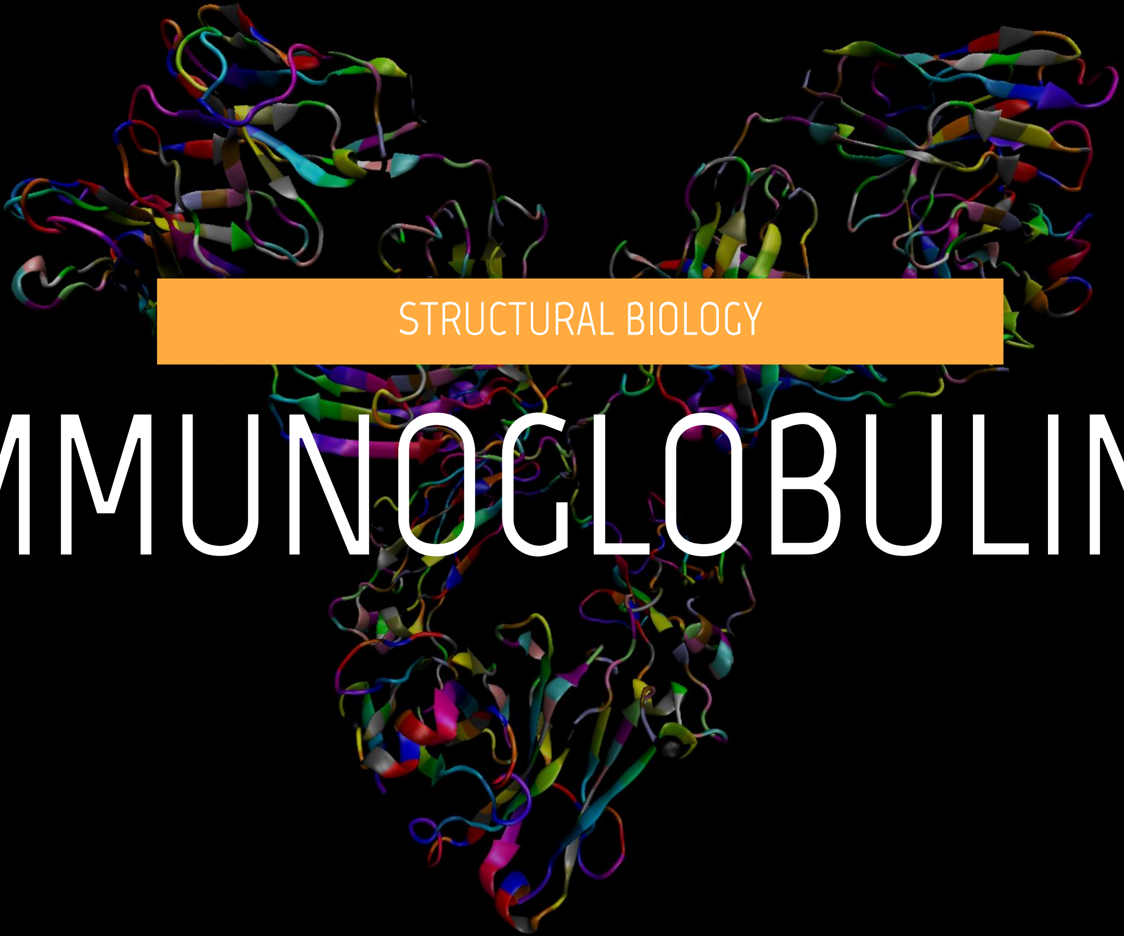


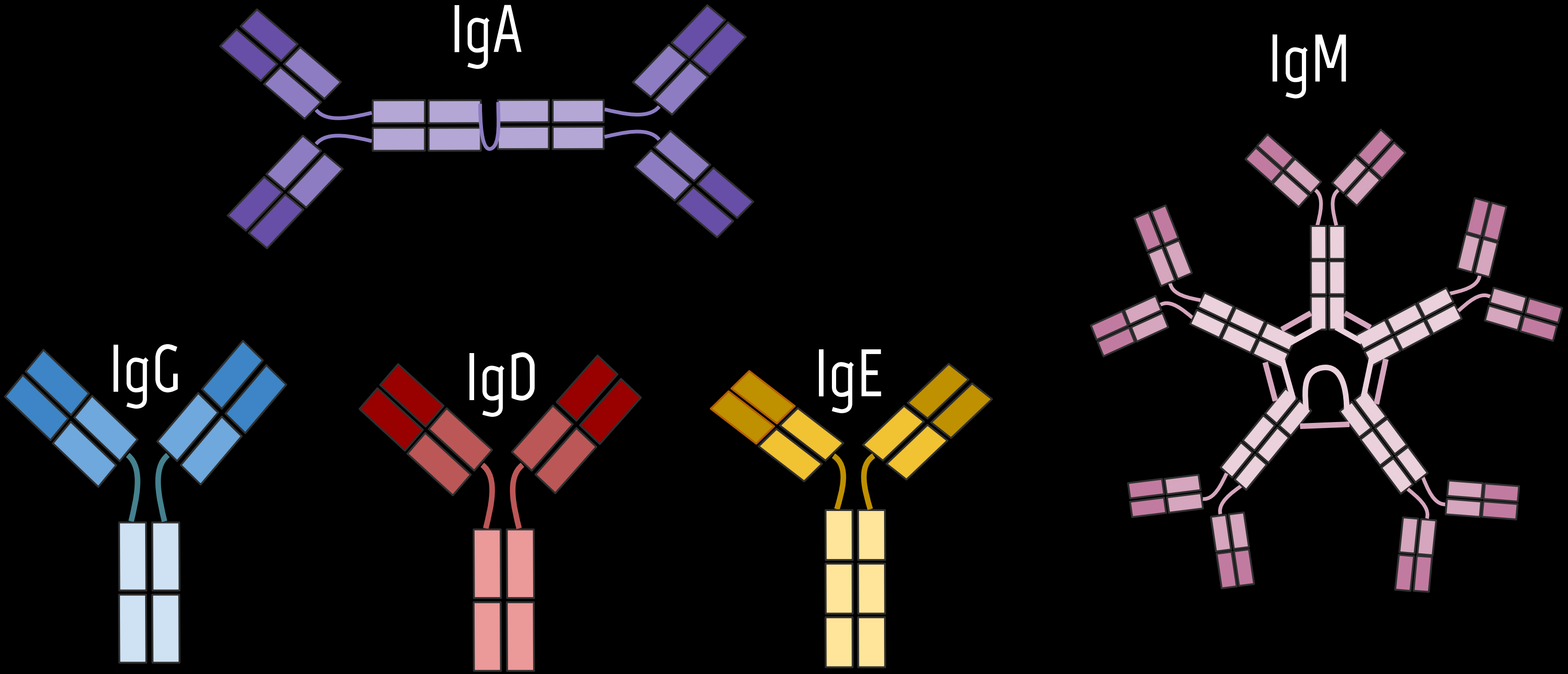
STRUCTURAL BIOLOGY

IMMUNOGLOBULINS

Júlia Brunet, Marta Nieto i Aina Pocurull

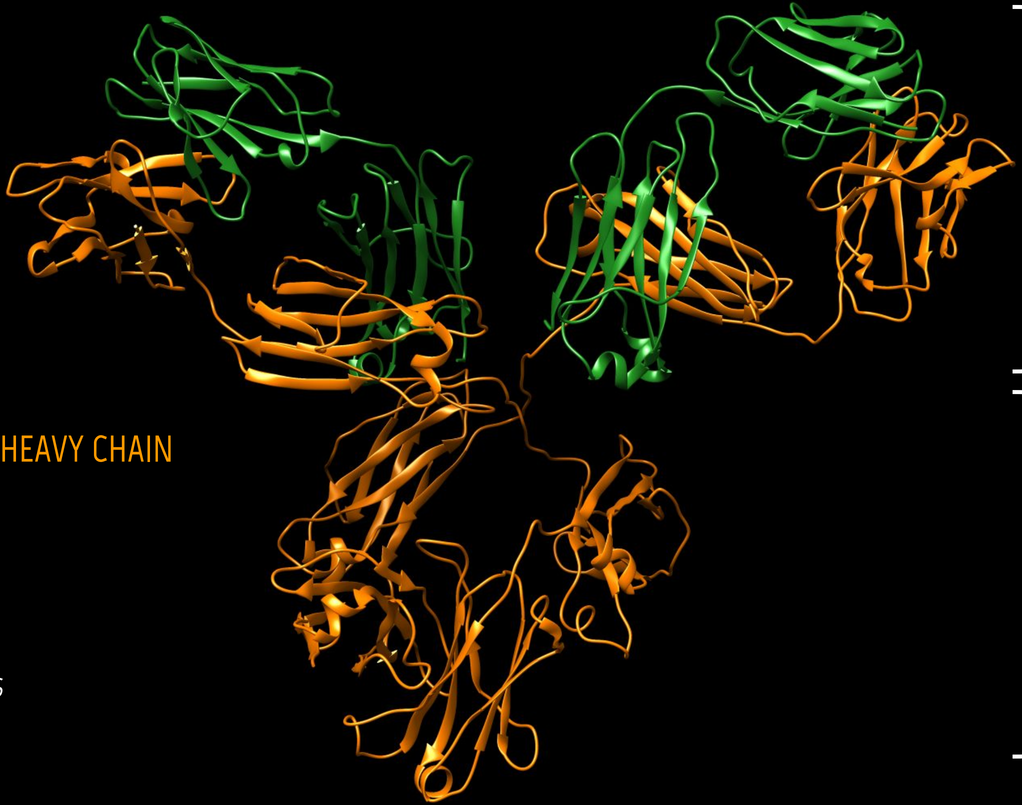
INTRODUCTION & CONCEPTS

ISOTYPES



STRUCTURE

LIGHT CHAIN
- Kappa
- Lambda



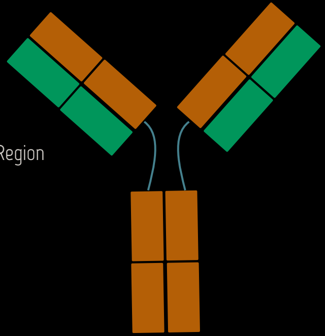
HEAVY CHAIN

1IGY
Mus musculus
3,2 Å

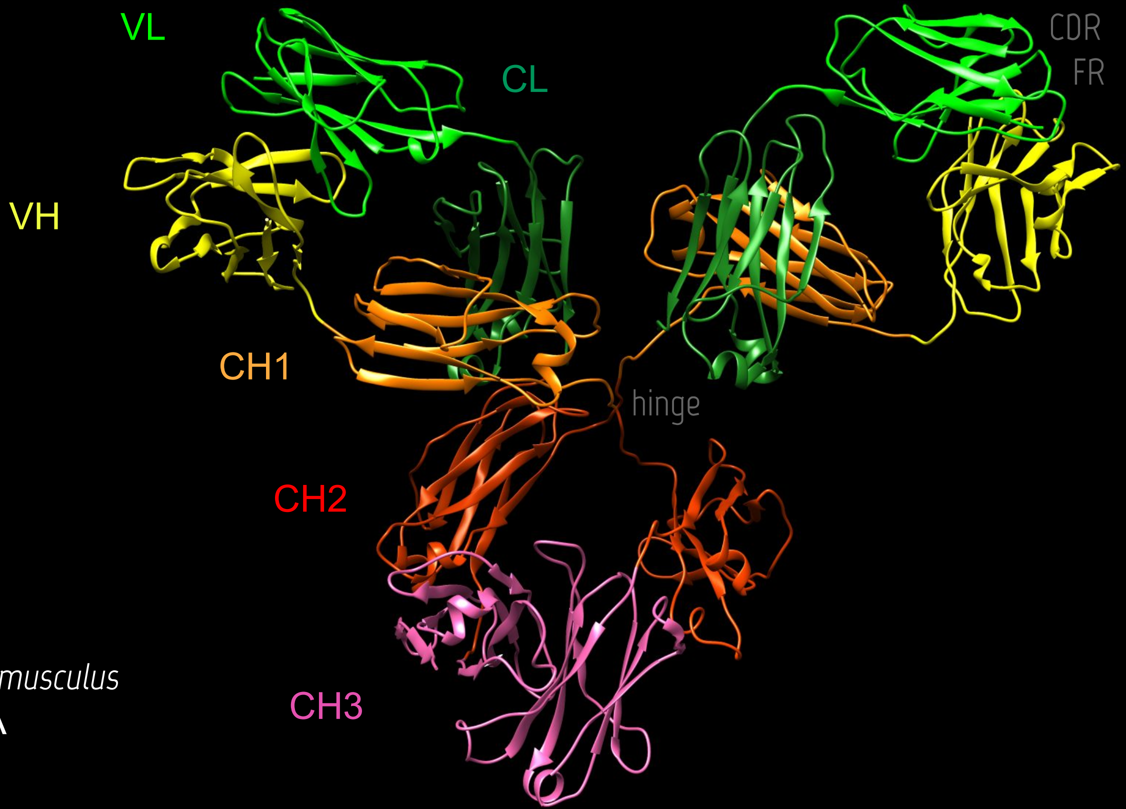
Fab
Antigen Binding

Fc
Crystallizable Region

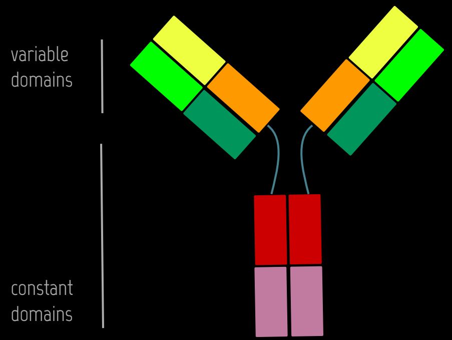
immunoglobulin *superfamily* (IgSF)



STRUCTURE

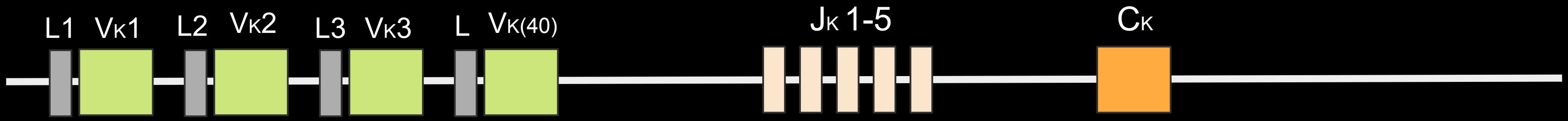


1IGY
Mus musculus
3,2 Å

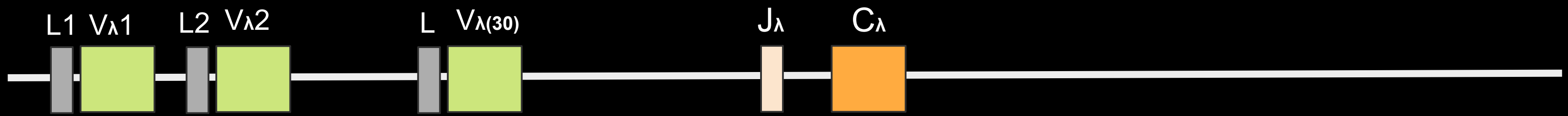


GERMLINE CONFIGURATION OF IMMUNOGLOBULIN GENES

Chromosome 2 - k light chain locus



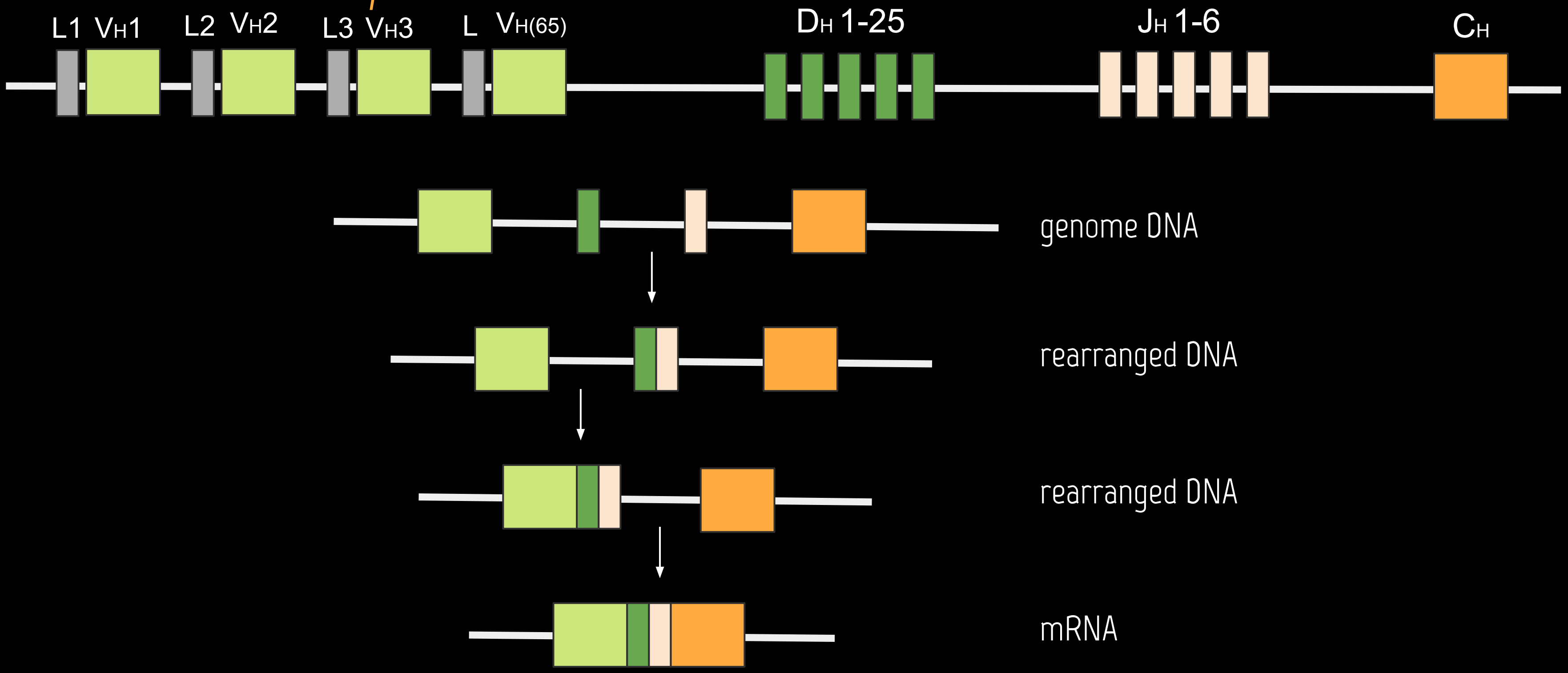
Chromosome 22 - λ light chain locus



Chromosome 14 - heavy chain locus

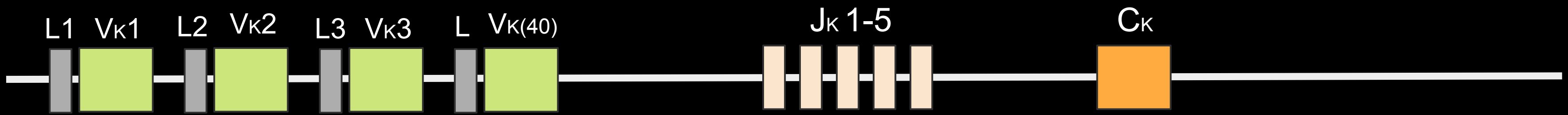
REARRANGEMENT

Chromosome 14 - heavy chain locus

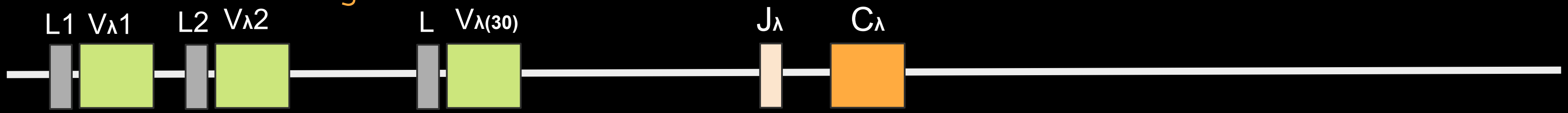


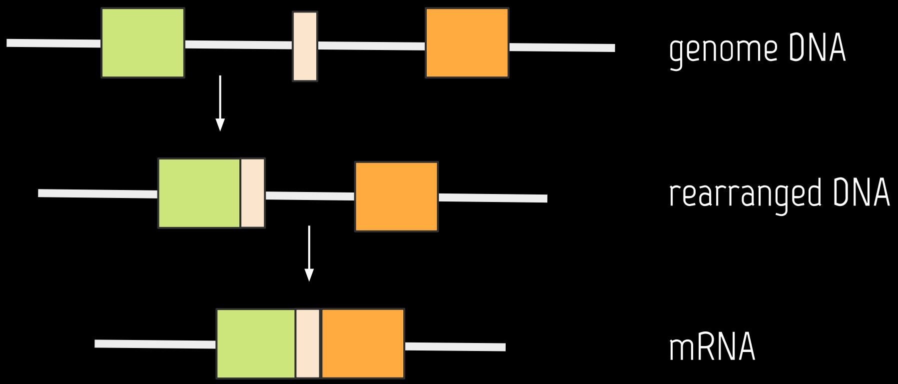
REARRANGEMENT

Chromosome 2 - k light chain locus

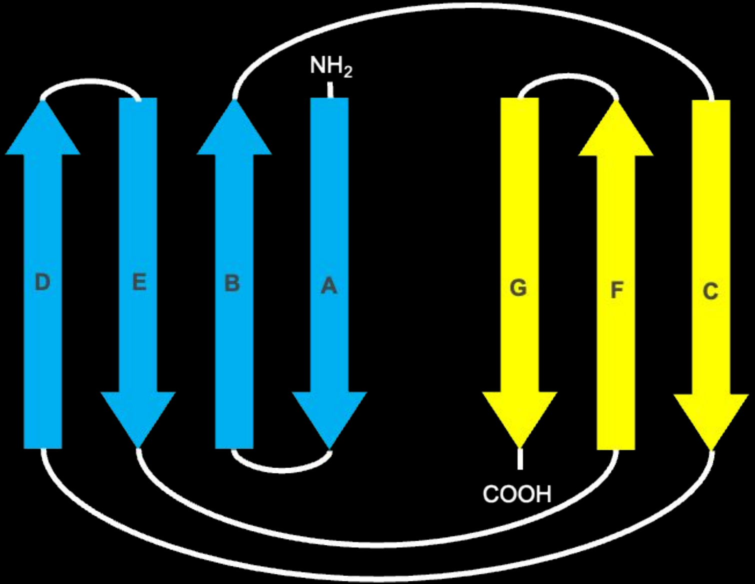


Chromosome 22 - λ light chain locus

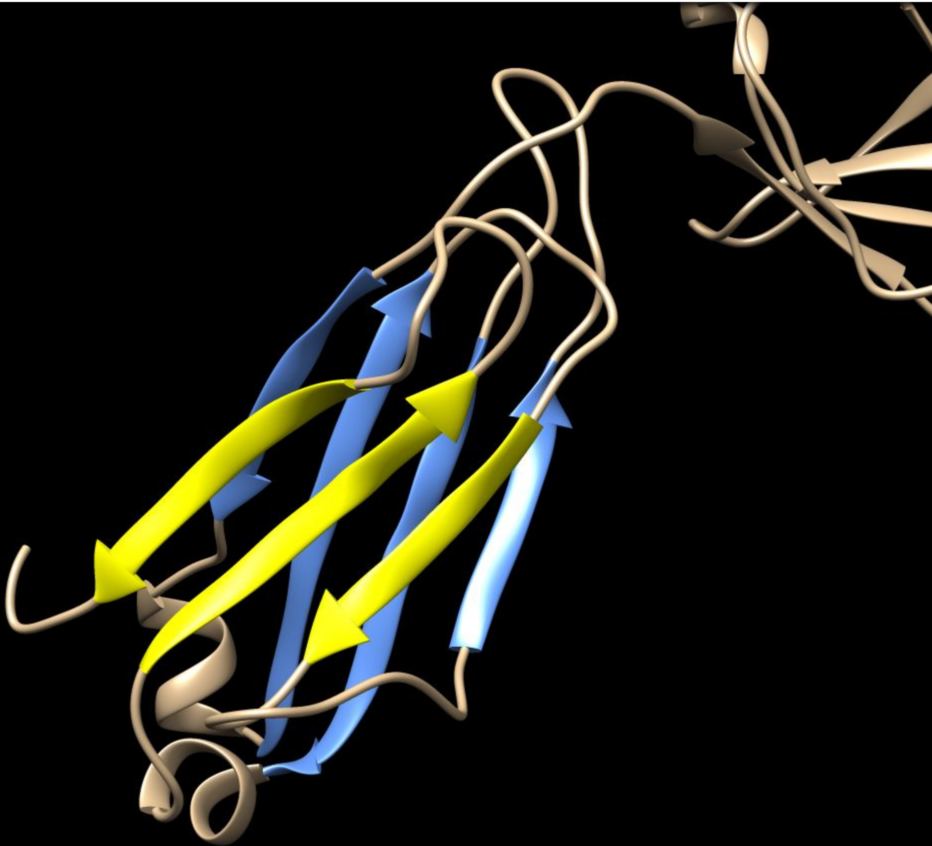




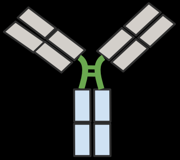
IMMUNOGLOBULIN LIKE-FOLD



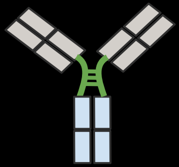
GREEK-KEY FOLD



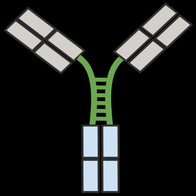
IMMUNOGLOBULIN G



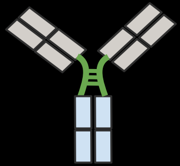
IgG1



IgG2



IgG3

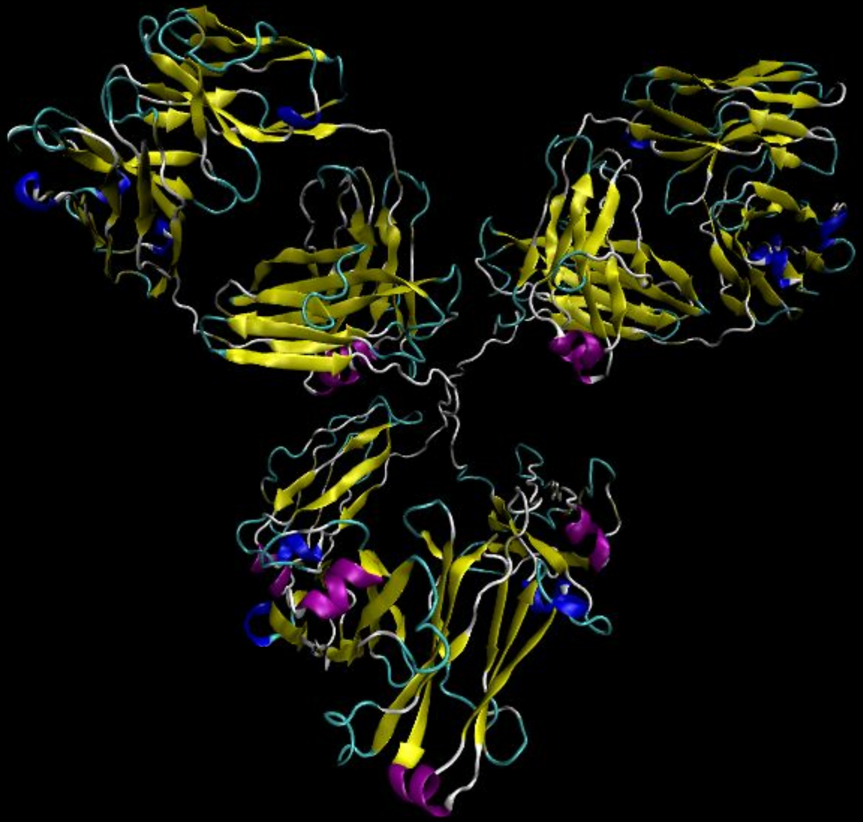


IgG4

SCOP CLASSIFICATION

CLASS	All beta proteins
FOLD	Immunoglobuline - like β - sandwich
SUPERFAMILY	Immunoglobulin
FAMILY	V set domains CI set domains C2 set domains

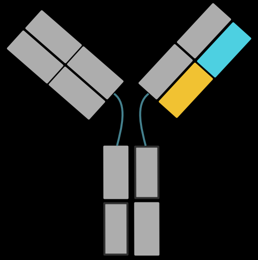
SECONDARY STRUCTURE



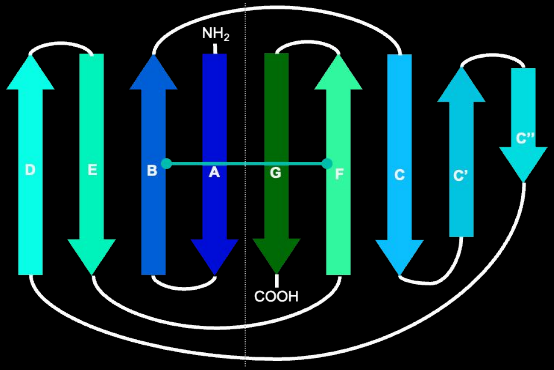
- ● beta-sheet
- ● alpha-helix
- ● helix
- ● turn
- ● coil

1IGY
Mus musculus
3,2 Å

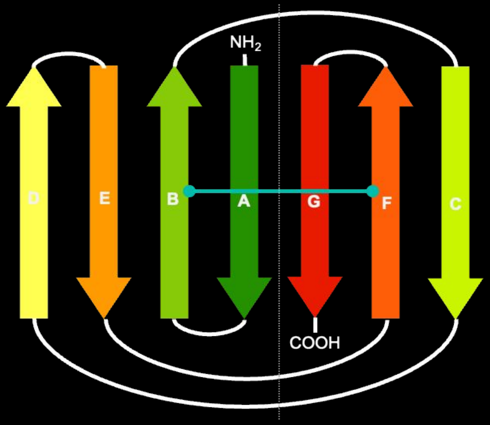
TOPOLOGY



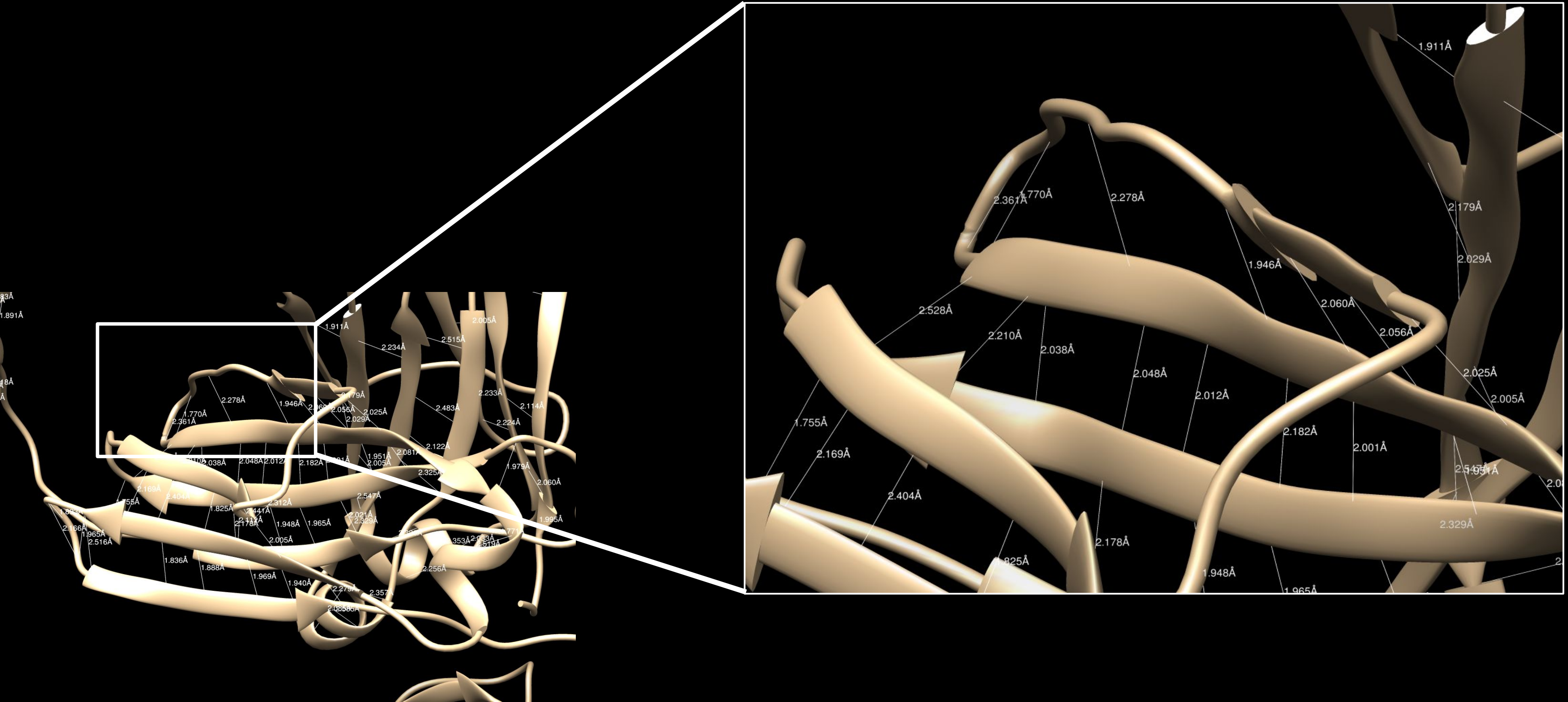
V Region



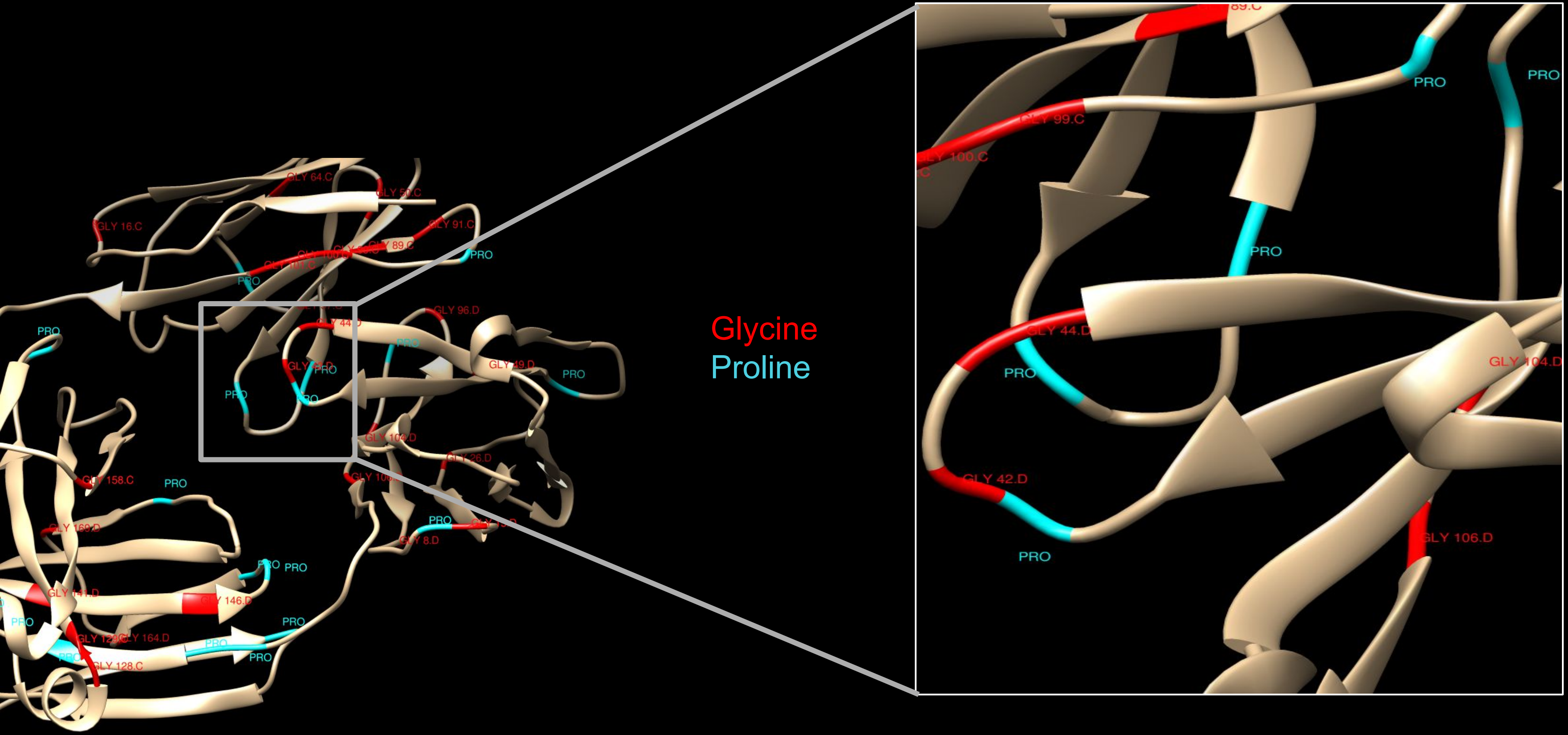
C Region



HYDROGEN BONDS

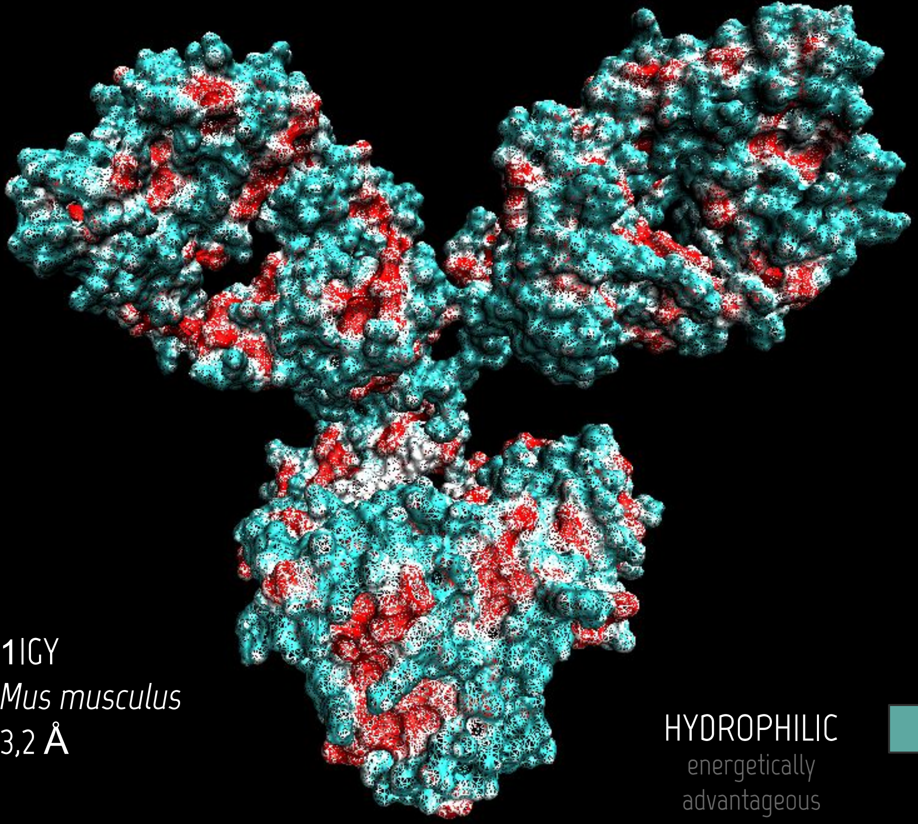


GLYCINE AND PROLINE



Glycine
Proline

HYDROPHOBIC AND HYDROPHILIC RESIDUES



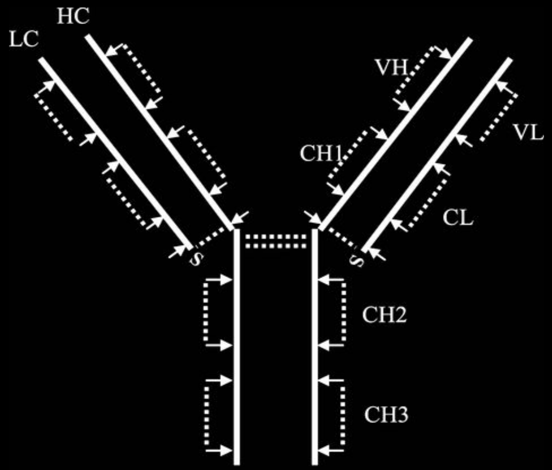
1IGY
Mus musculus
3,2 Å

HYDROPHILIC
energetically advantageous

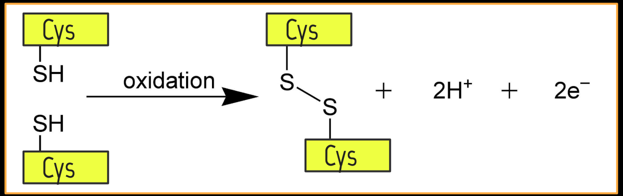
🟩 🟩 ⬜ 🟥 🟥

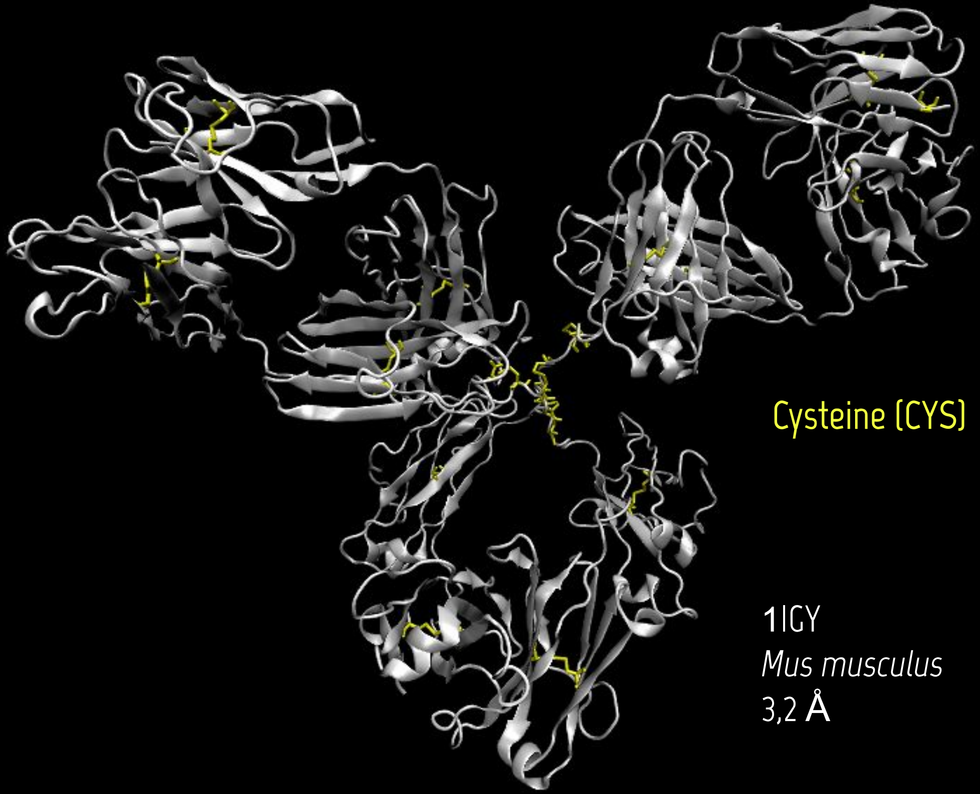
HYDROPHOBIC
repeal water

DISULPHIDE BRIDGES



IgG1

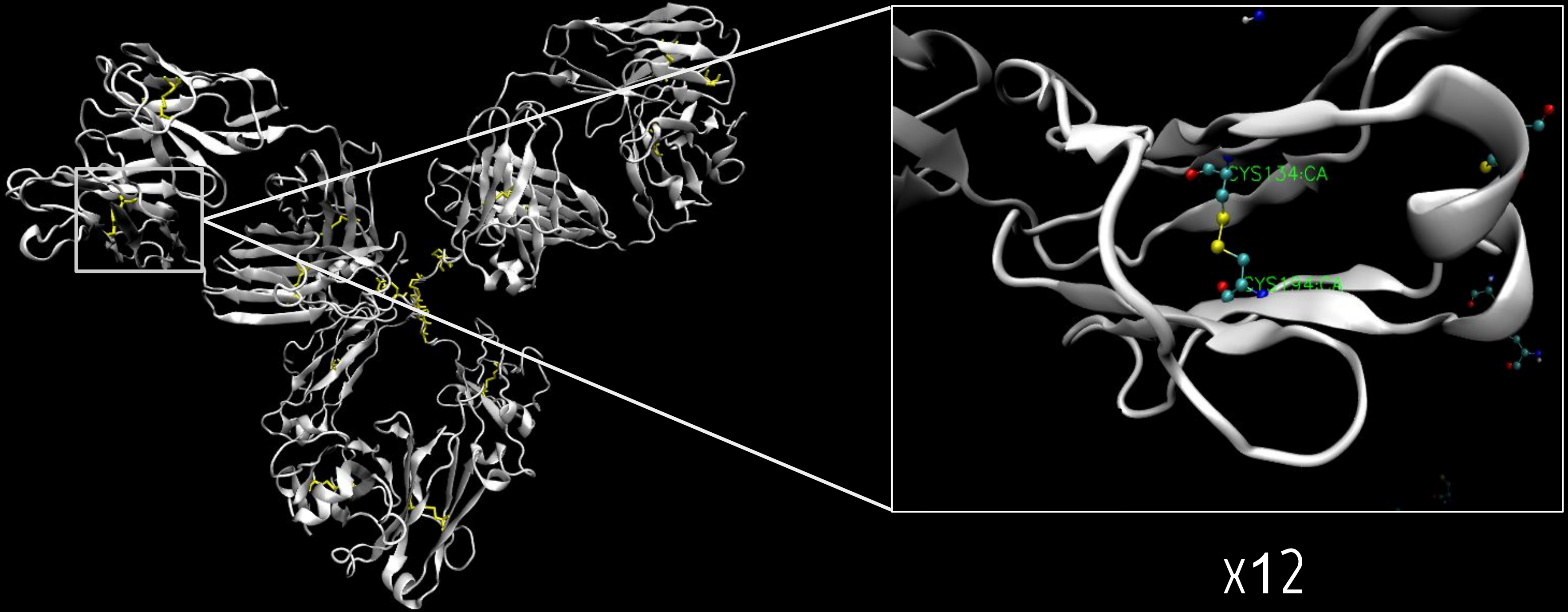




Cysteine (CYS)

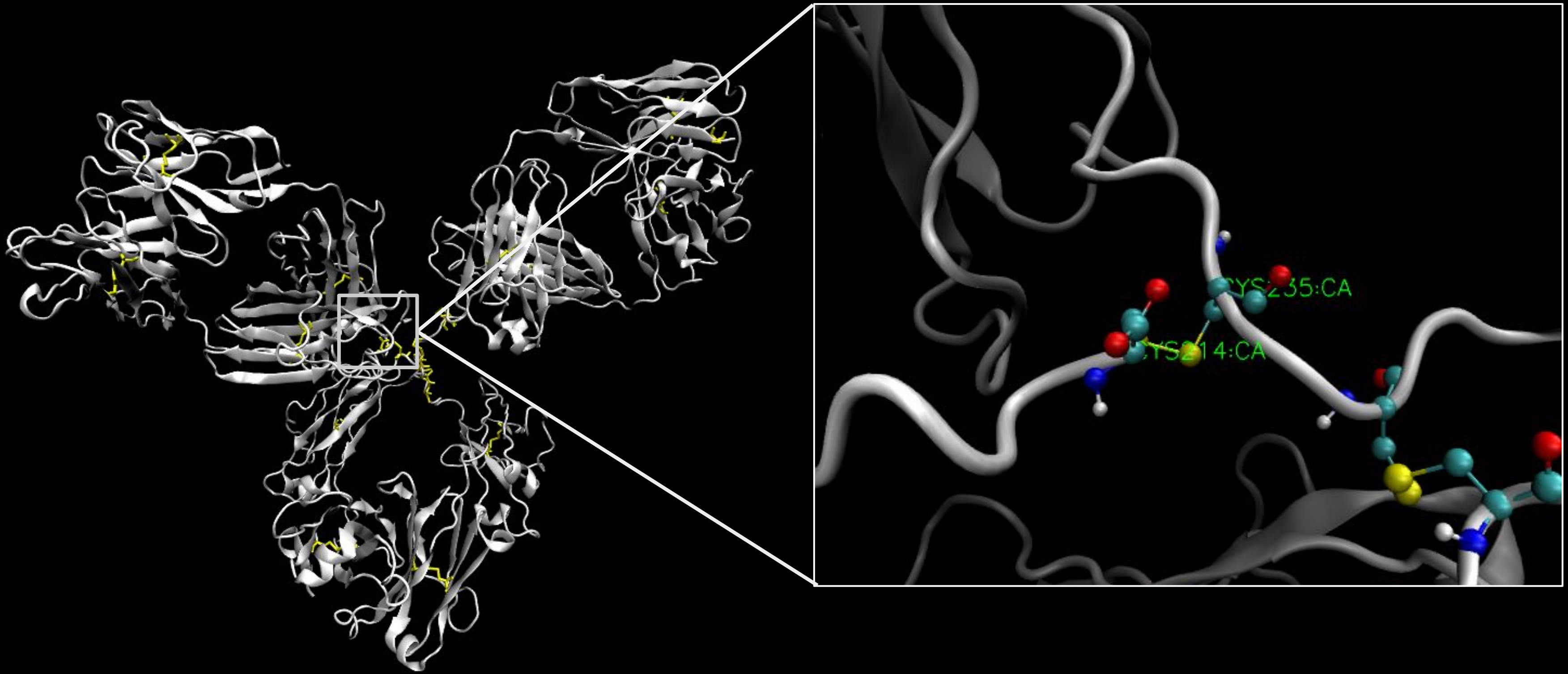
1IGY
Mus musculus
3,2 Å

DISULPHIDE BRIDGES ⟶ INTRA-CHAIN



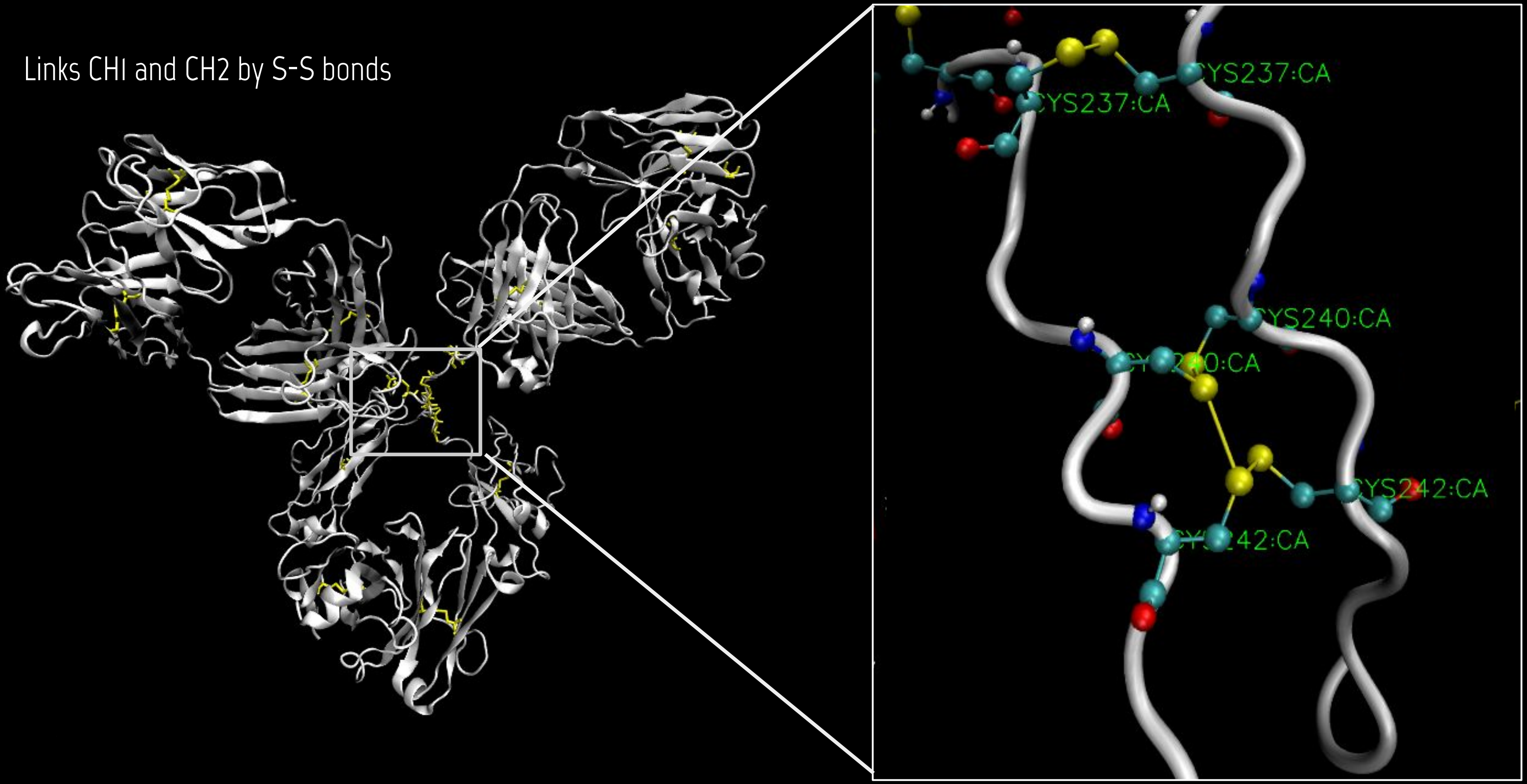
x12

DISULPHIDE BRIDGES ⟶ HEAVY AND LIGHT CHAIN

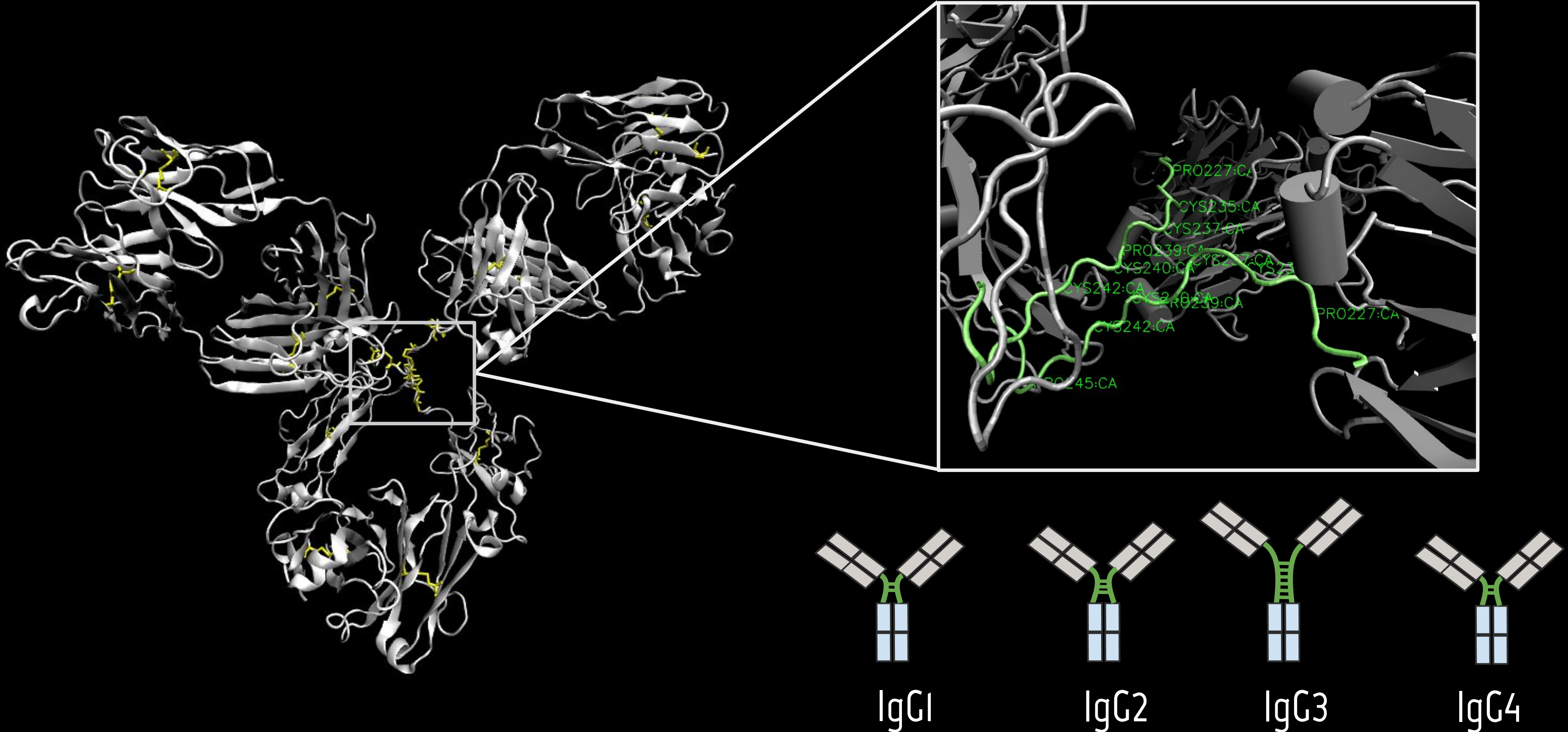


DISULPHIDE BRIDGES → HINGE

Links CHI and CH2 by S-S bonds

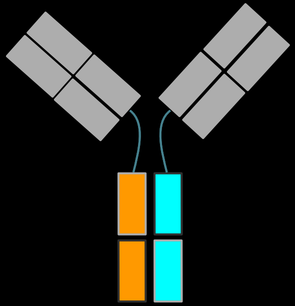


DISULPHIDE BRIDGES → HINGE



CONSTANT REGION

GLYCOSYLATION

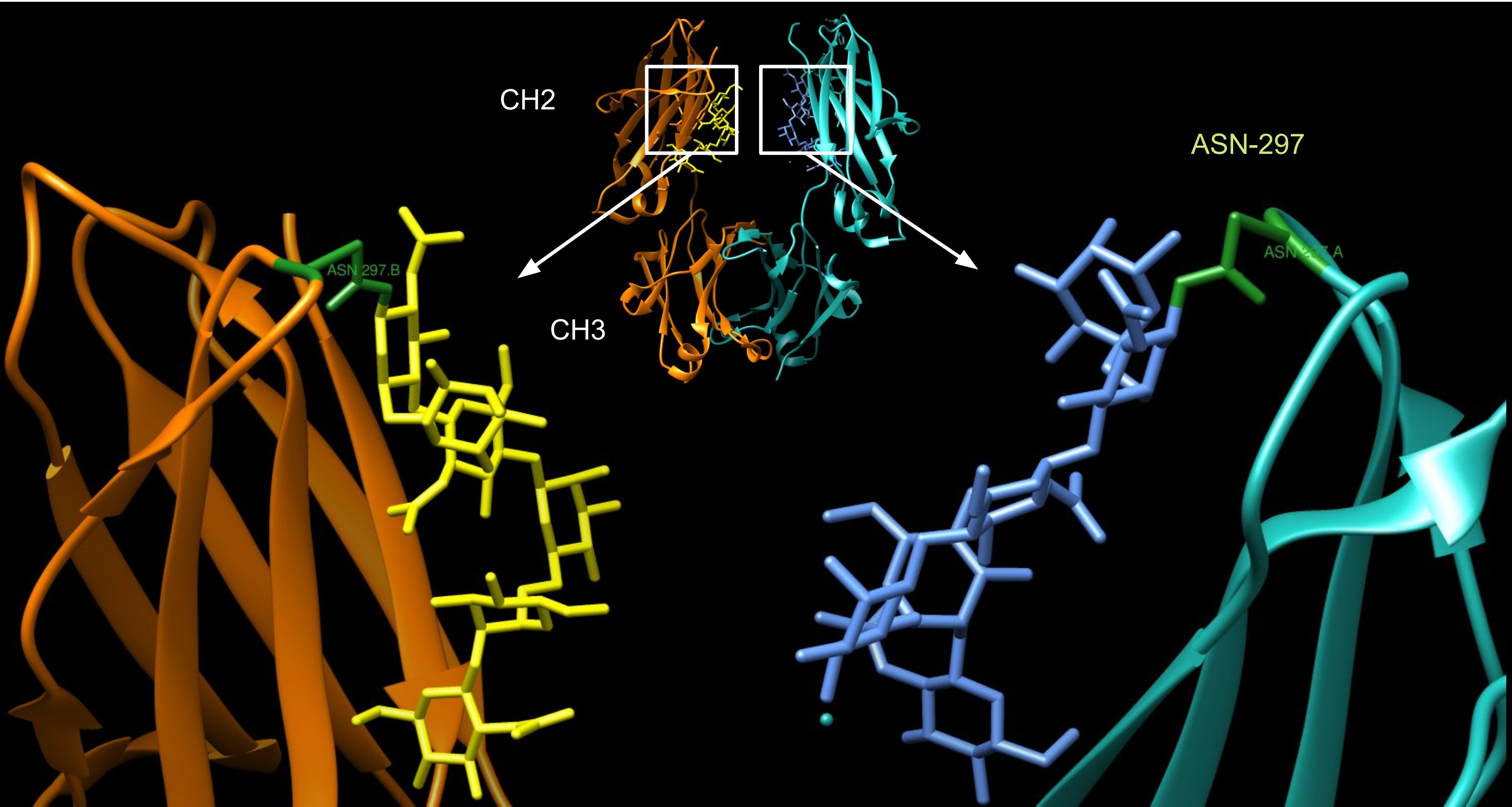


● ● Glycosilations

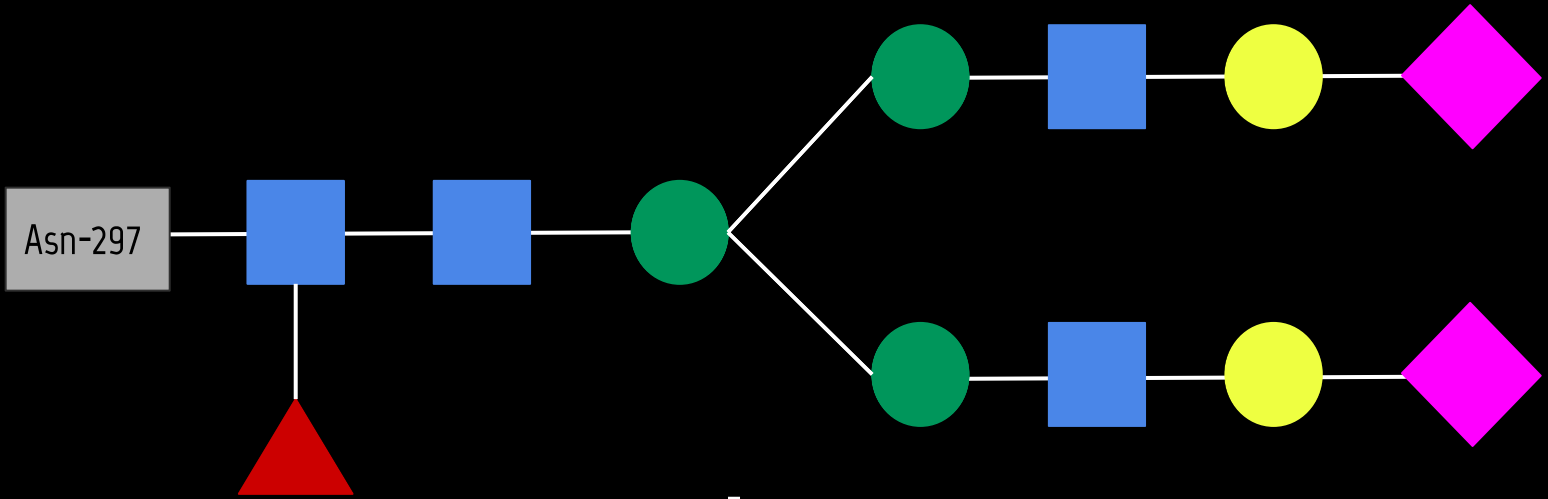
3AVE
Homo sapiens
2,00 Å

GLYCOSYLATION FUNCTIONS
- FcgR recognition
- Antibody dependent cellular cytotoxicity (ADCC)
- Complement dependent cytotoxicity

GLYCOSYLATION

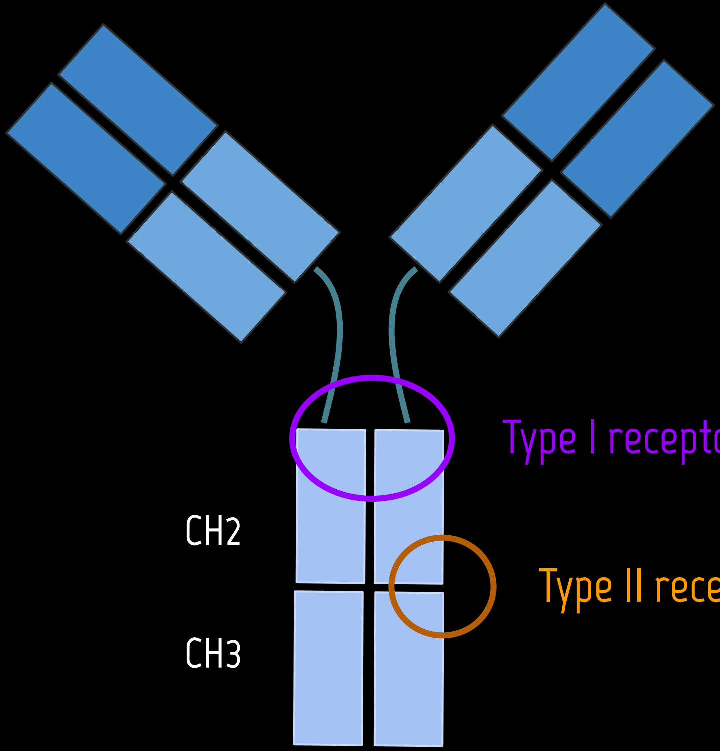


GLYCOSYLATION



- ▲ Fucose
- 🟦 GlcNAc
- 🟢 Mannosa
- 🟡 Galactose
- ◆ Sialic acid

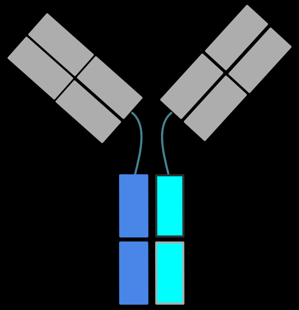
IgG RECEPTORS

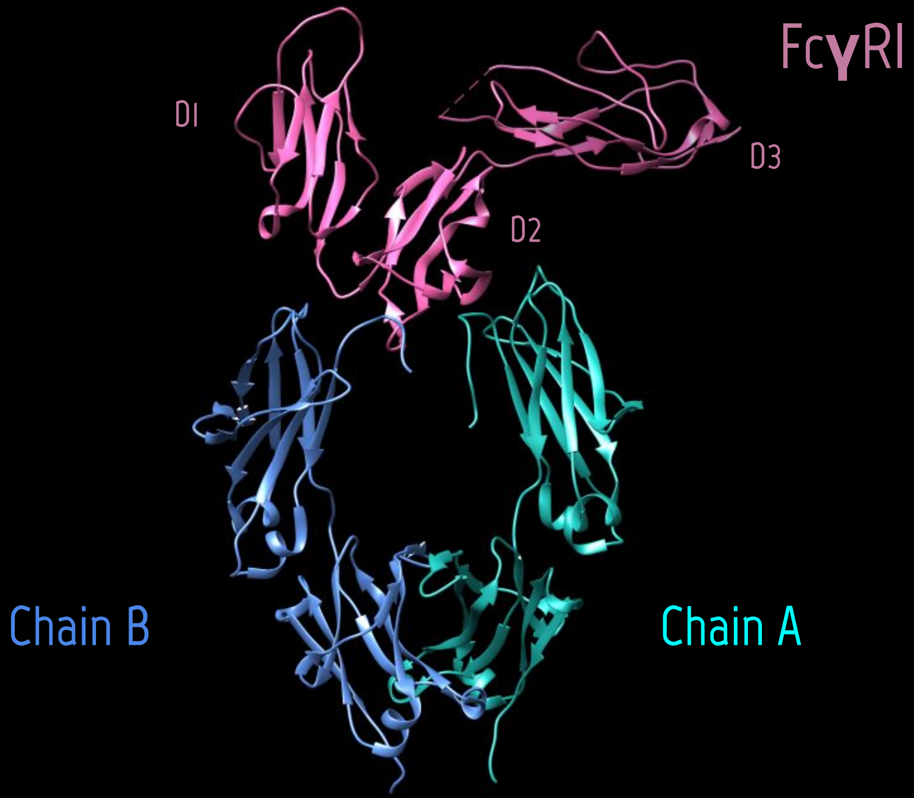


Type I receptors {
FcγR
Cql

Type II receptors {
FcRn
Protein A

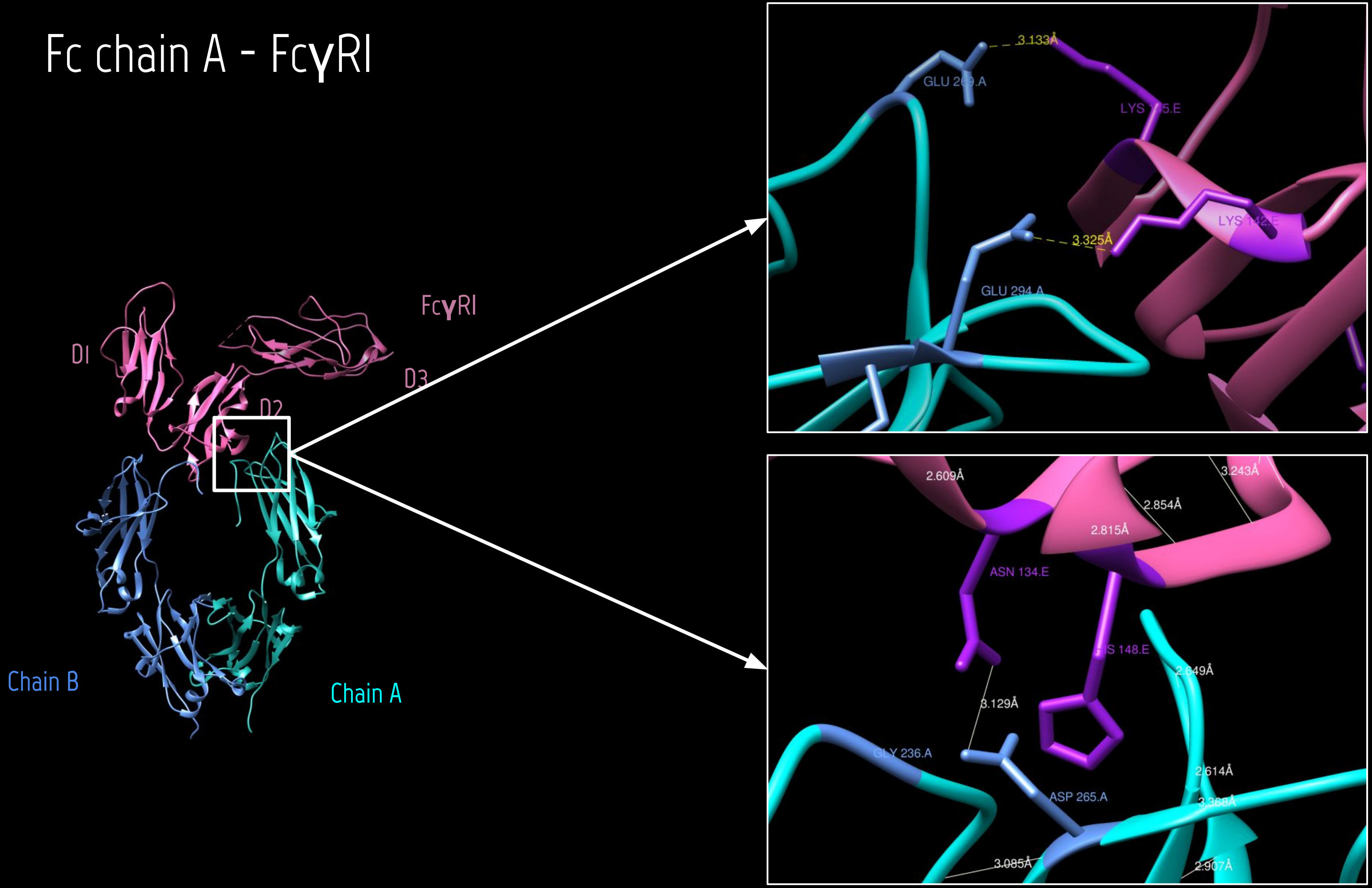
FcγR I



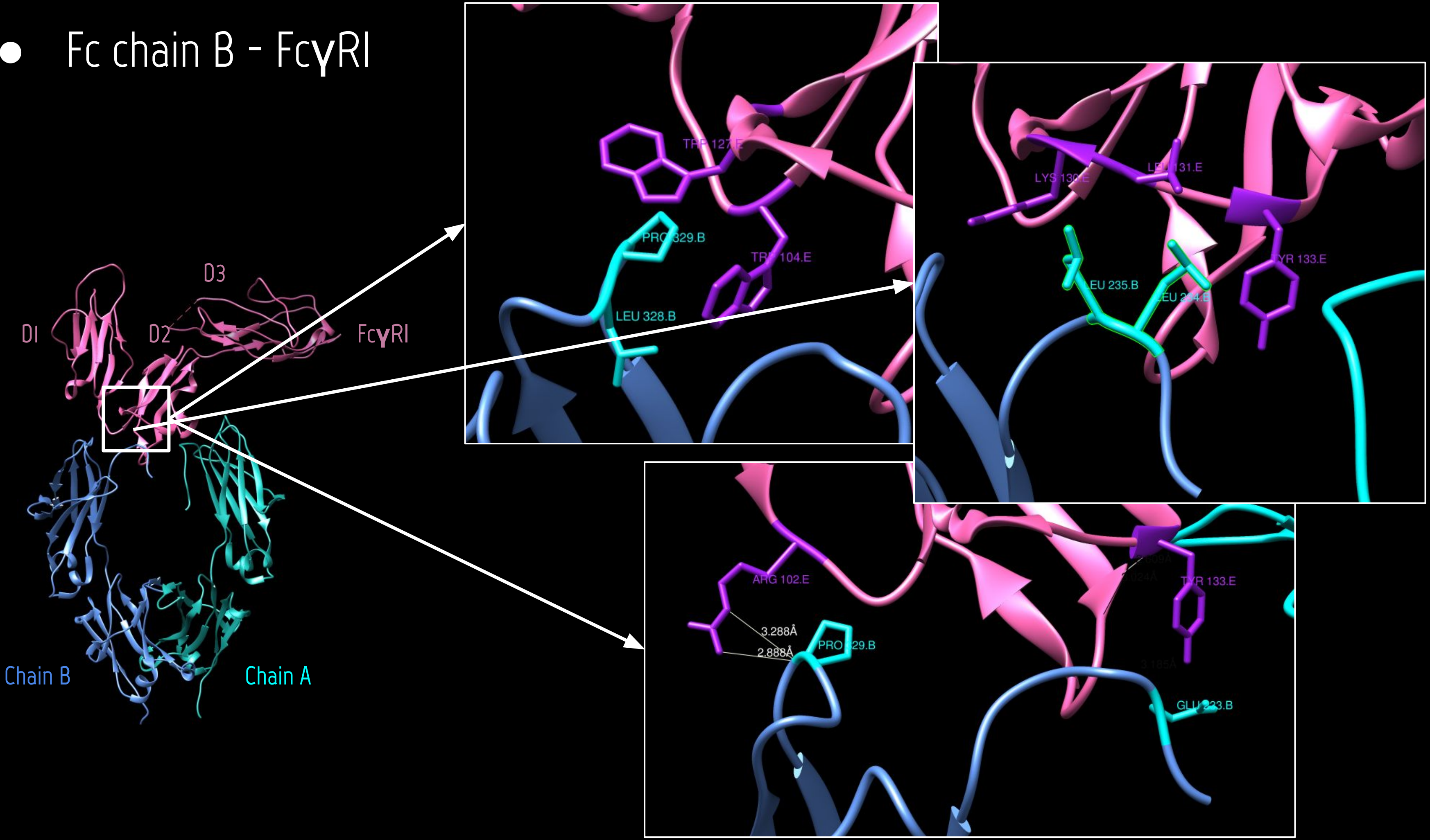


4X4M
Homo sapiens
3,48 Å

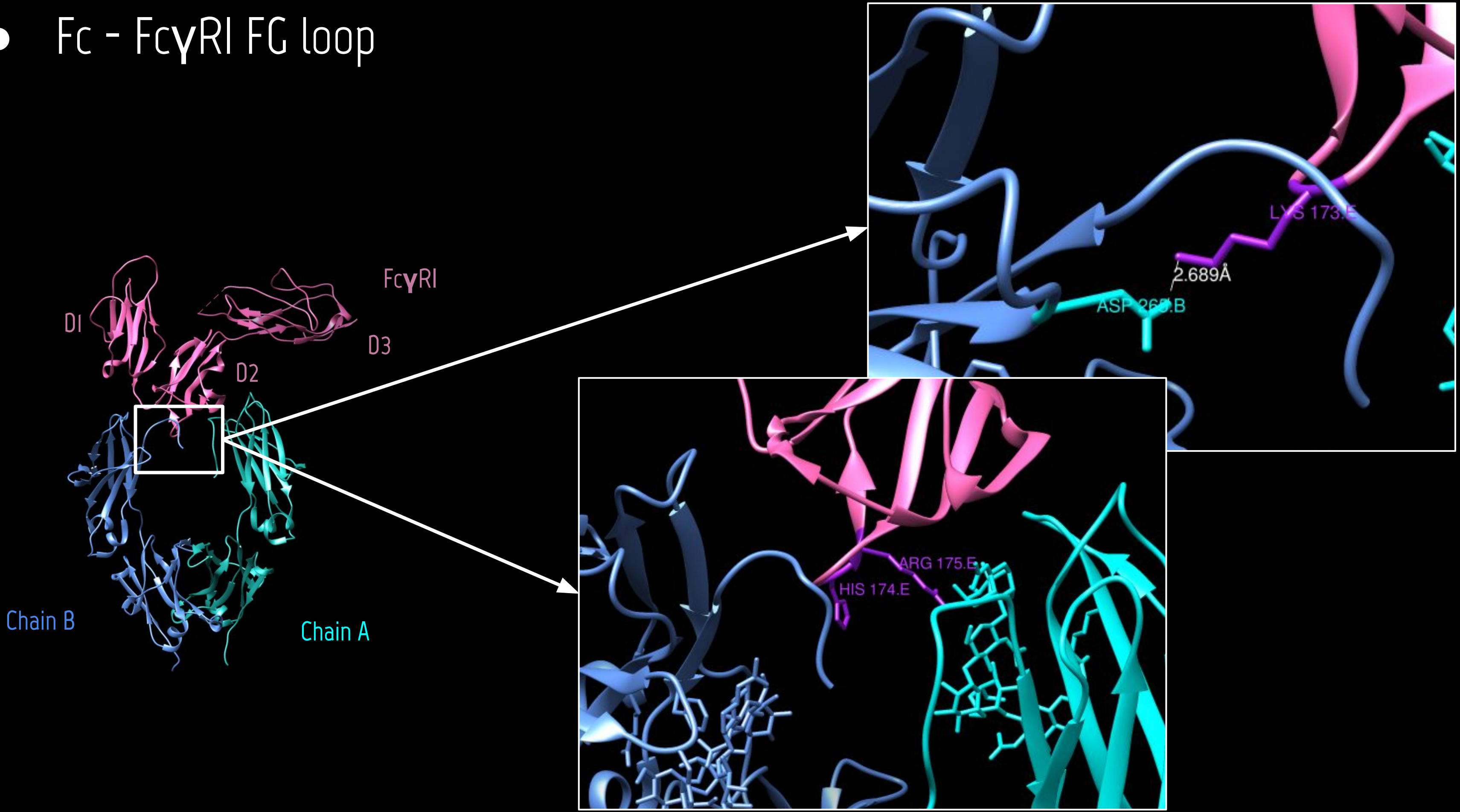
- Fc chain A - FcγRI



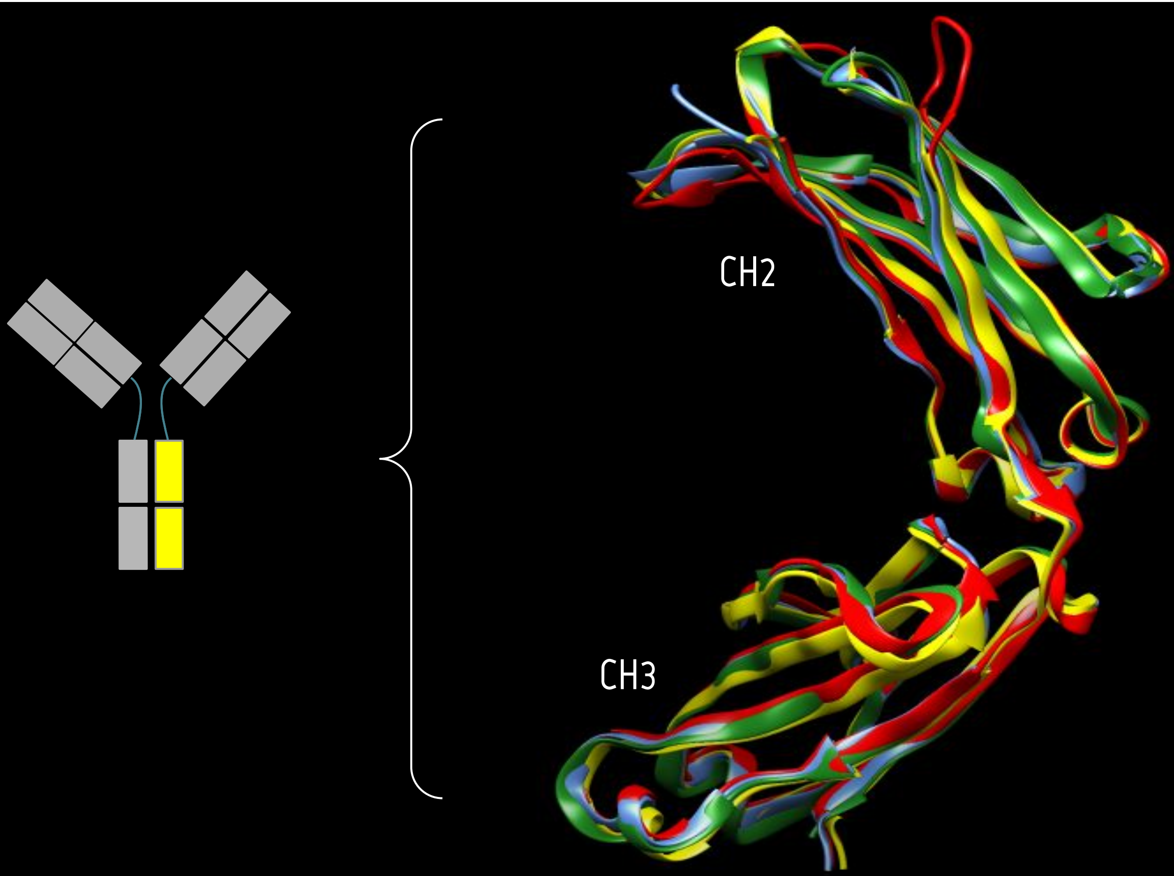
Fc chain B - FcγRI



- Fc - FcγRI FG loop



IgG SUBTYPES



3AVE - IgG1 2Å

4HAF - IgG2 2,04Å

6D58 - IgG3 2,39Å

4C55 - IgG4 2,35Å

IgG SUBTYPES

0,86 { 0,78 { 0,57 {

IgG4

IgG2

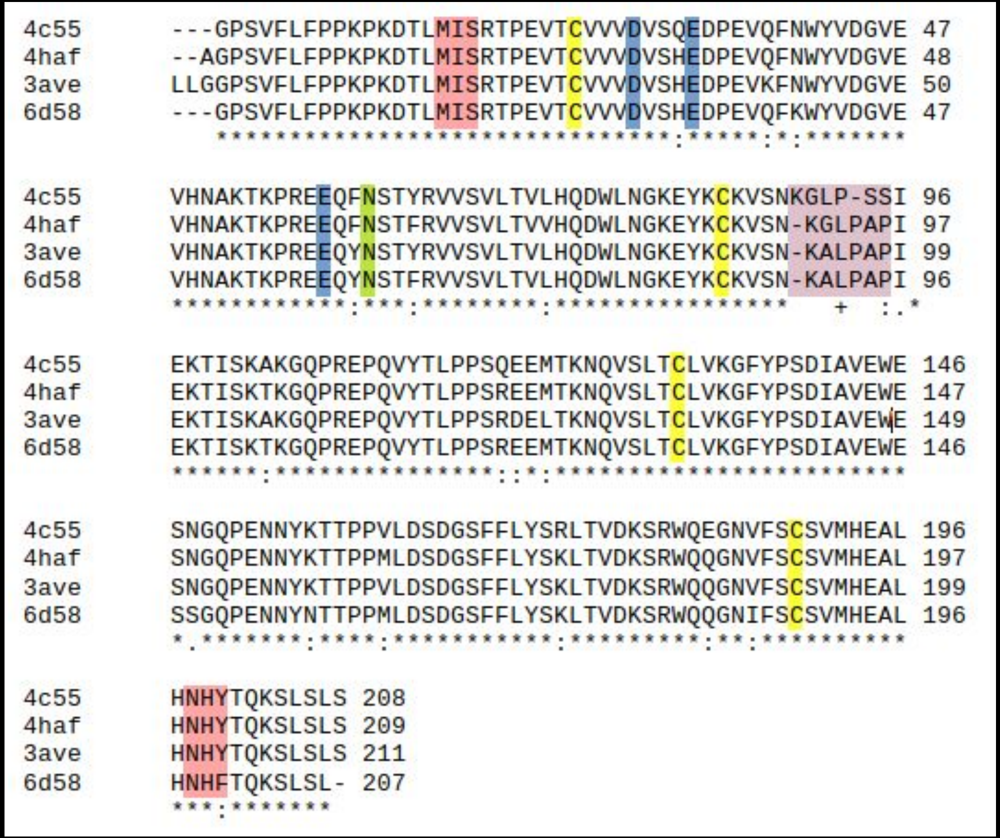
IgG1

IgG3

RMSD

Aspartate - 265

Glutamate - 269 and 294



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4haf        --AGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVE 48
3ave        LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE 50
6d58        ---GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVE 47
               *******************************:*****:*:*******

4c55        VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP-SSI 96
4haf        VHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSN-KGLPAPI 97
3ave        VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN-KALPAPI 99
6d58        VHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSN-KALPAPI 96
            ************:***:********:****************   +  :.*

4c55        EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE 146
4haf        EKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE 147
3ave        EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE 149
6d58        EKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE 146
            ******:***************::*:************************

4c55        SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL 196
4haf        SNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL 197
3ave        SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL 199
6d58        SSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEAL 196
            *.*******:****:***********:*********:**:**********

4c55        HNHYTQKSLSLS 208
4haf        HNHYTQKSLSLS 209
3ave        HNHYTQKSLSLS 211
6d58        HNHFTQKSLSL- 207
            ***:*******
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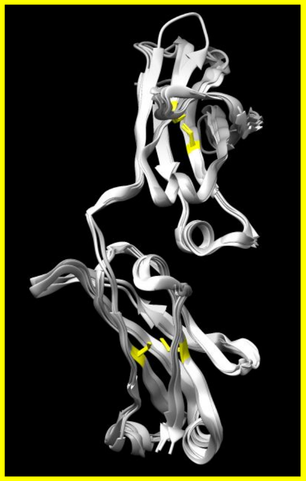
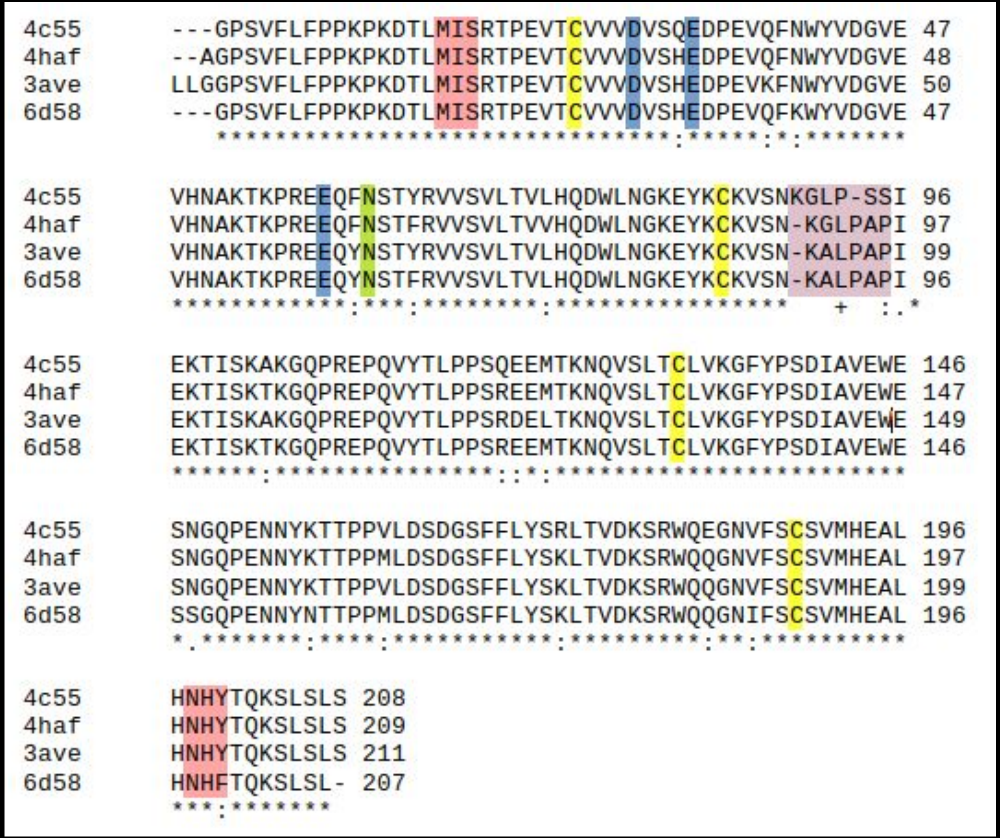


IgG SUBTYPES

IgG4
IgG2
IgG1
IgG3

0,86 { 0,78 { 0,57 {

RMSD



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4c55      ---GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE 47
4haf      --AGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVE 48
3ave      LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE 50
6d58      ---GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVE 47
             *******************************:*****:*:*******

4c55      VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP-SSI 96
4haf      VHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSN-KGLPAPI 97
3ave      VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN-KALPAPI 99
6d58      VHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSN-KALPAPI 96
          ************:***:********:****************    +  :.*

4c55      EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE 146
4haf      EKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE 147
3ave      EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE 149
6d58      EKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE 146
          ******:***************::*:************************

4c55      SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL 196
4haf      SNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL 197
3ave      SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL 199
6d58      SSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEAL 196
          *.*******:****:***********:*********:**:**********

4c55      HNHYTQKSLSLS 208
4haf      HNHYTQKSLSLS 209
3ave      HNHYTQKSLSLS 211
6d58      HNHFTQKSLSL- 207
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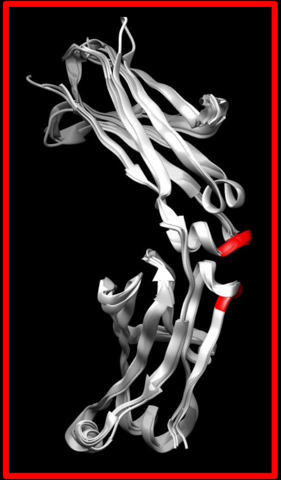
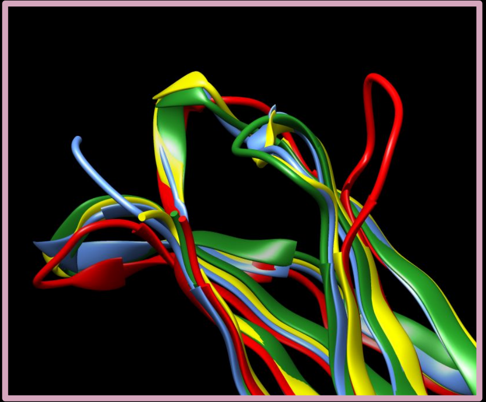
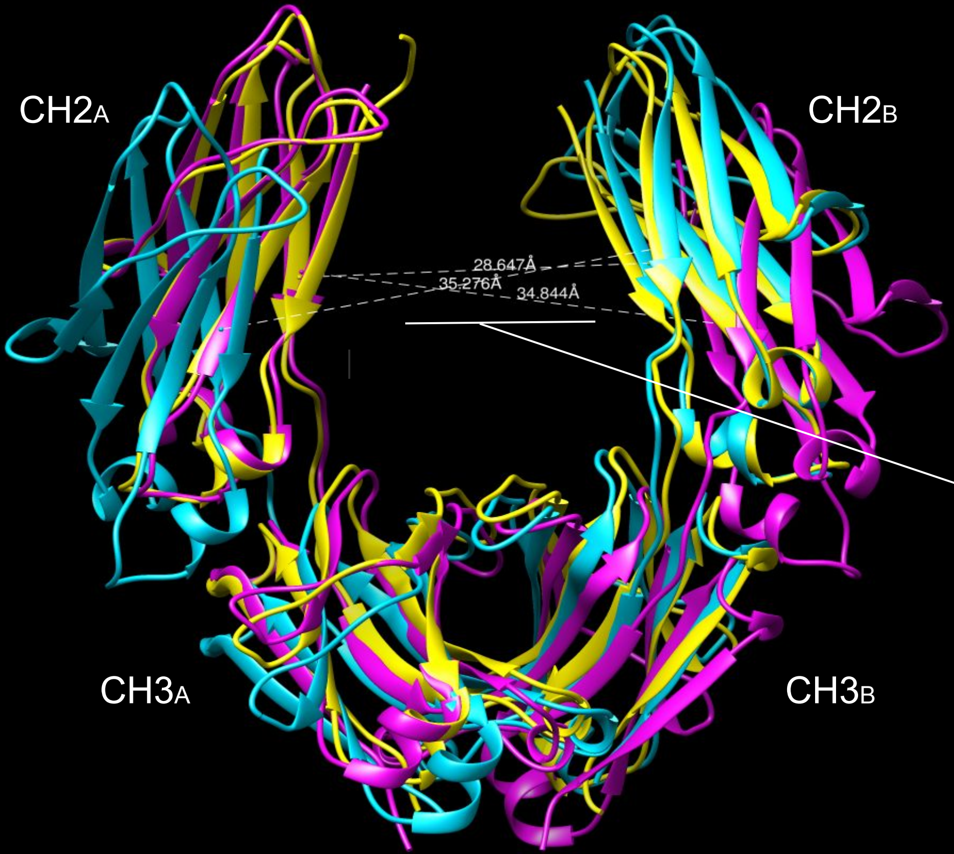




LALA MUTANTS



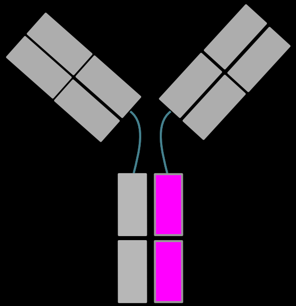
distance between
LEU 242 CB (CH2A) and
LEU 242 CB (CH2B)

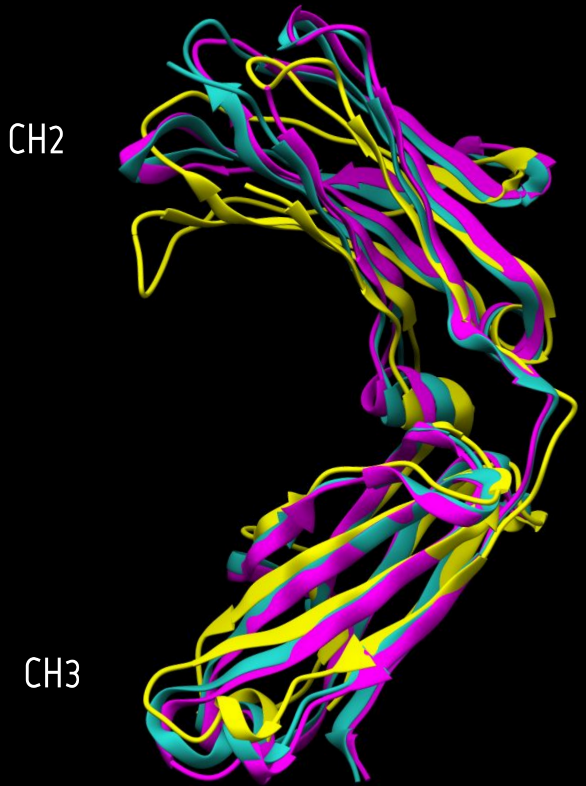
3ave (WT)⟶ 28.647 Å
6cjx (LALA)⟶ 34.844 Å
6cjc (LALA)⟶ 35.276 Å

LEU to ALA mutations at
positions 234 & 235 (HINGE)

6cjx & 6cjc ⟶ RMS 0.44
3ave & 6cjx 6cjc ⟶RMS 1.45

IgG1 AMONG SPECIES



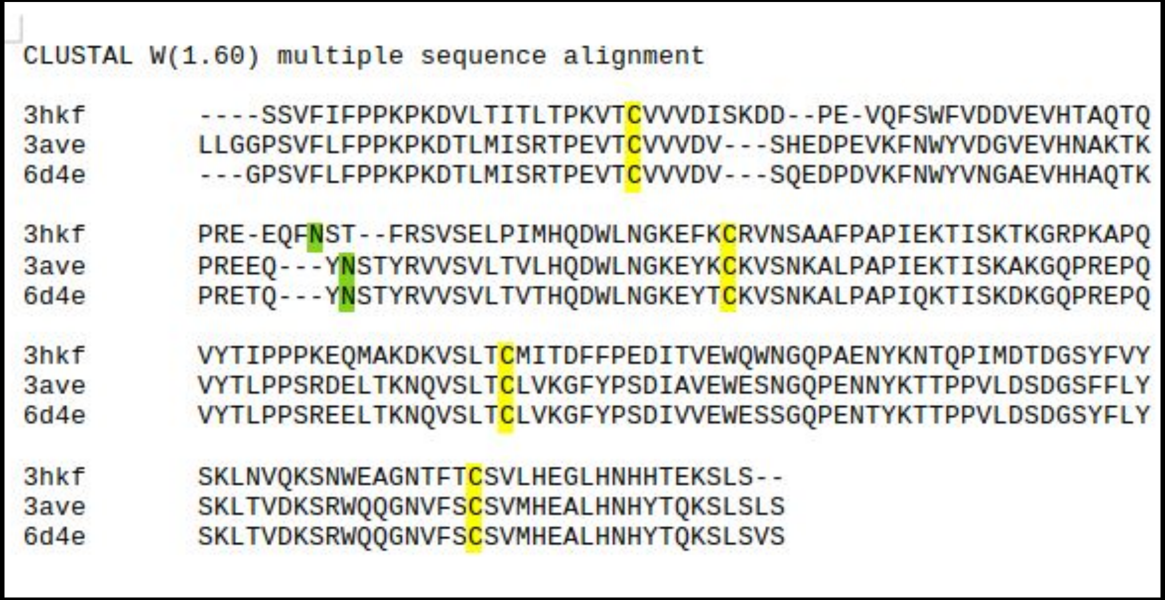


3HKF (*Mus musculus*) 2.50 Å
3AVE (*Homo sapiens*) 2.00 Å
6D4E (*Macaca mulatta*) 2.80 Å

IgG1 AMONG SPECIES

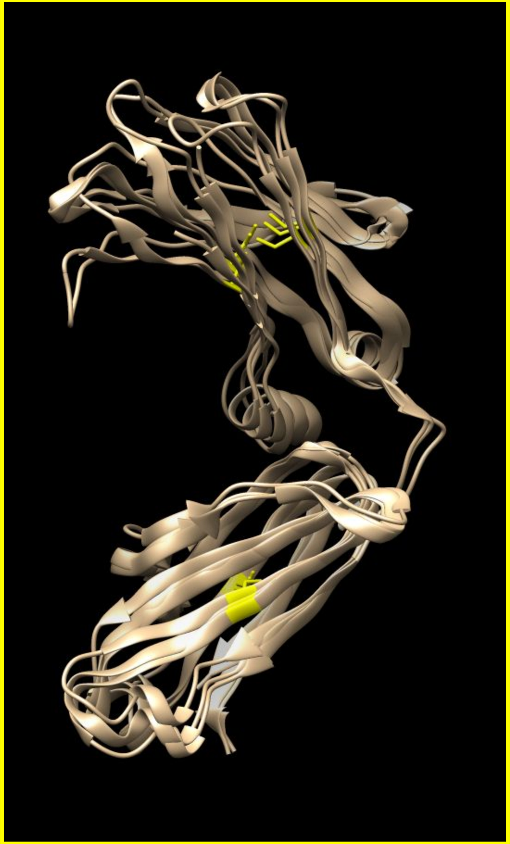
2,23
0,99
Mus musculus
Homo sapiens
Macaca mulatta

RMSD

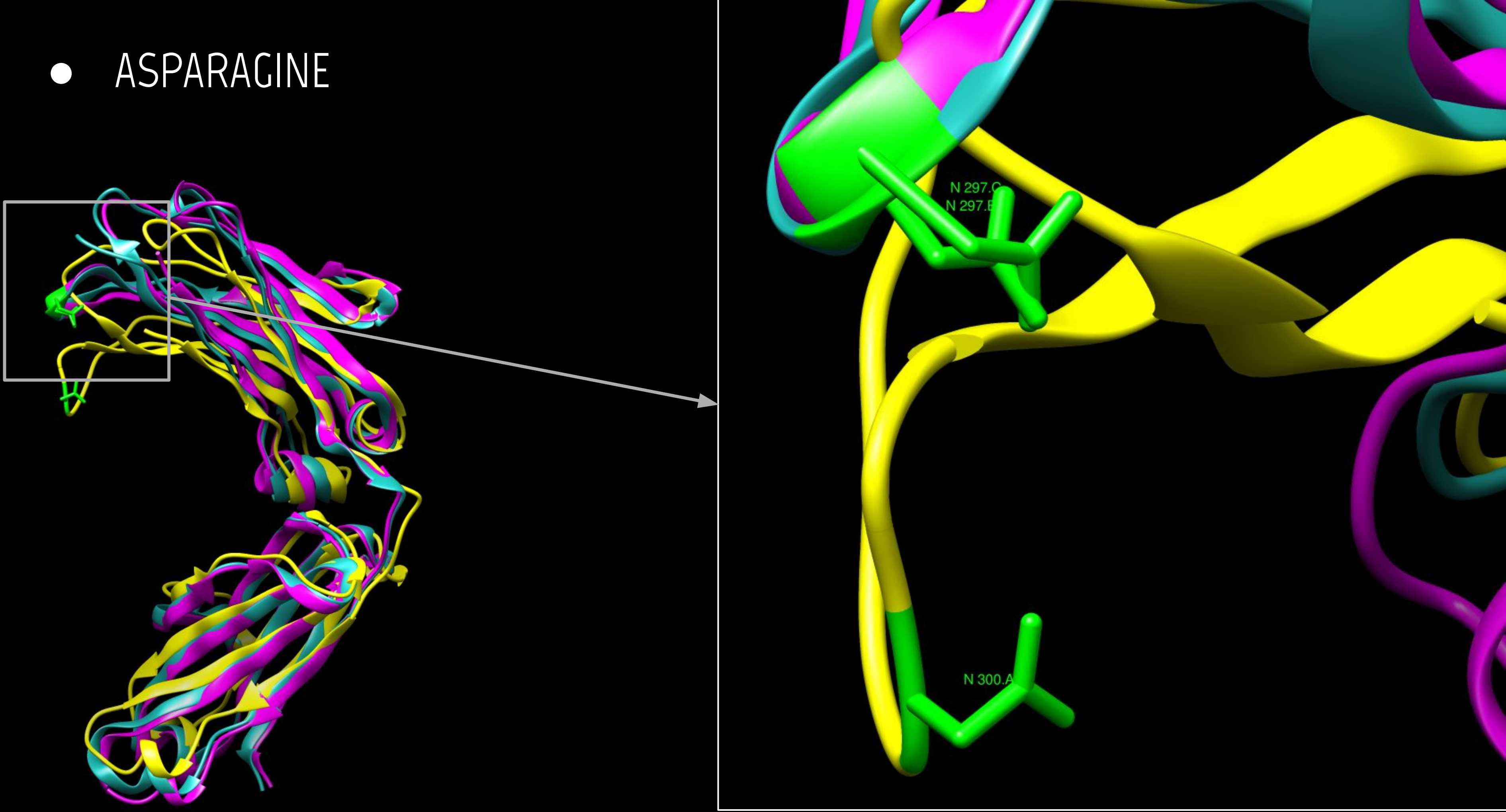


```
CLUSTAL W(1.60) multiple sequence alignment

3hkf        ----SSVFIFPPKPKDVLTITLTPKVTCVVVDISKDD--PE-VQFSWFVDDVEVHTAQTQ
3ave        LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV---SHEDPEVKFNWYVDGVEVHNAKTK
6d4e        ---GPSVFLFPPKPKDTLMISRTPEVTCVVVDV---SQEDPDVKFNWYVNGAEVHHAQTK

3hkf        PRE-EQFNST--FRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQ
3ave        PREEQ---YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
6d4e        PRETQ---YNSTYRVVSVLTVTHQDWLNGKEYTCKVSNKALPAPIQKTISKDKGQPREPQ

3hkf        VYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVY
3ave        VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
6d4e        VYTLPPSREELTKNQVSLTCLVKGFYPSDIVVEWESSGQPENTYKTTPPVLDSDGSYFLY

3hkf        SKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLS--
3ave        SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
6d4e        SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSVS
```

- DISULPHIDE BRIDGES

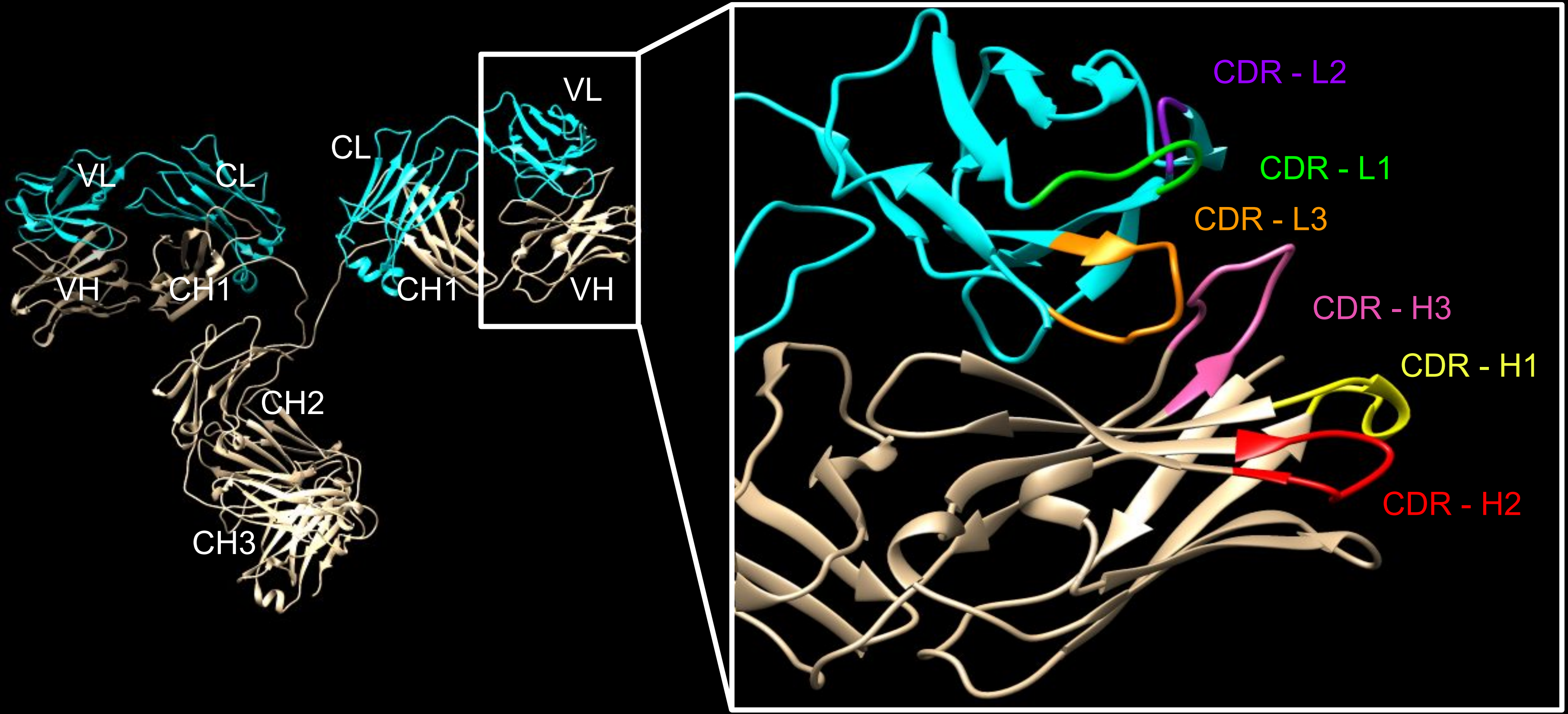


- ASPARAGINE

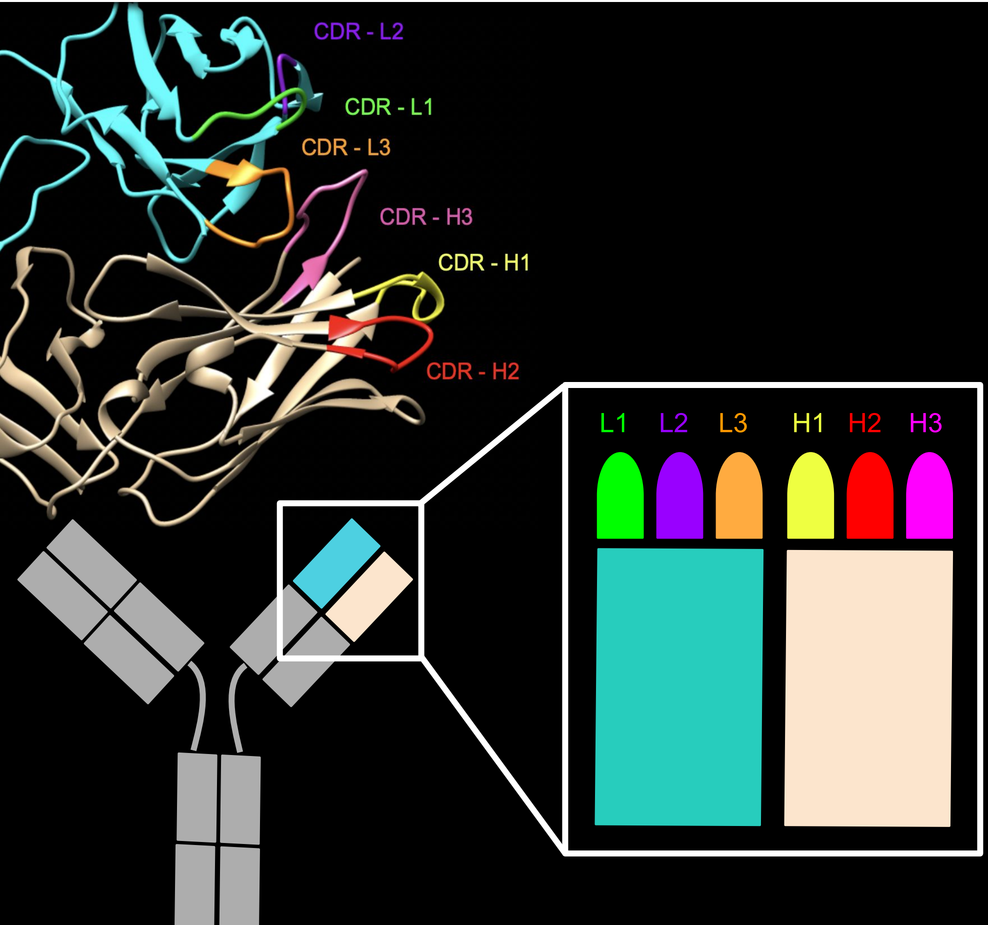


VARIABLE REGION

COMPLEMENTARITY-DETERMINING REGIONS (CDR)



CANONICAL STRUCTURES



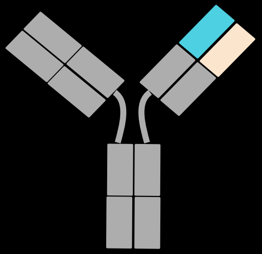
Chothia classification

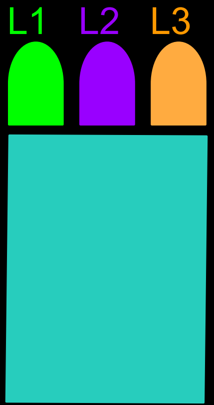
HEAVY CHAIN	canonical structure
H1	1, 2, 3
H2	1, 2a, 2b, 2c, 3a, 3b, 3c, 4
H3	-

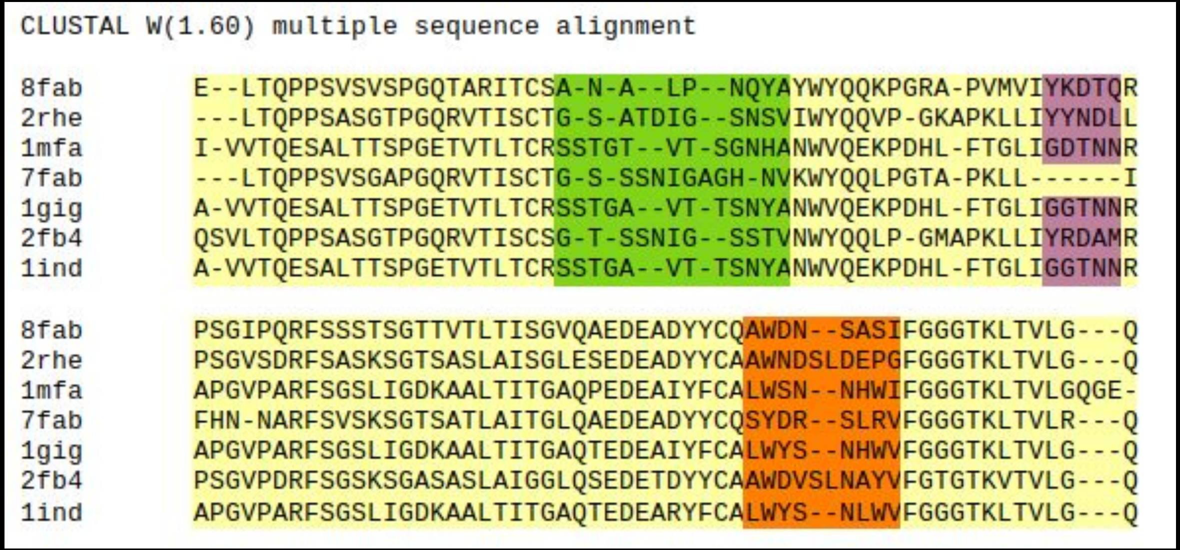
LIGHT CHAIN		canonical structure
L1	LAMBDA (λ)	1λ, 2λ, 3aλ, 3bλ, 4λ
	KAPPA (k)	1k, 2k, 3ak, 3bk, 4k
L2		1
L3	LAMBDA (λ)	1aλ, 1bλ, 1λc, 2λ
	KAPPA (k)	1k, 2k, 3k, 4k, 5k

LIGHT CHAIN (LAMBDA)

LIGHT CHAIN		canonical structure
L1	LAMBDA (λ)	1λ, 2λ, 3aλ, 3bλ, 4λ
	KAPPA (k)	1k, 2k, 3ak, 3bk, 4k
L2		1
L3	LAMBDA (λ)	1aλ, 1bλ, 1λc, 2λ
	KAPPA (k)	1k, 2k, 3k, 4k, 5k







```
CLUSTAL W(1.60) multiple sequence alignment

8fab        E--LTQPPSVSVSPGQTARITCSA-N-A--LP--NQYAYWYQQKPGRA-PVMVIYKDTQR
2rhe        ---LTQPPSASGTPGQRVTISCTG-S-ATDIG--SNSVIWYQQVP-GKAPKLLIYYNDLL
1mfa        I-VVTQESALTTSPGETVTLTCRSSTGT--VT-SGNHANWVQEKPDHL-FTGLIGDTNNR
7fab        ---LTQPPSVSGAPGQRVTISCTG-S-SSNIGAGH-NVKWYQQLPGTA-PKLL------I
1gig        A-VVTQESALTTSPGETVTLTCRSSTGA--VT-TSNYANWVQEKPDHL-FTGLIGGTNNR
2fb4        QSVLTQPPSASGTPGQRVTISCSG-T-SSNIG--SSTVNWYQQLP-GMAPKLLIYRDAMR
1ind        A-VVTQESALTTSPGETVTLTCRSSTGA--VT-TSNYANWVQEKPDHL-FTGLIGGTNNR

8fab        PSGIPQRFSSSTSGTTVTLTISGVQAEDEADYYCQAWDN--SASIFGGGTKLTVLG---Q
2rhe        PSGVSDRFSASKSGTSASLAISGLESEDEADYYCAAWNDSLDEPGFGGGTKLTVLG---Q
1mfa        APGVPARFSGSLIGDKAALTITGAQPEDEAIYFCALWSN--NHWIFGGGTKLTVLGQGE-
7fab        FHN-NARFSVSKSGTSATLAITGLQAEDEADYYCQSYDR--SLRVFGGGTKLTVLR---Q
1gig        APGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYS--NHWVFGGGTKLTVLG---Q
2fb4        PSGVPDRFSGSKSGASASLAIGGLQSEDETDYYCAAWDVSLNAYVFGTGTKVTVLG---Q
1ind        APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYS--NLWVFGGGTKLTVLG---Q
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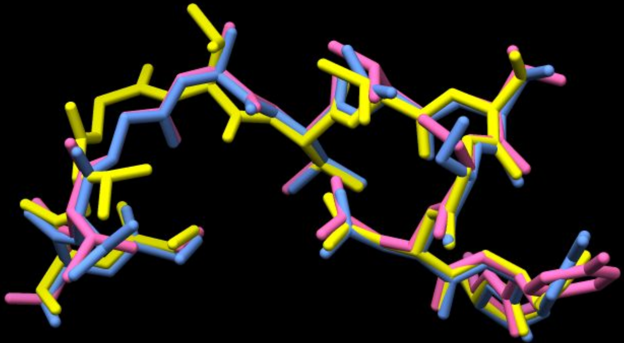
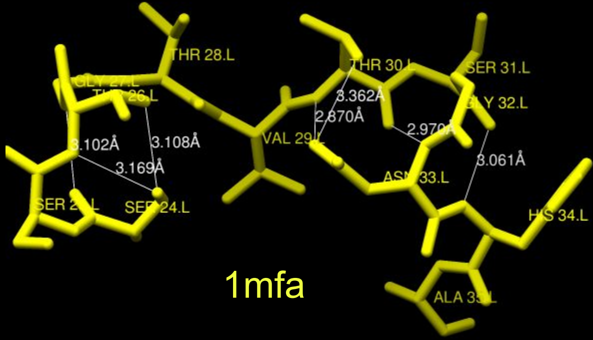
L1 - λ3

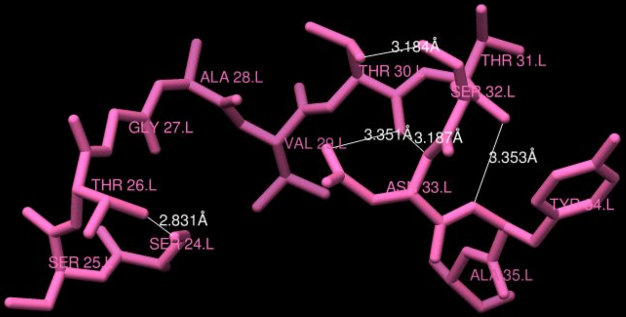
1ind TCRSSTGAVTTSNYAN
1mfa TCRSSTGTVTSGNHAN
1gig TCRSSTGAVTTSNYAN



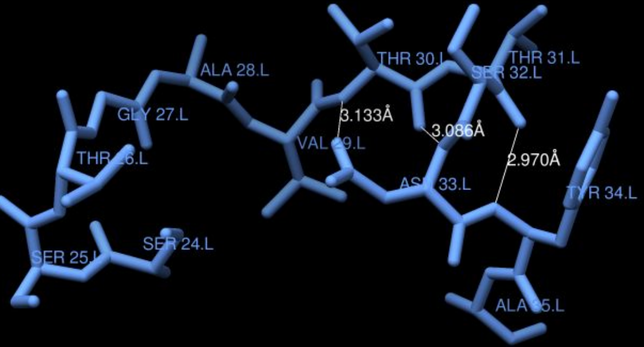


1mfa

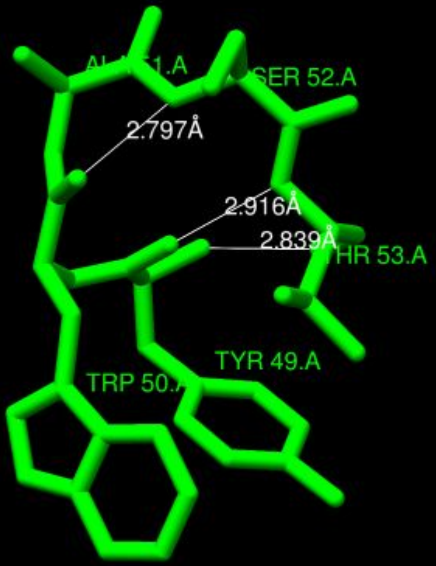
1ind



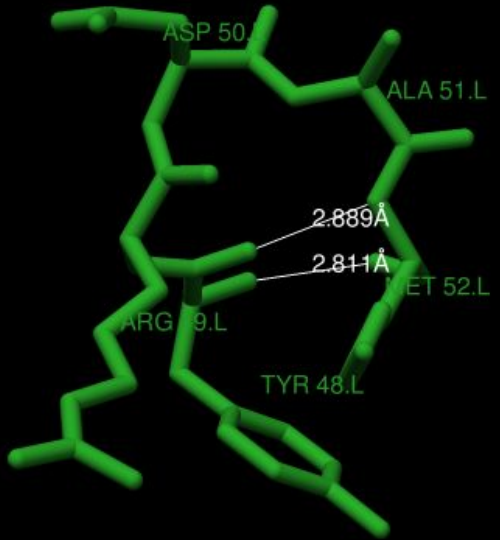
1gig



L2



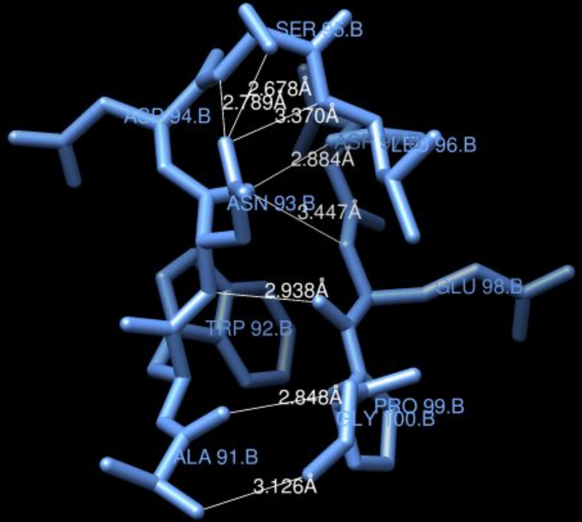
1hil
kappa



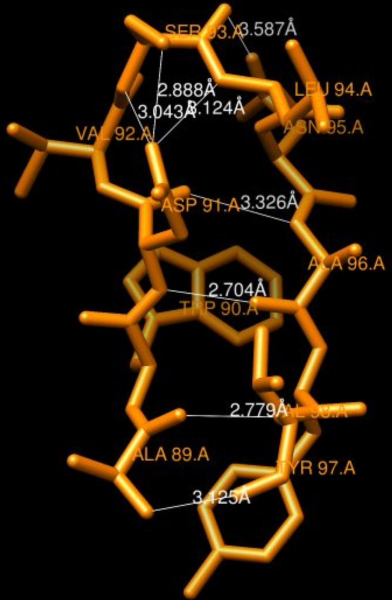
2fb4
lambda

L3 - λ2

2fb4 AAWDVSLNAYVF
2rhe AAWNDSLDEPGF



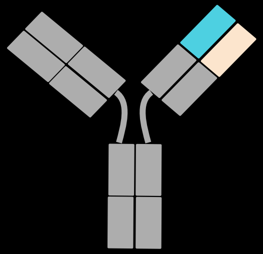
2rhe

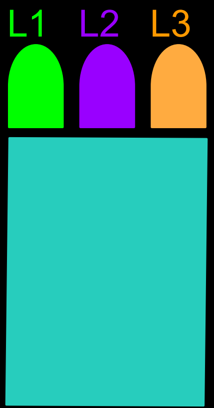


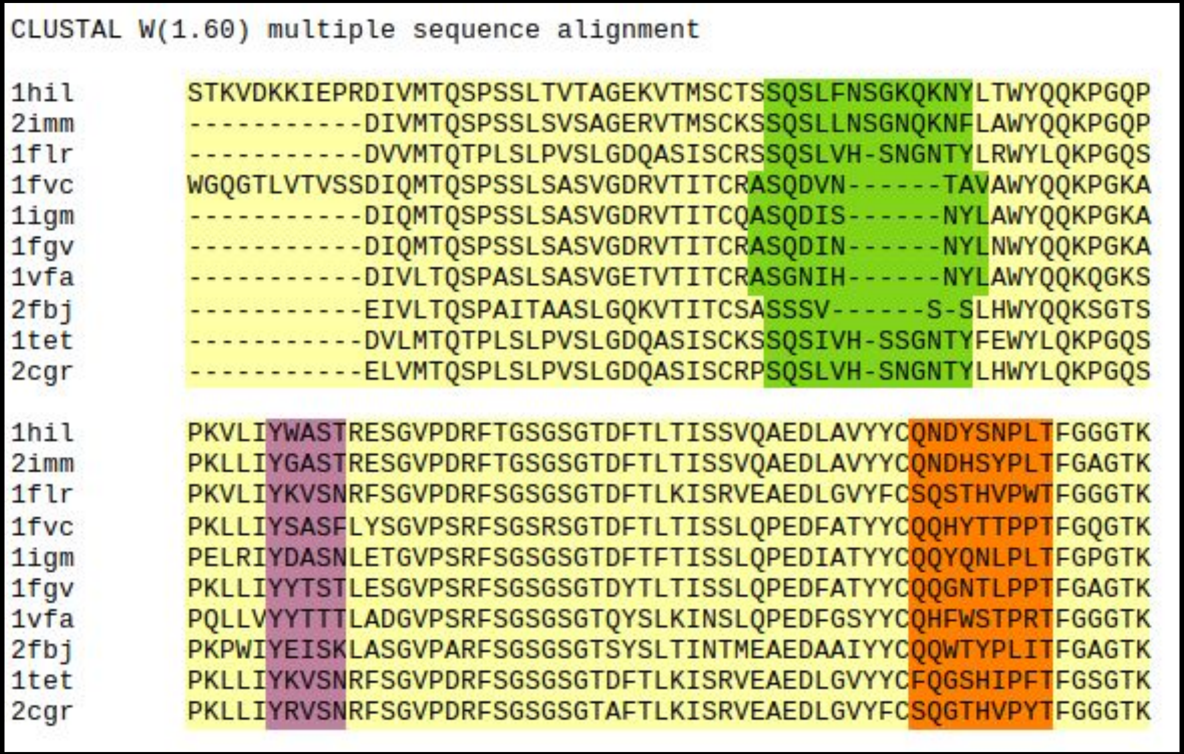
7fab

LIGHT CHAIN (KAPPA)

LIGHT CHAIN		canonical structure
LI	LAMBDA (λ)	Iλ, 2λ, 3aλ, 3bλ, 4λ
	KAPPA (k)	Ik, 2k, 3ak, 3bk, 4k
L2		I
L3	LAMBDA (λ)	Iaλ, Ibλ, Iλc, 2λ
	KAPPA (k)	Ik, 2k, 3k, 4k, 5k







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CLUSTAL W(1.60) multiple sequence alignment

1hil          STKVDKKIEPRDIVMTQSPSSLTVTAGEKVTMSCTSSQSLFNSGKQKNYLTWYQQKPGQP
2imm          -----------DIVMTQSPSSLSVSAGERVTMSCKSSQSLLNSGNQKNFLAWYQQKPGQP
1flr          -----------DVVMTQTPLSLPVSLGDQASISCRSSQSLVH-SNGNTYLRWYLQKPGQS
1fvc          WGQGTLVTVSSDIQMTQSPSSLSASVGDRVTITCRASQDVN------TAVAWYQQKPGKA
1igm          -----------DIQMTQSPSSLSASVGDRVTITCQASQDIS------NYLAWYQQKPGKA
1fgv          -----------DIQMTQSPSSLSASVGDRVTITCRASQDIN------NYLNWYQQKPGKA
1vfa          -----------DIVLTQSPASLSASVGETVTITCRASGNIH------NYLAWYQQKQGKS
2fbj          -----------EIVLTQSPAITAASLGQKVTITCSASSSV------S-SLHWYQQKSGTS
1tet          -----------DVLMTQTPLSLPVSLGDQASISCKSSQSIVH-SSGNTYFEWYLQKPGQS
2cgr          -----------ELVMTQSPLSLPVSLGDQASISCRPSQSLVH-SNGNTYLHWYLQKPGQS

1hil          PKVLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSNPLTFGGGTK
2imm          PKLLIYGASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGAGTK
1flr          PKVLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTK
1fvc          PKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTK
1igm          PELRIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYQNLPLTFGPGTK
1fgv          PKLLIYYTSTLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGAGTK
1vfa          PQLLVYYTTTLADGVPSRFSGSGSGTQYSLKINSLQPEDFGSYYCQHFWSTPRTFGGGTK
2fbj          PKPWIYEISKLASGVPARFSGSGSGTSYSLTINTMEAEDAAIYYCQQWTYPLITFGAGTK
1tet          PKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPFTFGSGTK
2cgr          PKLLIYRVSNRFSGVPDRFSGSGSGTAFTLKISRVEAEDLGVYFCSQGTHVPYTFGGGTK
```
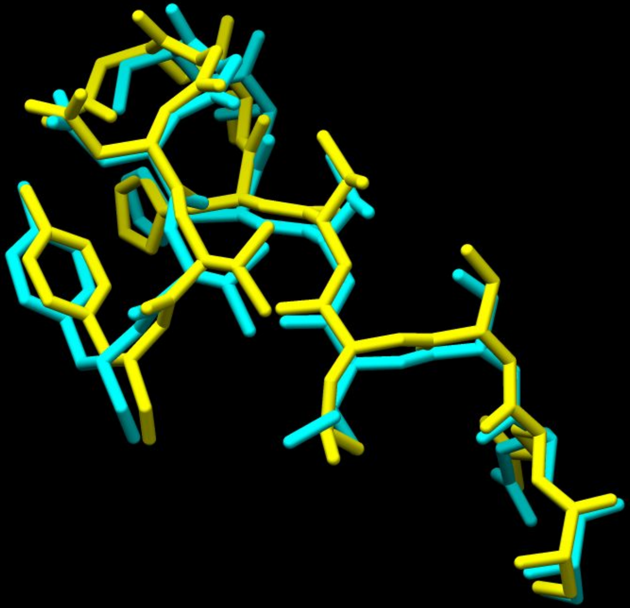
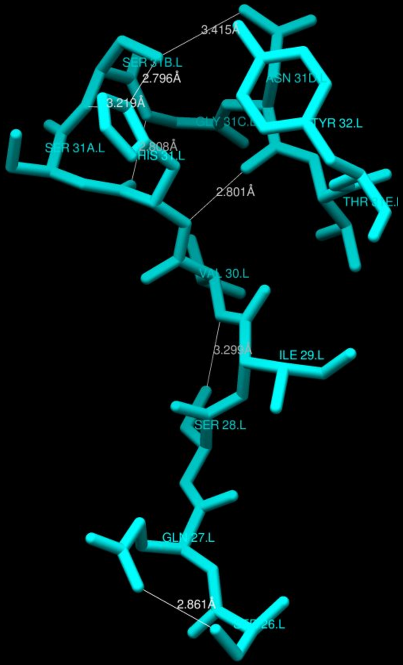

L1 - κ4

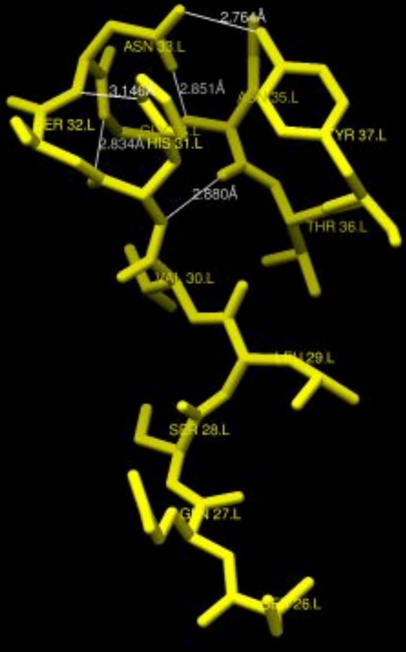
1tet	KSSQSIVHSSGNTYF
2cgr	RPSQSLVHSNGNTYL



1tet
2cgr



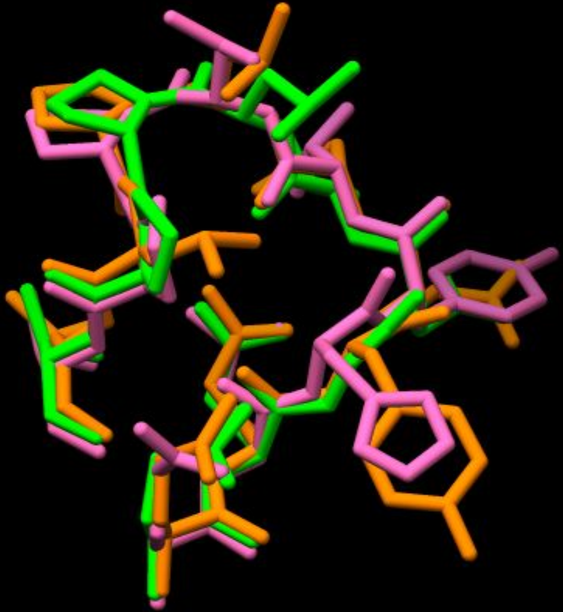
1tet



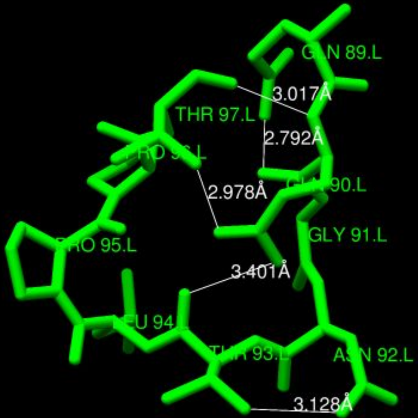
2cgr

L3 - κ1

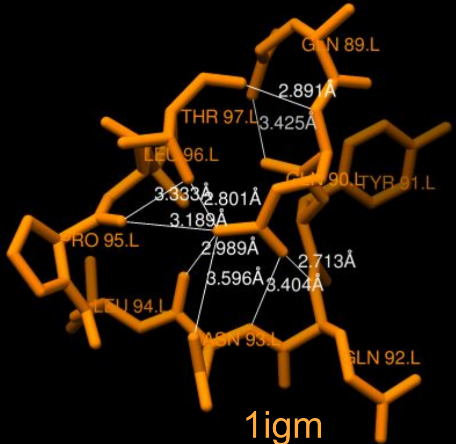
1fgv
1igm
1fvc



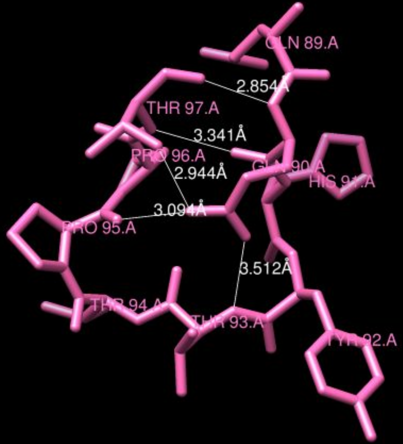
1fvc	CQQHYTTPPTF
1igm	CQQYQNLPLTF
1fgv	CQQGNTLPPTF



1fgv



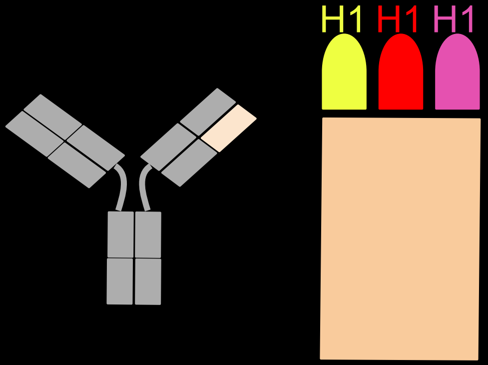
1igm



1fvc

HEAVY CHAIN

HEAVY CHAIN	canonical structure
H1	1, 2, 3
H2	1, 2a, 2b, 2c, 3a, 3b, 3c, 4
H3	-



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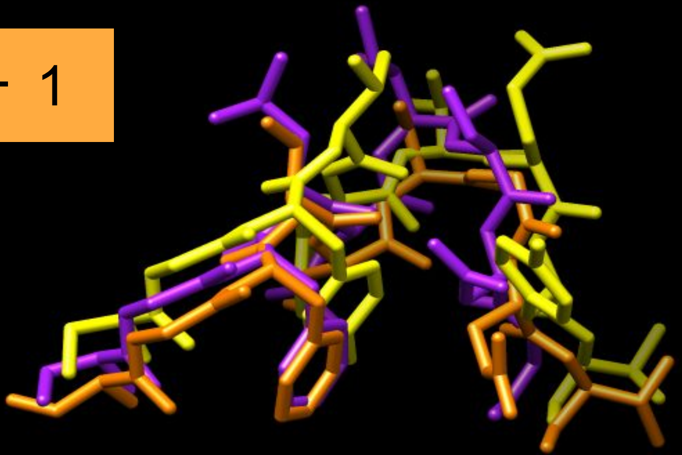
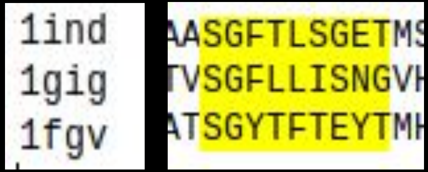
1hil         LVKPGGSLKLSCAASGFSFS-S-YGMSWVRQTPD-KRLEWVATISN---GGGY-TYYPDS
1fvc         LVQPGGSLRLSCAASGFNIK-D-TYIHWVRQAPG-KGLEWVARIYPT--NG-Y-TRYADS
1mfa         VARPGASVKMSCKASGYTFT-N-YWMHWIKQRPG-QGLEWIGAIYPG--NS-A-TFYNHK
1igm         LVQPGGSLRLSCAASGFTFN-I-FVMSWVRQAPG-KGLEWVSGVFG---SGGN-TDYADA
1fgv         LVQPGGSLRLSCATSGYTFT-E-YTMHWMRQAPG-KGLEWVAGINPK--NG-G-TSYADS
1vfa         LVAPSQSLSITCTVSGFSLT-G-YGVNWVRQPPG-KGLEWLGMIWG---DG-N-TDYNSA
2fb4         VVQPGRSLRLSCSSSGFIFS-S-YAMYWVRQAPG-KGLEWVAIIWD---DGSD-QHYADS
1gig         LVAPSQSLSITCTVSGFLLI-S-NGVHWVRQPPG-KGLEWLGVIWA---GG-N-TNYNSA
7fab         LVRPSQTLSLTCTVSGTSFD-D-YYWTWVRQPPG-RGLEWIGYVFY---TG-T-TLLDPS
1acy         VIKPSQSLSLTCIVSGFSITRTNYCWHWIRQAPG-KGLEWMGRICY---EG-S-IYYSPS
1ai1         VIKPSQSLSLTCIVSGFSITRTNYCWHWIRQAPG-KGLEWMGRICY---EG-S-IYYSPS
1tet         LKTPGETVRISCKASGYTFT-T-YGMSWVKQTPG-KGFKWMGWINTY--SG-V-PTYADD
2fbj         LVQPGGSLKLSCAASGFDFS-K-YWMSWVRQAPG-KGLEWIGEIHP---DS-GTINYTPS
1baf         LVKPSQSQSLTCTVTGYSITSD-YAWNWIRQFPGNK-LEWMGYMSY---SG-S-TRYNPS
2cgr         LMKPGASVQISCKATGYTFS-E-YWIEWVKERPG-HGLEWIGEILPG--SG-R-TNYREK
1ind         SVKPGGSLKLSCAASGFTLS-G-ETMSWVRQTPE-KRLEWVATTLSG--GG-F-TFYSAS
1flr         LVQPGRPMKLSCVASGFTFS-D-YWMNWVRQSPE-KGLEWVAQIRNKPYNY-E-TYYSDS
1ggc         ILQPSQTLSLTCSFSGFSLSTYGMGVSWIRQPSG-KGLEWLAHIFW---DG-D-KRYNPS

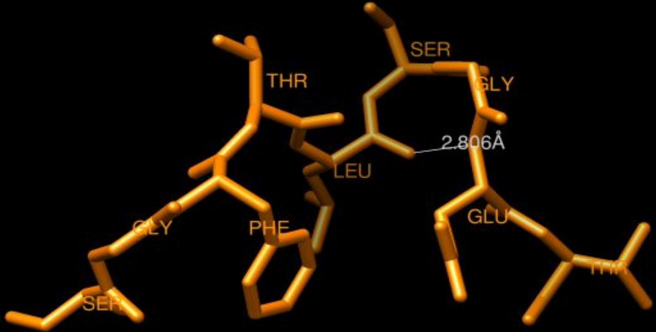
8fab         VKGRFTISRDNSKRTLYMQMNSLRTEDTAVYYCARDP-D-IL----------TAFS-FDY
1ggi         LKSRLKISKDTSNNQVFLKITSVDTADTATYYCVQE-------------------G-YIY
1hil         VKGRFTISRDNAKNTLYLQMSSLKSEDSAMYYCARRERY--------D----EN-G-FAY
1fvc         VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG-G--D-----G----FY-A-MDY
1mfa         FRAKTKLTAVTSTTTAYMELSSLTSEDSAVYYCTRGG-H--------------G-YYGDY
1igm         VKGRFTITRDNSKNTLYLQMNSLRAEDTAIYYCAKHR-VSYVL----------T-G-FDS
1fgv         VKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARWR-G--L-----D----VR-Y-FDV
1vfa         LKSRLSISKDNSKSQVFLKMNSLHTDDTARYYCARER-D--------------Y-R-LDY
2fb4         VKGRFTISRNDSKNTLFLQMDSLRPEDTGVYFCARDGGHGFC-SSAS-----CF-G-PDY
1gig         LMSRVSISKDNSKSQVFLKMKSLQTDDTAMYYCARDF-Y--------DYDVFYY-A-MDY
7fab         LRGRVTMLVNTSKNQFSLRLSSVTAADTAVYYCARNLIA--------------G-G-IDV
1acy         IKSRSTISRDTSLNKFFIQLISVTNEDTAMYYCSRENHM--------Y----ET-Y-FDV
1ai1         IKSRSTISRDTSLNKFFIQLISVTNEDTAMYYCSRENHM--------Y----ET-Y-FDV
1tet         FKGRFAFSLETSASTAYLQINNLKNEDTATYFCARR--S--------------W-Y-FDV
2fbj         LKDKFIISRDNAKNSLYLQMSKVRSEDTALYYCARLH-Y--------------YGY-NAY
1baf         LRSRISITRDTSKNQFFLQLKSVTTEDTATYFCARG-----------------W-P-LAY
2cgr         FKGKATFTADTSSNTAYMQLSSLTSEDSAVYYCTRG--Y--------------S-S-MDY
1ind         VKGRFTISRDNAQNNLYLQLNSLRSEDTALYFCASH-------------------R-FVH
1flr         VKGRFTISRDD--SSVYLQMNNLRVEDMGIYYCTGSY-Y----------------G-MDY
1ggc         LKSRLKISKDTSNNQVFLKITSVDTADTATYYCVQE-------------------G-YIY
```

H1 - 1

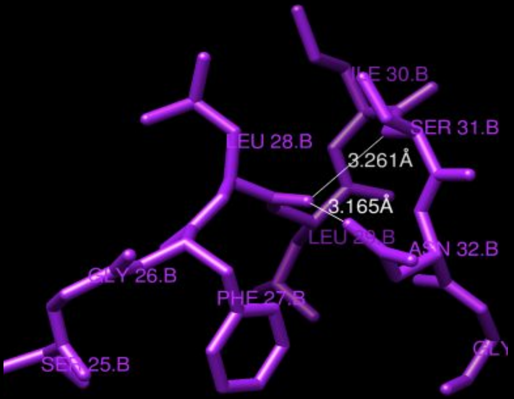




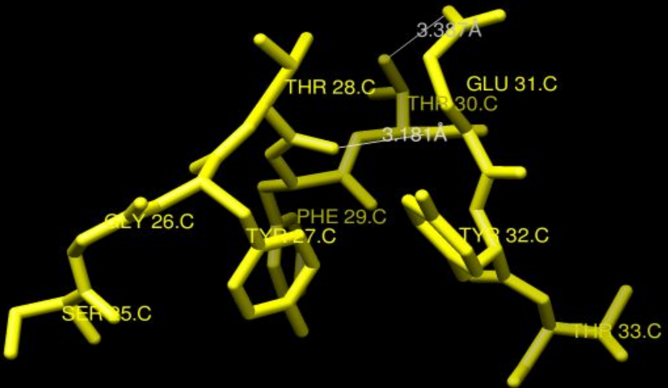
1ind	AASGFTLSGETMS
1gig	TVSGFLLISNGVH
1fgv	ATSGYTFTEYTMH



1ind

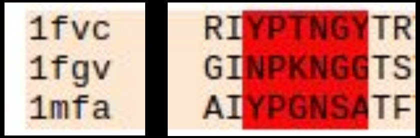


1gig

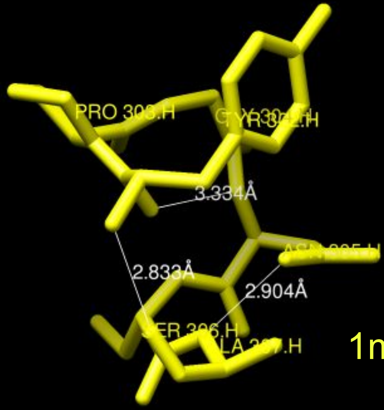


1fgv

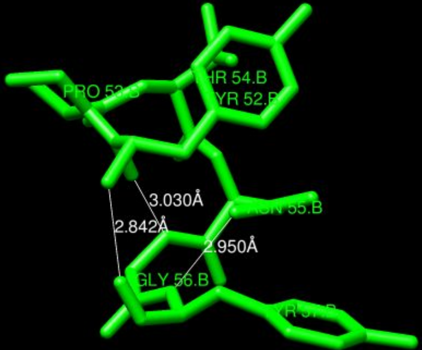
H2 - 2a



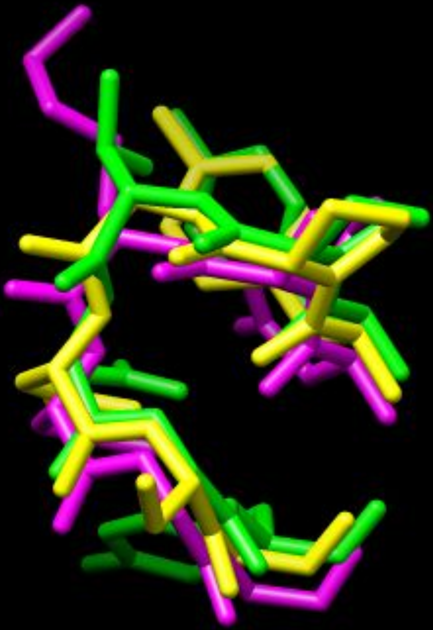
1fvc	RIYPTNGYTR
1fgv	GINPKNGGTS
1mfa	AIYPGNSATF



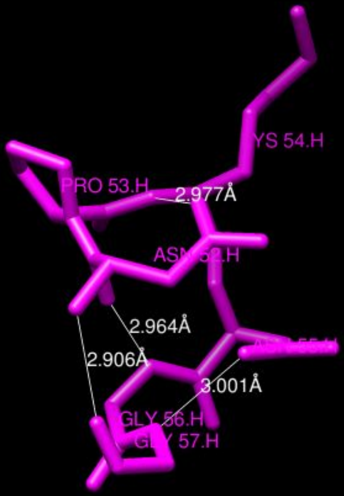
1mfa



1fvc



1fvc
1fgv
1mfa



1fgv

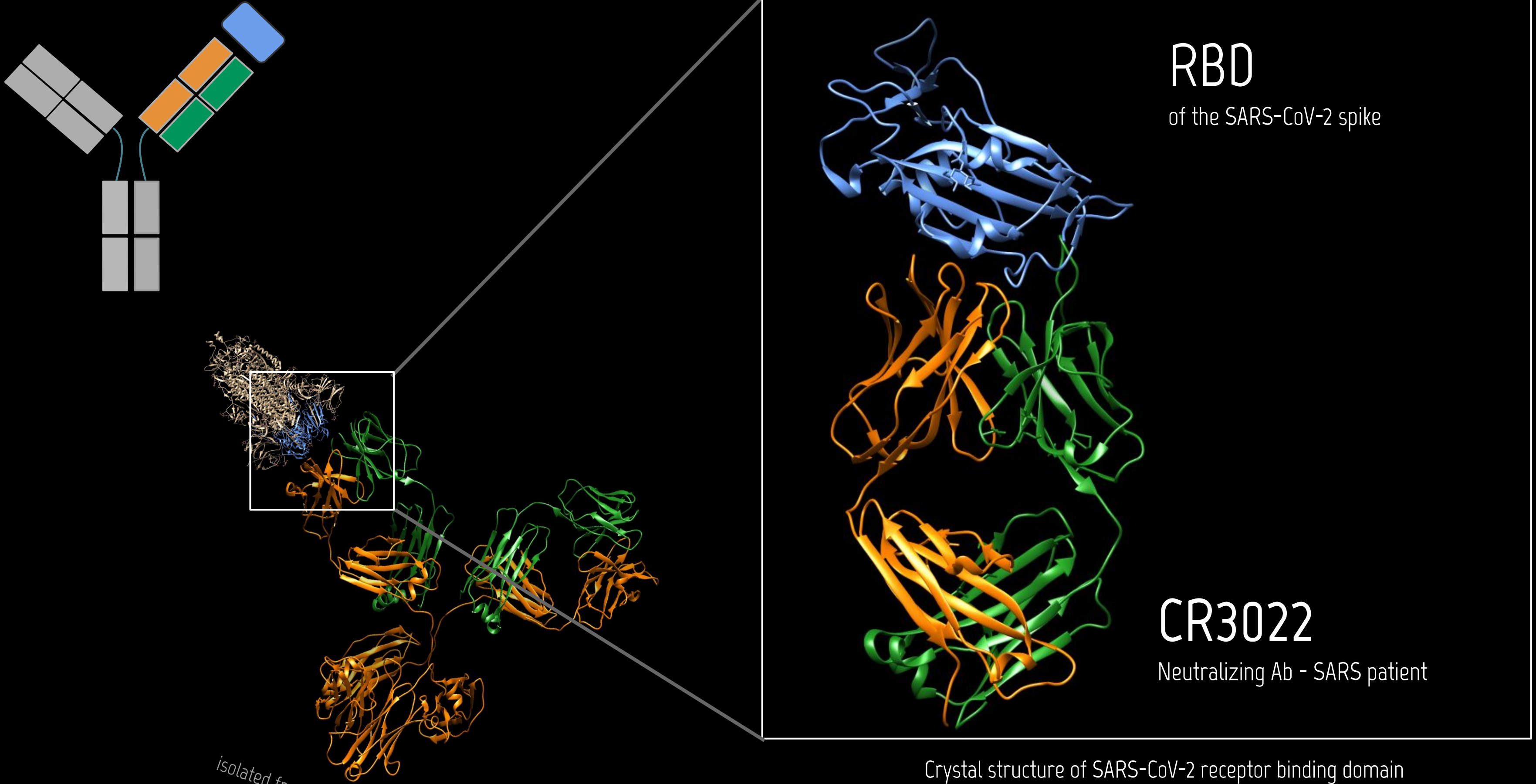
Fab → CDR → hotspot hypothesis

↓ residues determine the binding energy

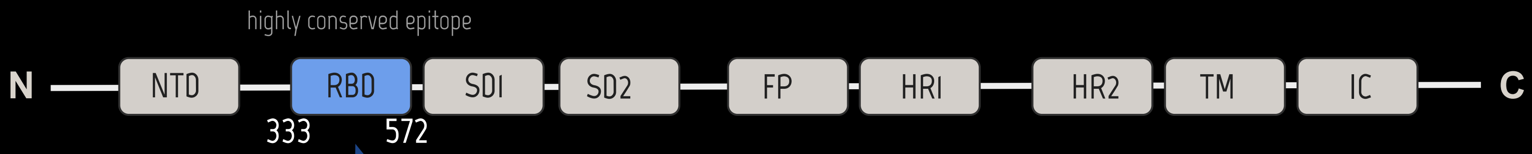
ANTIBODY-ANTIGEN INTERACTION

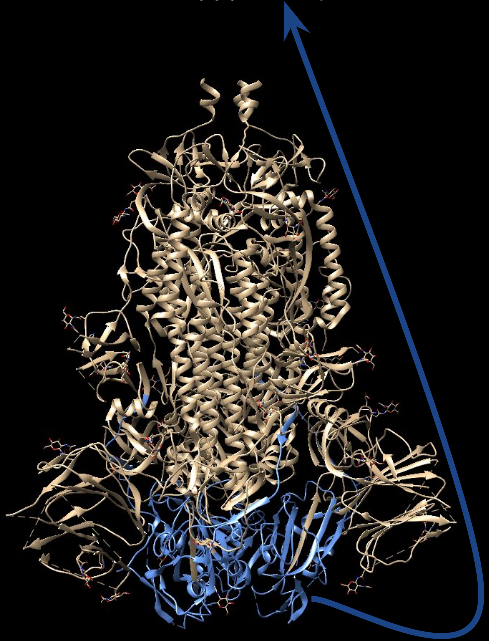
Reversible noncovalent interaction forces

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



Crystal structure of SARS-CoV-2 receptor binding domain
in complex with human antibody CR3022 - 3.08 Å

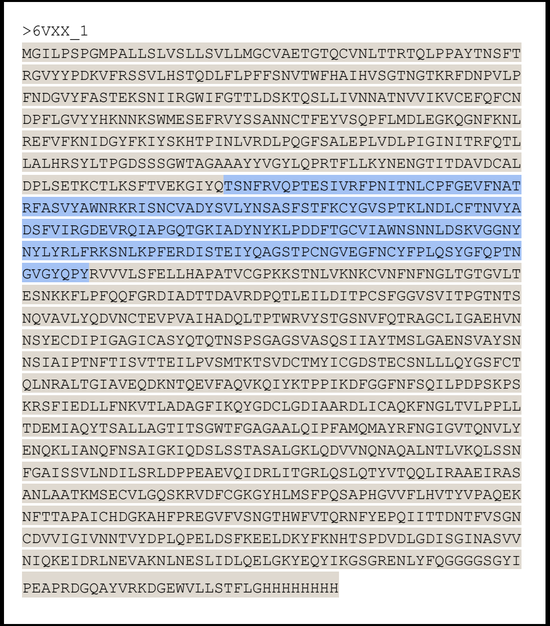




SPIKE

Structure of the SARS-CoV-2 spike glycoprotein

(closed state) 2.80 Å



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>6VXX_1
MGILPSPGMPALLSLVSLLSVLLMGCVAETGTQCVNLTTRTQLPPAYTNSFT
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FNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCN
DPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNL
REFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTL
LALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCAL
DPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNAT
RFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYA
DSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNY
NYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTN
GVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLT
ESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTS
NQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN
NSYECDIPIGAGICASYQTQTNSPSGAGSVASQSIIAYTMSLGAENSVAYSN
NSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCT
QLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPS
KRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLL
TDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLY
ENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSN
FGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRAS
ANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEK
NFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGN
CDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKGSGRENLYFQGGGGSGYI
PEAPRDGQAYVRKDGEWVLLSTFLGHHHHHHHH
```
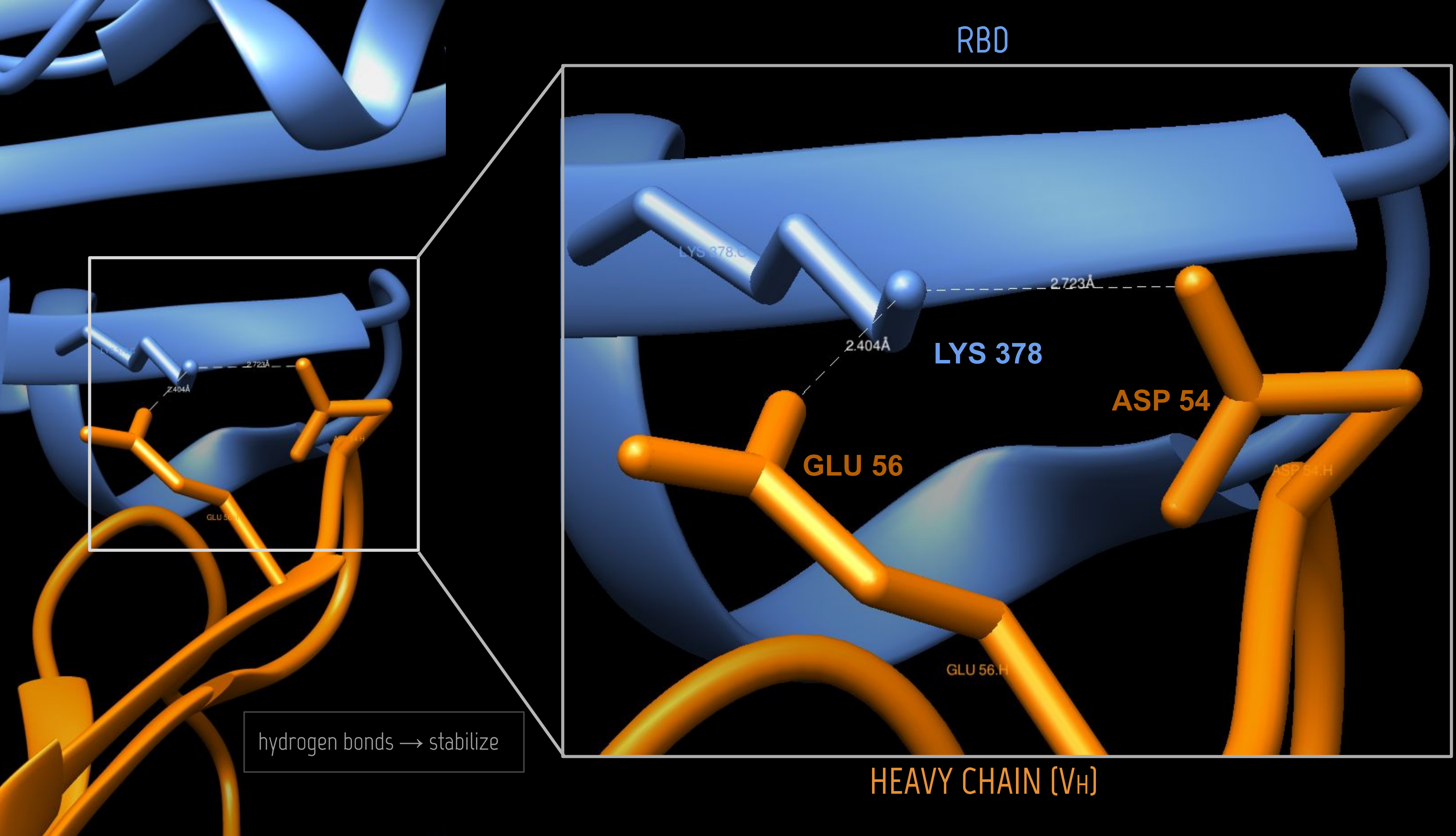
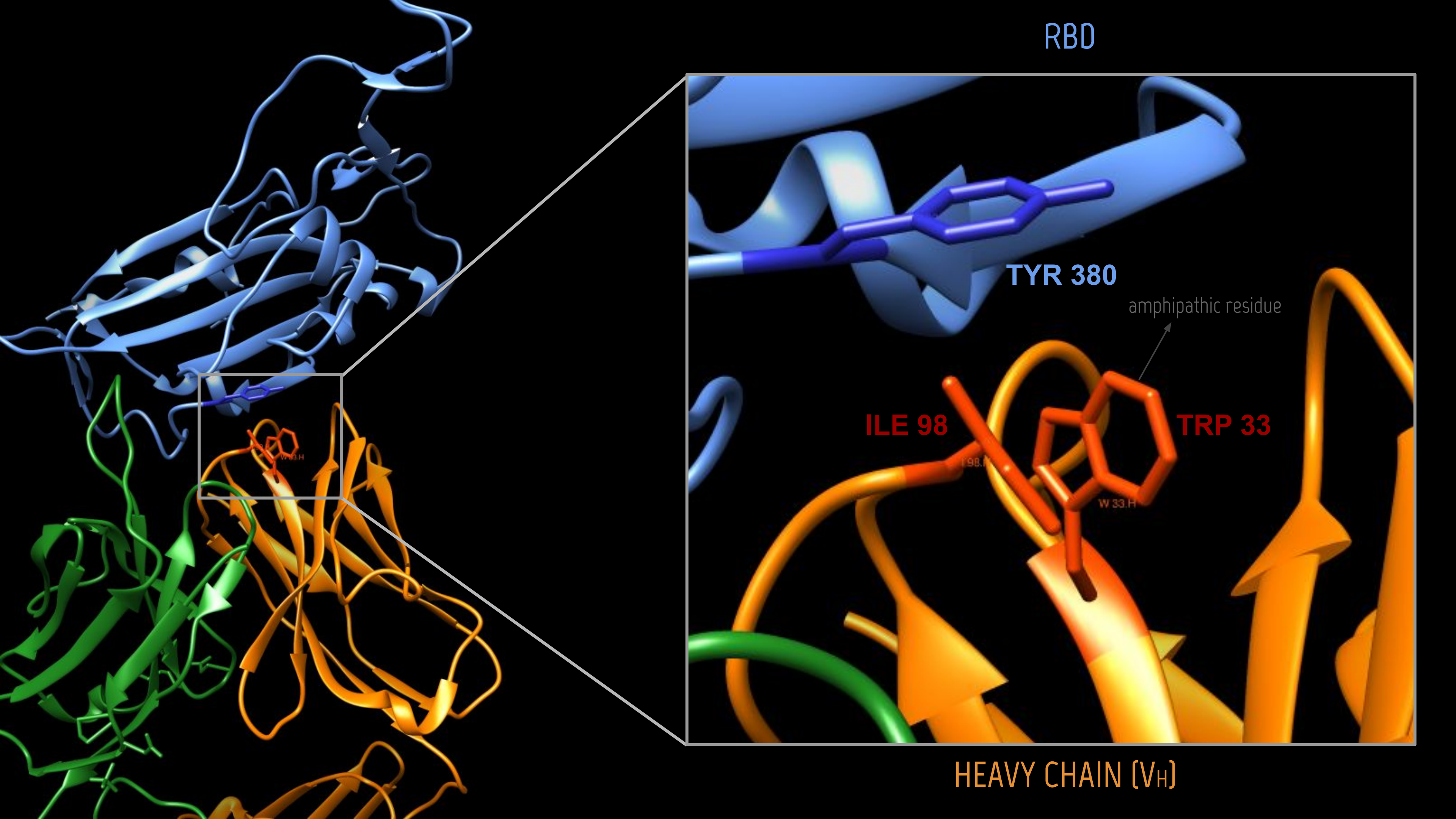
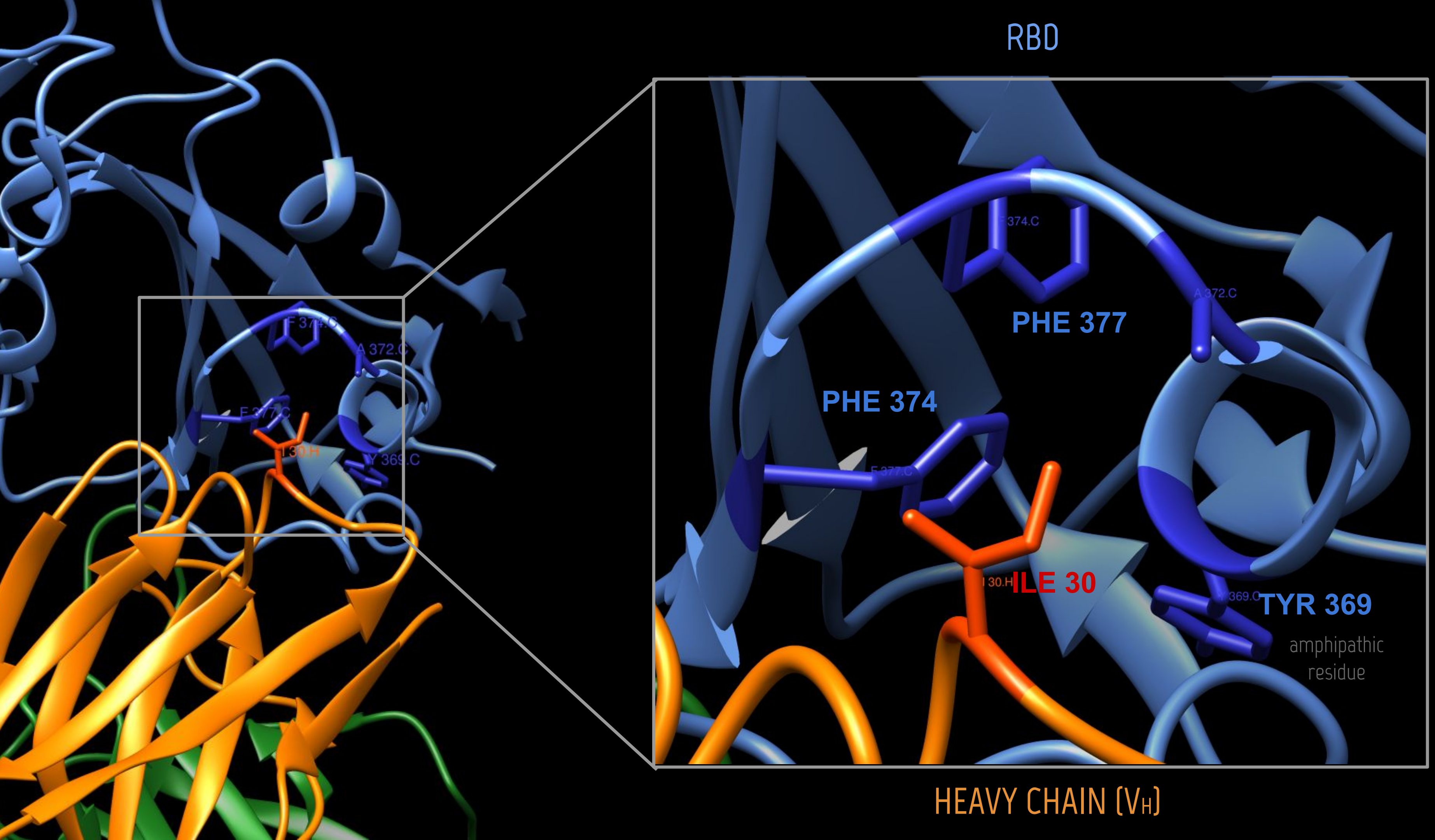
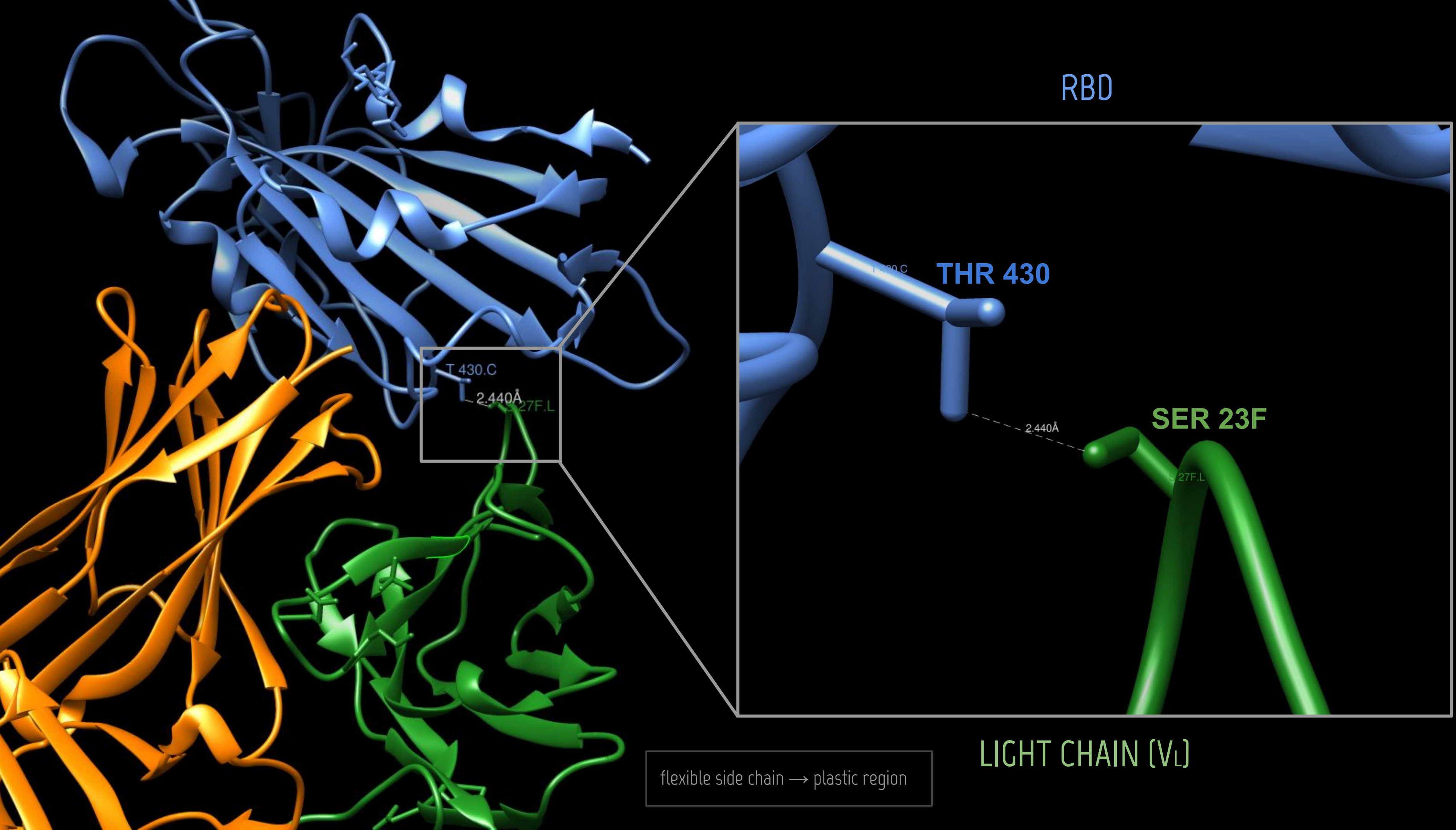



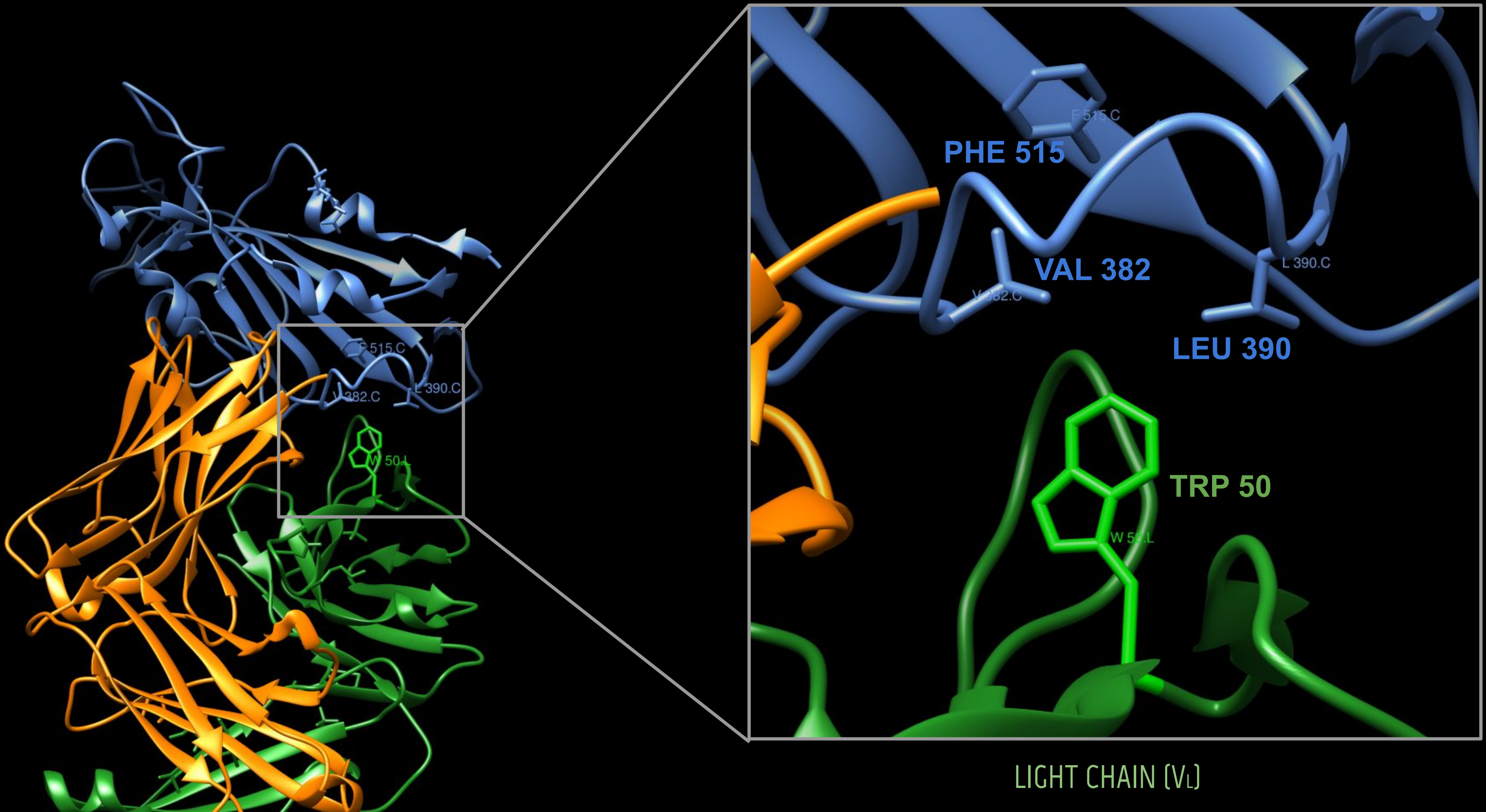
hydrogen bonds ⟶ stabilize


RBD
TYR 380
amphipathic residue
ILE 98
TRP 33
I 98.H
W 33.H
HEAVY CHAIN (V$_H$)


RBD
F 374.C
A 372.C
F 377.C
I 30.H
Y 369.C
F 374.C
A 372.C
PHE 377
PHE 374
F 377.C
I 30.H
ILE 30
Y 369.C
TYR 369
amphipathic residue
HEAVY CHAIN (V_H)



flexible side chain → plastic region


RBD
F 515.C
V 382.C
L 390.C
W 50.L
PHE 515
VAL 382
LEU 390
TRP 50
LIGHT CHAIN (VL)

CONCLUSIONS

WE CONCLUDE THAT...

- Immunoglobulins are essential for antigen recognition and immune response
- The basic immunoglobulin fold is a greek key motif beta sandwich
- Disulfide bridges are important for the maintenance of the Ig structure
- Glycosylations at ASN-297 play an essential role in structure integrity and Fc receptor recognition.
- Mutations at the hinge region (LALA mutants) lead to widening of the Fc opening.
- There are different subtypes of IgG, all of them with similar Fc regions
- The Fc region of IgG1 is conserved among the species analyzed
- Fab interacts with the antigen through hypervariable regions (CDRs), which conform the antigen binding site
- There are conserved CDR structures which are called canonical structures.
- RBD is a highly conserved epitope from Spike that interacts with the Immunoglobulin G

PEM QUESTIONS

1. Which is the main fold of immunoglobulins?

a. Jelly roll
[✓ b. Greek key sandwich]
c. TIM barrel
d. Rossman fold
e. beta-barrel

2. In which immunoglobulin domain do IgG1 LALA mutants have the mutations?

a. CDR1
b. CDR2
c. CDR3
d. Fc region
[✓ e. Hinge region]

3. How many canonical structures have L2 (CDR2 of light chain) domain?

[✓ a. 0]
b. 1
c. 2
d. 3
e. 4

4. Mark the wrong answer about complementary determining regions (CDRs):

a) There are CDR in both the light and heavy chains.
b) Canonical structures refer to a limited number of conformations of the CDRs
c) They are involved in binding of antigens
d) They are part of the variable region of immunoglobulins
e) There are only 2 CDRs for each, the light and heavy chain.

5. **Related to the hydrophobicity of immunoglobulins, indicate the true sentences:**

1) The hydrophobic residues are mostly inside, as they repel water.
2) Hydrophilic residues on the surfaces contact with water as it is energetically advantageous
3) Hydrophobicity decreases along with distance from the center of the domain
4) Light-chain IgG domains are found not to contain regular hydrophobic cores

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

6. The variable chains in immunoglobulins are essential for the recognition of the antigens. Its acronym CDR comes from te words:

a. Committee in Defense of the Republic
b. Complementarity-determining region
c. Complex-determining region
d. Canonical-detecting receptor
e. Common dual region

7. Mark the wrong answer related to the antigen-antibody interaction:

a) RBD is an epitope from Spike (SARS CoV-2) that interacts with the Immunoglobulin G
b) Some of the interactions that stabilize the structure are hydrogen bonds
c) The antigen interacts with both the heavy and the light chain
d) The binding Ab-Ag is based on reversible covalent interaction forces.
e) It is based on electrostatic forces, Van der Waals, hydrogen bonds and hydrophobic forces.

8. Mark the correct answer about immunoglobulin structure

a) The hinge region is the same length in all IgG subtypes (IgG1, IgG2, IgG3 and IgG4) and all the Ig isotypes (IgG, IgM, IgE, IgD and IgA)
b) Disulfide bonds are not conserved in different IgG subtypes and isotypes
c) Glycosylation of the Fc region of Immunoglobulins is essential for their correct function
d) Proline and glycine are mainly found in the beta strands and they are not present in the turns and coils.
e) The immunoglobulin structure is conformed by multiple alpha helices and none beta-sheets.

9. FcγRI…

a) Binds to Fc region
b) Binds to Fab region
c) Binds to CDRs
d) Does not bind to any part of immunoglobulines
e) Binds to antigens

10. Which is the most abundant immunoglobuline in human serum?

a) Ig A
b) Ig E
c) Ig D
d) Ig G
e) Ig M

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