The structural biology landscape in South Africa: what role do synchrotrons play in African science?

Dr Thandeka Moyo

National Institute for Communicable Diseases, South Africa

AfLS2020 Virtual Event, 20th November 2020

SYNCHROTRON TECHNIQUES FOR AFRICAN RESEARCH AND TECHNOLOGY

START

diamond

Presentation Overview

1. The importance of structural biology in Africa
2. Structural biology highlights from South African researchers
3. The role of Light Sources in tackling Africa’s HIV disease burden

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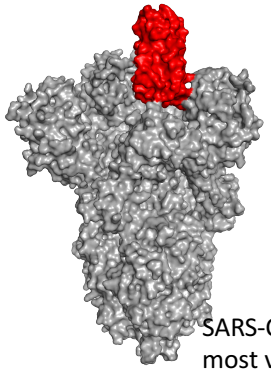
Contribution of Light Sources to Biological and Medical Sciences

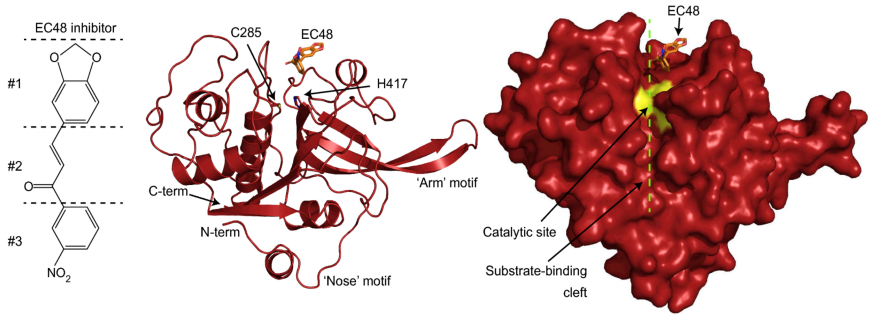
Structural biology helps us understand the **structure and function of macromolecules** including proteins, DNA and RNA

Aids in vaccine design



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

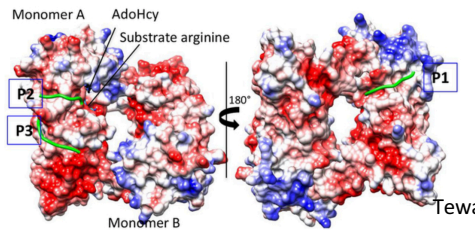
Provides information on protein-inhibitor interactions for drug, herbicide and pesticide design



Malaria protein bound by inhibitor

Machin et al 2019, *Malaria Journal*

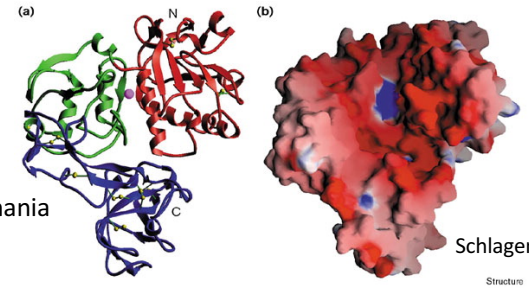
Provides insight into the mechanism of enzymes and is an enabler for industrial enzymology



Active site binding of protein arginine methyltransferases

Tewaryy 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*

Africa is the only continent without a Light Source but may be the continent that needs it the most!

Georges Salloum-Abou-Jaoude

JOURNAL OF SYNCHROTRON RADIATION

ISSN: 1600-5775

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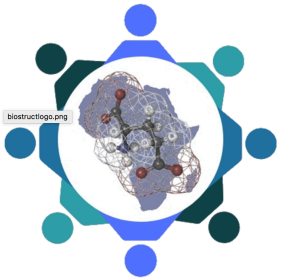
BioStruct-Africa: empowering Africa-based scientists through structural biology knowledge transfer and mentoring – recent advances and future perspectives

Emmanuel Nji,[a,b*] Daouda A. K. Traore,[c,d,e,f‡] Mama Ndi,[a‡] Carolyn A. Joko[g‡] and Declan A. Doyle[h]



BioStruct-Africa

Empowering Africa-based Scientists
through Structural Biology knowledge

Springer Link

Editorial | Published: 15 July 2019

The workshop on "Biophysics and Structural Biology at Synchrotrons" presented at the University of Cape Town from 16–24 January 2019

Bryan Trevor Sewell

COVID-19 Corona Virus
South African Resource Portal

CCP4 Crystallographic School in South Africa

Data Collection to Structure Refinement and Beyond

University of Cape Town, South Africa

~~18 – 26 November 2020~~ TBD in 2021

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GCRF START
Synchrotron Techniques for African Research and Technology

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Anton Hamman

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UNIVERSITEIT STELLENBOSCH UNIVERSITY

Albie van Dijk

Ana Ebrecht

NWU NORTH-WEST UNIVERSITY NOORDWES-UNIVERSITEIT YUNIBESITI YA BOKONE-BOPHIRIMA

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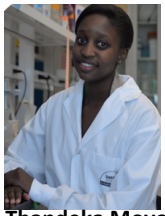
Lynn Morris

Penny Moore



Thandeka Moyo

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division in the National Health Laboratory Service

Trevor Sewell

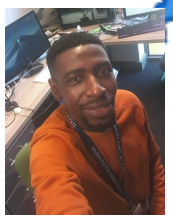
Lauren Arendse

Ed Sturrock

Lizelle Lubbe

Jeremy Woodward

Andani Mulelu



Stanley Makumire

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Dirk Opperman

Carmien Tolmie

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
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Albie van Dijk

Ana Ebrecht

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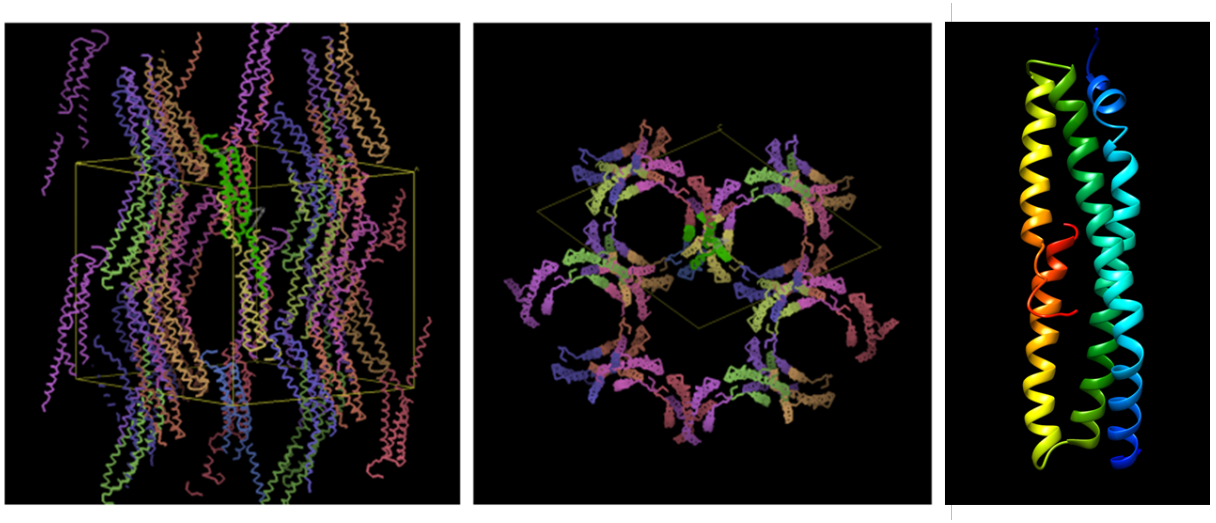
Non-structural protein 4 (NS4) from African horse sickness virus

-NS4 is a key virulence factor → target for a vaccine development
- Function of NS4 in AHSV unclear

Data collection statistics	
Unit cell parameters (a b c, α β γ)	101.084 101.084 113.872 90 90 120
Space group	$P6_522$ or $P6_122$
Resolution range (Å)	∞ – 3.36 (3.40 – 3.36)
Completeness (%)	99.8 (100.0)
$< I/\sigma >$	18.93 (1.37)
R-merge	0.1021 (0.9470)
$CC_{1/2}$	0.93(0.35)



- Data collected for a truncated version of the protein
- Coiled-coil structure
- Analysis of the structure currently underway to predict interaction with DNA and other proteins
- Understanding the protein structure **can shed light on the molecular virulence mechanism and host-virus interaction**

CYTOCHROME P450 REDUCTASE (CPR)

- CPR plays a pivotal role in primary and secondary metabolism of bacteria, plants and animals
-It supplies electrons to enzymes that are vital for the survival of the organism

- The structural characterization of the **CPR helps to understand how this process occurs** opening the possibility to use it as a drug target

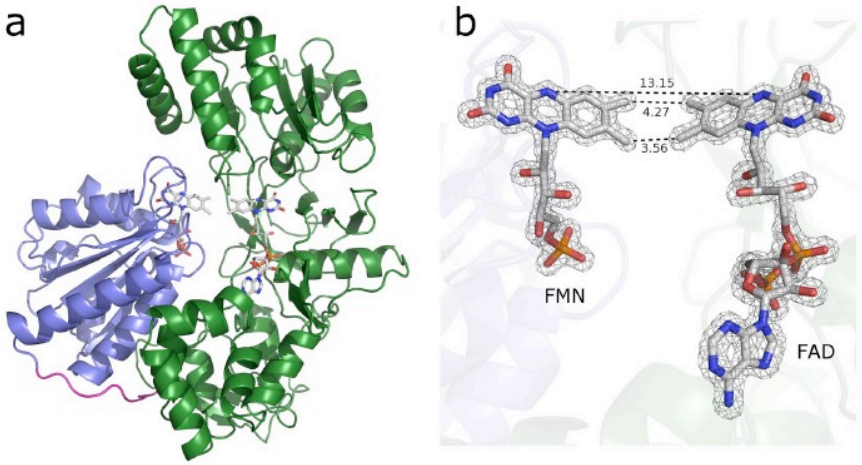
www.nature.com/scientificreports

SCIENTIFIC REPORTS
nature research

OPEN

Biochemical and structural insights into the cytochrome P450 reductase from *Candida tropicalis*

Ana C. Ebrecht[1,3], Naadia van der Bergh[2,3], Susan T. L. Harrison[2,3], Martha S. Smit[1,3], B. Trevor Sewell[4*] & Diederik J. Opperman[1,3*]



Structure solved with and without cofactor (NADPH)

Structure solved in collaboration with the University of Free State and University of Cape Town

Dirk Opperman

Carmien Tolmie

UNIVERSITY OF THE
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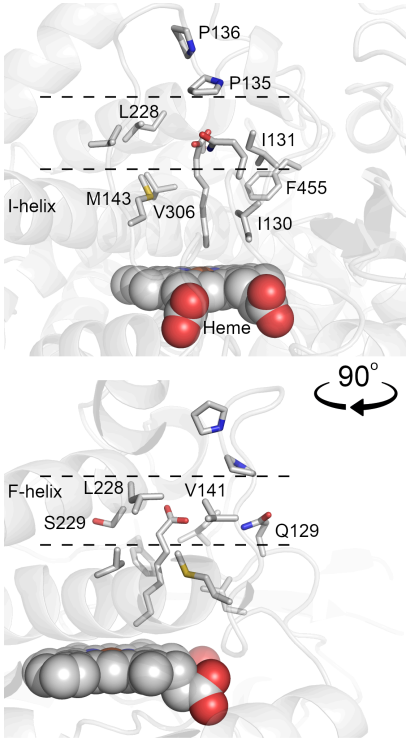
Cytochrome P450 monooxygenases

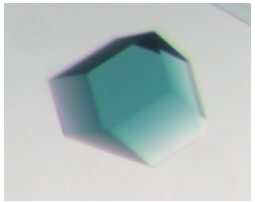
-These proteins are heme-thiolate enzymes that catalyse a range of reactions
-The research focuses on CYPs that perform regioselective hydroxylations of fatty acids and alkanes.

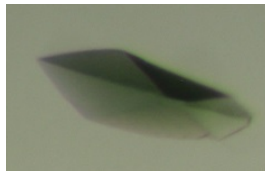


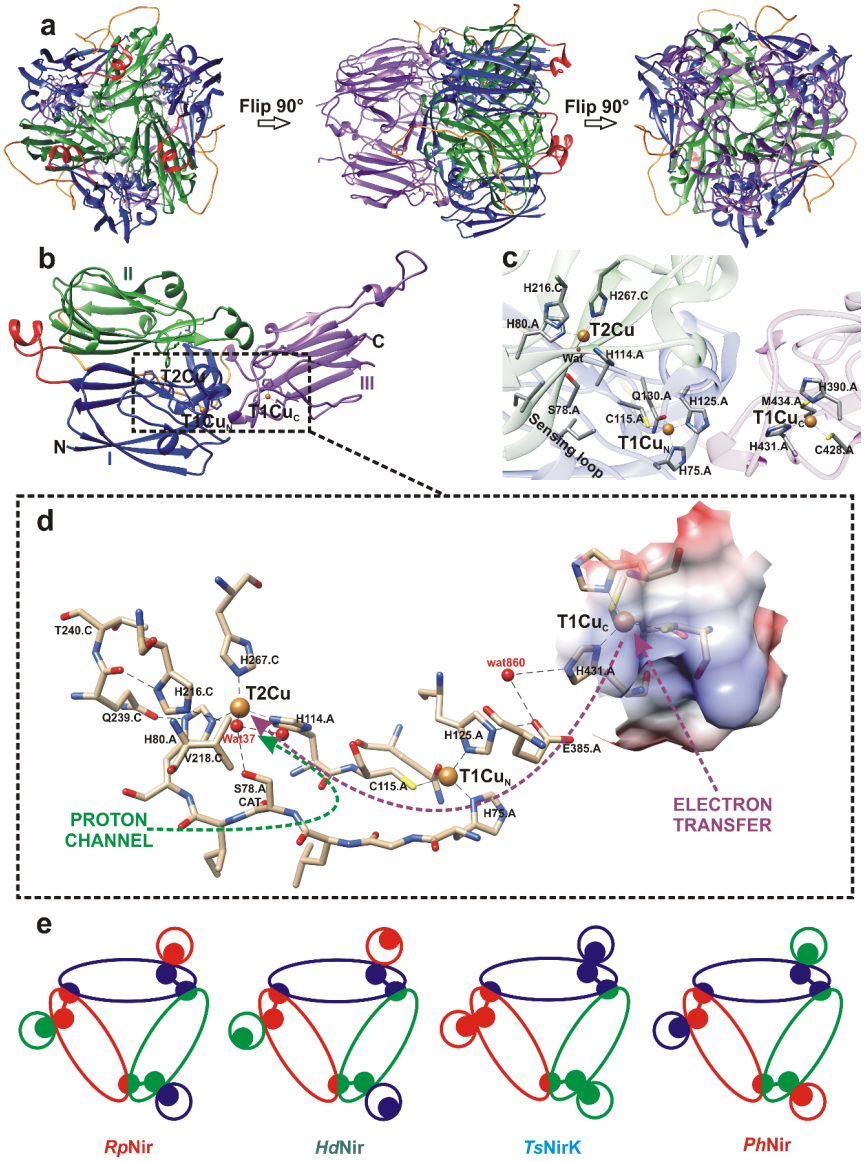
- Using X-ray crystallography, the 3D structure of the CYPs are solved to gain insight into how the **active site determines the regioselectivity** of the enzymes

Nitrite reductases

-Nitrite reductases are key enzymes in the denitrification pathway.
-The copper-containing nitrite reductase from a thermophilic bacterium was solved.
-The structure showed a **unique distribution of domains and subunit interactions** as well as an unusual copper-coordination, which indicates a novel nitrite-reduction mechanism







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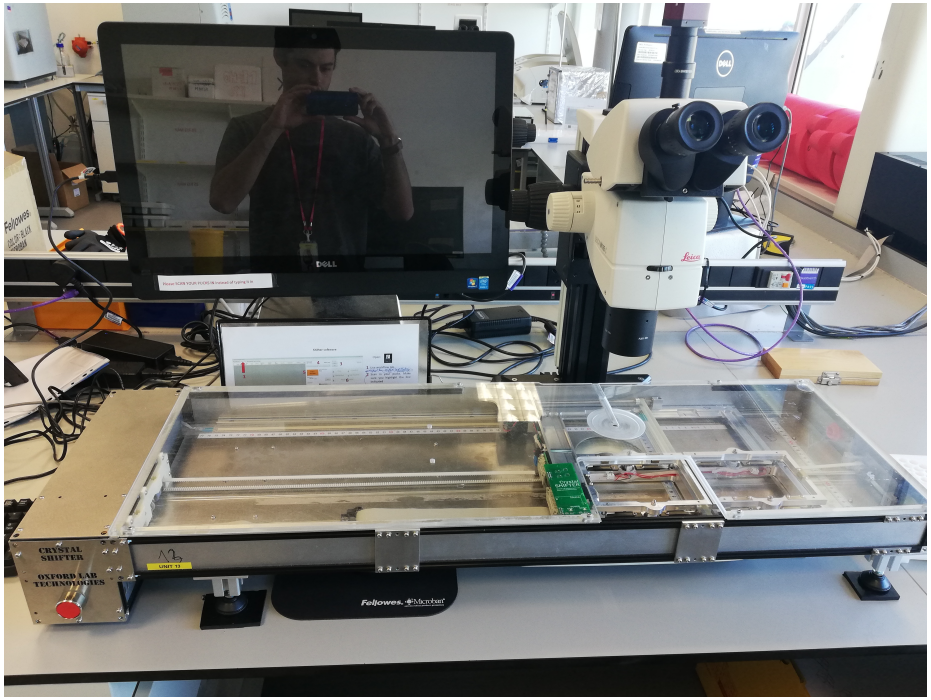
Xchem at the Diamond Light Source to solve a protein structure

-The research focuses on developing antimicrobial inhibitors **against Staphylococcus aureus**.
-XtalShifter is semi-automatic machine allowing fishing of over 100 crystals in less than an hour
-The crystals were soaked with different fragments (inhibitors) prior to fishing.
-Soaked crystals were then put on the beamline to collect diffraction data.



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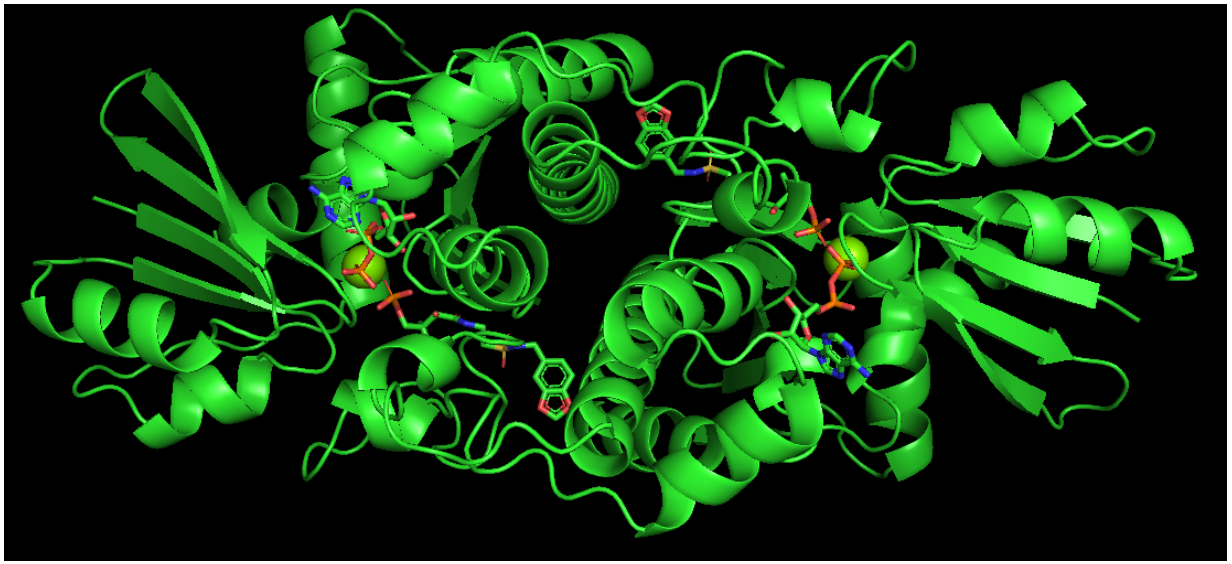
Crystal structure of SaPanK with inhibitor

-Solved crystal structure of the Pantothenate kinase with a bound inhibitor at 1.44 A resolution.



-From this crystal structure, it was determined that the **inhibitor is phosphorylated by the ATP** and subsequently **trapped inside the active site**.

-This gives invaluable information on how the inhibitor interacts with the active site and this knowledge can be use it to **develop improved versions of this inhibitor with better potency**.

Wolf-Dieter Schubert

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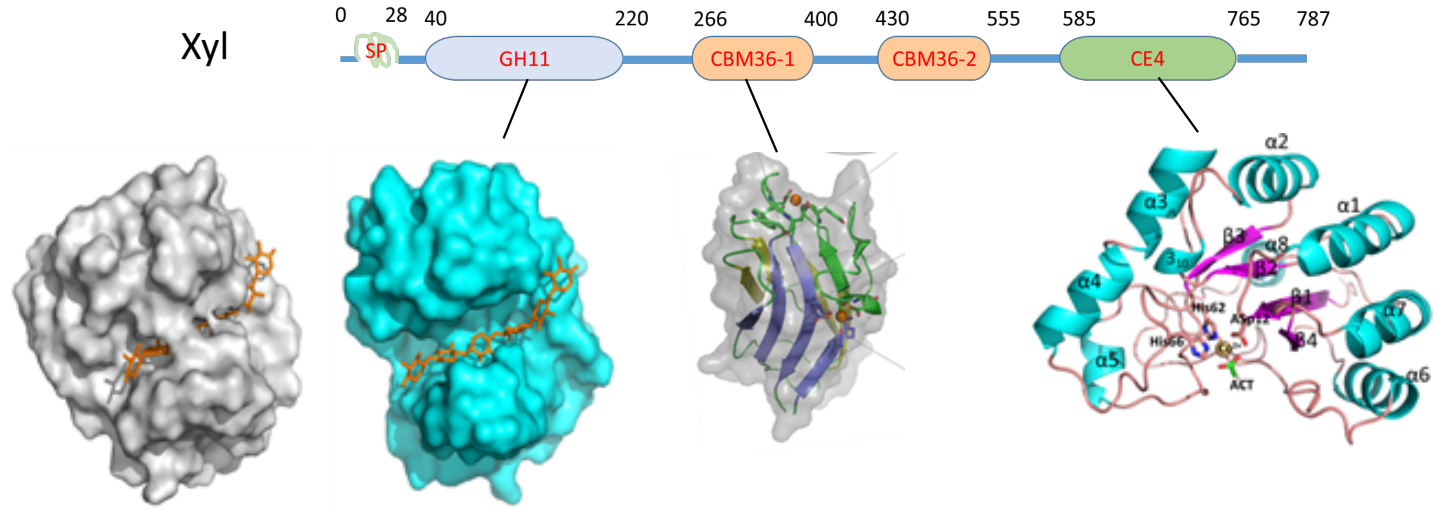
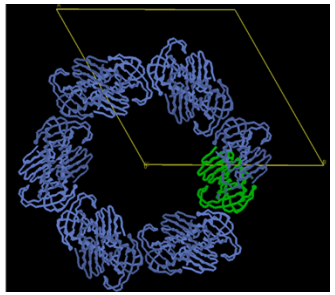
Structural characterization of a multidomain xylanase from a termite metagenome



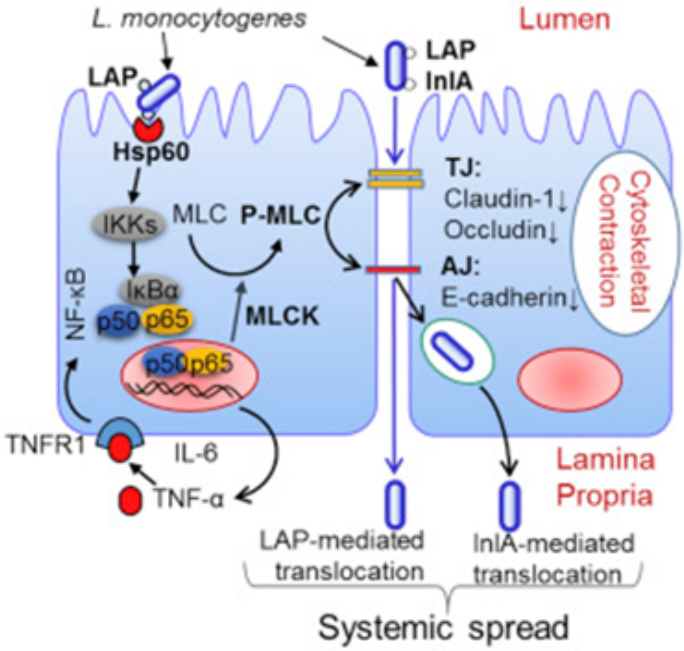


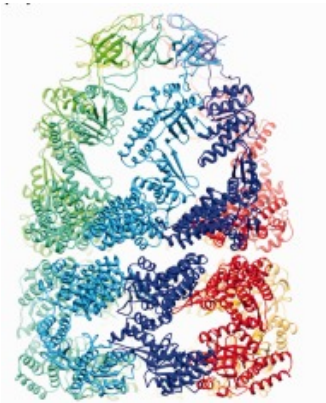
-Individual domains have been analysed structurally and kinetically.

-pH and temperature optima of the two catalytic domains indicate **Xyl to be a mesophilic enzyme** working at neutral pH.

-Despite initial indication of interdependence of domains, data indicate **distinct domains connected by flexible linkers**.

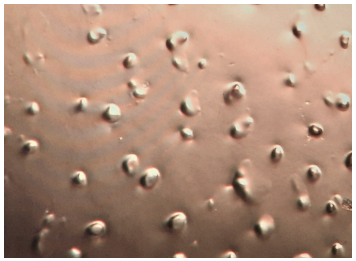
Characterizing the interaction of human heat shock protein 60 (HSP60) with listerial adhesion protein (LAP)



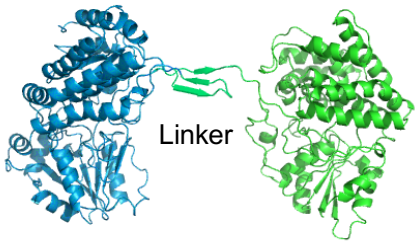


Mitochondrial HSP60 (GroEL)

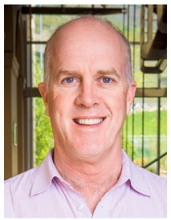
-Both HSP60 and LAP (AdhE) are normally located far away from the cell surface.
-Both proteins have **additional functions in listerial infection**.
-Structure proposes the two proteins are in complex on the surface of epithelial cells.



LAP Microcrystals



Bifunctional alcohol and aldehyde dehydrogenase (AdhE)



Ed Sturrock

Lizelle Lubbe

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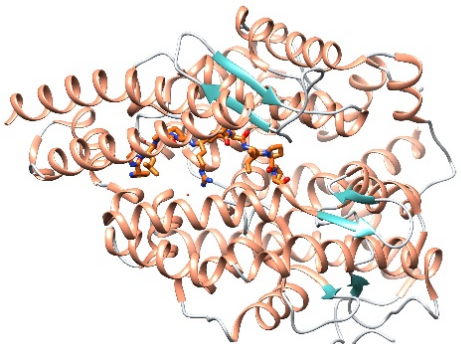
Structural insight into angiotensin converting enzyme function

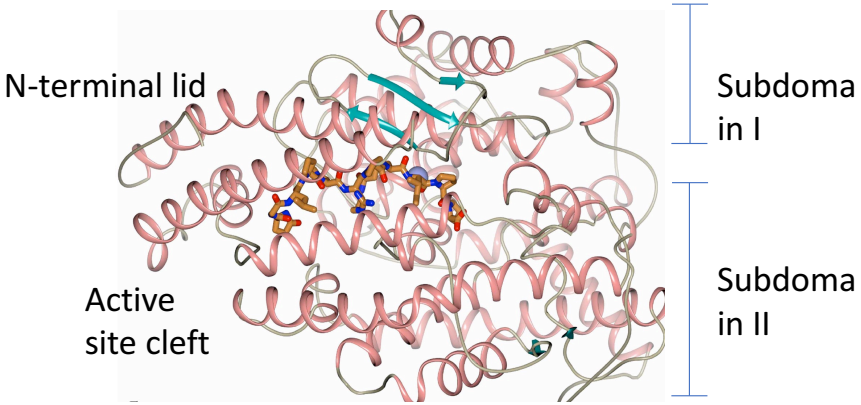
-The enzymatic activity of Angiotensin Converting Enzyme (ACE) causes tightening of blood vessels and a raise in blood pressure

-Large proline-rich peptides (BPPs) found in snake venom cause hypotensive shock of the prey upon envenoming

-The research investigates if the interaction of BPPS with ACE can be be used as a **template for designing antihypertensive drugs**?



C-domain co-crystallized with BPPb



N-domain co-crystallized with BPPb
(PDB ID: 6QS1) 1.8Å resolution

PORTLAND PRESS

Structural basis for the C-domain-selective angiotensin-converting enzyme inhibition by bradykinin-potentiating peptide b (BPPb)

Edward D. Sturrock[1], Lizelle Lubbe[1], Gyles E. Cozier[2], Sylva L.U. Schwager[1], Afolake T. Arowolo[1], Lauren B. Arendse[1], Emma Belcher[1] and K. Ravi Acharya[2]

Mechanism of domain-selective inhibition

Biochemical Journal (2020) **477** 1241–1259
https://doi.org/10.1042/BCJ20200060

PORTLAND PRESS

Research Article

ACE-domain selectivity extends beyond direct interacting residues at the active site

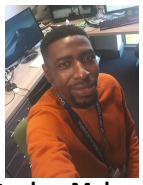
Gyles E. Cozier[1,*], Lizelle Lubbe[2,*], Edward D. Sturrock[2] and K. Ravi Acharya[1]

The 8 unique active site residues affect binding of:

- Ndom inhibitors (33RE, SG6, ketoACE13)
- Cdom inhibitor (BPPb)

-These residues can be **targeted for the design of drug-like domain-selective inhibitors** to treat hypertension and fibrosis (without inducing side-effects)

Trevor Sewell



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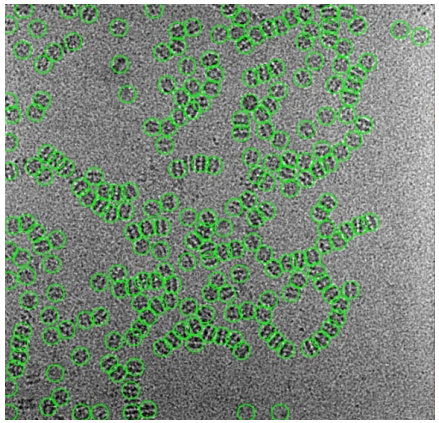
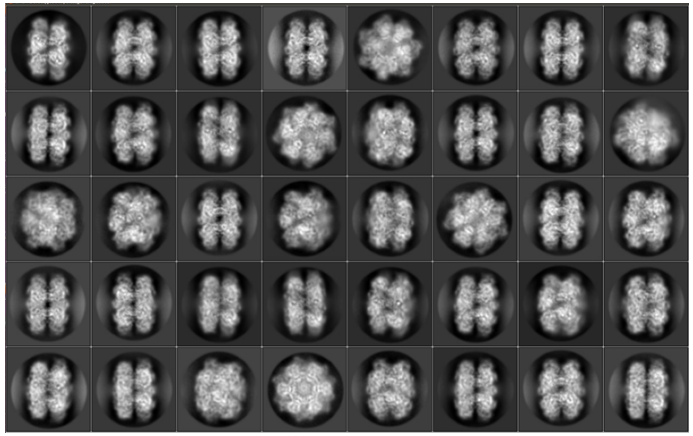
Plasmodium falciparum Glutamine Synthetase

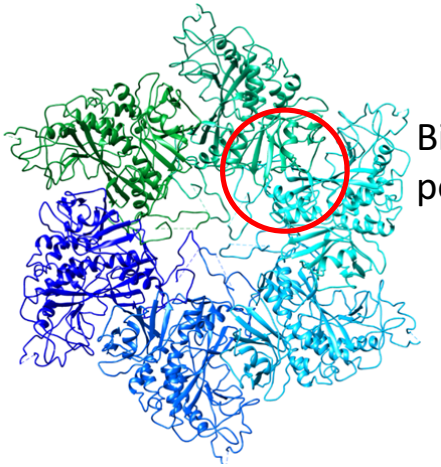
$$Glutamate + ATP + NH_3 \overset{2M^{2+}}{\longleftrightarrow} Glutamine + ADP + P_i$$



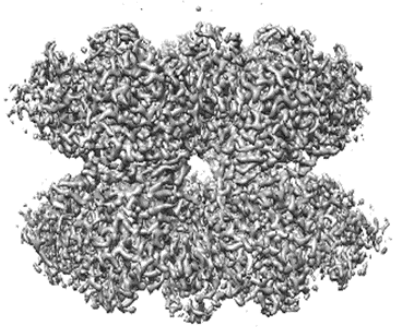
Relion processing



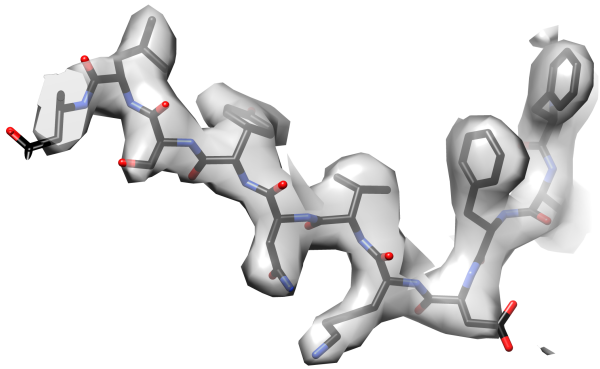
Refined final atomic model

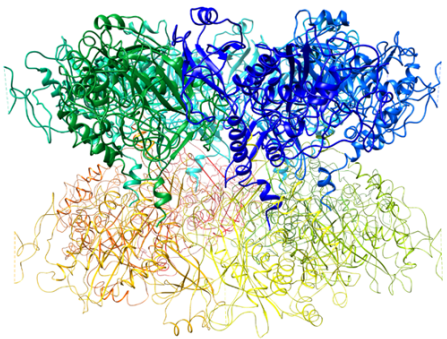


Binding pocket

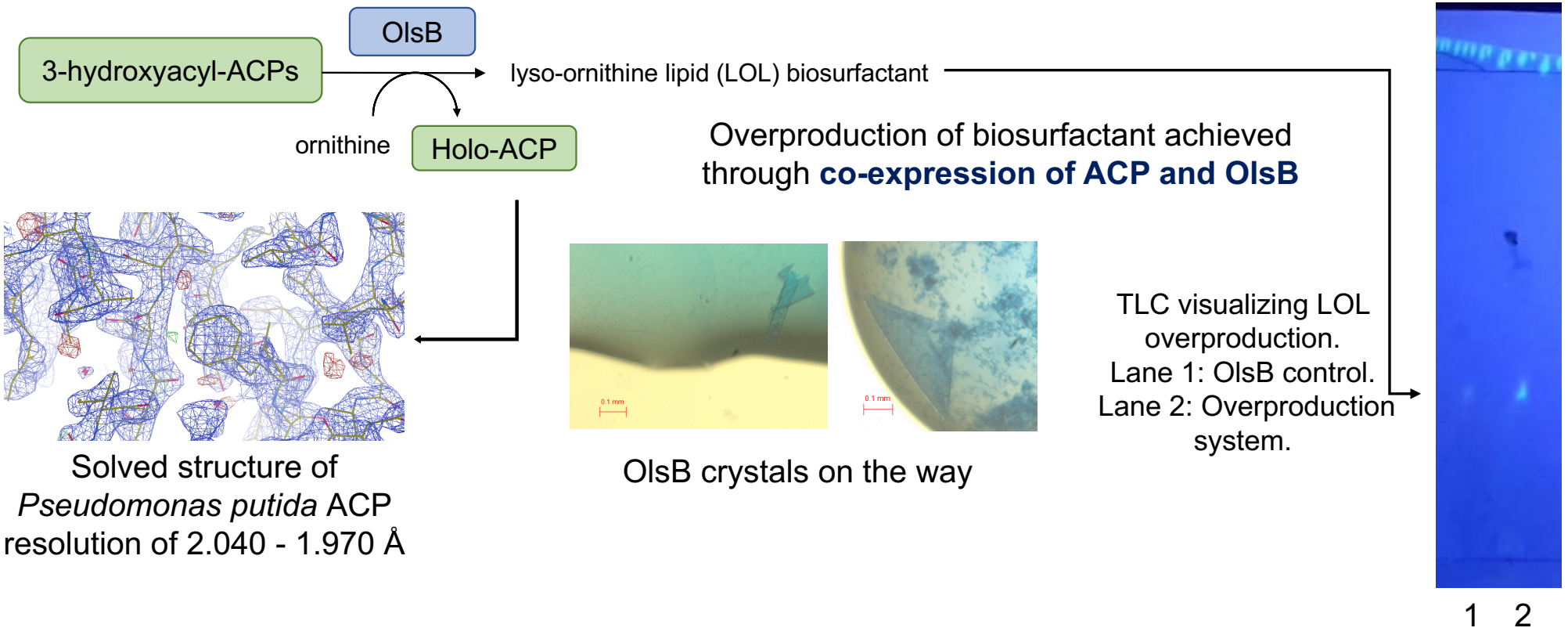


3.2 Å Resolution map!

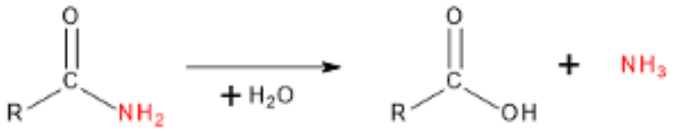


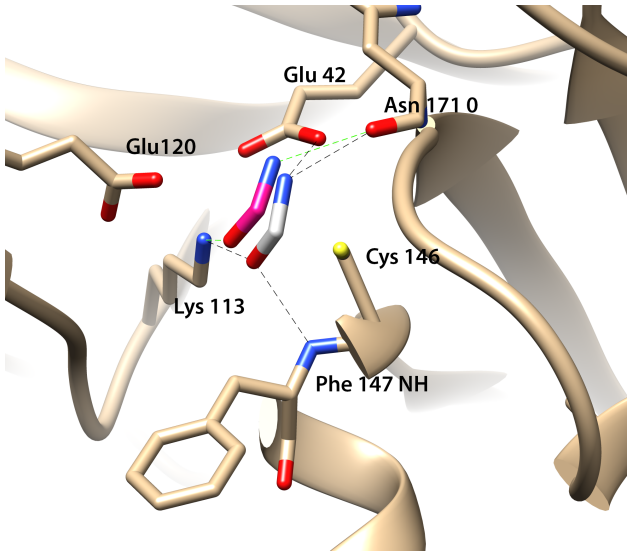


Characterization of a novel ornithine acyl-ACP N-acyltransferase (OlsB) and overexpression of its biosurfactant product

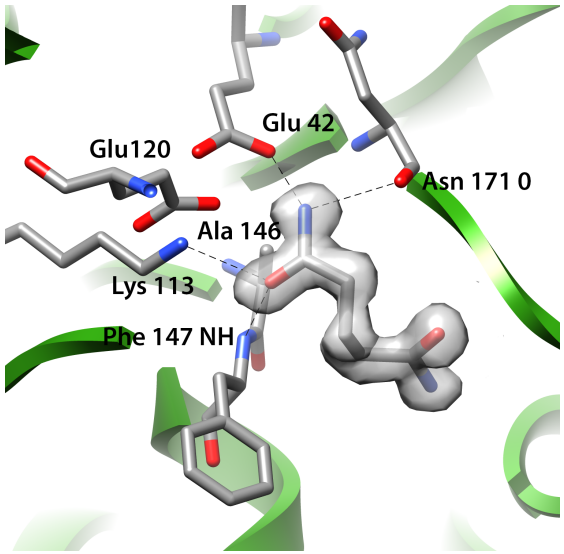


Substrate position and hydrolysis in the amidases

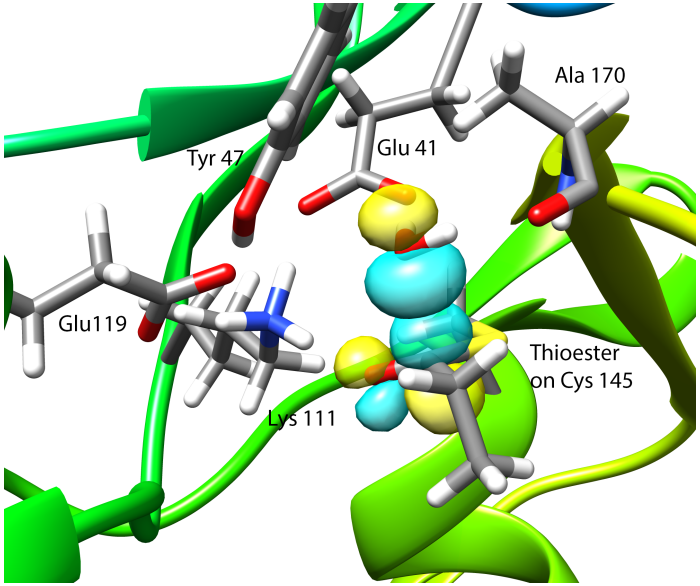




Docking of 14 amides predicts the substrate amide hydrogen bonding in both WT and C146A variants. The involvement of the backbone carbonyl of Asn 171 was unknown.



Crystal structure of glutaramide in the C146A amidase variant **verifies the prediction**. This location leads to the formation of a thioester Intermediate.



Quantum mechanical calculations show orbital overlap with a water positioned by the carboxyl of the active site glutamate and the same backbone carbonyl leading to hydrolysis and product formation.

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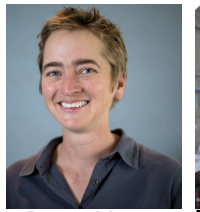
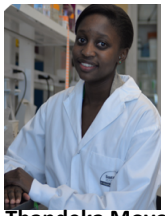
3. The role of Light Sources in tackling Africa’s HIV disease burden

Lynn Morris



Penny Moore



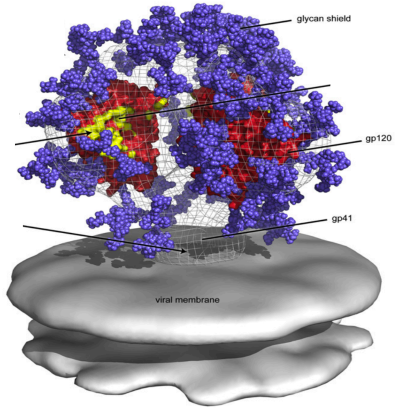
Thandeka Moyo

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division in the National Health Laboratory Service

SYNCHROTRON TECHNIQUES FOR AFRICAN RESEARCH AND TECHNOLOGY
START

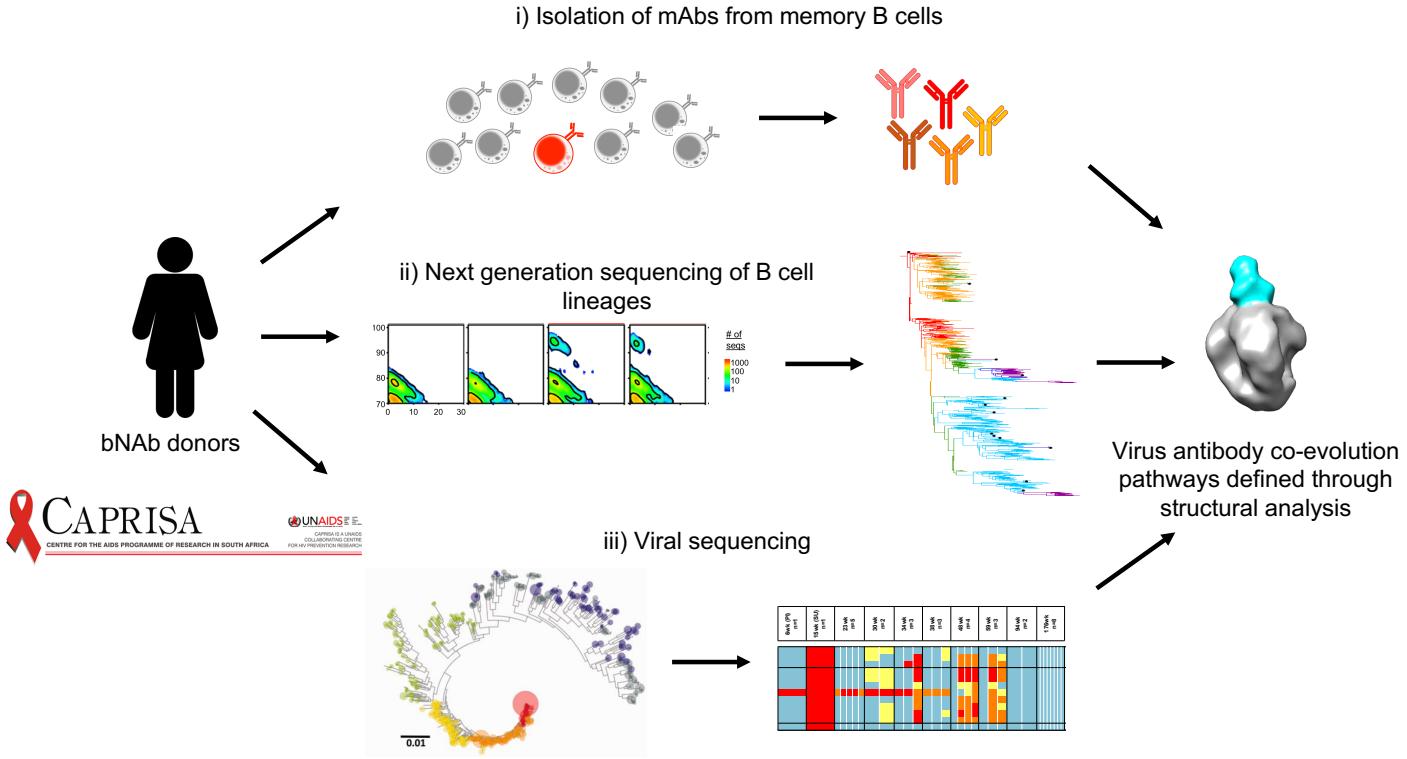
Neutralizing antibodies in HIV vaccine development

- **Broadly neutralizing antibodies (bNAbs)** are of interest in the HIV vaccine field
- These antibodies neutralize various HIV-1 strains and inhibit entry into cells
- Sole target: HIV Envelope



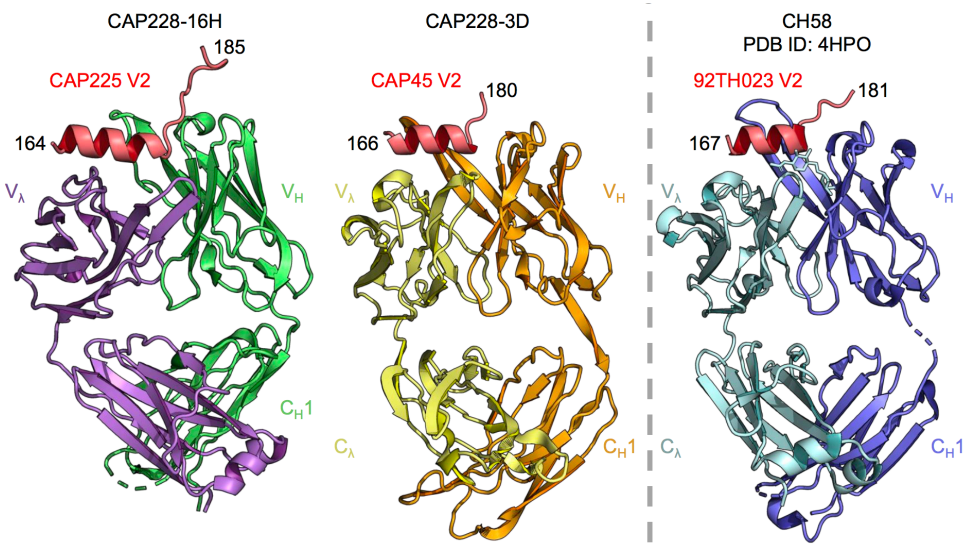
Adapted from Burton et al., 2012

Virus-antibody co-evolution studies



Adapted from Moore, CHIVR, 2018

Crystallization of antibodies in complex with HIV scaffolds and peptides



V1/V2 scaffold subdomain

2.3 Å

V2 peptide

2.6 Å

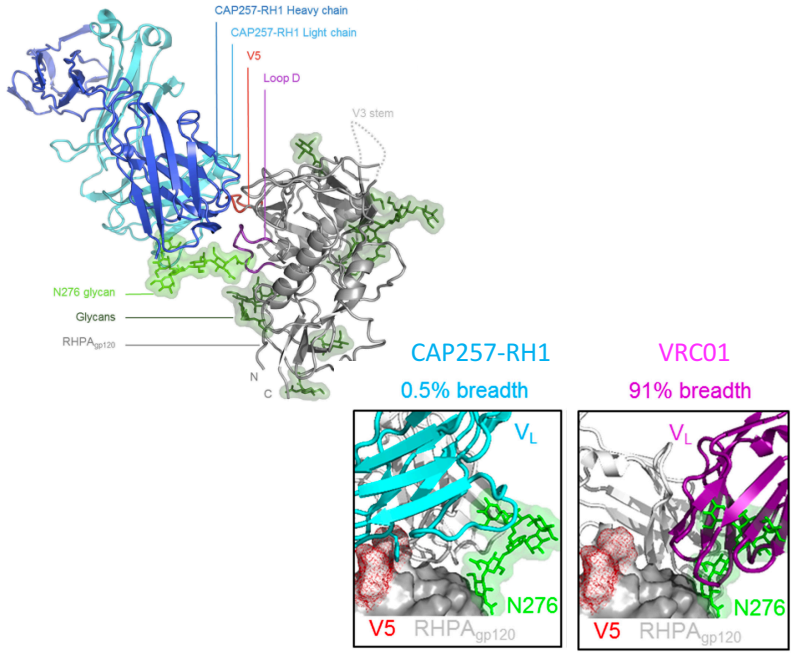
- Co-crystal structures of two antibodies in complex with V1/V2 scaffold and V2 peptide
- These antibodies have **similar target to a vaccine elicited antibody**, CH58

Atomic structure explains why an antibody is narrowly-neutralizing

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

Structure of an N276-Dependent HIV-1 Neutralizing Antibody Targeting a Rare V5 Glycan Hole Adjacent to the CD4 Binding Site

Constantinos Kurt Wibmer,[a,b,c] Jason Gorman,[c] Colin S. Anthony,[d] Nonhlanhla N. Mkhize,[a,b] Aliaksandr Druz,[c] Talita York,[d] Stephen D. Schmidt,[c] Phillip Labuschagne,[e] Mark K. Louder,[c] Robert T. Bailer,[c] Salim S. Abdool Karim,[f,g] John R. Mascola,[c] Carolyn Williamson,[d,f] Penny L. Moore,[a,b,f] Peter D. Kwong,[c] Lynn Morris[a,b,f]



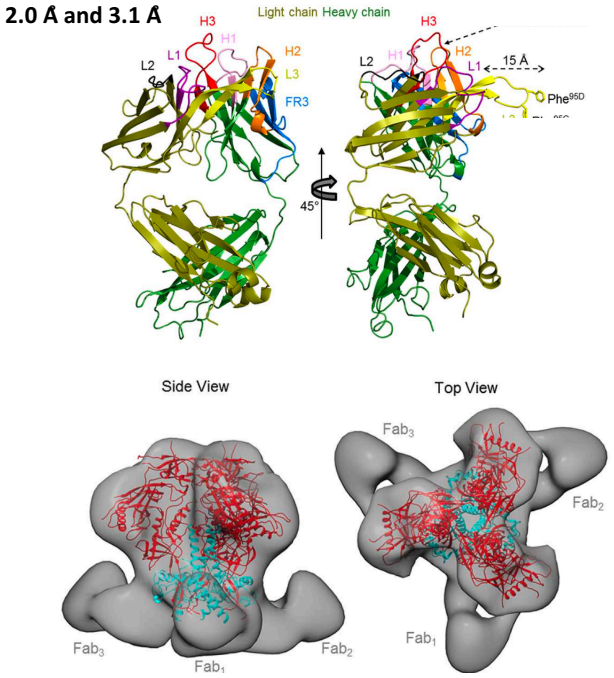
- Isolated a CD4bs-specific antibody, CAP257-RH1
- Narrowly-neutralizing antibody (0.5% breadth)
- Co-crystallization with gp120 revealed **binding angle was incompatible with glycosylated V5 loops** present in almost all HIV strains

Defining a novel antibody binding target

PLOS | PATHOGENS

Structure and Recognition of a Novel HIV-1 gp120-gp41 Interface Antibody that Caused MPER Exposure through Viral Escape

Constantinos Kurt Wibmer[1,2], Jason Gorman[3], Gabriel Ozorowski[4], Jinal N. Bhiman[1,2], Daniel J. Sheward[5], Debra H. Elliott[6], Julie Rouelle[6], Ashley Smira[6], M. Gordon Joyce[3], Nonkululeko Ndabambi[5], Aliaksandr Druz[3], Mangai Asokan[3], Dennis R. Burton[7,8], Mark Connors[9], Salim S. Abdool Karim[10,11], John R. Mascola[3], James E. Robinson[6], Andrew B. Ward[4], Carolyn Williamson[5,10], Peter D. Kwong[3], Lynn Morris[1,2,10]*, Penny L. Moore[1,2,10]*

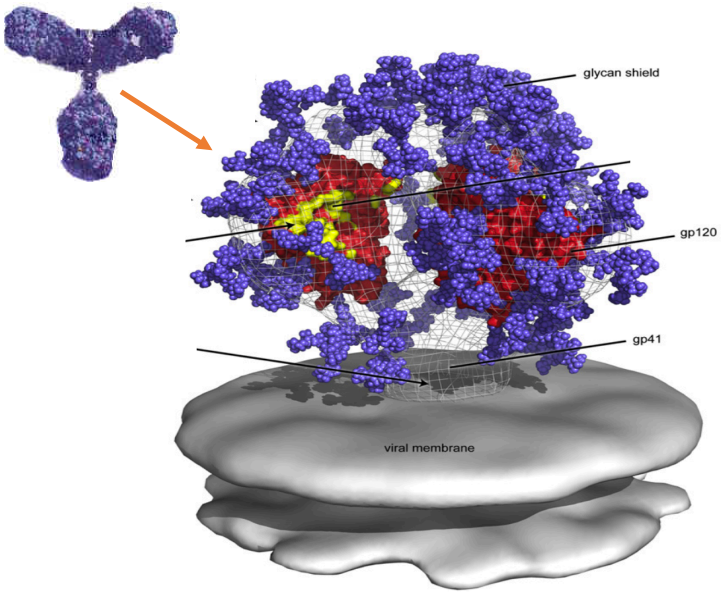


- Isolated the neutralizing monoclonal antibody CAP248-2B
- X-ray crystallography and NS-EM show antibody binds to a **novel target**

Structural characterization of antibody lineages from single donor

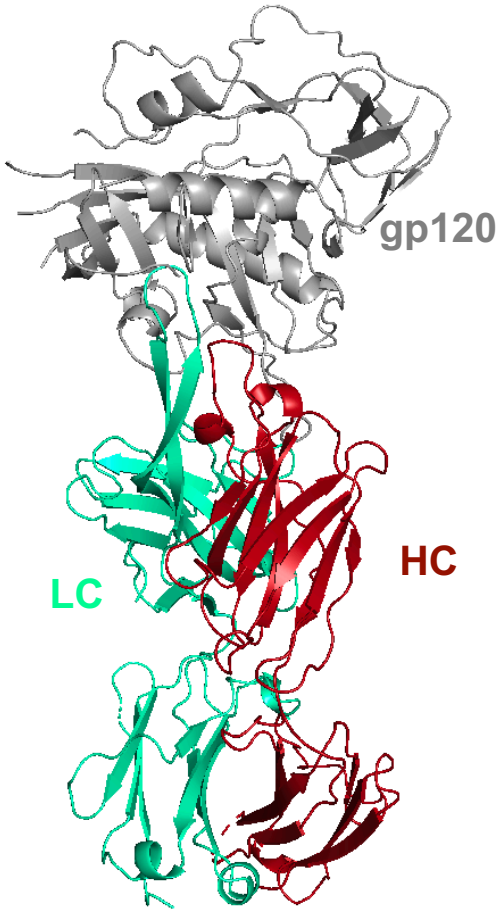
CAP314 – HIV-infected donor who developed bNAbs within 2 years post-infection

Isolated and characterized three antibody lineages

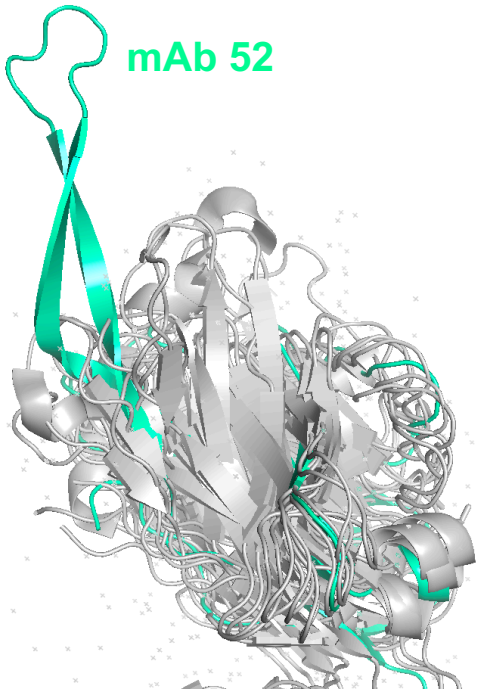


Adapted from Burton et al., 2012

Crystallization of an antibody with an unusually long “binding” arm



29 aa insertion



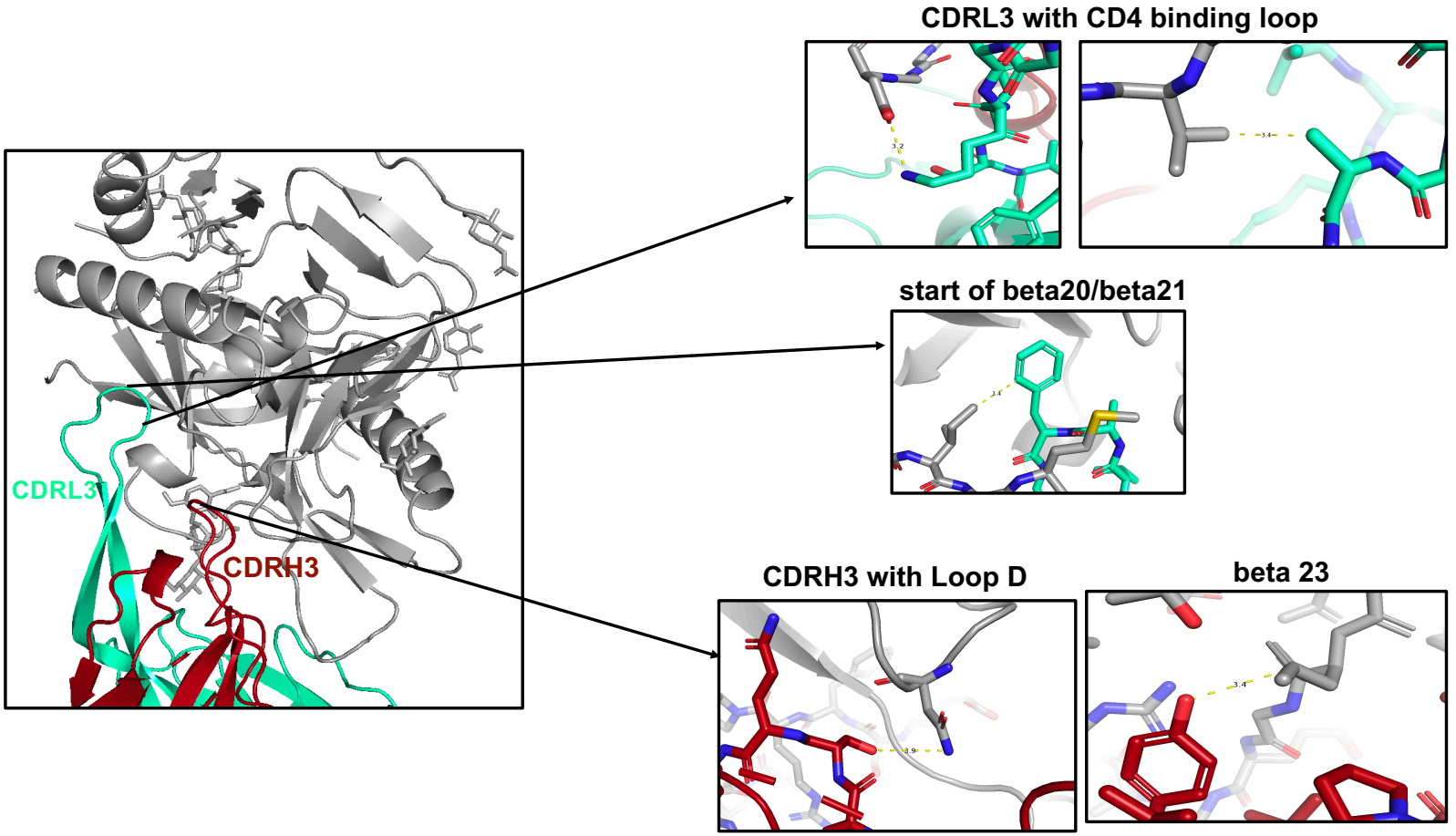
mAb52 light chain aligned to 9 HIV antibodies

Table 1 | Breadth, potency and select characteristics of bnAbs

Epitope	Ab	IC_{50} geometric mean (μg/ml)	Coverage	Number of viruses tested	HC V gene	LC V gene	CDRH3 length (aa)	CDRL3 length (aa)	$V_{HH}J$ + $V_{κH}J$ or $V_{λH}J$ (%nt)	Insertions (+) or deletions (–)	Refs.
V2 apex	VRC26.25	0.002	59%	174	*HV3-30*03*	*λV1-51*02*	38	12	10		22
V2 apex	PCT64-24E	0.911	33%	115	*HV3-15*01*	*κV3-20*01*	25	8	8		25
V2 apex	VRC38.01	0.361	31%	210	*HV3-13*01*	*κV2-28*01*	18	10	13		24
V2 apex	PG9	0.118	84%	200	*HV3-33*05*	*λV2-14*01*	30	10	11		17
V2 apex	PGDM1400	0.017	81%	194	*HV1-8*01*	*κV2-14*01*	34	9	18		12
V2 apex	CH01	1.007	52%	195	*HV3-20*01*	*κV3-20*01*	26	9	13		102
V3 glycan	BG18	0.032	62%	119	*HV4-4*02*	*λV3-25*03*	23	11	18		35
V3 glycan	DH270.1	0.510	42%	179	*HV1-2*02*	*λV2-23*02*	20	10	6		34
V3 glycan	DH270.6	0.151	57%	179	*HV1-2*02*	*λV2-23*02*	20	10	11		34
V3 glycan	PGDM12	0.134	57%	111	*HV3-11*03*	*κV2-24*01*	21	9	16	CDRH1 (-2 aa)	12
V3 glycan	VRC41.01	0.275	53%	107	*HV4-39*07*	*κV3-20*01*	21	9	16		103
V3 glycan	PGDM21	0.139	50%	111	*HV4-34*08*	*κV3-20*01*	20	9	18	CDRH2 (+4 aa)	12,44
V3 glycan	PCDN-33A	0.410	49%	125	*HV4-34*01*	*κV3-20*01*	22	8	11		104
V3 glycan	BF520.1	2.600	44%	34	*HV1-2*02*	*κV3-15*01*	20	11	6		32
V3 glycan	VRC29.03	1.276	28%	179	*HV4-59*08*	*κV3-20*01*	20	9	12	CDRH2 (+6 aa)	31
V3 glycan	PGT121	0.048	66%	200	*HV4-59*01*	*λV3-21*01*	26	12	17	L-FR3 (+3 aa), L-FR1 (-7 aa)	2,17,27
V3 glycan	10-1074	0.039	68%	200	*HV4-59*01*	*λV3-21*01*	26	12	NA	L-FR3 (+3 aa), L-FR1 (-4 aa)	105
CD4bs	N49-P7	0.100	100%	117	*HV1-2*02*	*λV2-11*01*	21	5	19	CDRL1 (-6 aa)	41
CD4bs	N6	0.058	98%	327	*HV1-2*02*	*κV1-33*01*	15	5	25		40
CD4bs	NC-Cow1	0.028	72%	117	*HV1-7*01*	-	62	-	-		53
CD4bs	IOMA	2.320	50%	118	*HV1-2*02*	*λV2-23*02*	19	8	10		52
CD4bs	CH235	5.927	19%	176	*HV1-46*01*	*κV3-15*01*	15	8	6		106
CD4bs	CH235.12	0.650	90%	199	*HV1-46*01*	*κV3-15*01*	15	8	19		51
CD4bs	b12	2.204	47%	200	*HV1-3*01*	*κV3-20*01*	20	9	12		48
CD4bs	VRC01	0.329	91%	196	*HV1-2*02*	*κV3-20*01*	14	5	23	CDRL1 (-3 aa)	61,76
CD4bs	3BNC117	0.097	85%	198	*HV1-2*02*	*κV1-33*01*	12	5	19	H-FR3 (+4 aa), CDRL1 (-4 aa)	56
CD4bs	CH103	0.699	85%	150	*HV4-61*08*	*λV3-1*01*	15	10	15	CDRL1 (-3 aa)	50
Silent face	VRC-PG05	3.714	53%	78	*HV3-7*01*	*κV4-1*01*	19	8	9		15
Interface- FP	VRC34.01	0.310	49%	179	*HV1-2*02*	*κV1-9*01*	15	9	11		59
Interface- FP	ACS202	0.140	44%	81	*HV3-30*03*	*κV1-33*01*	24	9	15	CDRH2 (+1 aa)	60
Interface- FP	PGT151	0.023	72%	200	*HV3-30*03*	*κV2D-29*02*	28	9	16		55
Interface- FP	35O22	0.151	56%	200	*HV1-18*03*	*λV2-14*02*	16	10	22	H-FR3 (+8 aa)	57
Interface- FP	8ANC195	1.115	66%	200	*HV1-3*03*	*κV1-5*03*	22	9	21	CDRH1 (+1 aa), H-FR3 (+4 aa), CDRH2 (-2 aa), HCDRL1 (+1 aa)	56
MPER	DH511.11P	0.674	99%	180	*HV3-15*01*	*κV1-39*01*	23	11	15		65
MPER	4E10	1.765	98%	200	*HV1-69*17*	*κV3-20*01*	20	9	10		107
MPER	10E8	0.299	98%	199	*HV3-15*05*	*λV3-19*01*	22	12	17		57

Neutralization breadth and potency were captured mainly from the tool CATNAP[100] or, in some cases, the primary manuscript (far right column). Overall nucleotide mutation (%nt) was calculated as the mutation frequency given the genes encoding the heavy-chain variable (V) region and joining (J) region (HC V gene), as well as those encoding the light-chain V and J regions (LC V gene). The neutralization IC_{50} values reported are Ab inhibitory concentrations that reduce viral infectivity by 50% relative to neutralization in the absence of Ab (measured on TZM-bl cells). Geometric mean neutralization IC_{50} was calculated by exclusion of resistant viruses. Neutralization breadth was calculated on the basis of a neutralization IC_{50} cut-off of between 10 μg/ml and 50 μg/ml.

Novel mode of binding to HIV CD4 binding site



Conclusion

- The future of African structural biology is bright
- Even without own our light source we are able to generate invaluable data to help solve African problems
- The biggest challenge we currently face is a lack of funding and expertise in the field
- With initiatives such as START and other collaborations we are able to overcome many of these challenges and grow structural biology capacity

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- Jean-Philippe Julien, Canada
- Peter Kwong - USA
- Andrew Ward –USA

CAPRISA participants

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diamond

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SYNCHROTRON TECHNIQUES FOR AFRICAN RESEARCH AND TECHNOLOGY START