

# The role of synchrotrons in African Bio-science

**Thandeka Moyo-Gwete**

Senior Medical Scientist

National Institute for Communicable Diseases

Johannesburg, South Africa



# Who are we??



Medical Research Council Antibody Immunity  
Research Unit

Based in Johannesburg, South Africa

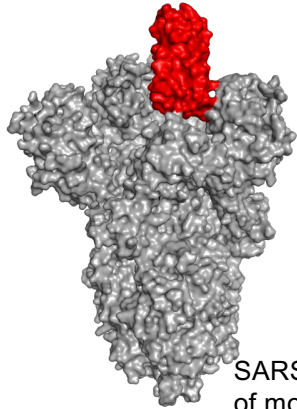
Virology, Immunology and Structure

HIV, Influenza, CMV, SARS-CoV-2

# Contribution of Light Sources to Biological and Medical Sciences

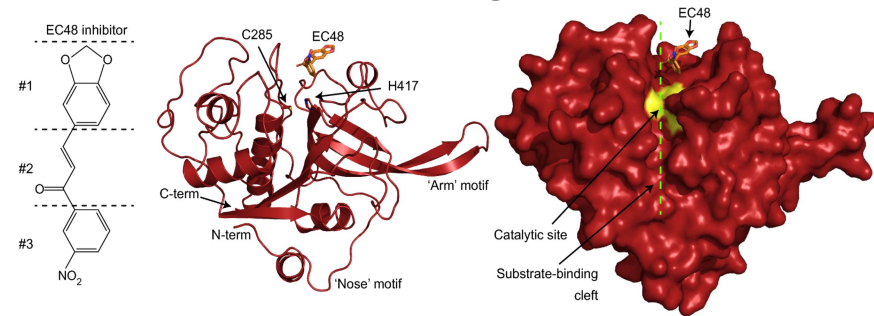
Structural biology helps us understand the **structure and function of macromolecules** including proteins, DNA and RNA

**Aids in vaccine design**



SARS-CoV-2 spike protein – basis of most vaccine candidates

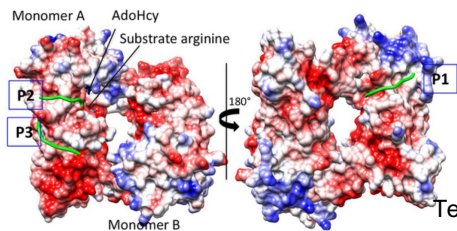
**Provides information on protein-inhibitor interactions for drug, herbicide and pesticide design**



Malaria protein bound by inhibitor

Machin et al 2019, *Malaria Journal*

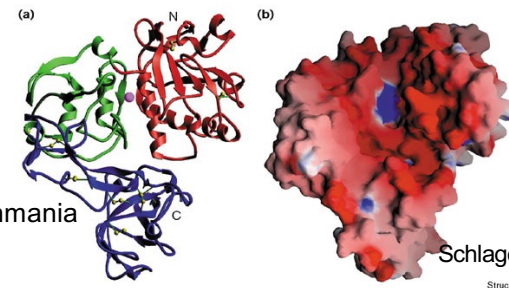
**Provides insight into the mechanism of enzymes and is an enabler for industrial enzymology**



Active site binding of protein arginine methyltransferases

Tewary et al., 2019, *Cell Mol Life Sci.*

**Reveal the structure and therefore vulnerable regions of proteins from pathogens**



Structure of the main leishmania surface antigen

Schlagenhauf et al, 1998, *Structure*

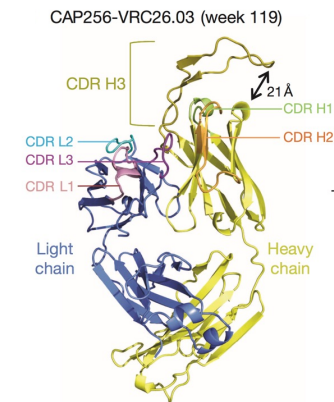


# Importance of structural biology in virology research: **HIV**, SARS-CoV-2 and beyond



# Neutralizing antibodies in HIV vaccine development

- **Broadly neutralizing antibodies (bNAbs)** are required for an effective HIV vaccine
- They neutralize various global HIV-1 strains and inhibit entry into cells
- However, HIV infection has shown us:
  - Rare – 20% HIV infected individuals develop them
  - Unusual features
  - Take long to appear – chronic infection
- Therefore, studying bNAbs and their targets may aid in immunogen design to elicit bNAbs



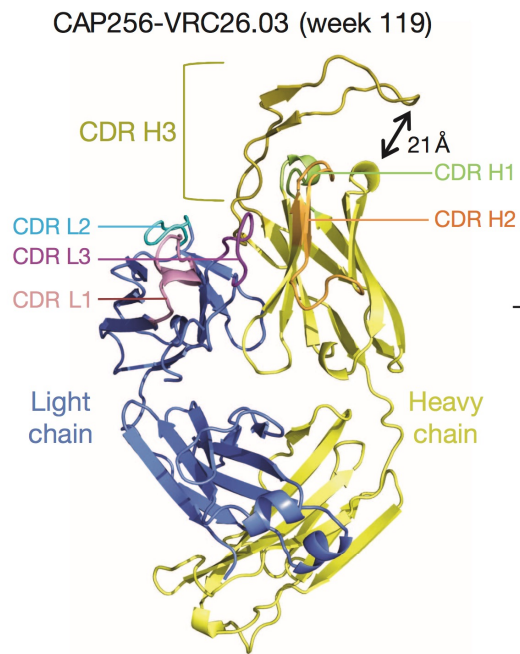
# Importance of structural biology in bNAb research

Why we study the structure of antibody-HIV Envelope complexes?

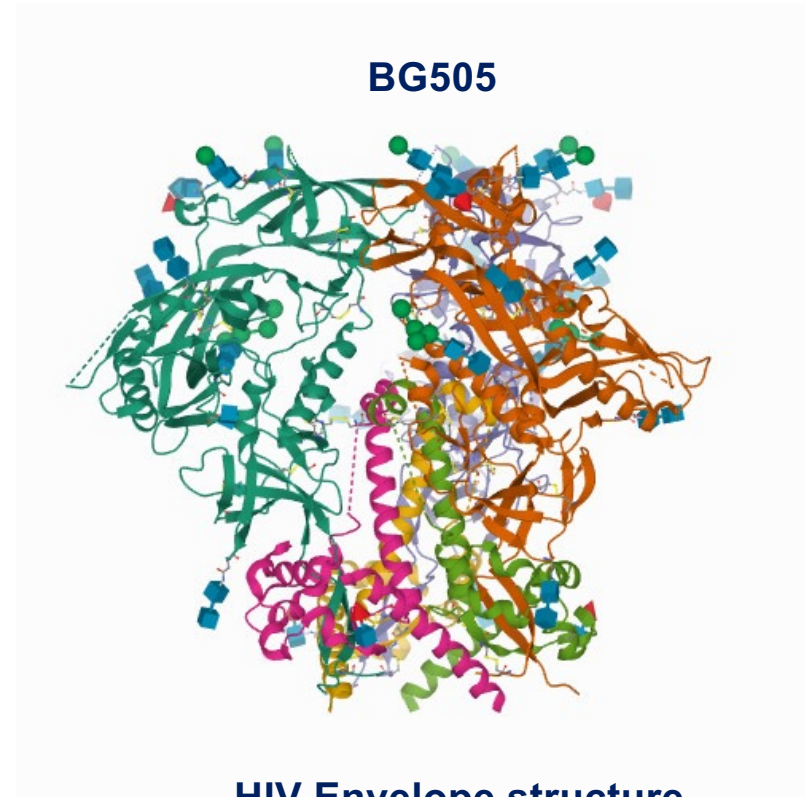
- define novel epitopes targeted by bNAbs
- discover key residues important for the neutralization of HIV by bNAbs
- define structural attributes of “special” viral strains
- inform design of immunogens which will elicit bNAbs



# Technique to obtain the high resolution structure of proteins:

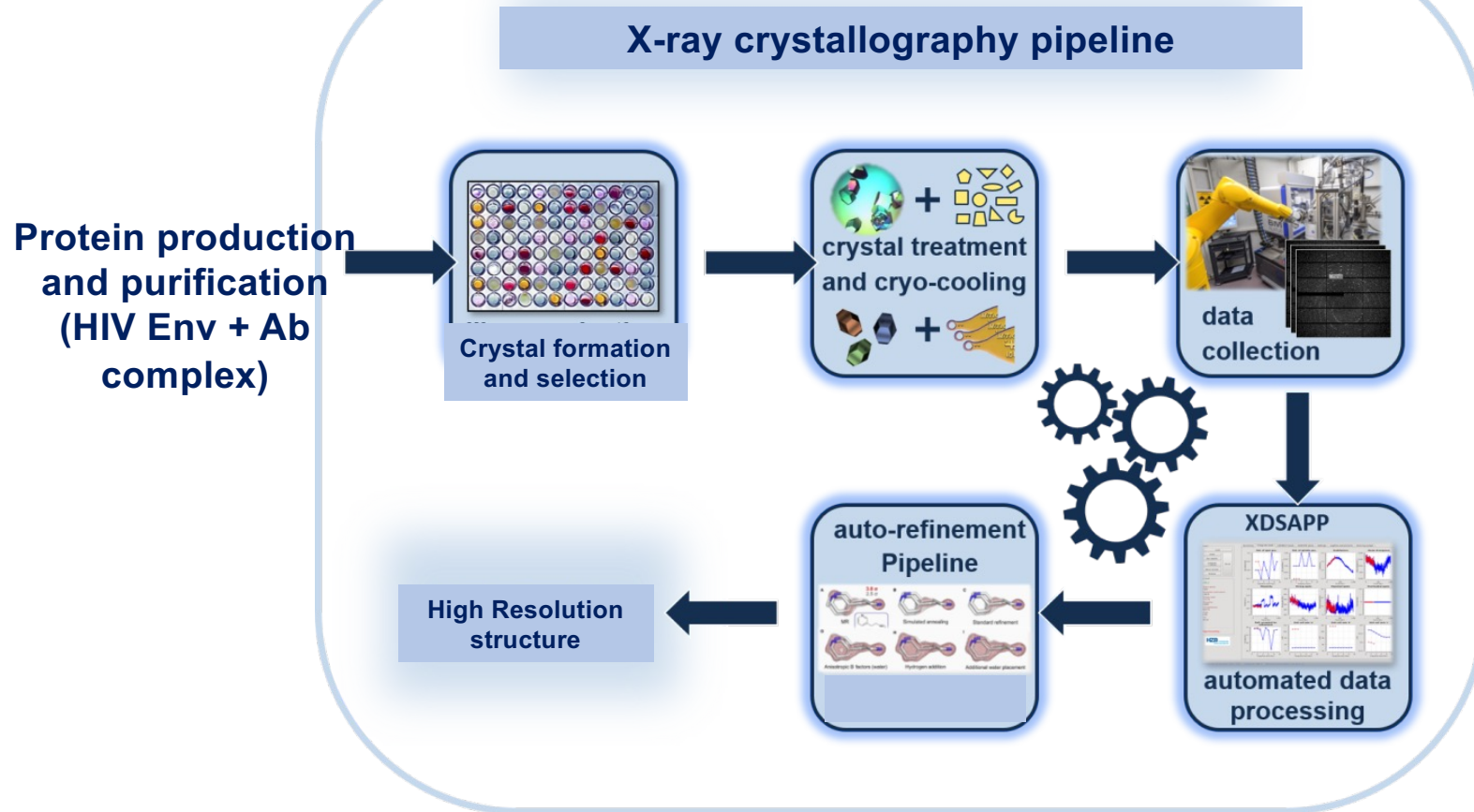


**Antibody structure**



**HIV Envelope structure**

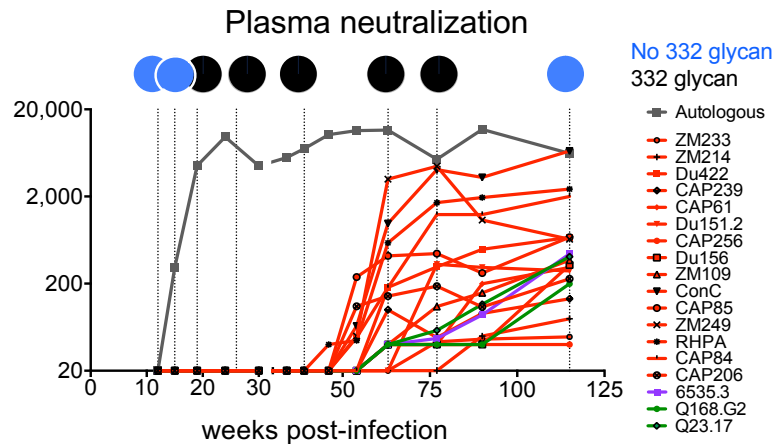




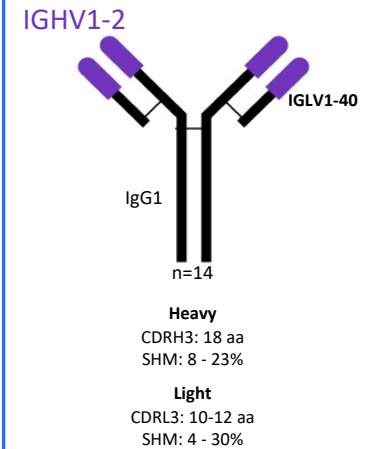
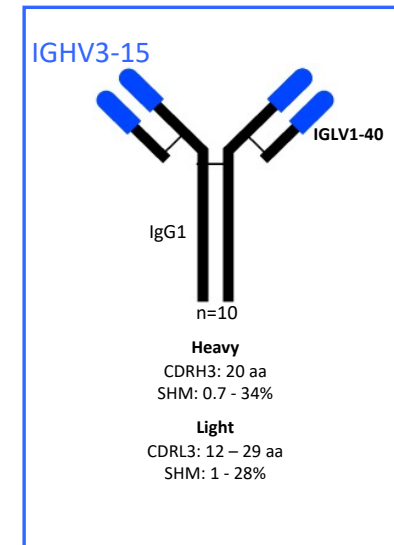
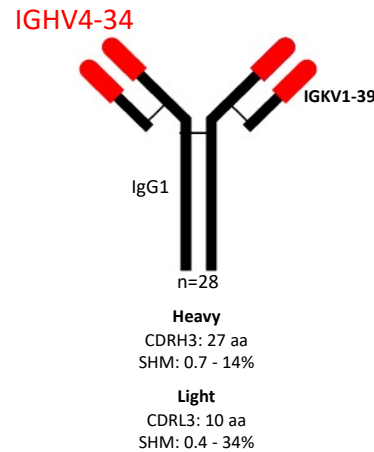
# Structural characterization of antibody lineages from single donor

**CAP314** – HIV-infected donor who developed bNAbs within 2 years post-infection

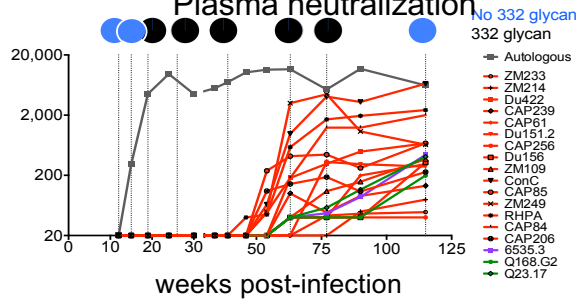
Isolated and characterized three antibody lineages (families)



Moore et al., Nature Medicine, 2012

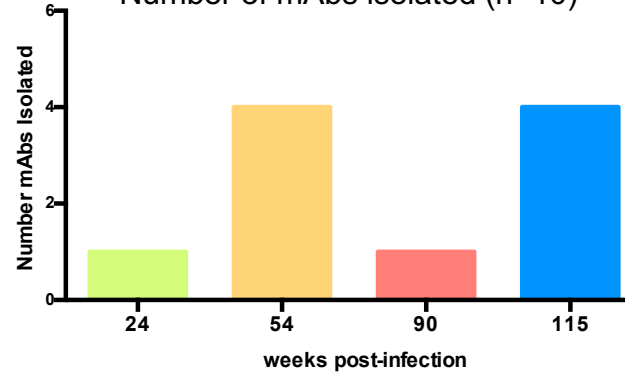


### Plasma neutralization

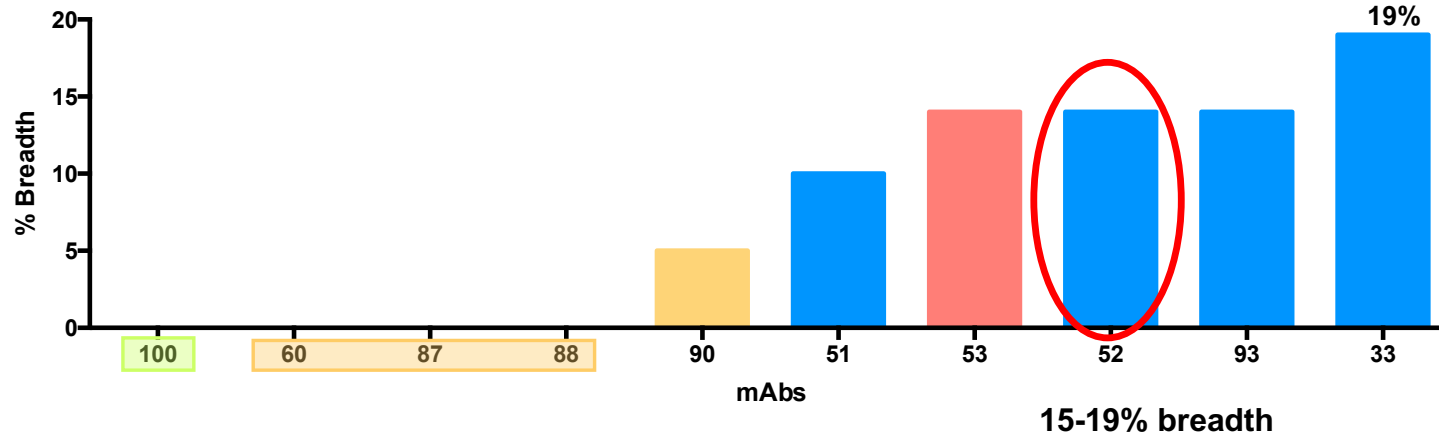


Moore et al., Nature Medicine, 2012

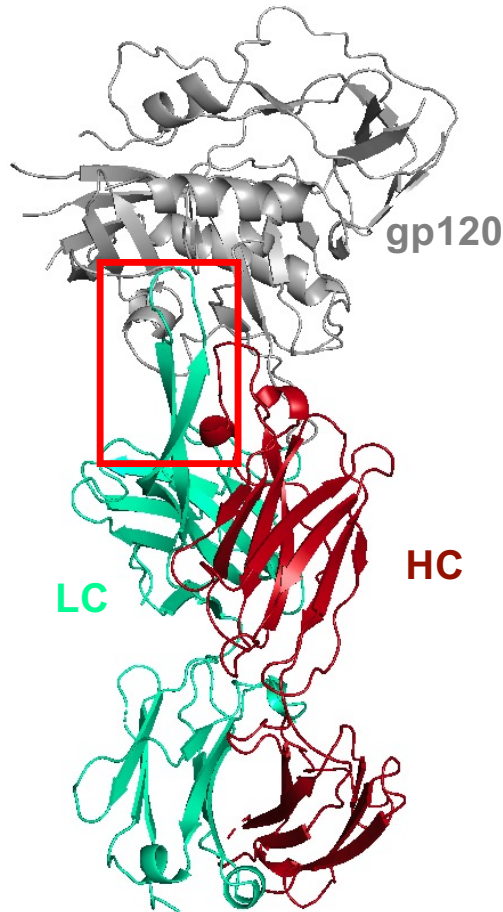
### Number of mAbs isolated (n=10)



### Breadth of the IGHV3-15 family based on heterologous viruses neutralized by the plasma (n=21)



# CDRL3 insertion (29 aa) associated with increased breadth over time



## 3-15 lineage Light Chain

IGLV1-40*01
IGLJ2*01
100_24wpi_1.0%
90_54wpi_3.1%
88_54wpi_3.5%
60_54wpi_3.8%
87_54wpi_4.2%
53_90wpi_6.3%
51_115wpi_7.3%
33_115wpi_7.3%
52_115wpi_7.6%
93_115wpi_28.6%

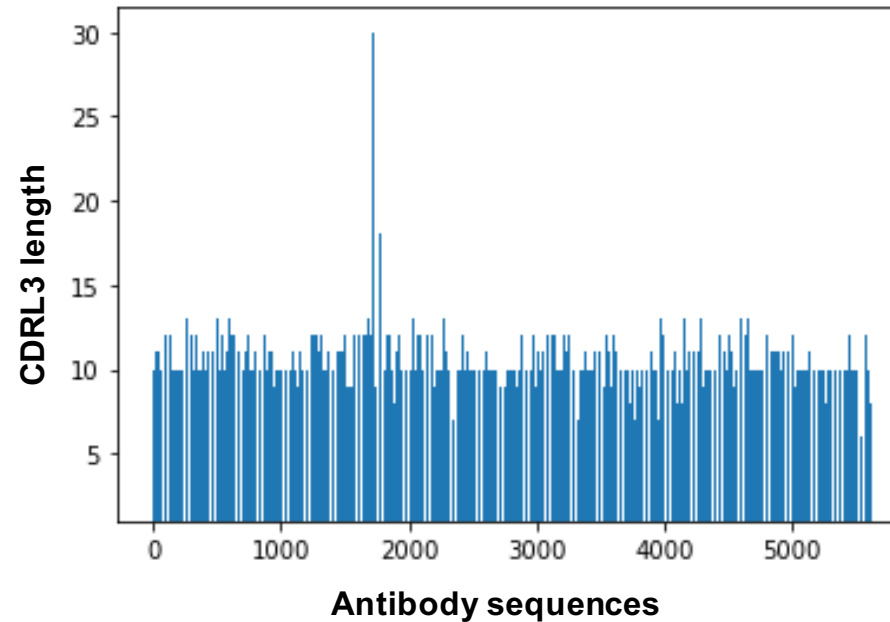
SHM

## Deletion in FR1 and CDRL1

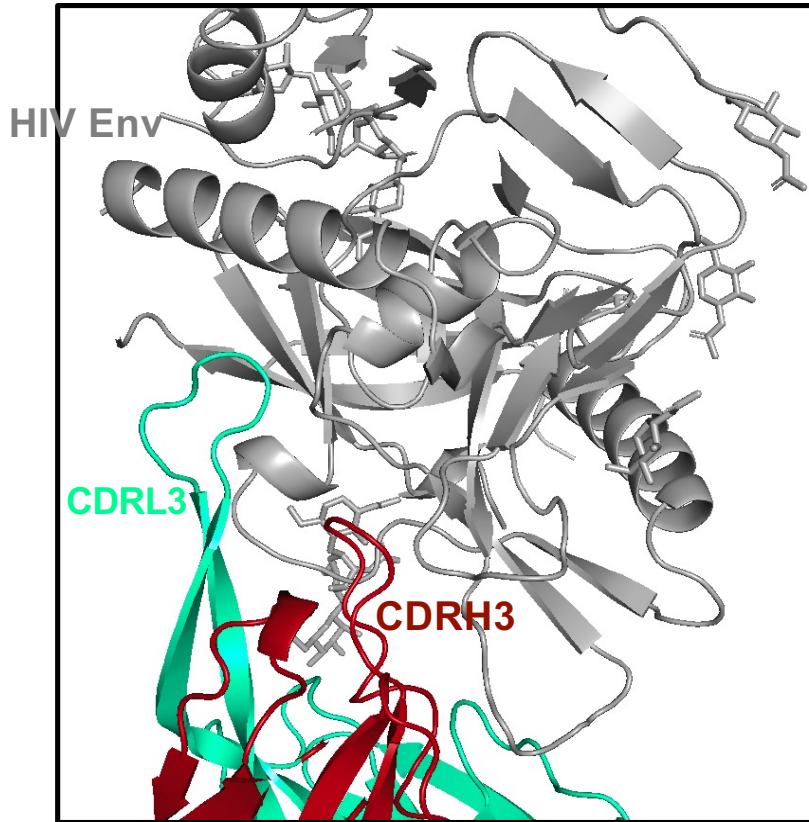
FR1	CDRL1	FR2	CDRL2	FR3	CDRL3	FR4
QSVLTQPPSVSGAPGQRVTISCTGS	SSNIGAGYD	VHWYQQLPGTAPKLLIY	GNS	NRPSGVDRFSGSKSGTSASLAITGLQAEDEADYYC	SYDSSLG	GGGKLTVL
.....R.....	.....P.....	.....N.....	.....R.....	.....R.....	.....Y.....	.....I.....
.....R.....Y.....	.....C.....	.....N.....	.....R.....	.....R.....KSH	.....VVF	.....VVF
.....FE.....H.....C.....	.....RR.....	.....DN.....	.....R.....	.....NR.....RSY	.....VVF	.....VVF
.....R.....	.....RR.....	.....DN.....	.....R.....	.....N.....KSY	.....FVF	.....FVF
.....R.....G.....K.....R.....	.....DN.....	.....R.....	.....R.....	.....A.....NT.....KTY	.....VFP	.....VFP
.....R.....NG.....K.....R.....	.....DN.....	.....R.....	.....R.....	.....A.....NT.....KTY	.....LVF	.....LVF
.....T.....R.....NG.....K.....P.....R.....V.....	.....DN.....	.....R.....	.....R.....	.....A.....K.....D.....G.....	.....KTH	.....KTH
.....R.....NG.....K.....R.....	.....DN.....	.....R.....	.....R.....	.....A.....NT.....KTY	.....LVF	.....LVF
.....FE.....H.....R.....S.....	.....DN.....	.....R.....	.....R.....	.....A.....DR.....RSY	.....FVF	.....FVF

## 29aa Insert in CDRL3

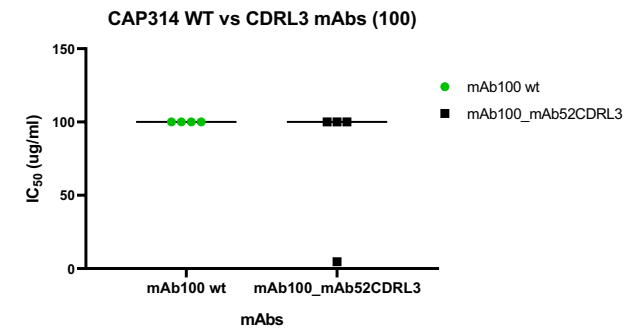
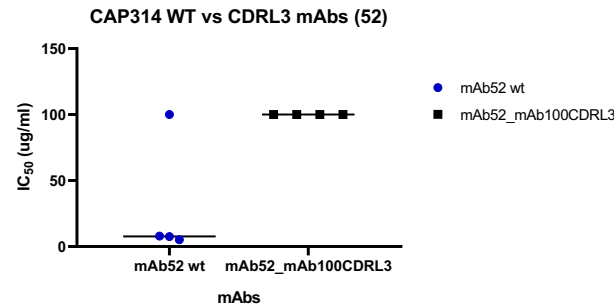
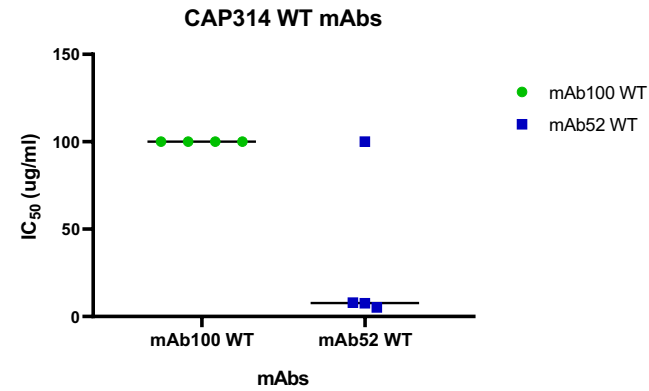
.....VVF
.....VVF
.....VVF
.....FVF
.....VFP
.....LVF
.....KTH
.....LVF
.....FVF



# Novel mode of binding to HIV CD4 binding site

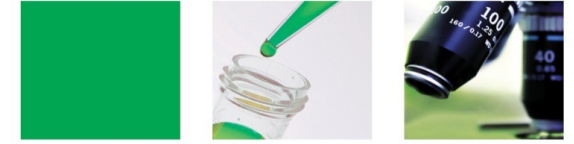


Binds to common HIV bNAb epitope (CD4 binding site) in a unique way



# Using structural biology our lab can now:

- i) Learn more about the structure and function of bNAbs – what unusual features do they have? how can we elicit them in a vaccine?
- ii) explore vaccine elicited antibody responses – HIV trimer trials: study the structure of these Abs and how they interact with full HIV trimers and other immunogens
- iii) Discover and characterize unique HIV strains – learn more about unique features of Env strains that have enhanced capacity to elicit bNAbs



# Importance of structural biology in virology research: HIV, **SARS-CoV-2** and beyond



# The answer to the vaccine question: the spike protein

Corona = crown or circle of light

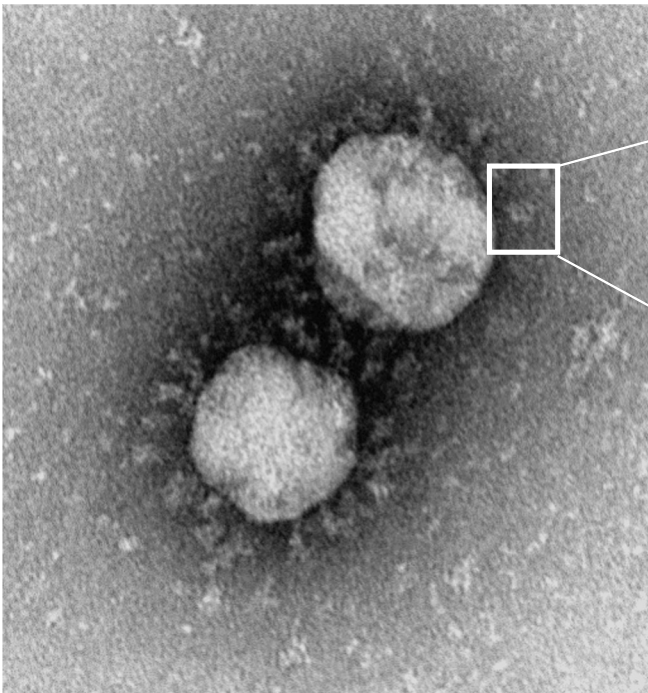
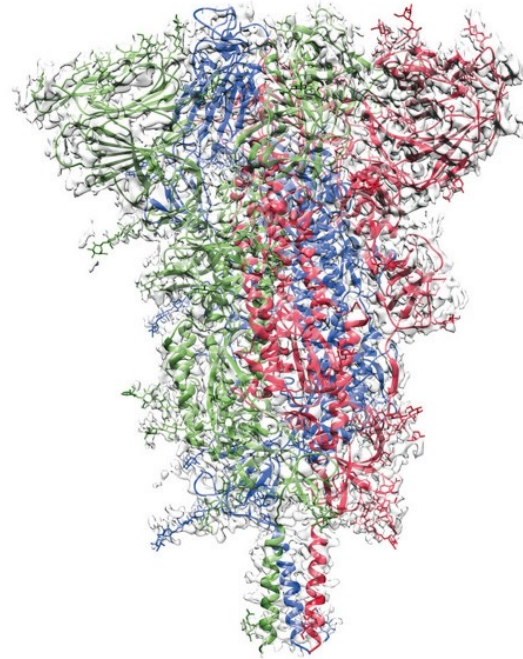


Image: Monica Birkhead  
CEZPD, NICD

SARS-CoV-2 prefusion spike

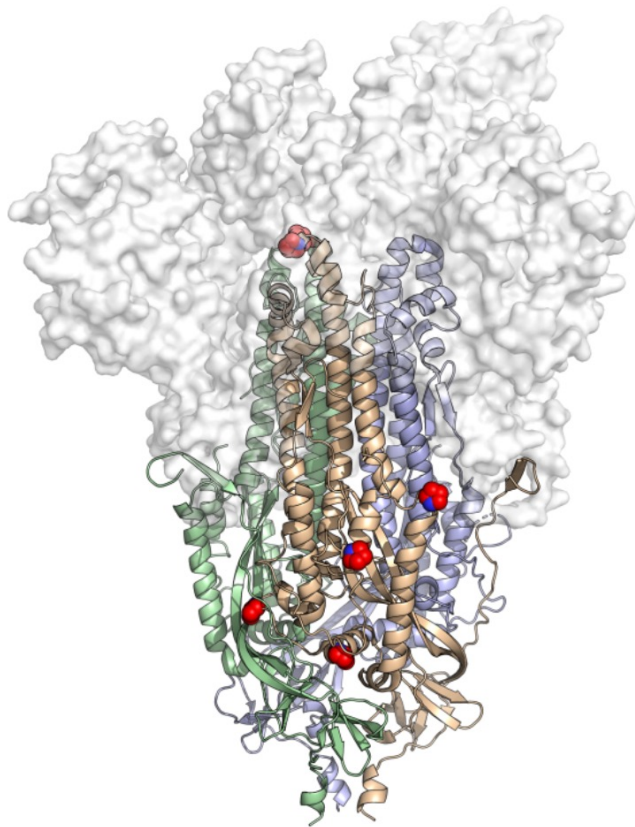


SARS-CoV-2 postfusion spike





# Using structural biology to make the best vaccine candidate

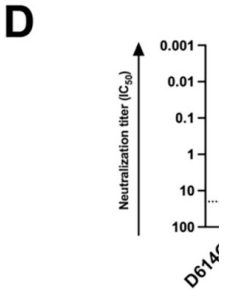
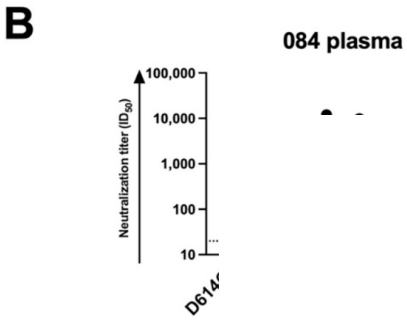
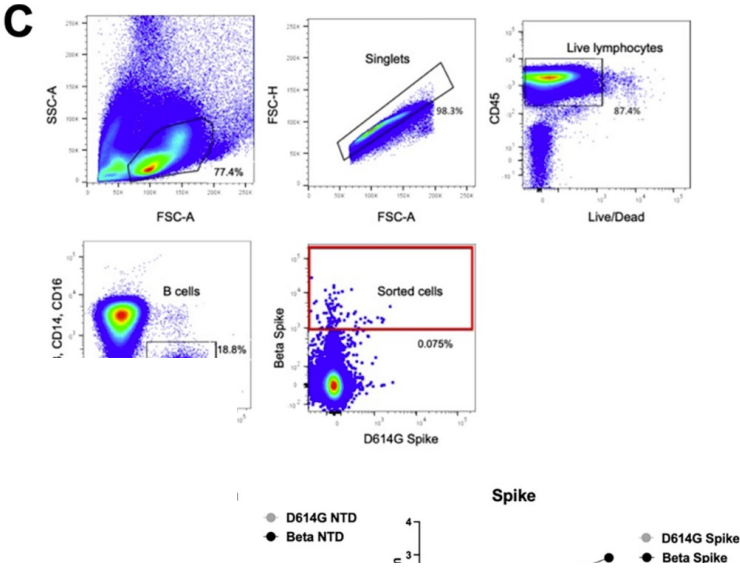


Moderna  
Pfizer  
JnJ  
Novavax

# Isolation of a cross-reactive SARS-CoV-2 antibody

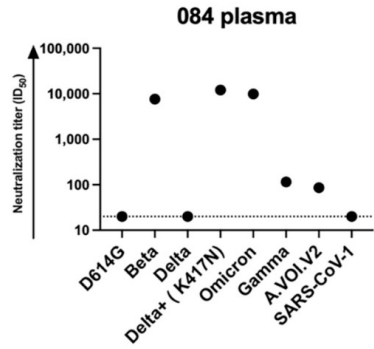
**A**

Sample ID	SA-01-0084
Sex	Female
Age	55-60
HIV status	Uninfected
Days post-positive RT-PCR test	2 days
Date blood sample collected	January 2021
Beta unfection sequence confirmed	Yes

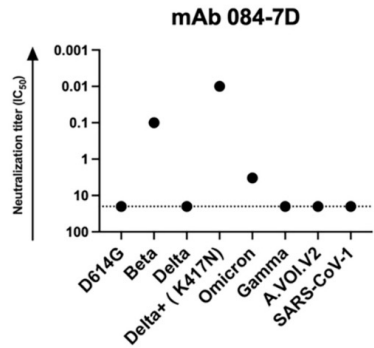


# Isolation of a cross-reactive SARS-CoV-2 antibody

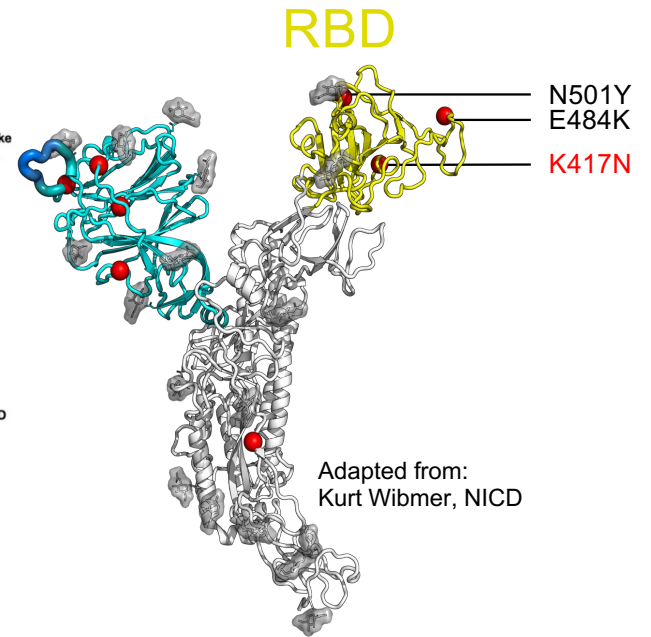
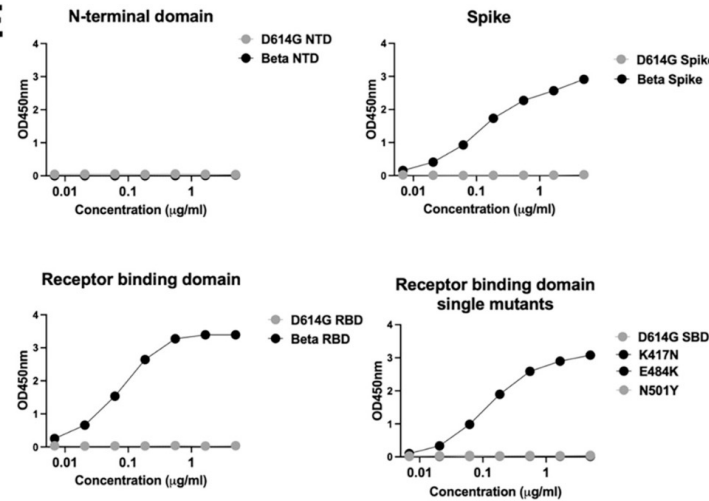
**B**

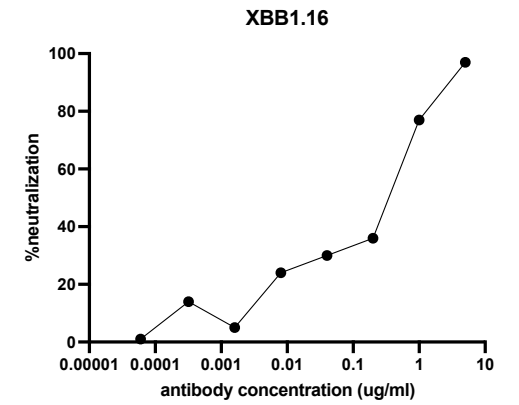
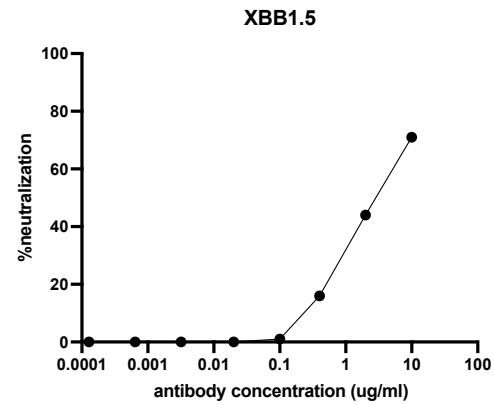
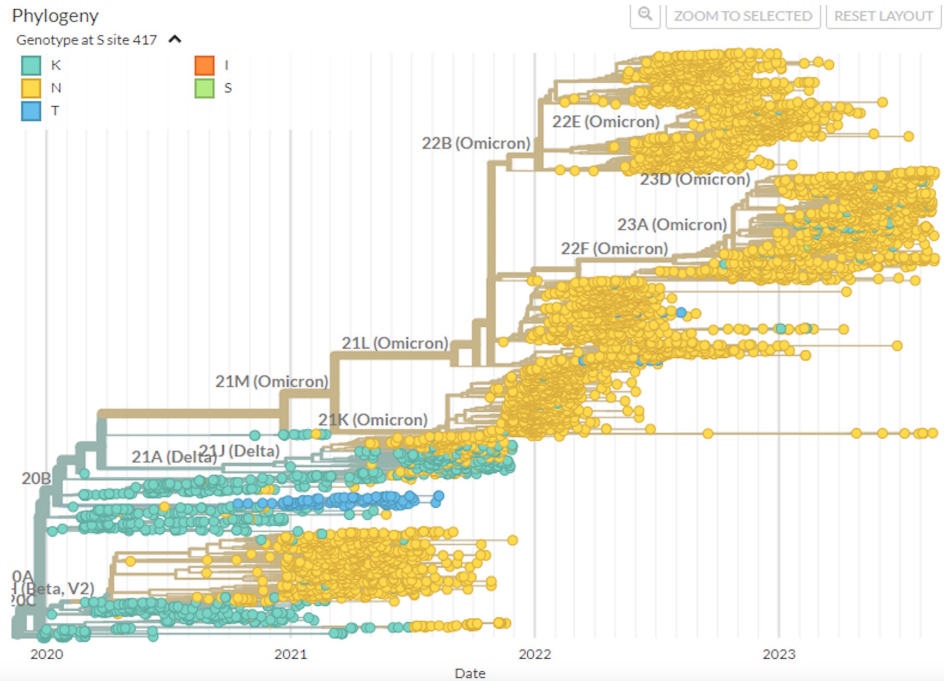


**D**



**E**

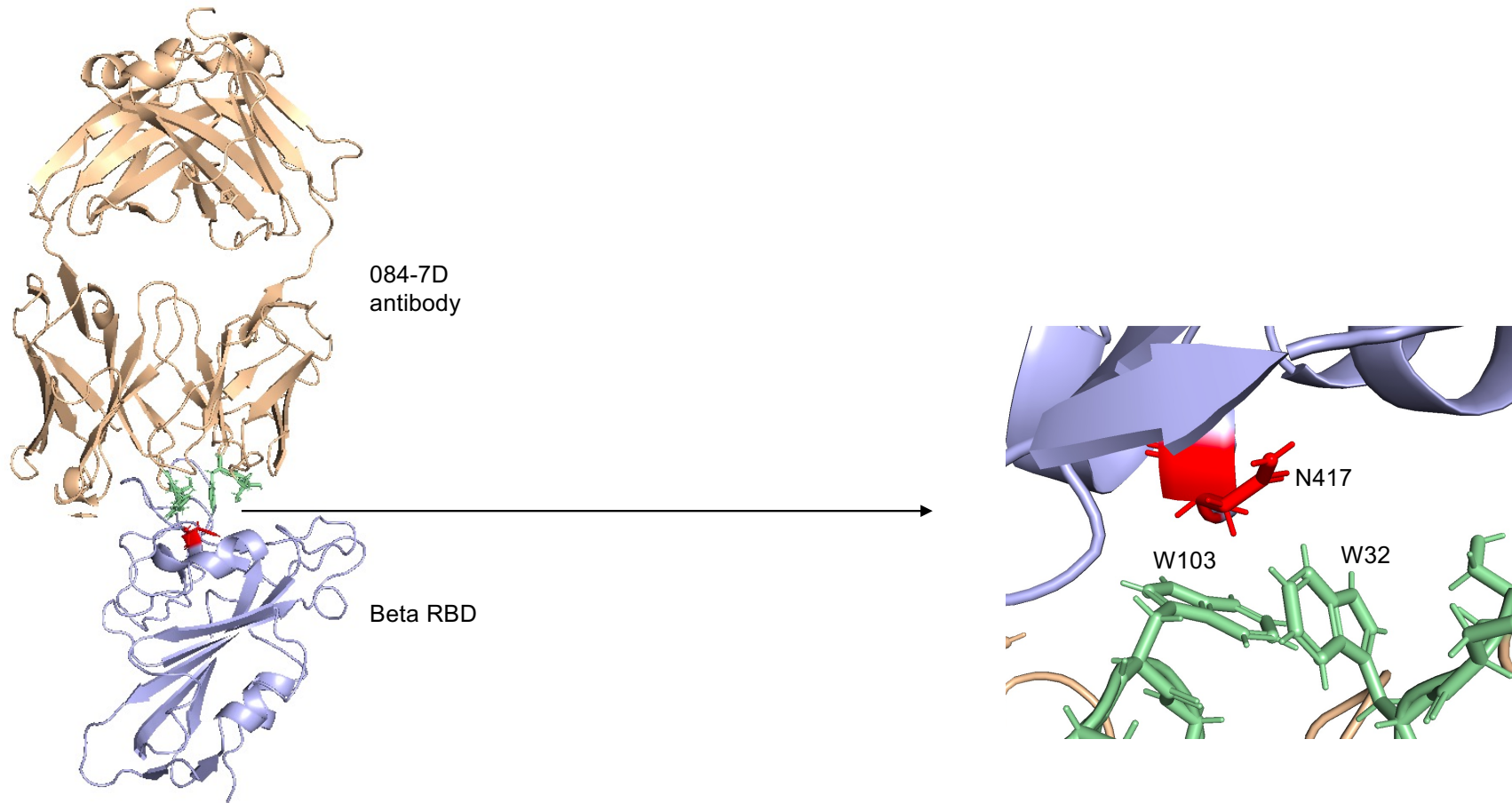




Palesa Masake, Jayde Polley

N417 is still present in new variants

# Structure of 084-7D in complex with Beta RBD



**A**

**mAb 084-7D heavy chain**

mAb ID	Isolated as	Cloned as	Gene usage			V-gene Mutation frequency	CDRH3 length	CDRH3 AA Sequence
			VH	D	J			
084-7D	IgG1, IgA1, IgM	IgG1	3-23*01	2-8*01	1*01	5,9%	13	AKDHPSWGSSFLN

```

1           20           40
VH3-23*01 EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYAMSWVRQA PGKGLEWVSA
VH3-53*01 EVQLVESGGG LIQPGGSLRL SCAASGFTVS SNYMSWVRQA PGKGLEWVSV
CAB-A17-HC DVHLVESGG- LIQPGGSLRL SCAASEFIVS ANYMSWVRQA PGEGLQWVSV
084-7D HC  EVQLLESGGG LVQPGGSLRL SCAASGFSFS SYAMNWRQA PGKGLEWVSA

60           80
VH3-23*01 ISGSGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAK
VH3-53*01 IY-SGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAR
CAB-A17-HC IY-PGGSTFY AESVKGRFTI SRDNSRNTLY LQMNSLRAED TGVYYCAR
084-7D HC  ISGGGDNIYY ADSVKGRFTI SRDNYKNTLH LQMKSLRAED TAVYYCAK
  
```

**B**

**mAb 084-7D light chain**

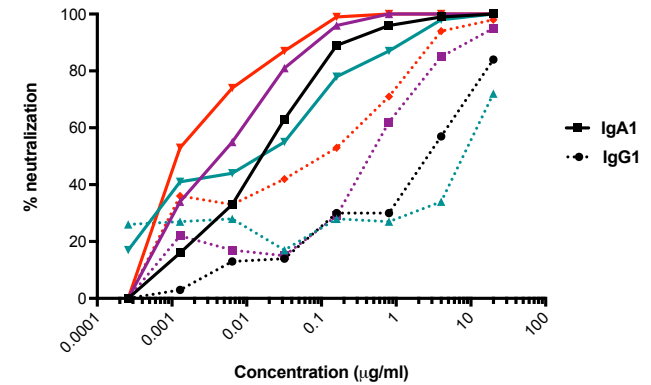
mAb ID	Gene usage		V-gene Mutation frequency	CDRL3 length	CDRL3 AA Sequence
	VK	J			
084-7D	1-5*03	4*01	2,9%	9	QQYNSYSLT

```

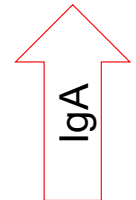
1           20           40
VK1-5*03  DIQMTQSPST LSASVGDRVT ITCRASQSI- SSWLAWYQOK PGKAPKLLIY
VK3-20*01 EIVLTQSPGT LSLSPGERAT LSCRASQSVS SSYLAWYQOK PGQAPRLLIY
CAB-A17-LC EIVLTQSPGT LSLSPGERAS LSCRASQSLS -TYLAWYQOK PGQAPRLLIF
084-7D LC  DIQMTQSPST LSASVGDRVT ITCRASQSI- SSWLAWYQOK PGRAPKLLIY

60           80
VK1-5*03  KASSLESGVP SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNSYS
VK3-20*01 GASSRATGIP DRFSGSGSGT DFTLTISRLQ PEDFAVYYCQ QYGSPP
CAB-A17-LC GASSRASGIP DRFSGGGSGT DFTLTISRLQ PEDFAVYYCQ QYGSPP
084-7D LC  TASNLESGVP SRFSGSGSGT EFSLTISSLQ PDDFATYYCQ QYNSYS
  
```

084-7D IgA vs IgG1

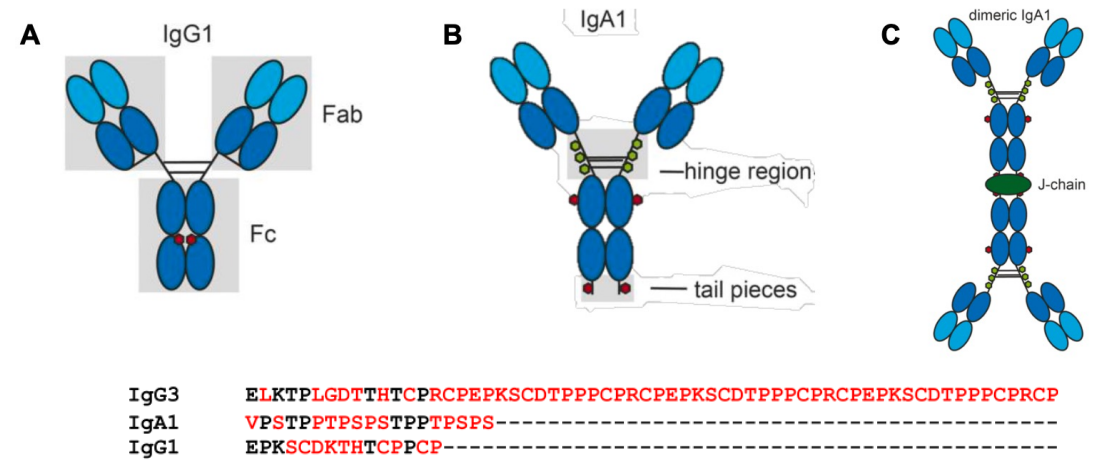
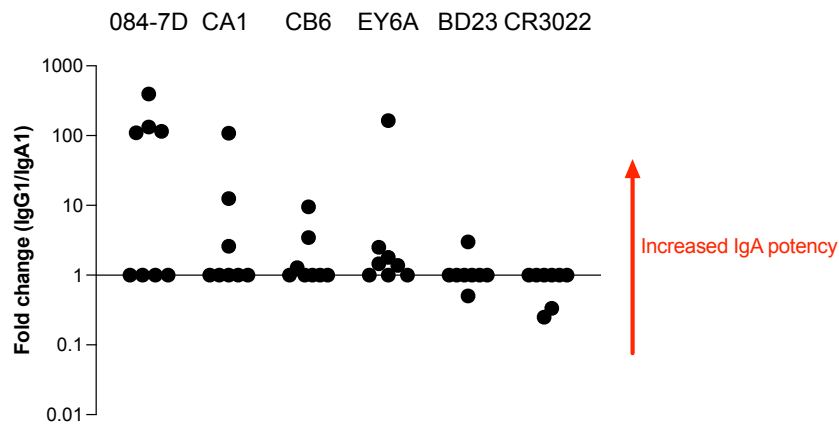


Pseudovirus	084-7D IgG1	084-7D IgA1
Beta	0,11	0,001
BA.1	2,65	0,02
BA.4	0,46	0,004
XBB.1.16	0,35	0,02
PANG-17	7,87	0,02



**IgA version is more potent than the IgG1 version**

# Is IgA more potent in other SARS-CoV-2 mAbs?



CH1 domain appears to play a role in modulating antibody affinity as a consequence of the greater CH1 alpha flexibility, which confers to the IgA a higher kinetically competent form to bind the antigen (Tudor, 2012, PNAS)

Greater hinge region flexibility due to O-linked glycosylation

# Final conclusions

- Structural biology is a key field for both virology and immunology – **without it we would not have a SARS-CoV-2 vaccine**
- Understanding the structure of a protein helps us understand its function and how to manipulate the protein to get a desired effect
- Although there is still no HIV vaccine, major strides have taken place over the last 40 years bringing us closer to this goal
- Structural biology will continue to be a relevant even in the face of the next global pandemic



# Acknowledgements

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## Clinical teams and participants

