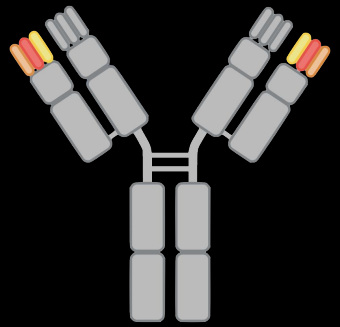


2 RHE
1.60 Å, *Homo sapiens*

Fv region - Light chain k

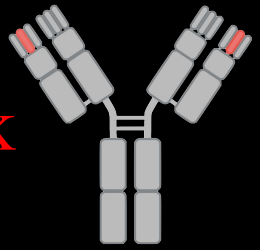
Multiple sequence alignment

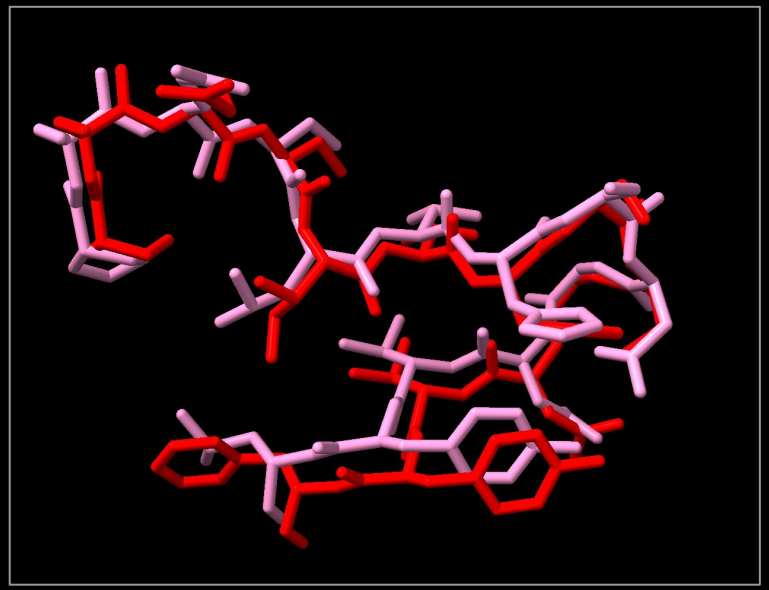
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 5 1FVC            DIQMTQSPSSLSASVGDRVTITCRASQDVNT------AVAWYQQKPGKAPKLLIYSASFL
 6 1IGM            DIQMTQSPSSLSASVGDRVTITCQASQDISN------YLAWYQQKPGKAPELRIYDASNL
 7 1VFA            DIVLTQSPASLSASVGETVTITCRASGNIHN------YLAWYQQKQGKSPQLLVYYTTTL
 8 1FLR            DVVMTQTPLSLPVSLGDQASISCRSSQSLVHS-NGNTYLRWYLQKPGQSPKVLIYKVSNR
 9 2CGR            ELVMTQSPLSLPVSLGDQASISCRPSQSLVHS-NGNTYLHWYLQKPGQSPKLLIYRVSNR
10 1TET            DVLMTQTPLSLPVSLGDQASISCKSSQSIVHS-SGNTYFEWYLQKPGQSPKLLIYKVSNR
11 2FBJ            EIVLTQSPAITAASLGQKVTITCSASSSVSS-------LHWYQQKSGTSPKPWIYEISKL
12 1HIL            DIVMTQSPSSLTVTAGEKVTMSCTSSQSLFNSGKQKNYLTWYQQKPGQPPKVLIYWASTR
13 2IMM            DIVMTQSPSSLSVSAGERVTMSCKSSQSLLNSGNQKNFLAWYQQKPGQPPKLLIYGASTR
14                 :: :**:*   ..: *: .:::* .* .:         . ** ** * .*:  :*  :
15
16 1FGV            ESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGAGTKVEIKRT-----
17 1FVC            YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT-----
18 1IGM            ETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYQNLPLTFGPGTKVDIKRTVAAPS
19 1VFA            ADGVPSRFSGSGSGTQYSLKINSLQPEDFGSYYCQHFWSTPRTFGGGTKLEIKR------
20 1FLR            FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKLEIKRADAAPT
21 2CGR            FSGVPDRFSGSGSGTAFTLKISRVEAEDLGVYFCSQGTHVPYTFGGGTKLELKRADAAPT
22 1TET            FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPFTFGSGTKLEIKRADAAPT
23 2FBJ            ASGVPARFSGSGSGTSYSLTINTMEAEDAAIYYCQQWTYPLITFGAGTKLELKRADAAPT
24 1HIL            ESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSNPLTFGGGTKLELKRADAAPT
25 2IMM            ESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGAGTKLELKR------
26                   *** **:** *** :::.*. ::.** . *:* :     *** ***:::**
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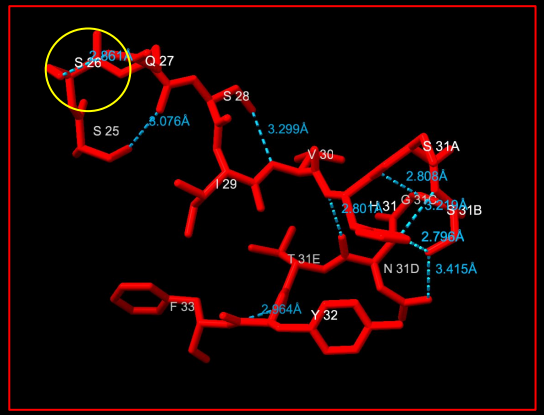


L1 k
L2 k
L3 k

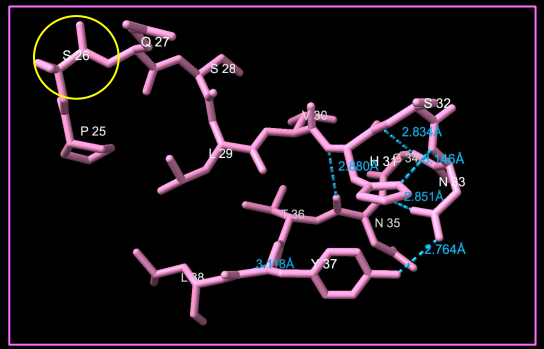
Fv region - L1-4k





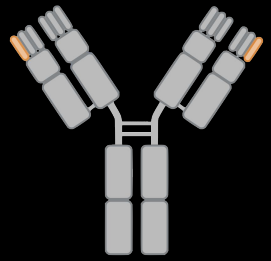


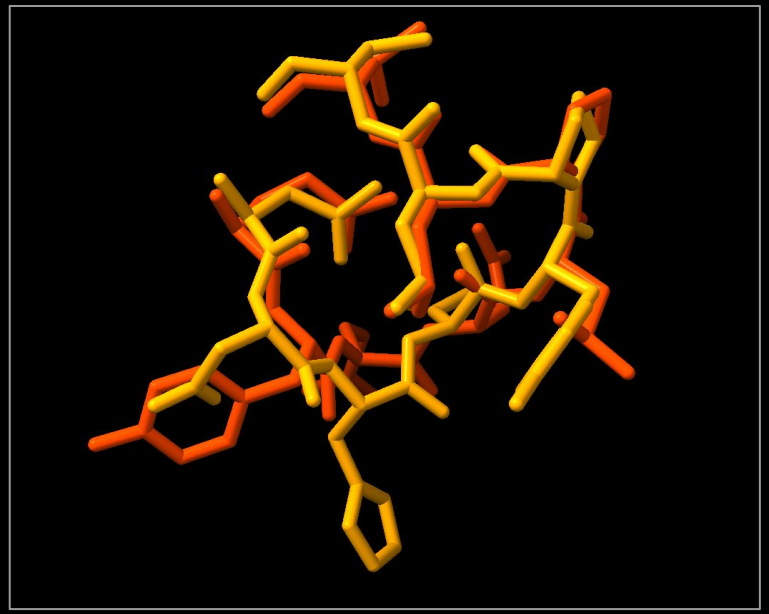
1 TET
2.20 Å, *Mus musculus*

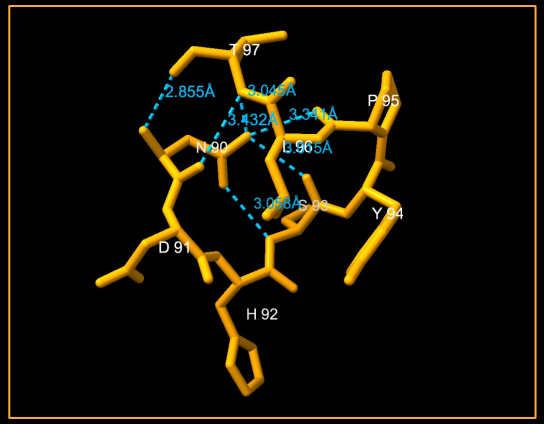


2 CGR
2.20 Å, *Mus musculus*

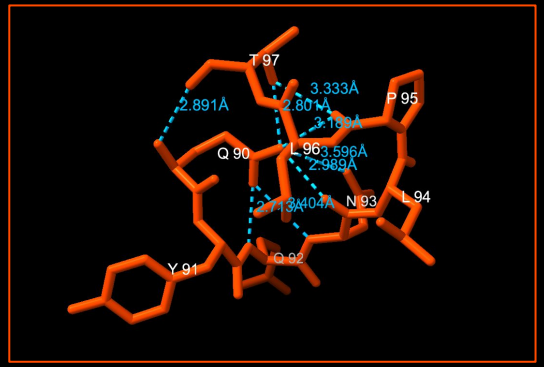
Fv region - L3-1k





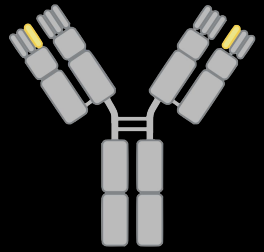


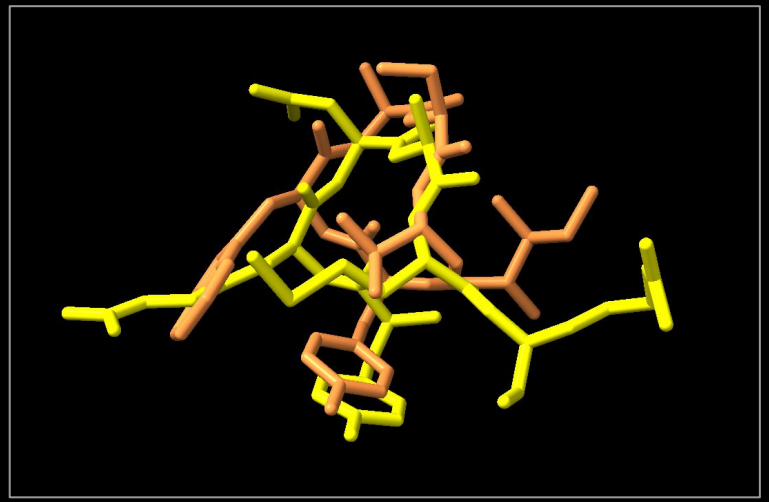
2 IMM
2.00 Å, *Mus musculus*

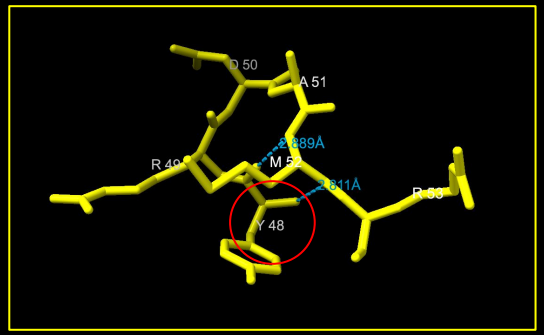


1 IGM
2.30 Å, *Homo sapiens*

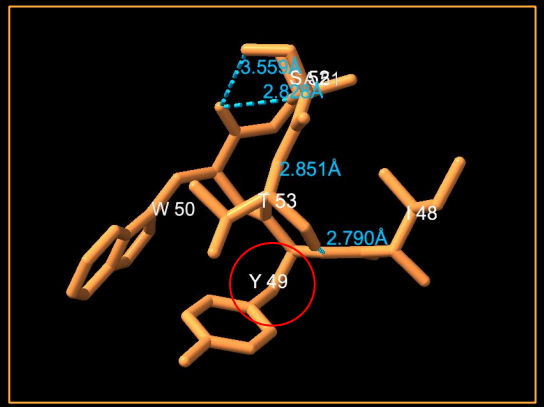
Fv region - L2 λ-k







2 FB4 (λ)
1.90 Å, *Homo sapiens*

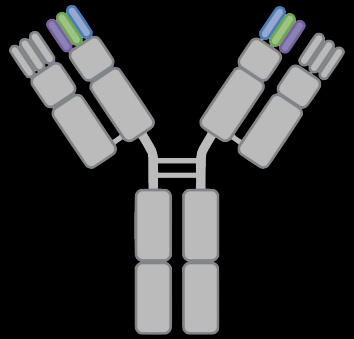


1 HIL (k)
2.00 Å, *Mus musculus*

Fv region - Heavy chain

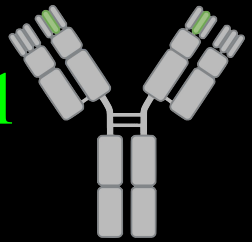
Multiple sequence alignment

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 6 1IND          EVTLVESGGDSVKPGGSLKLSCAASGFTLSGETMSWVRQTPEKRLEWVATTLSG--GGFT
 7 1TET          QIQLVQSGPELKTPGETVRISCKASGYTFTTYGMSWVKQTPGKGFKWMGWINTY--SGVP
 8 1FGV          EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPK--NGGT
 9 1FVC          EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT--NGYT
10 1IGM          EVHLLESGGNLVQPGGSLRLSCAASGFTFNIFVMSWVRQAPGKGLEWVSGVFGS--GGNT
11 2FBJ          EVKLLESGGGLVQPGGSLKLSCAASGFDFSKYWMSWVRQAPGKGLEWIGEIHPD--SGTI
12 2FB4          EVQLVQSGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGKGLEWVAIIWDD--GSDQ
13 8FAB          AVKLVQAGGGVVQPGRSLRLSCIASGFTFSNYGMHWVRQAPGKGLEWVAVIWYN--GSRT
14 7FAB          AVQLEQSGPGLVRPSQTLSLTCTVSGTSFDDYYWTWVRQPPGRGLEWIGYVFYT--GT-T
15 1GIG          QVQLKESGPGLVAPSQSLSITCTVSGFLLISNGVHWVRQPPGKGLEWLGVIWAG--GN-T
16 1VFA          QVQLQESGPGLVAPSQSLSITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGD--GN-T
17 1MFA          EVQVQQSGTVVARPGASVKMSCKASGYTFTNYWMHWIKQRPGQGLEWIGAIYPG--NSAT
18 2CGR          RVQLLESGAELMKPGASVQISCKATGYTFSEYWIEWVKERPGHGLEWIGEILPG--SGRT
19                : : ::*     *. .: ::*  :*  :      *::: * : ::*:.       .
20
21 1FLR          YYSDSVKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTG----------SYYGMDYWGQ
22 1HIL          YYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDSAMYYCARRERY------DENGFAYWGQ
23 1IND          FYSASVKGRFTISRDNAQNNLYLQLNSLRSEDTALYFCA------------SHRFVHWGH
24 1TET          TYADDFKGRFAFSLETSASTAYLQINNLKNEDTATYFCAR----------RSWYFDVWGT
25 1FGV          SYADSVKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWRGLN--YGFDVRYFDVWGQ
26 1FVC          RYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD----GFYA--MDYWGQ
27 1IGM          DYADAVKGRFTITRDNSKNTLYLQMNSLRAEDTAIYYCAKHRVS-----YVLTGFDSWGQ
28 2FBJ          NYTPSLKDKFIISRDNAKNSLYLQMSKVRSEDTALYYCARLHY------YGYNAY--WGQ
29 2FB4          HYADSVKGRFTISRNDSKNTLFLQMDSLRPEDTGVYFCARDGGHGFCSSASCFGPDYWGQ
30 8FAB          YYGDSVKGRFTISRDNSKRTLYMQMNSLRTEDTAVYYCARDP-----DILTAFSFDYWGQ
31 7FAB          LLDPSLRGRVTMLVNTSKNQFSLRLSSVTAADTAVYYCARNL--------IAGGIDVWGQ
32 1GIG          NYNSALMSRVSISKDNSKSQVFLKMKSLQTDDTAMYYCARDFYD---YDVFYYAMDYWGQ
33 1VFA          DYNSALKSRLSISKDNSKSQVFLKMNSLHTDDTARYYCARERD---------YRLDYWGQ
34 1MFA          FYNHKFRAKTKLTAVTSTTTAYMELSSLTSEDSAVYYCTRGGH--------GYYGDYWGQ
35 2CGR          NYREKFKGKATFTADTSSNTAYMQLSSLTSEDSAVYYCTRG----------YSSMDYWGQ
36                     .  :  :    :     :.:..:   * . *:*:                 **
37
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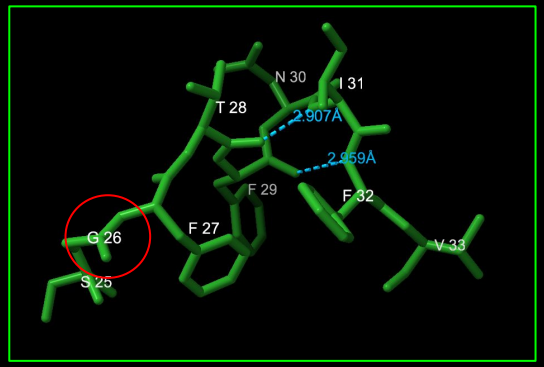


H1
H2
H3

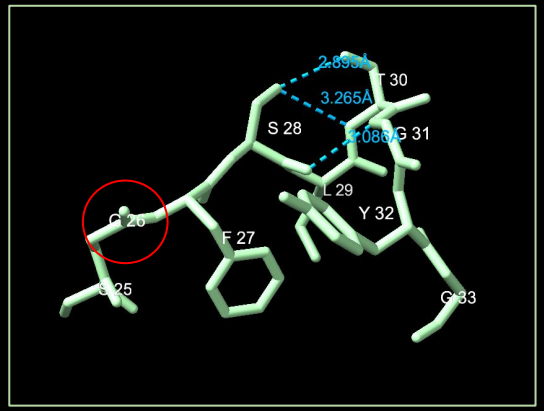
Fv region - H1-1





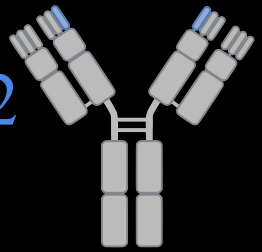


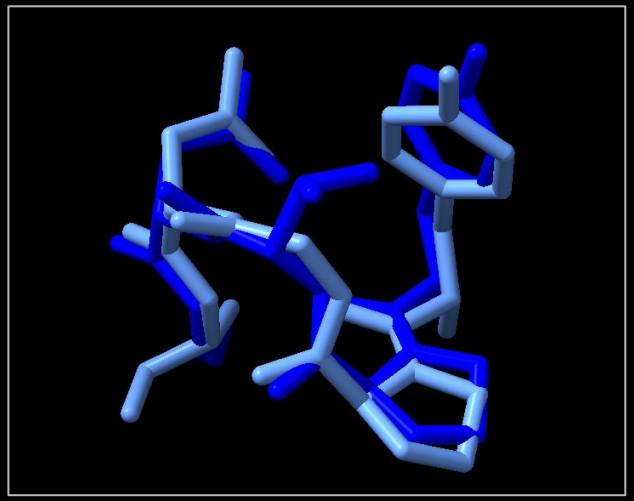
1 IGM
2.30 Å, *Homo sapiens*

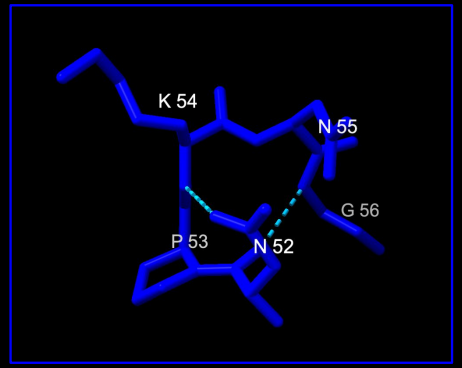


1 VFA
1.80 Å, *Mus musculus*

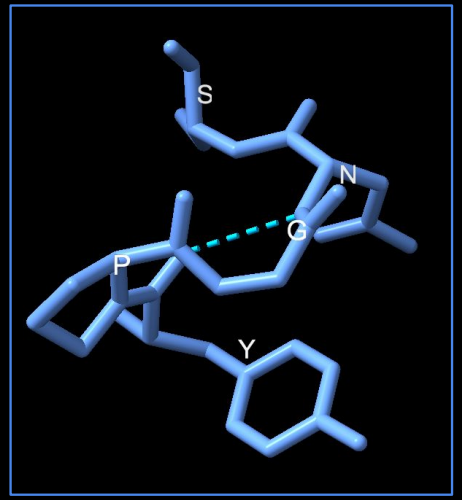
Fv region - H2-2







1 FVGV
1.90 Å, *Homo sapiens*



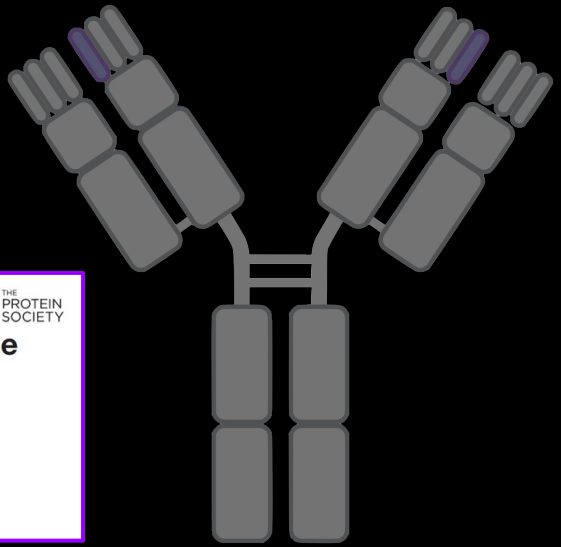
1 MFA
1.70 Å, *Mus musculus*

Fv region - H3

PROTEINS

The H3 loop of antibodies shows unique structural characteristics

Cristian Regep,[1,2] Guy Georges,[3] Jiye Shi,[4] Bojana Popovic,[5] and Charlotte M. Deane[1]*



THE PROTEIN SOCIETY

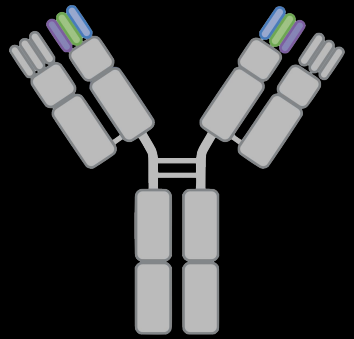
The diversity of H3 loops determines the antigen-binding tendencies of antibody CDR loops

Yuko Tsuchiya[1]* and Kenji Mizuguchi[1]

Fv region - Heavy chain

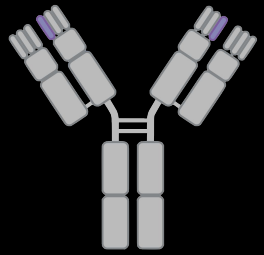
Multiple sequence alignment

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 4 1FLR          EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYET
 5 1HIL          EVQLVESGGDLVKPGGSLKLSCAASGFSFSSYGMSWVRQTPDKRLEWVATISNG--GGYT
 6 1IND          EVTLVESGGDSVKPGGSLKLSCAASGFTLSGETMSWVRQTPEKRLEWVATTLSG--GGFT
 7 1TET          QIQLVQSGPELKTPGETVRISCKASGYTFTTYGMSWVKQTPGKGFKWMGWINTY--SGVP
 8 1FGV          EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPK--NGGT
 9 1FVC          EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT--NGYT
10 1IGM          EVHLLESGGNLVQPGGSLRLSCAASGFTFNIFVMSWVRQAPGKGLEWVSGVFGS--GGNT
11 2FBJ          EVKLLESGGGLVQPGGSLKLSCAASGFDFSKYWMSWVRQAPGKGLEWIGEIHPD--SGTI
12 2FB4          EVQLVQSGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGKGLEWVAIIWDD--GSDQ
13 8FAB          AVKLVQAGGGVVQPGRSLRLSCIASGFTFSNYGMHWVRQAPGKGLEWVAVIWYN--GSRT
14 7FAB          AVQLEQSGPGLVRPSQTLSLTCTVSGTSFDDYYWTWVRQPPGRGLEWIGYVFYT--GT-T
15 1GIG          QVQLKESGPGLVAPSQSLSITCTVSGFLLISNGVHWVRQPPGKGLEWLGVIWAG--GN-T
16 1VFA          QVQLQESGPGLVAPSQSLSITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGD--GN-T
17 1MFA          EVQVQQSGTVVARPGASVKMSCKASGYTFTNYWMHWIKQRPGQGLEWIGAIYPG--NSAT
18 2CGR          RVQLLESGAELMKPGASVQISCKATGYTFSEYWIEWVKERPGHGLEWIGEILPG--SGRT
19                : : ::*     *. .: ::*  :*  :      *::: * : ::*:.       .
20
21 1FLR          YYSDSVKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTG----------SYYGMDYWGQ
22 1HIL          YYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDSAMYYCARRERY------DENGFAYWGQ
23 1IND          FYSASVKGRFTISRDNAQNNLYLQLNSLRSEDTALYFCA------------SHRFVHWGH
24 1TET          TYADDFKGRFAFSLETSASTAYLQINNLKNEDTATYFCAR----------RSWYFDVWGT
25 1FGV          SYADSVKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWRGLN--YGFDVRYFDVWGQ
26 1FVC          RYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD----GFYA--MDYWGQ
27 1IGM          DYADAVKGRFTITRDNSKNTLYLQMNSLRAEDTAIYYCAKHRVS-----YVLTGFDSWGQ
28 2FBJ          NYTPSLKDKFIISRDNAKNSLYLQMSKVRSEDTALYYCARLHY------YGYNAY--WGQ
29 2FB4          HYADSVKGRFTISRNDSKNTLFLQMDSLRPEDTGVYFCARDGGHGFCSSASCFGPDYWGQ
30 8FAB          YYGDSVKGRFTISRDNSKRTLYMQMNSLRTEDTAVYYCARDP-----DILTAFSFDYWGQ
31 7FAB          LLDPSLRGRVTMLVNTSKNQFSLRLSSVTAADTAVYYCARNL--------IAGGIDVWGQ
32 1GIG          NYNSALMSRVSISKDNSKSQVFLKMKSLQTDDTAMYYCARDFYD---YDVFYYAMDYWGQ
33 1VFA          DYNSALKSRLSISKDNSKSQVFLKMNSLHTDDTARYYCARERD---------YRLDYWGQ
34 1MFA          FYNHKFRAKTKLTAVTSTTTAYMELSSLTSEDSAVYYCTRGGH--------GYYGDYWGQ
35 2CGR          NYREKFKGKATFTADTSSNTAYMQLSSLTSEDSAVYYCTRG----------YSSMDYWGQ
36                     .  :  :    :     :.:..:   * . *:*:                **
37                                                  92                103 104
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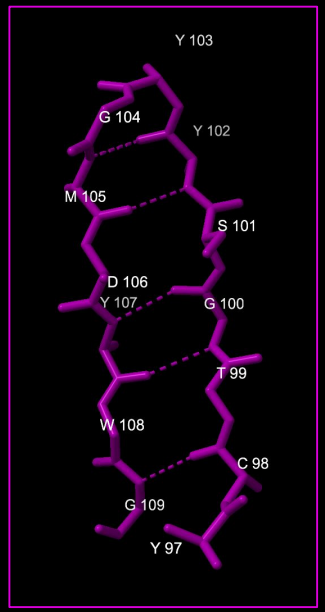


H1
H2
H3

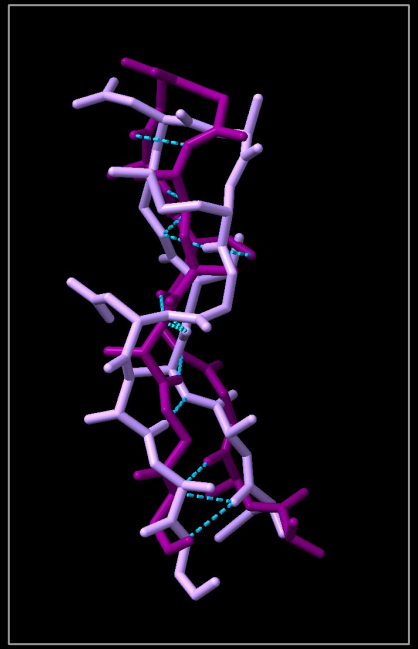
Fv region - H3



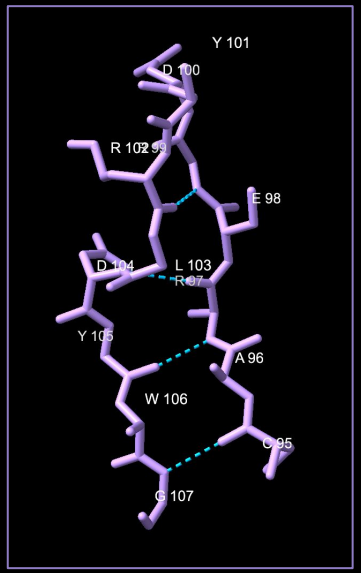
1 FLR - extended



1.85 Å, *Mus musculus*



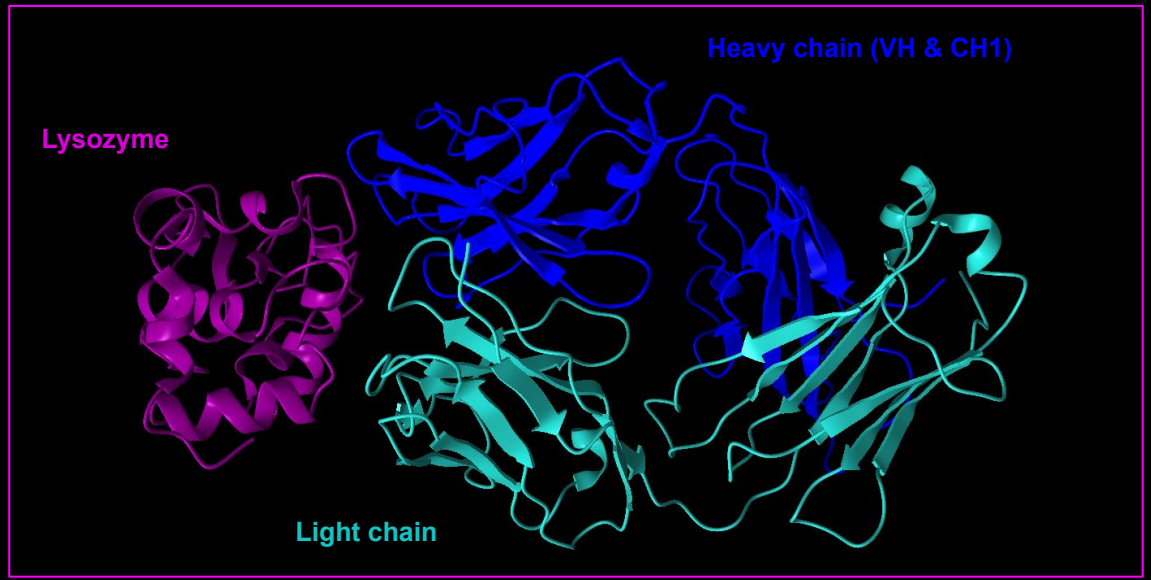
1 VFA - kinked



1.80 Å, *Mus musculus*

Interaction antigen-antibody

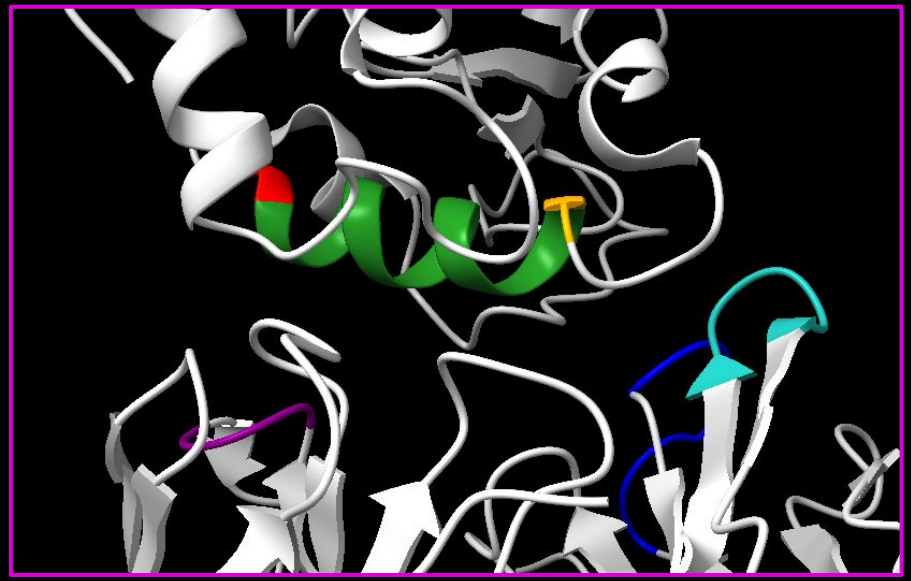
HyHEL-10 Fab - lysozyme complex



3HFM
3.00 Å, *Mus musculus*

Interaction antigen-antibody

HyHEL-10 Fab - lysozyme complex

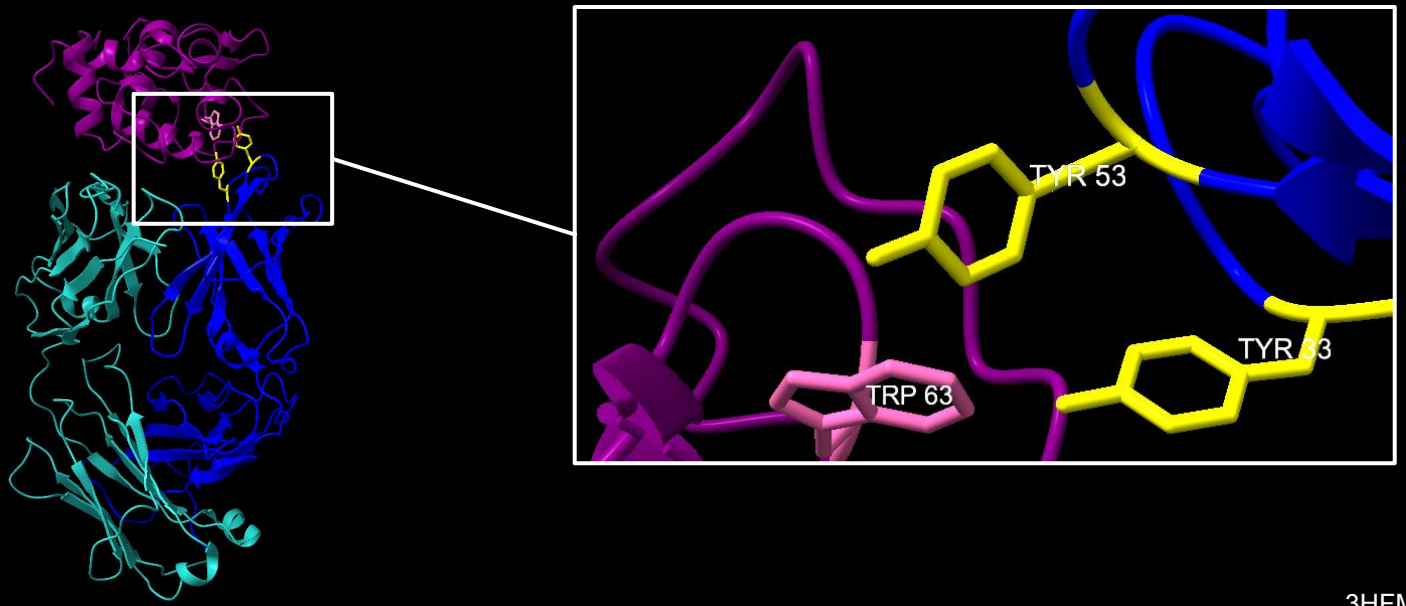


Lysozyme helix (residues 88-99)
N terminus
C terminus
CDR1-H
CDR2-Lk
CDR2-H

3HFM
3.00 Å, *Mus musculus*

Interaction antigen-antibody

Structure of the binding site



3HFM
3.00 Å, *Mus musculus*

Interaction antigen-antibody

HyHEL-10 Fab - lysozyme complex



- Discontinuous epitope
- Mostly accessible surface

3HFM
3.00 Å, *Mus musculus*

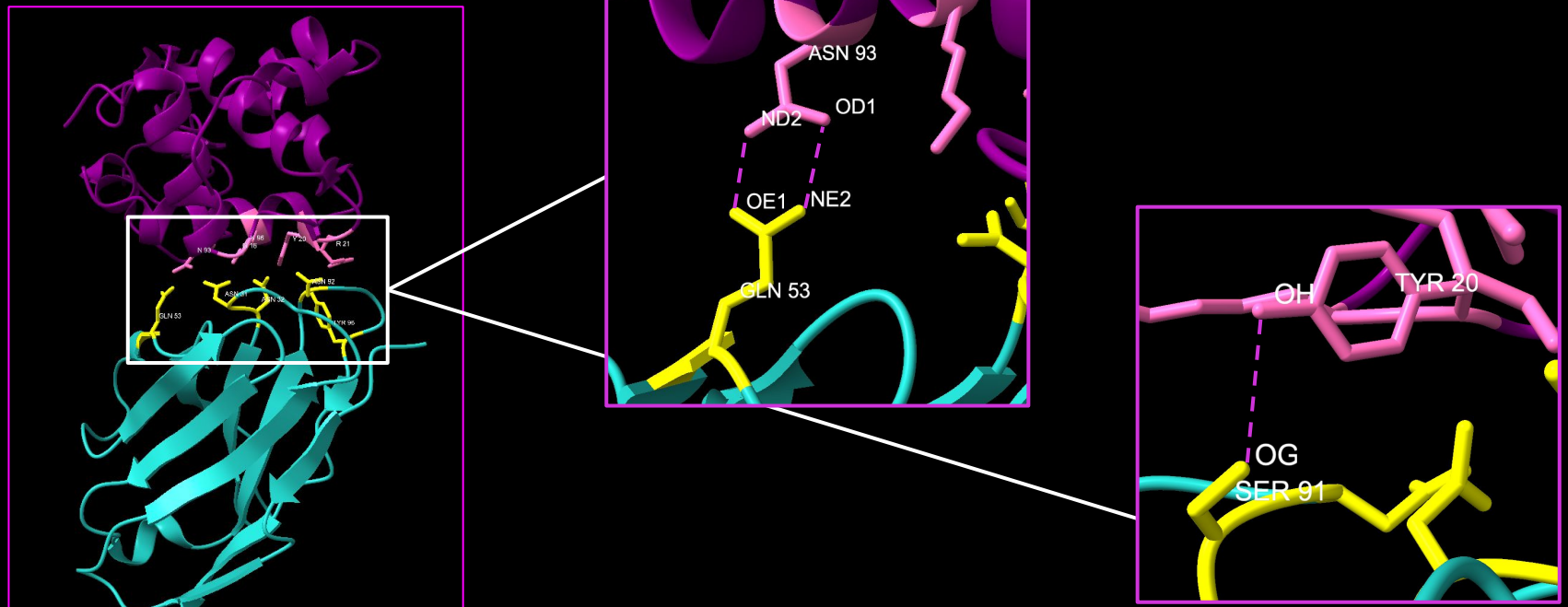
Interaction antigen-antibody

HyHEL-10 Fab - lysozyme complex

126 pairwise atomic contacts

- 111 **van der Waals** contacts
- 14 **hydrogen-bonding** contacts
- 1 **salt bridge**

Interaction antigen-antibody: H bonds Light chain

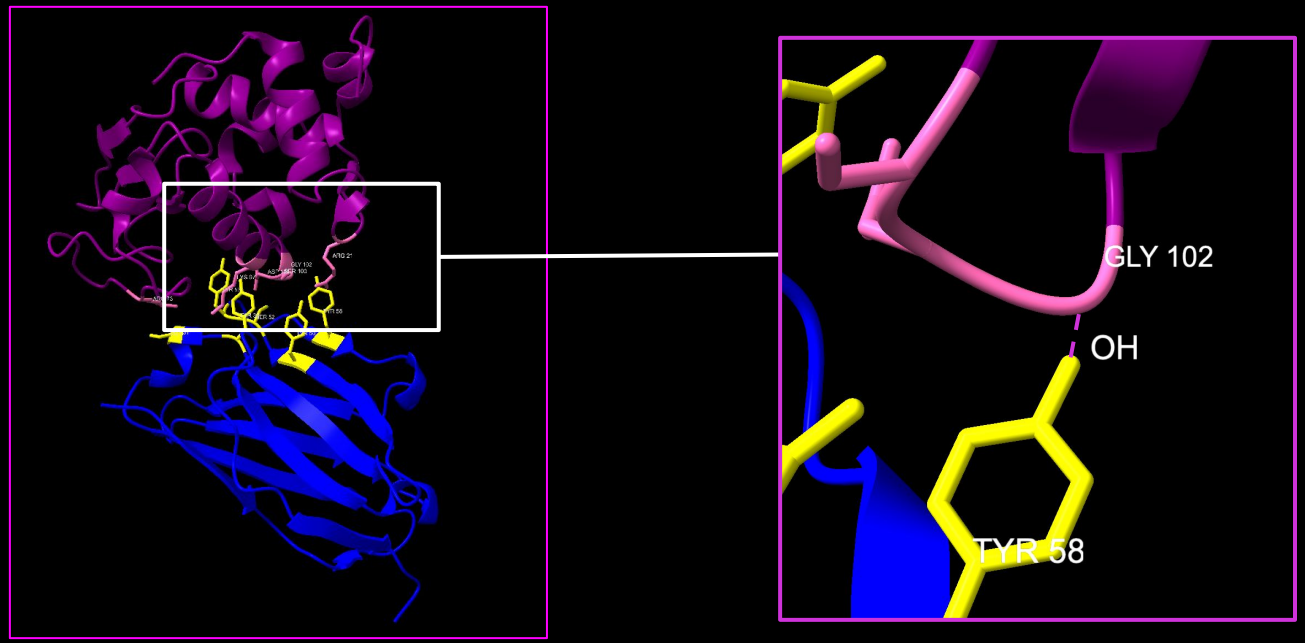


3HFM
3.00 Å, *Mus musculus*

VL (chain **L**): Asn-3, Asn-32, Gln-53, Asn-92, Tyr-96

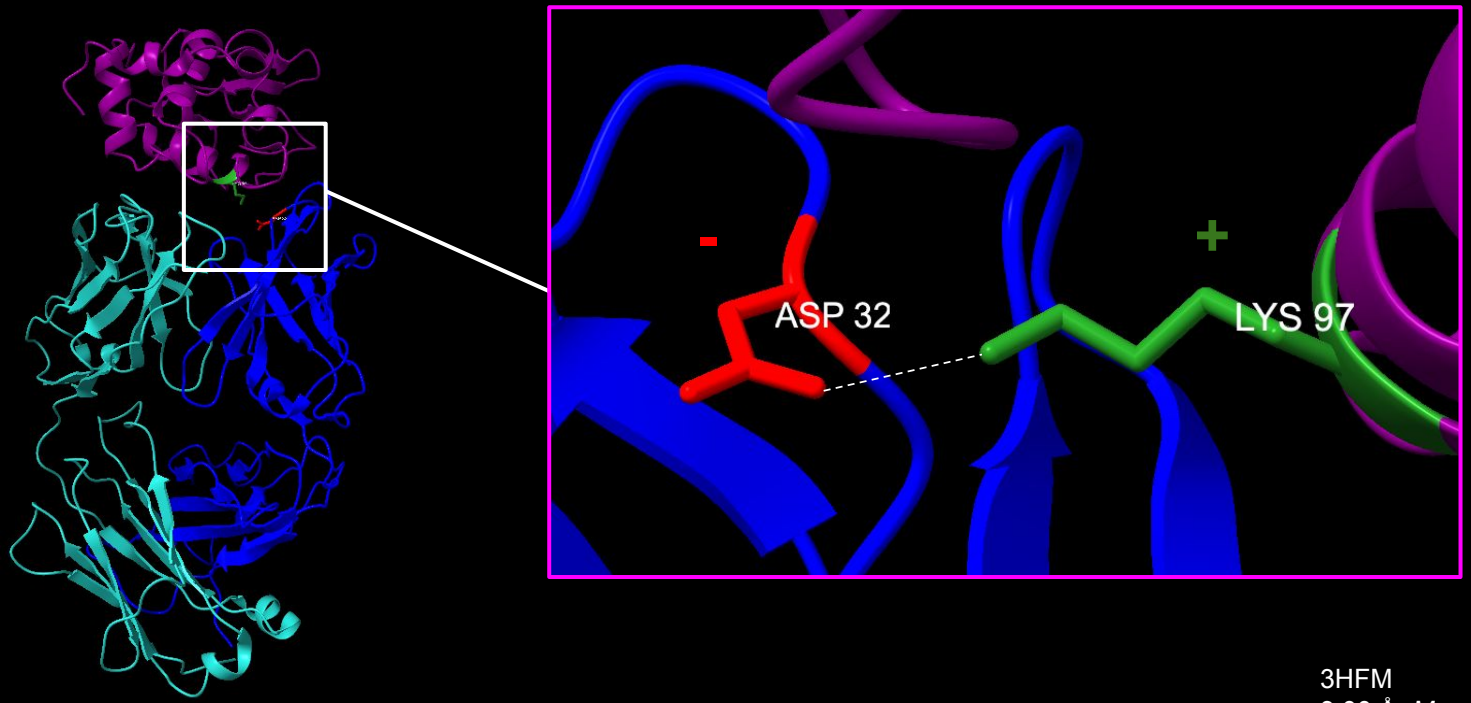
Interaction antigen-antibody: H bonds Heavy chain

VH (chain **H**): Ser-31, Tyr-33, Tyr-50, Ser-52, Tyr-53, Tyr-58



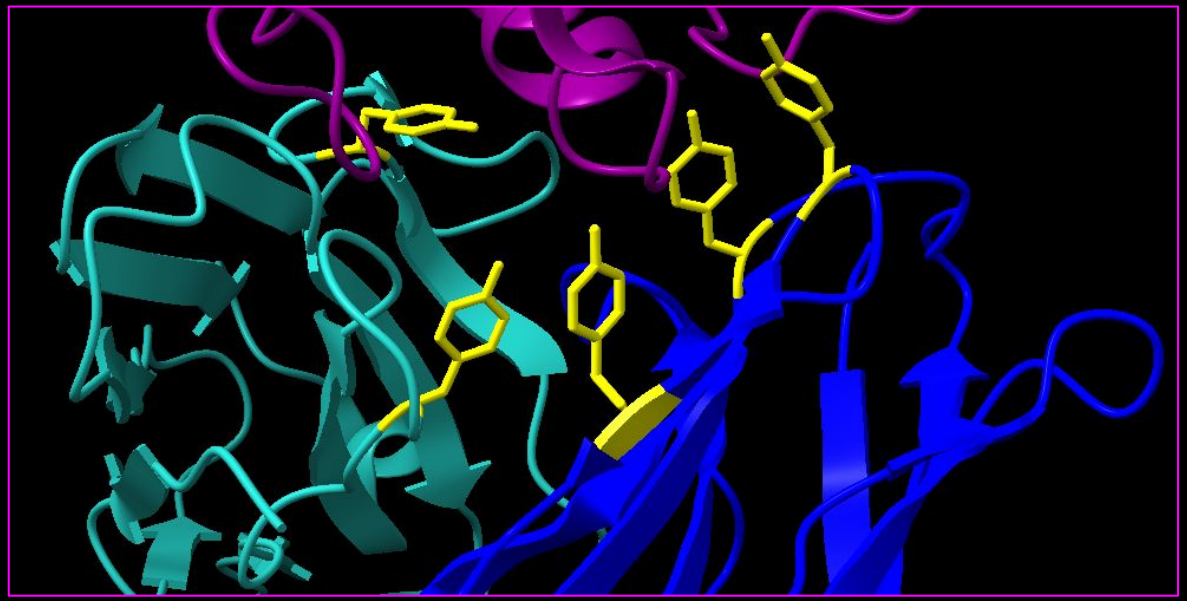
3HFM
3.00 Å, *Mus musculus*

Interaction antigen-antibody: Salt bridges



3HFM
3.00 Å, *Mus musculus*

Interaction antigen-antibody: Aromatic-rich surface



3HFM
3.00 Å, *Mus musculus*

CONCLUSIONS

- Immunoglobulins are a really diverse group of proteins
- Protein structure and stabilization is really dependent on specific residues
- The basic immunoglobulin fold is disposed in a greek key topology
- Gene rearrangement is one of the several factors that contributes to the diversity of immunoglobulin V regions
- The interaction between FcγRI–IgG1 Fc confirms a conserved Fcγ receptor-IgG binding mode
- Additionally, a unique FG loop interaction with Fc and its glycans explains the high affinity of FcγRI for IgG
- CDRs form the antigen-binding site, with CDR-H3 being the most variable and central contributor to specificity and affinity
- Even though CDRs display a great variation, most of them adopt canonical structures determined by loop length and key residues
- The HyHEL-10-lysozyme complex shows that antigen-antibody binding relies on structural complementarity and is stabilized mainly by van der Waals contacts and hydrogen bonds, especially involving tyrosines

QUESTIONS

1. Which structural motif characterizes the immunoglobulin fold?
   a. TIM barrel
   b. Rossmann fold
   c. Greek key β-sandwich
   d. α-helical bundle
   e. β-propeller

2. What is the structural role of glycine and proline in immunoglobulin domains?
   a. They stabilize α-helices
   b. They promote β-sheet formation exclusively
   c. They favor sharp turns and loop formation
   d. They form disulfide bridges
   e. They bind directly to Fc receptors

3. Which CDR does NOT follow canonical structure classification?
   a. L1
   b. L2
   c. L3
   d. H1
   e. H3

4. The conserved glycosylation site in IgG Fc is located at:
   a. Cys 297
   b. Asn 297
   c. Lys 142
   d. Tyr 53
   e. Asp 265

5. Regarding IgG subclasses, which statement is TRUE?
   a. IgG4 strongly activates complement
   b. IgG3 has the shortest hinge region
   c. IgG2 has additional disulfide bonds in the hinge compared to IgG1
   d. All subclasses have identical half-lives
   e. IgG1 is the least abundant subclass

6. Compared to low-affinity Fcγ receptors, FcγRI has:
   a. A longer FG loop positioned further from Fc glycans
   b. No interaction with Fc glycans
   c. A shorter FG loop positioned closer to Fc glycans
   d. No salt-bridge interactions
   e. Only hydrophobic contacts

7. In the HyHEL-10–lysozyme complex, most atomic contacts between antigen and antibody are:
   a. Disulfide bonds
   b. Salt bridges
   c. Hydrogen bonds
   d. Van der Waals interactions
   e. Covalent peptide bonds

8. Which statement about complementarity-determining regions (CDRs) is correct?
   a. CDRs are completely conserved in all antibodies
   b. CDRs form the antigen-binding site of antibodies
   c. CDRs determine the antibody isotype
   d. CDRs are only found in the Fc region
   e. CDRs have no influence on antigen specificity

9. The flexibility of the immunoglobulin is conferred in large part by:
   a. The constant domain
   b. The variable domain
   c. The hinge
   d. The light chain
   e. The heavy chain

10. FcγRI:
   a. Binds to the CDRs
   b. Binds directly to antigens
   c. Binds to the Fab region
   d. Binds to the Fc region of IgG
   e. Does not bind immunoglobulins

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