
SYNCHROTRON TECHNIQUES FOR AFRICAN RESEARCH AND TECHNOLOGY
START
South African Structural Biology
The relative importance of the different available technologies
GCRF
Global Challenges Research Fund
UKRI
Science and Technology Facilities Council
diamond

Structural biology is in its infancy in South Africa

Fewer than a dozen PIs are currently active in the field
Fewer than 10 labs in which protein can be prepared for for structural work
Four “protein capable” ”home-source” X-ray diffractometers
One cryoEM (>30 yr old platform) with new direct electron detector
No “protein capable” NMR spectrometers
Four Universities in which there are Biochemistry courses in which Structural Biology is taught.
Limited local research funding for Structural Biology

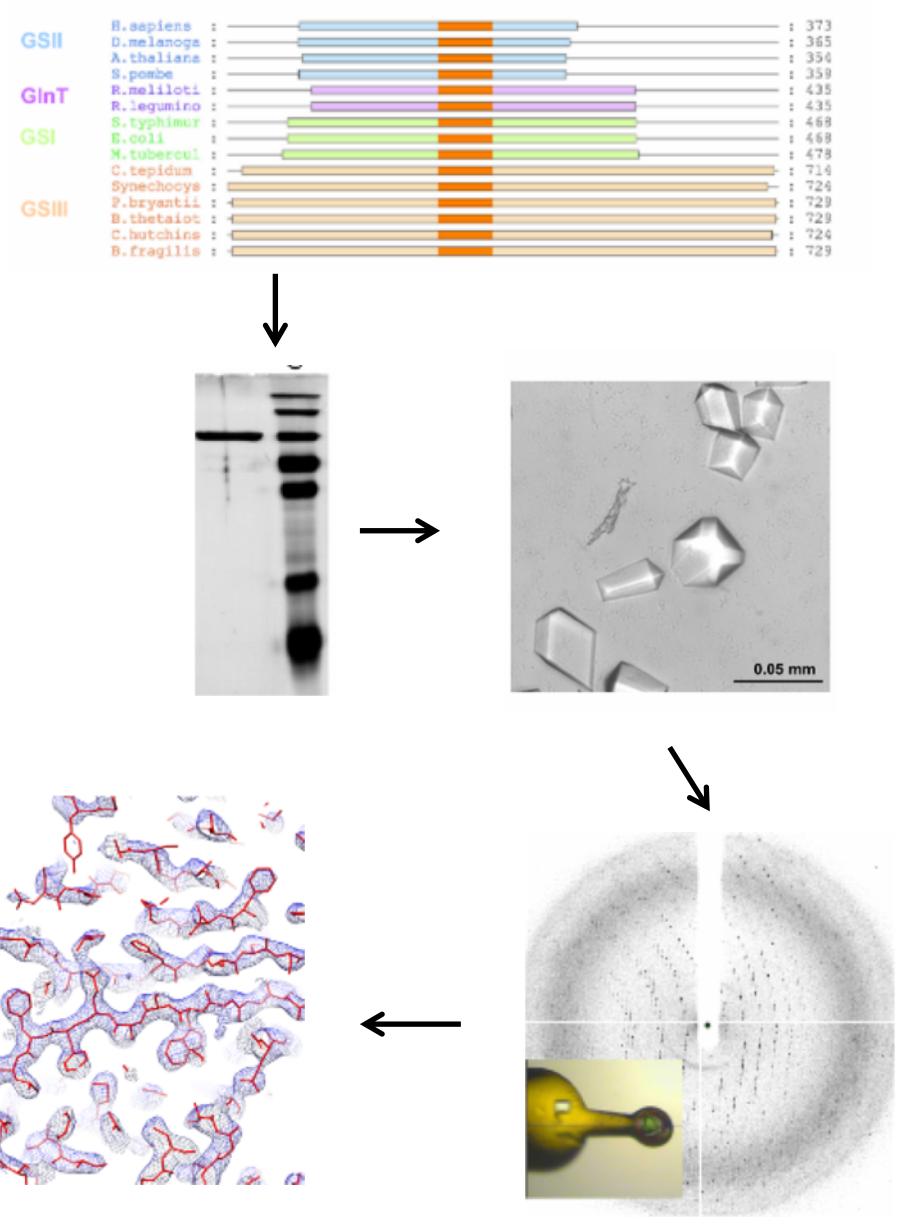
The situation in the rest of Africa is considerably worse. Only in Egypt have students returning from abroad been able to establish themselves in their home countries.

Structural Biology in Africa is a major development opportunity

SYNCHROTRON TECHNIQUES FOR AFRICAN RESEARCH AND TECHNOLOGY
START

Structural biology … pipeline

1. Sample selection
2. Soluble expression
3. Purification
4. (Crystallization)
5. Data collection
6. Data processing
7. Interpretation
8. Publication



What problems faced scientists wanting to solve protein structures in South Africa?

No existing community
Lack of appreciation of the value of structures
No supportive funding environment

In 1990 South Africa was isolated from all this.

START created a UK-Africa partnership to develop a programme of world class research based around energy materials (strand 1) and protein structure determination (strand 2).

The grant funded:

- Extended training in Synchrotron techniques - The nexus of START is Diamond Light Source, the UK synchrotron.
- Researchers who studied emerging and neglected diseases of direct importance to the African continent through an extensive programme of structural biology.

diamond

GCRF
Global Challenges Research Fund

UKRI Science and Technology Facilities Council

Reminder of motivation for hosting START?

- Interesting science and global collaboration
- A desire to open up synchrotron techniques to researchers who find access difficult
- Underpinning training to support an African Light Source
    - Established Researchers
    - The next generation

- Lab visits and lectures to larger groups
- Individual focused secondments
- A two-way process – PDRA's in UK and Africa working together
- Building a network of UK and African researchers with the synchrotron as the central hub. Strengthening links across the board.
- Training opportunities – good for African and UK researchers

The success of START was due to pre-existing infrastructure built by 9 co-investigators at 7 institutions

Trevor Sewell
University of Cape Town

Ed Sturrock
University of Cape Town

Jeremy Woodward
University of Cape Town

Wolf-Dieter Schubert
University of Pretoria

Yasien Sayed
University of the Witwatersrand

Dirk Opperman
University of the Free State

Erick Strauss
Stellenbosch University

Lynn Morris
National Inst for Communicable Diseases

Albie van Dijk
North West University

The START program has given rise to spectacular growth in the last three years

Anton Hamman

Erick Strauss

UNIVERSITEIT STELLENBOSCH UNIVERSITY

Albie van Dijk

Ana Ebrecht

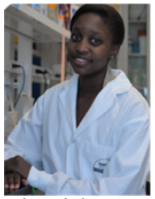
NWU NORTH-WEST UNIVERSITY NOORDWES-UNIVERSITEIT YUNIBESITI YA BOKONE-BOPHIRIMA

Wolf-Dieter Schubert

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Lynn Morris

Penny Moore



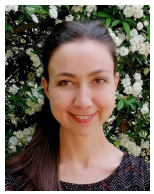
Thandeka Moyo

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

Trevor Sewell

Jeremy Woodward

Ed Sturrock

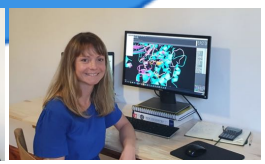


Lizelle Lubbe

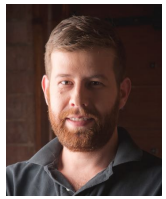
UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

Stanley Makumire

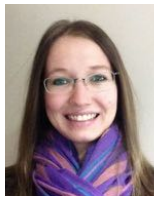
Andani Mulelu



Lauren Arendse



Dirk Opperman



Carmien Tolmie

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

UFS UV

SYNCHROTRON TECHNIQUES FOR AFRICAN RESEARCH AND TECHNOLOGY
START

What was funded for three years:

- Nine Research Assistants (post-docs), including a research budget and travel costs
- A local resource centre in Cape Town
- Two posts in the resource centre
- Annual workshops in South Africa
- Annual meetings in South Africa
- Travel between Diamond and South Africa for both South African and British participants

Post docs

Ana Ebrecht
University of North West

Ramesh Pandian
University of the Witwatersrand

Stanley Makumire
Lauren Arendse
Andani Mulelu
Lizelle Lubbe
University of Cape Town

Thandeka Moyo
NICD

Rodolpho do Aido Machado
Carmien Tolmie
University of the Free State

Blake Balcomb
Anton Hamann
University of Stellenbosch

Extension

Paul Kappo
University of Zululand

Kevin Naidoo
University of Cape Town

Anwar Jardine
University of Cape Town

Brandon Weber
University of Cape Town

Penny Moore
NICD

Nigel Makoah
UFS

Portia Maumela
Rhodes University

BIOPHYSICS & STRUCTURAL BIOLOGY AT SYNCHROTRONS

17 - 24 JANUARY 2019

University of Cape Town

CAPE TOWN

Western Cape, South Africa

Introducing bioscientists to synchrotron-based facilities, focusing on the structure determination and other biophysical resources required for vaccine design, drug discovery, industrial enzymology and agrochemicals.

The course will trace the technology required to go from gene to protein structure, as well as synchrotron based techniques for imaging cells. Topics covered will include advanced strategies for crystallization, high-throughput data collection by X-ray diffraction, single particle cryo-EM, structure refinement, X-ray tomography, circular dichroism and spectroscopy. Students will learn how to access synchrotron based resources and will get practical experience of working with proteins, data collection/processing, interpretation & complex experimental strategies, with time reserved for students to collect their own data using remote access of an MX beamline at the Diamond Light Source.

BURSARIES AVAILABLE

Post-doctoral fellows, emerging scientists and post-graduate students may apply for partial or full-cost bursaries to attend the workshop.

For more information
www.**biophysicsworkshop**.co.za

PRESENTERS

Gwyndaf Evans, Diamond Light Source
Margot Frangakis, Goethe University Frankfurt
Elspeth Garman, University of Oxford
Richard Garratt, University of São Paulo
Lars-Oliver Kautshor, Zeiss Microscopy
Michael Lawrence, WEHIMR
Barend H. Lich, Thermo Fisher Scientific
Sylvia Onesti, Elettra Sincrotrone Trieste
Eva Pereiro, ALBA Synchrotron
Helen Saibil, Birkbeck University of London
Wolf-Dieter Schubert, University of Pretoria
Frances Separovic, University of Melbourne
Trevor Sewell, University of Cape Town
Ramaswamy Subramanian, inStem
Frank von Delft, University of Oxford
Bonnie Wallace, Birkbeck University of London
Jeremy Woodward, University of Cape Town

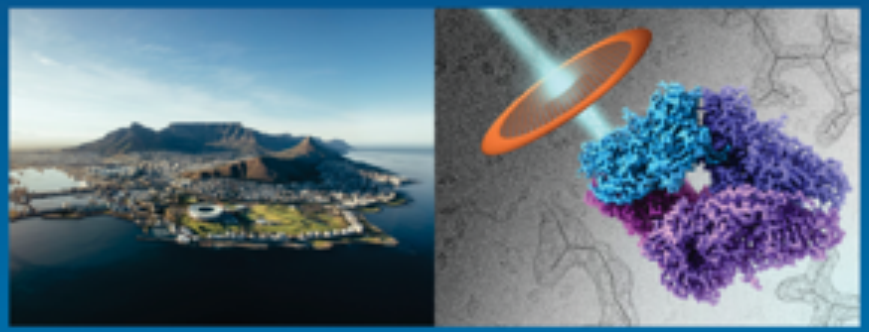
IUPAB

SOUTH AFRICAN INSTITUTE OF PHYSICS

Cryo-EM

MSSA pre-conference workshop

29th-30th November 2019 | University of Cape Town



We welcome PhDs, Postdocs and young scientists new to the field of single-particle cryo-EM to join us for a 2-day hands-on workshop at the Electron Microscopy Unit, University of Cape Town.

The workshop includes theoretical and practical aspects of single particle cryo-EM and screening by negative staining. Participants will receive hands-on sample preparation, imaging and processing training from Electron Microscopy Unit staff and Dr Eaazhisai Kandiah (ESRF).

Organised in conjunction with the Microscopy Society of Southern Africa conference (https://www.mssaconference.co.za/) by the European Synchrotron Radiation Facility in Grenoble and the Electron Microscope Unit, University of Cape Town.

For registration or enquiries, please contact: jeremy.woodward@uct.ac.za
A limited number of participants will be selected on the basis of a brief motivation.

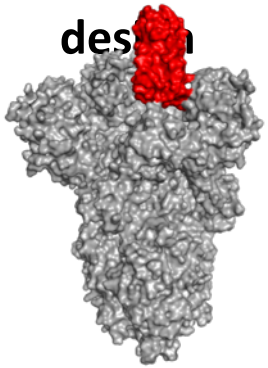
Application deadline: 30th October

Generously sponsored by:

Contributions of Light Sources to Biomedical science

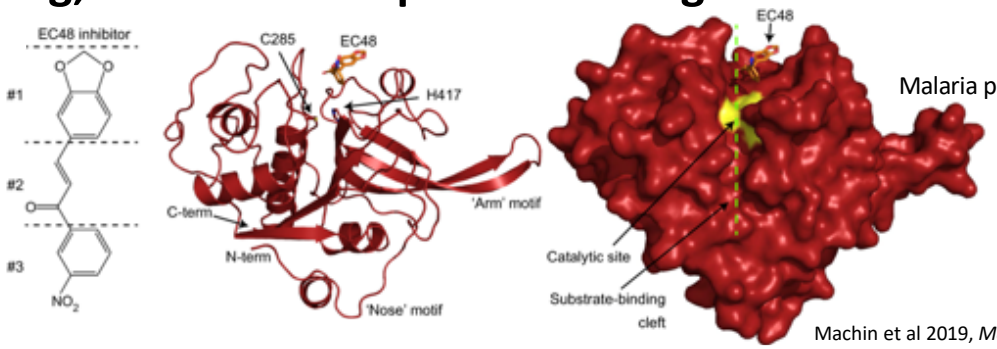
Structural biology helps us understand the **structure and function of macromolecules** including proteins, DNA and RNA

Aids in vaccine design



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

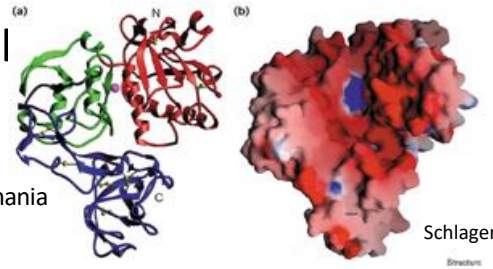
Provides information on protein-inhibitor interactions for drug, herbicide and pesticide design



Malaria protein bound by inhibitor

Machin et al 2019, *Malaria Journal*

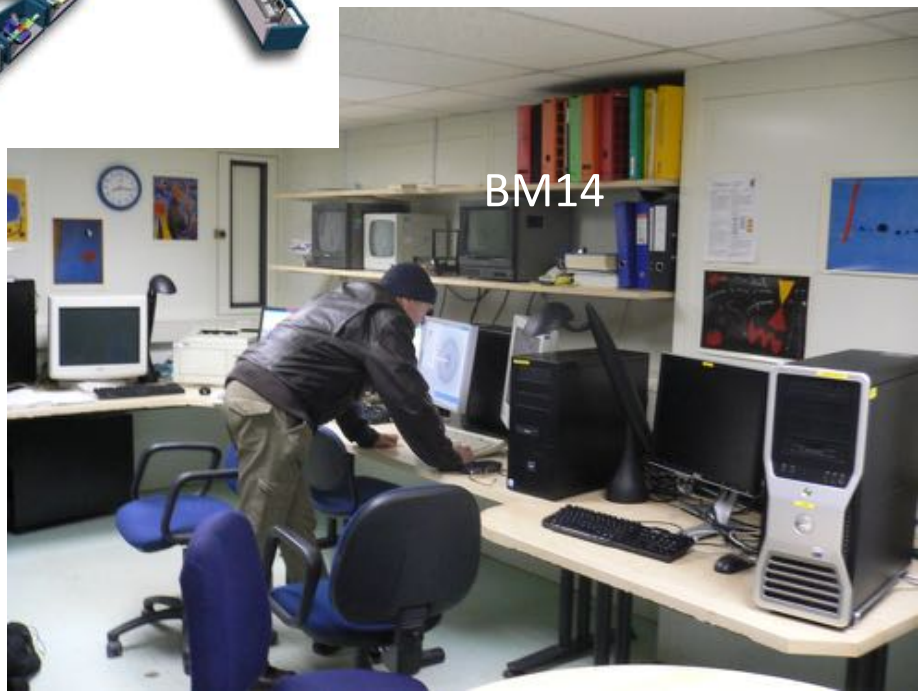
Reveal the structure and therefore vulnerable regions of proteins from p



Structure of the main leishmania surface antigen

Schlagenhauf et al, 1998, *Structure*

Synchrotron access was provided via an MoU with EMBL Grenoble

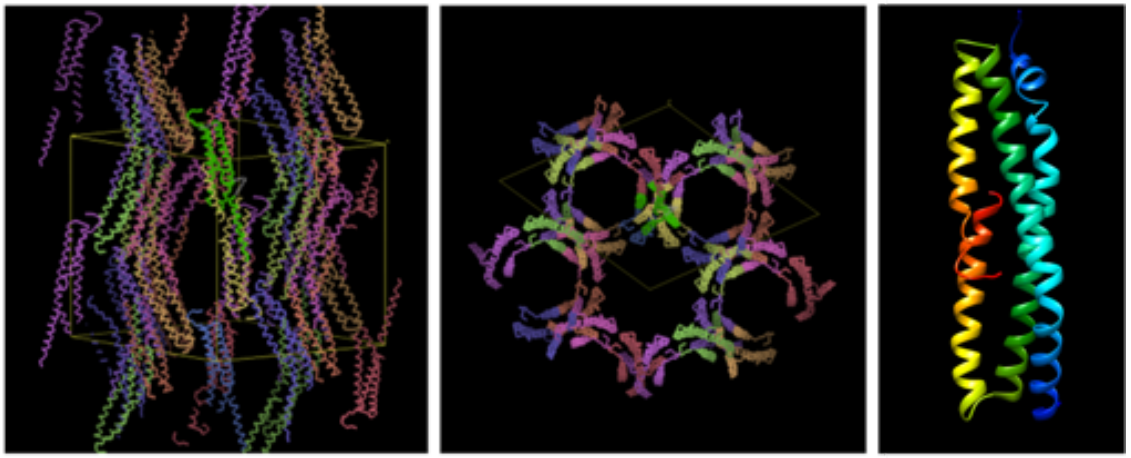


BM14

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)

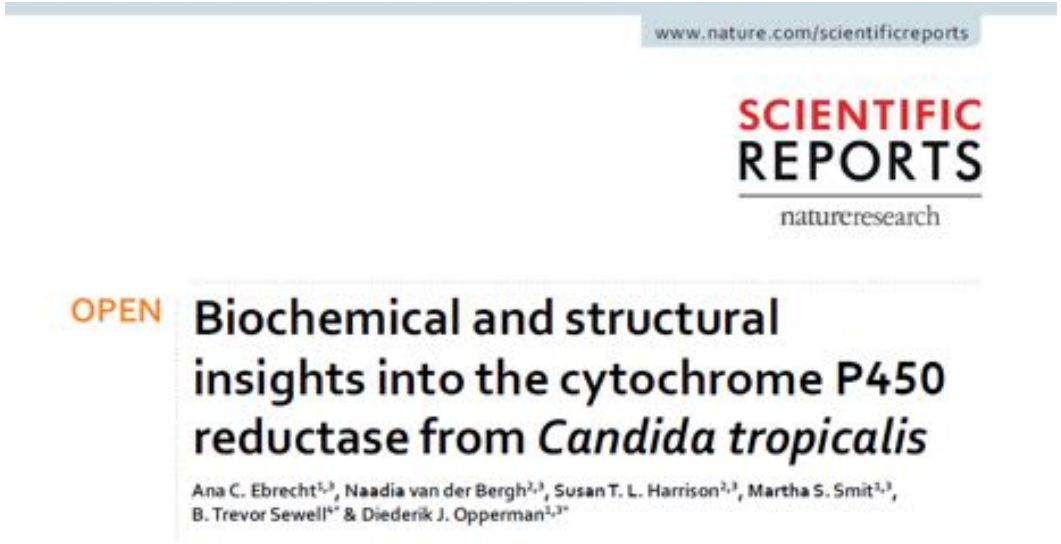


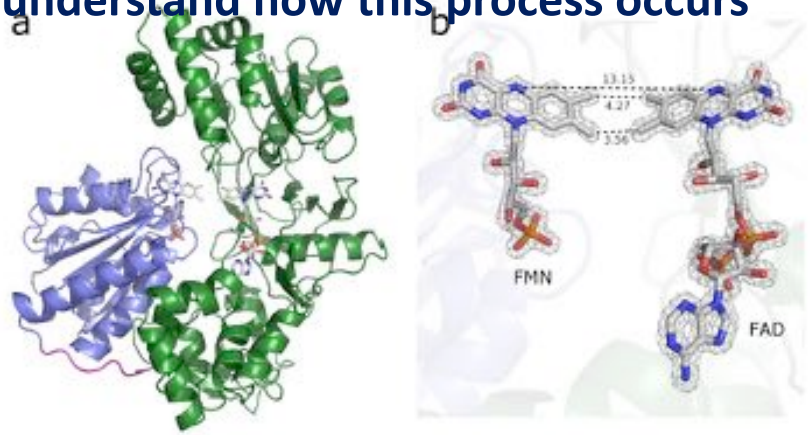
- Data collected for a truncated version of the protein
- Coiled-coil structure
- Analysis of the structure currently underway to predict interaction with DNA and other proteins
- Understanding the protein structure **can shed light in the molecular virulence mechanism and host-virus interaction**

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



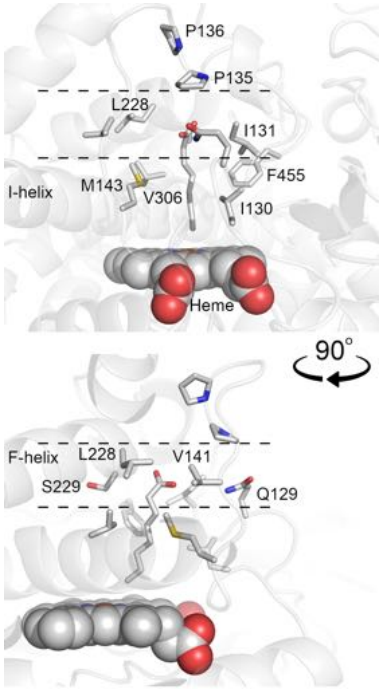


Structure solved with and without cofactor (NADPH)

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

Cytohrome P450 monooxygenases

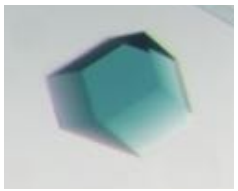
-These proteins are heme-thiolate enzymes that catalyse a range of reactions
-The research focuses on CYPs that perform regioselective hydroxylations of fatty acids and alkanes.

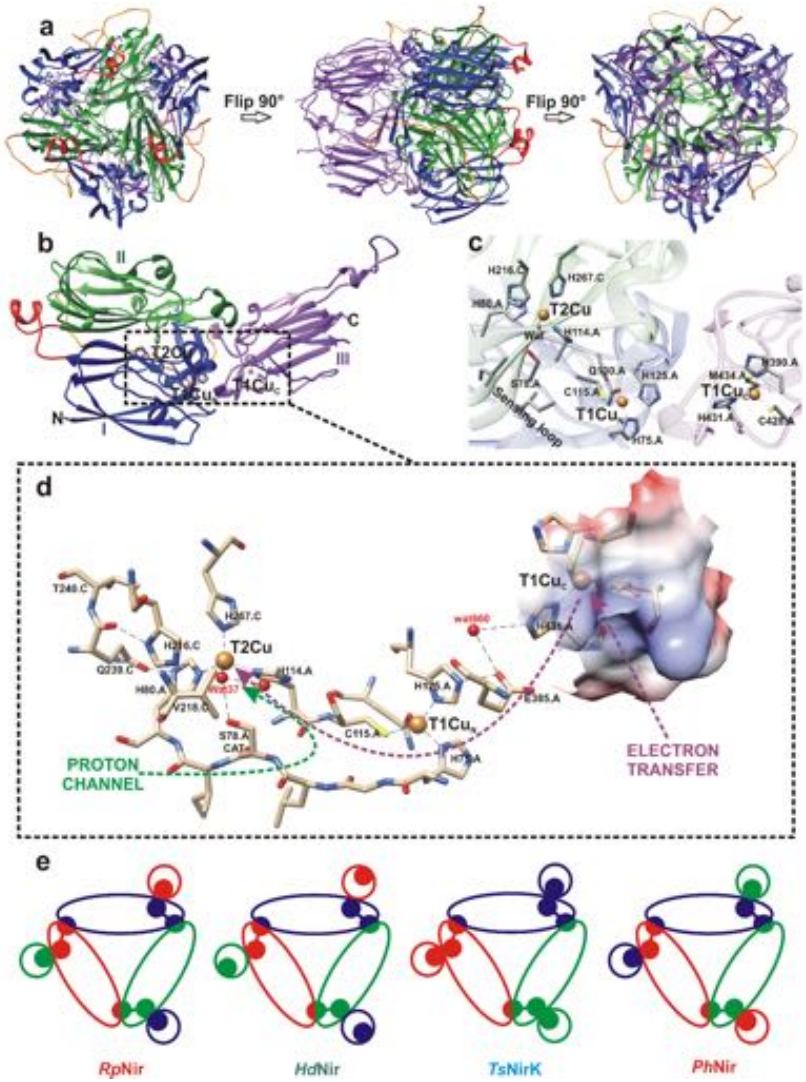


- Using X-ray crystallography, the 3D structure of the CYPs are solved to gain insight into how the **active site determines the regioselectivity** of the enzymes

Nitrite reductases

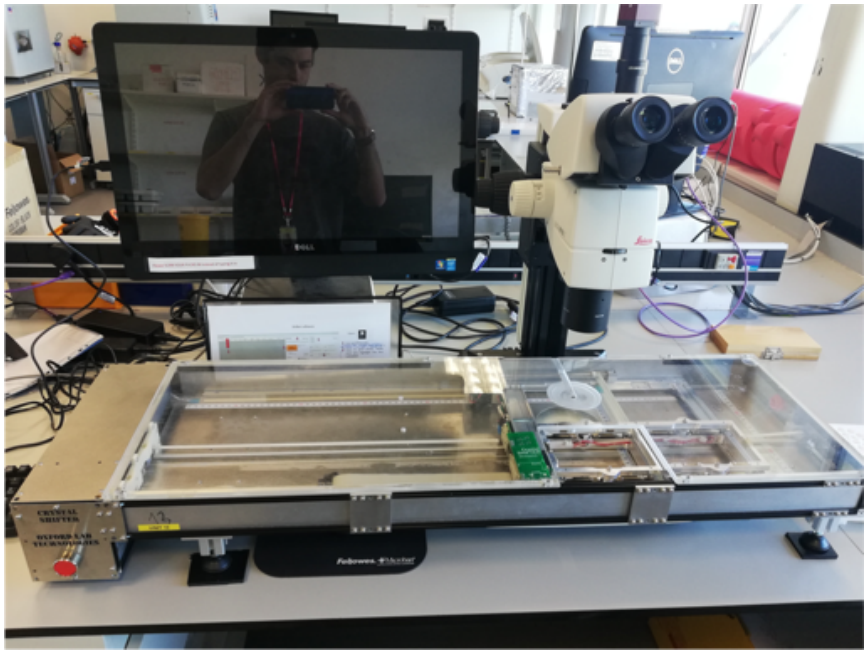
-Nitrite reductases are key enzymes in the denitrification pathway.
-The copper-containing nitrite reductase from a thermophilic bacterium was solved.
-The structure showed a **unique distribution of domains and subunit interactions** as well as an unusual copper-coordination, which indicates a novel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





Xchem at the Diamond Light Source to solve a protein structure

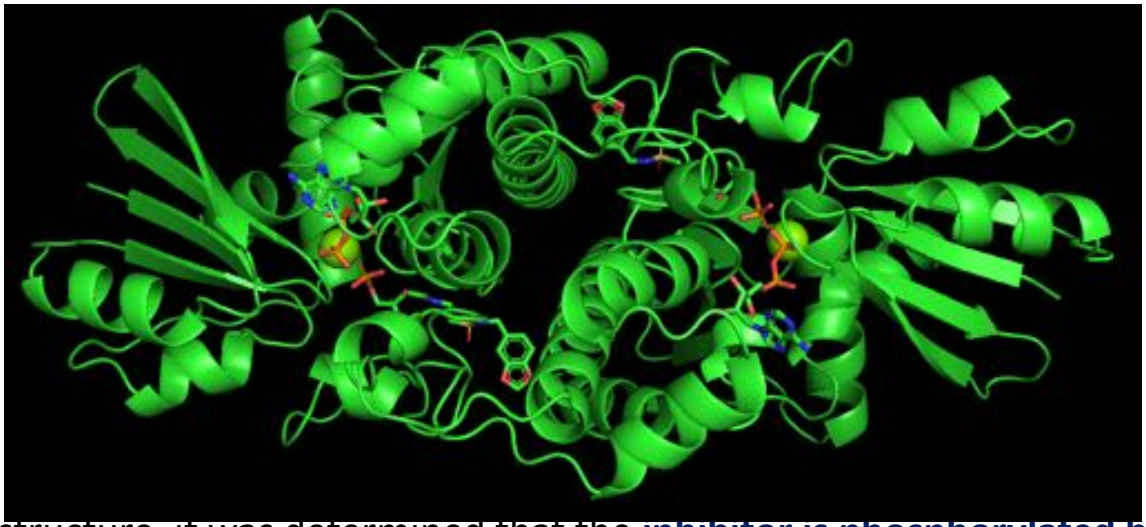
-The research focuses on developing antimicrobial inhibitors **against Staphylococcus aureus**.
-XtalShifter is semi-automatic machine allowing fishing of over 100 crystals in less than an hour
-The crystals were soaked with different fragments (inhibitors) prior to fishing.
-Soaked crystals were then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



diamond

Crystal structure of SaPanK with inhibitor

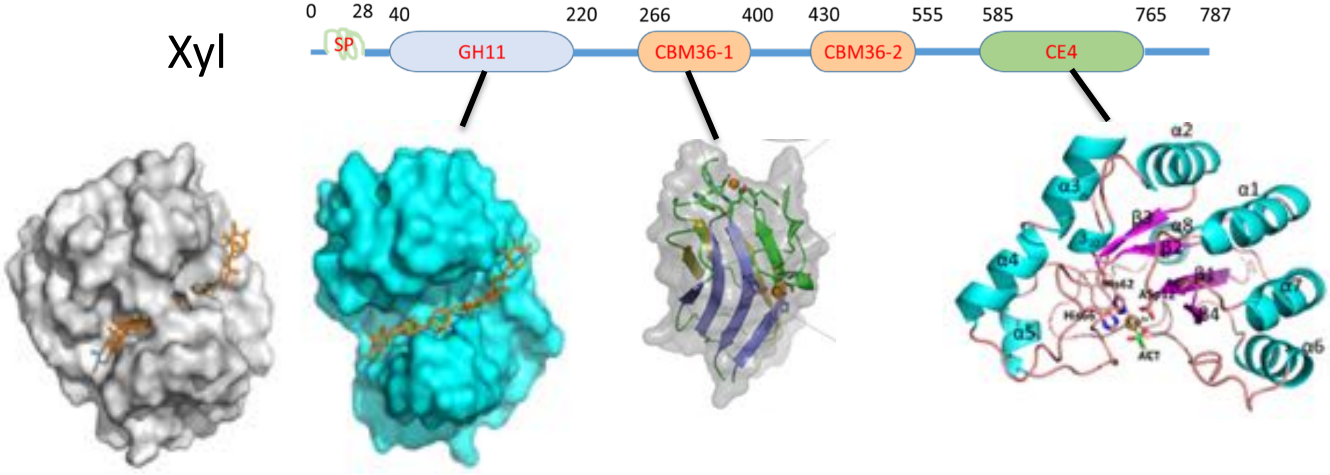
-Solved crystal structure of the Pantothenate kinase with a bound inhibitor at 1.44 A resolution.

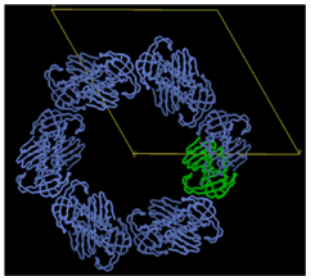


-From this crystal structure, it was determined that the **inhibitor is phosphorylated by the ATP** and subsequently **trapped inside the active site**.

-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**.

Structural characterization of a multidomain xylanase from a termite metagenome



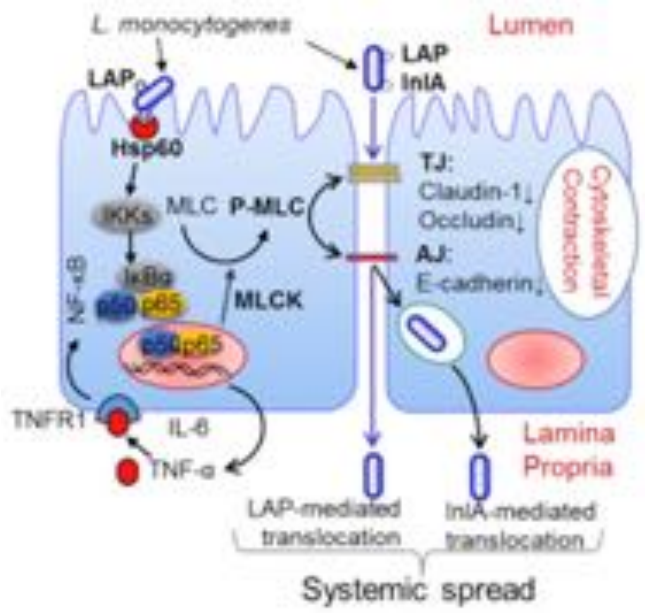


-Individual domains have been analysed structurally and kinetically.

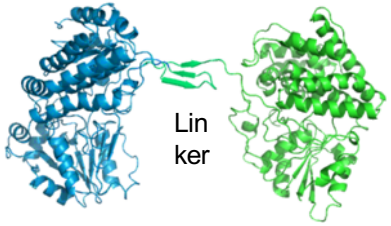
-pH and temperature optima of the two catalytic domains indicate Xyl to be a mesophilic enzyme working at neutral pH.

-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)

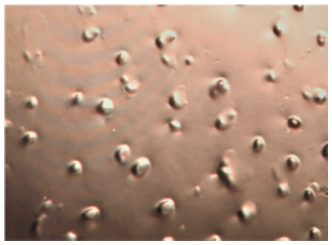


Mitochondrial HSP60 (GroEL)



Bifunctional alcohol and aldehyde dehydrogenase (AdhE)

-Both HSP60 and LAP (AdhE) are normally located far away from the cell surface.
-Both proteins have moonlighting functions in listerial infection.
-Proposed to form a complex on the surface of epithelial cells.



LAP Microcrystals

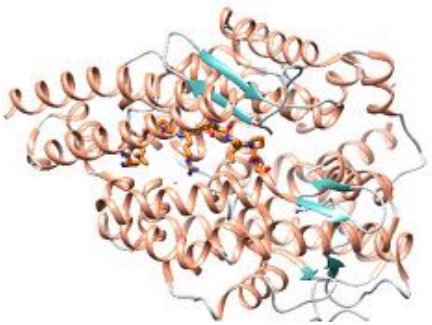
UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Structural insight into angiotensin converting enzyme function

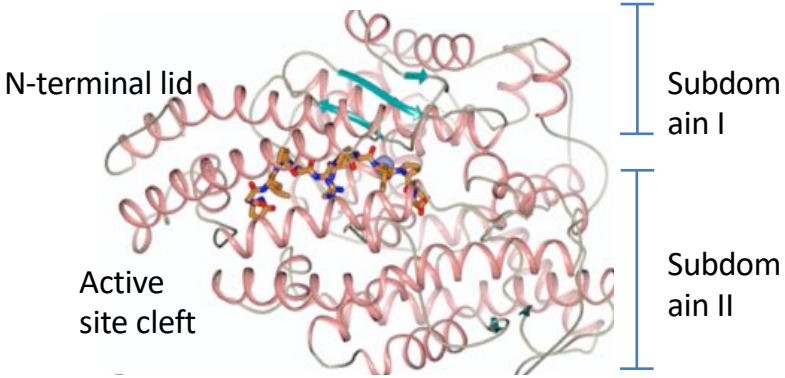
-The enzymatic activity of Angiotensin Converting Enzyme (ACE) causes tightening of blood vessels and a raise in blood pressure

-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 to determine if BPPs 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

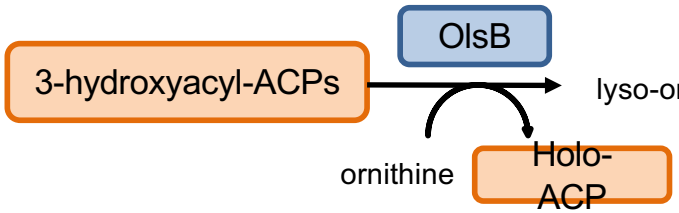
Gyles E. Cozier[1,*], Lizelle Lubbe[2,*], Edward D. Sturrock[2] and K. Ravi Acharya[1]

The 8 unique active site residues affect binding of:

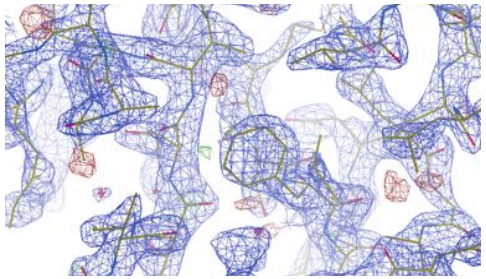
- Ndom inhibitors (33RE, SG6, ketoACE13)
- Cdom inhibitor (BPPb)

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

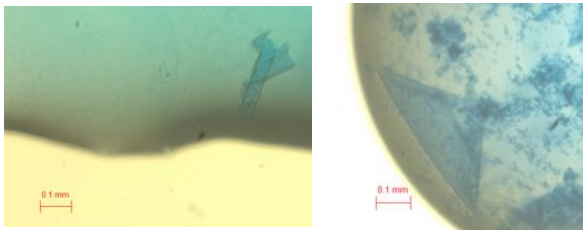
Characterization of a novel ornithine acyl-ACP N-acyltransferase (OlsB) and overexpression of its biosurfactant product



Overproduction of biosurfactant achieved through co-expression of ACP and OlsB



Solved structure of *Pseudomonas putida* ACP resolution of 2.040 - 1.970 Å



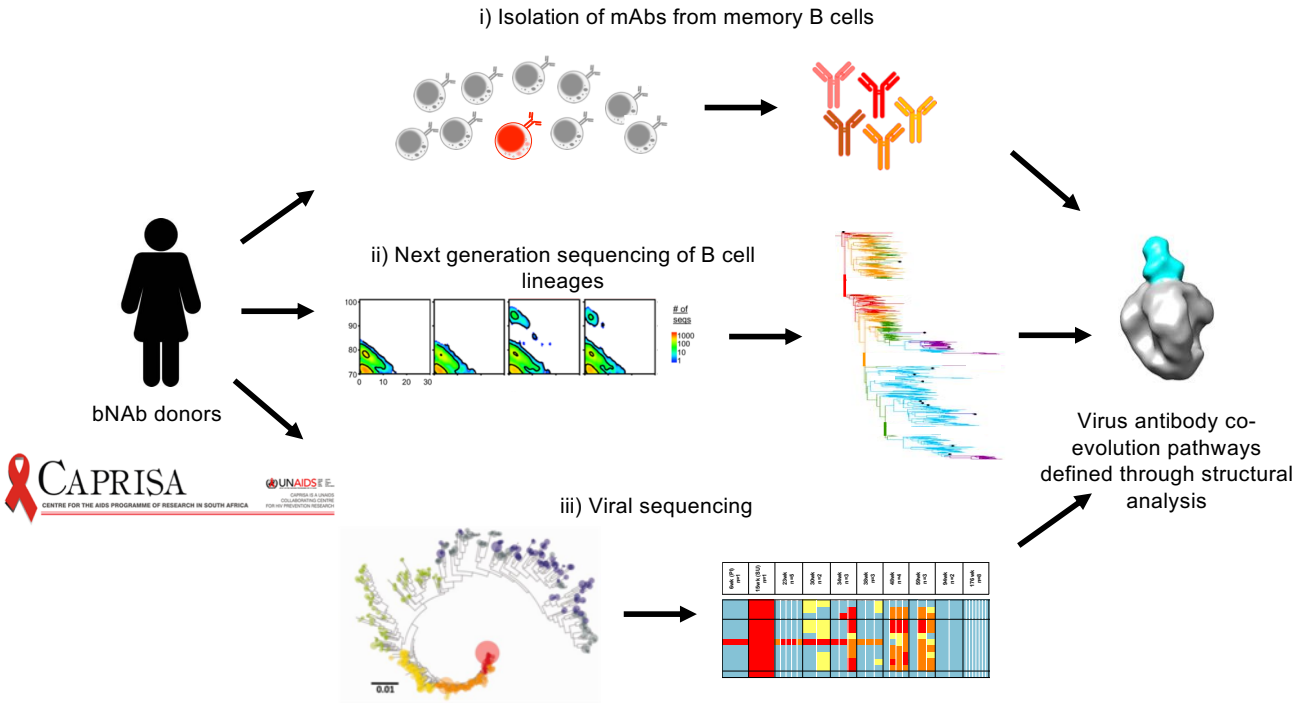
OlsB crystals on the way

TLC visualizing LOL overproduction. Lane 1: OlsB control. Lane 2: Overproduction system.

1 2

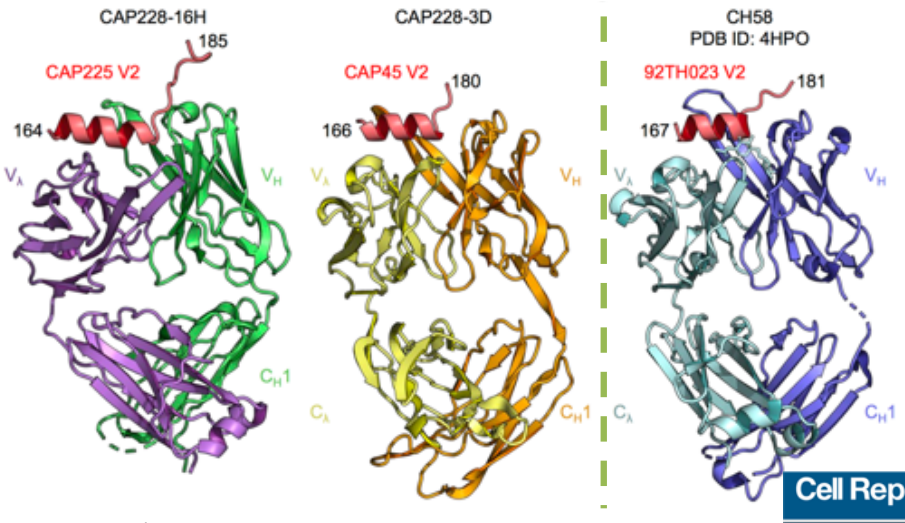
Phillip Venter
Supervisor: Prof. Trevor Sewell (UCT)
Co-supervisor: Prof. Marla Trindade (UWC)

Virus-antibody co-evolution studies



Adapted from Moore, CHIVR, 2018

Crystallization of antibodies in complex with HIV scaffolds and peptides



V1/V2 scaffold subdomain

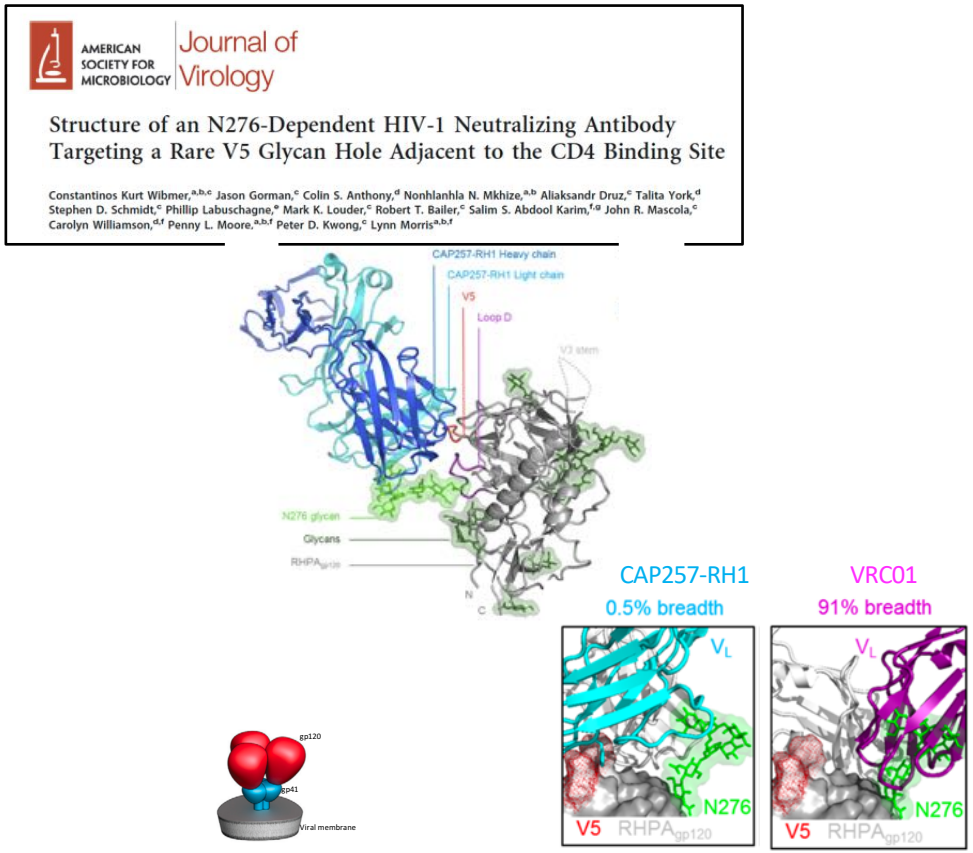
2.3 Å

V2 peptide

2.6 Å

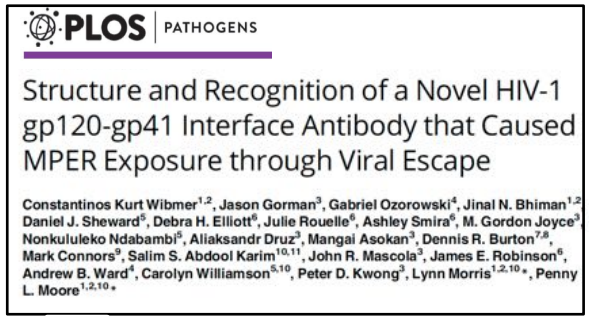
- Co-crystal structures of two antibodies in complex with V1/V2 scaffold and V2 peptide
- These antibodies have similar epitope to a vaccine elicited antibody, CH58

Atomic structure explains why an antibody is narrowly-neutralizing

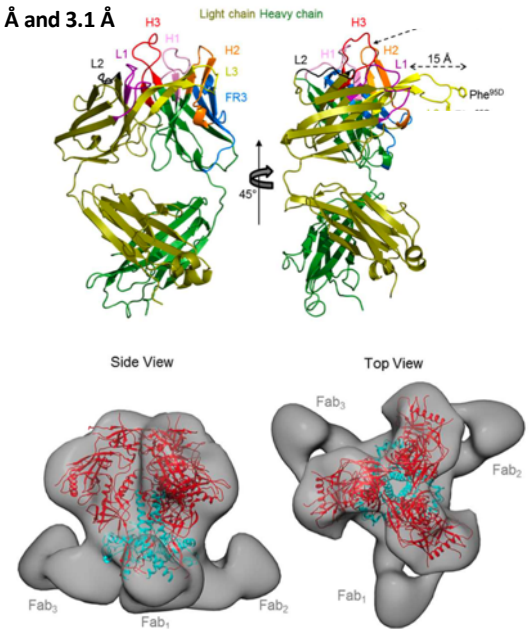


- Isolated a CD4bs-specific antibody, CAP257-RH1
- Narrowly-neutralizing antibody (0.5% breadth)
- Co-crystallization with gp120 revealed binding angle was incompatible with glycosylated V5 loops present in almost all HIV strains

Defining a novel antibody binding target



2.0 Å and 3.1 Å

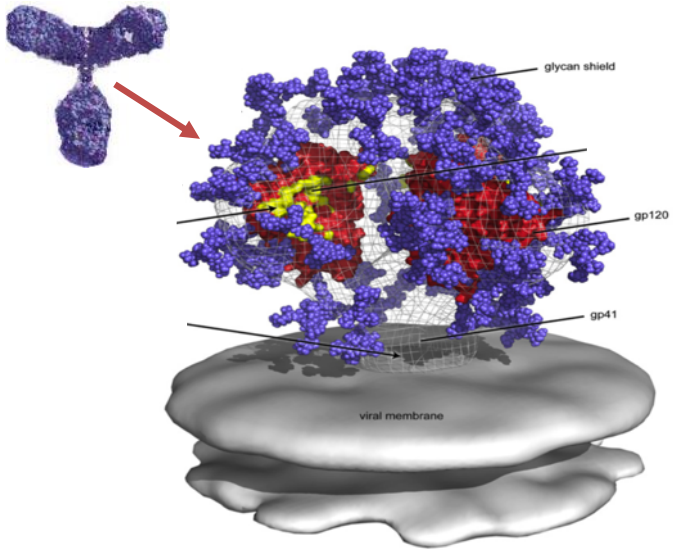


- Isolated the neutralizing monoclonal antibody CAP248-2B
- X-ray crystallography and NS-EM show antibody binds to a novel epitope

Structural characterization of antibody lineages from single donor

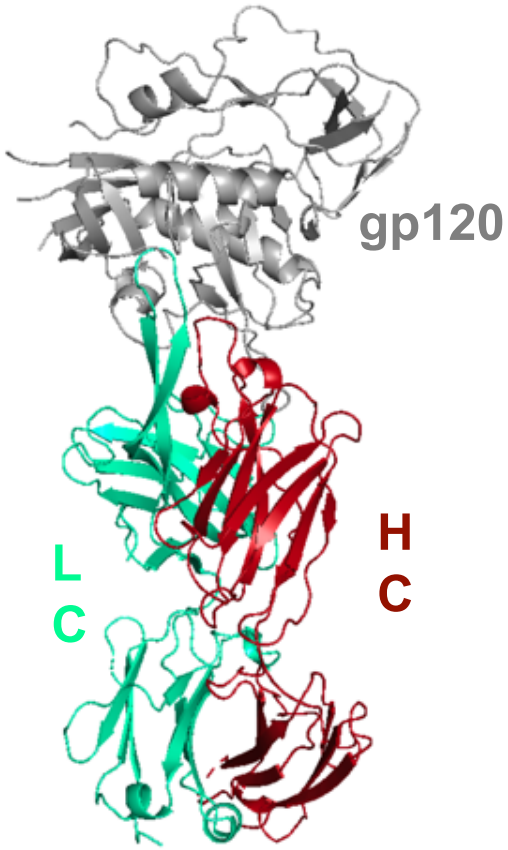
CAP314 – HIV-infected donor who developed bNAbs within 2 years post-infection

Isolated and characterized three antibody lineages

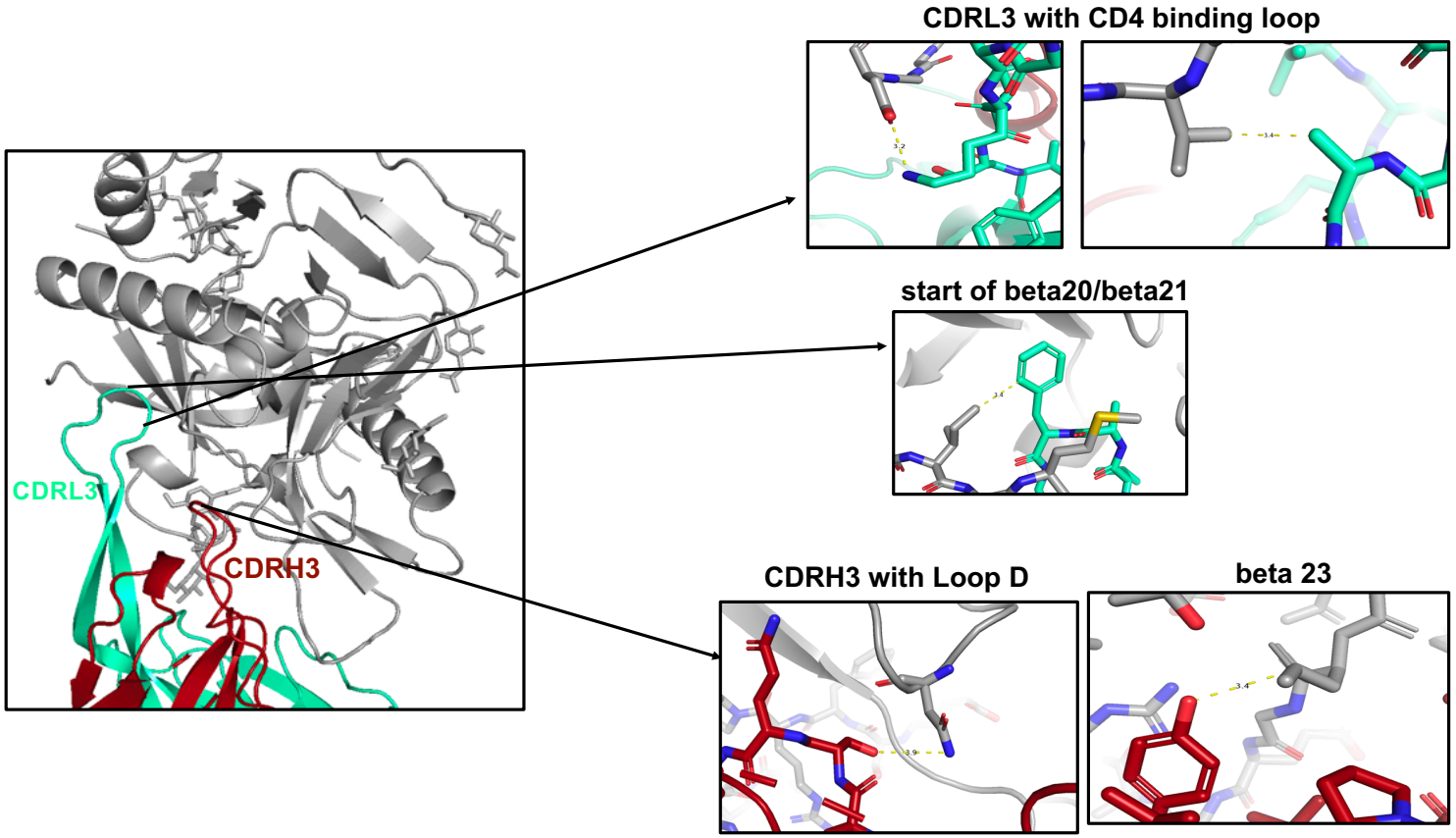


Adapted from Burton et al., 2012

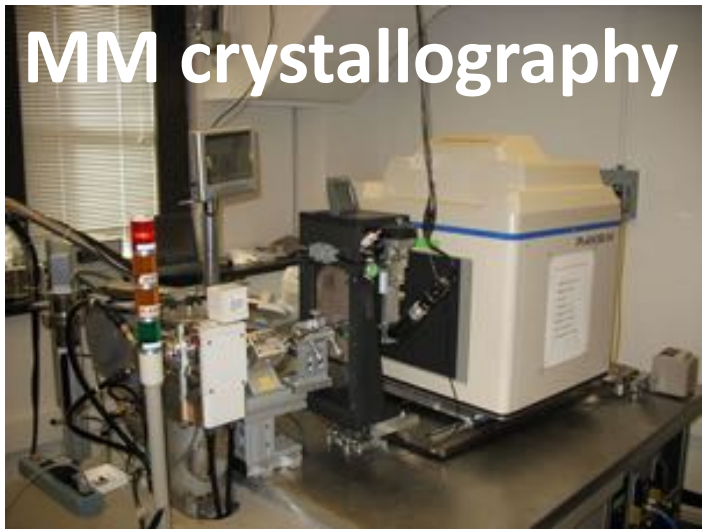
Crystallization of an antibody with an unusually long “binding” arm

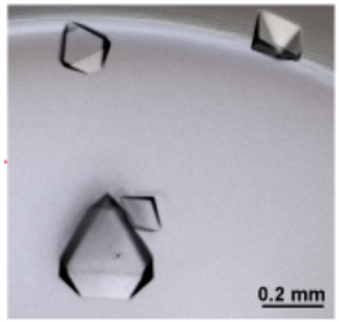


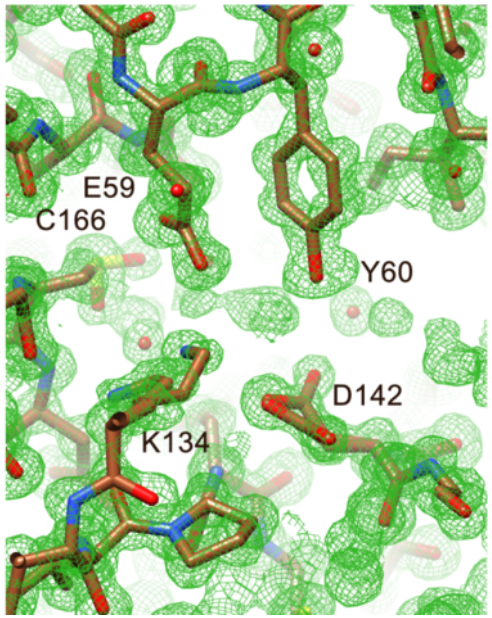
Novel mode of binding to HIV CD4 binding site

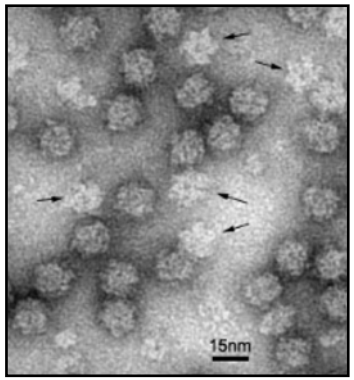


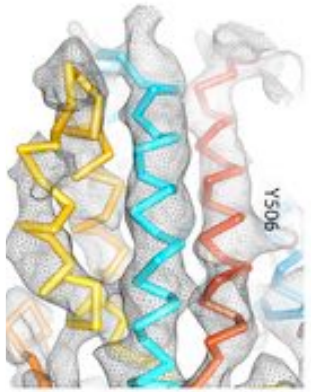
Convergent techniques





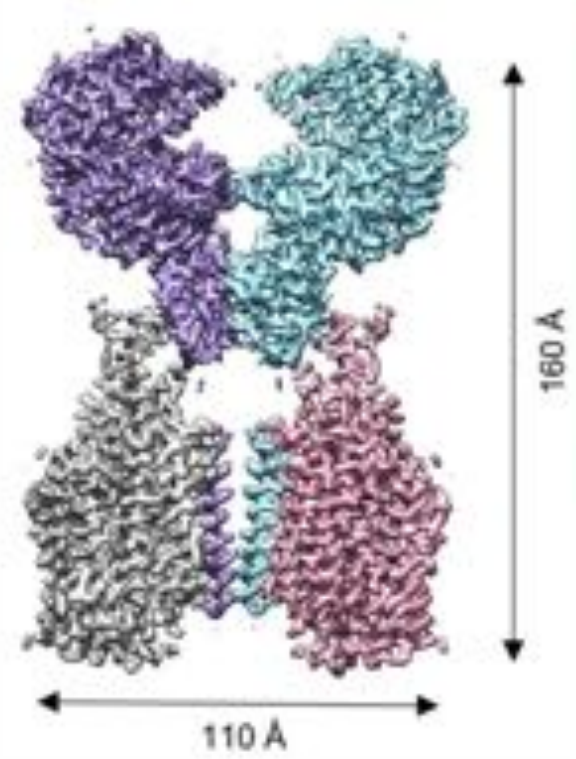


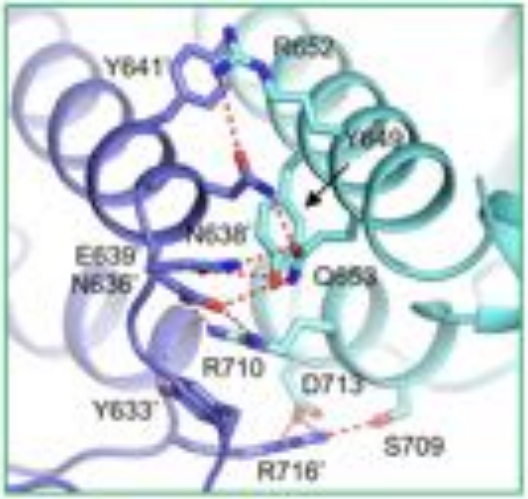




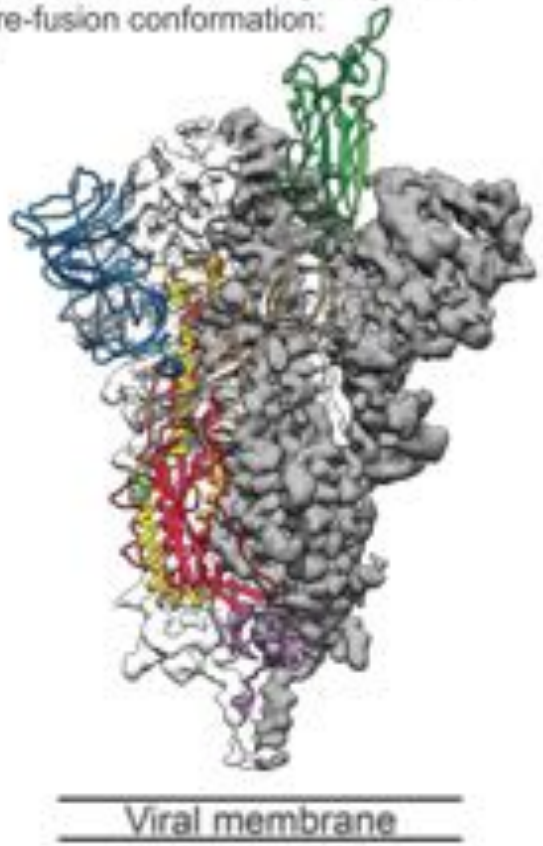
Cryo-EM is no longer a niche technique:

The new coronavirus spike protein interacting with it's human receptor:





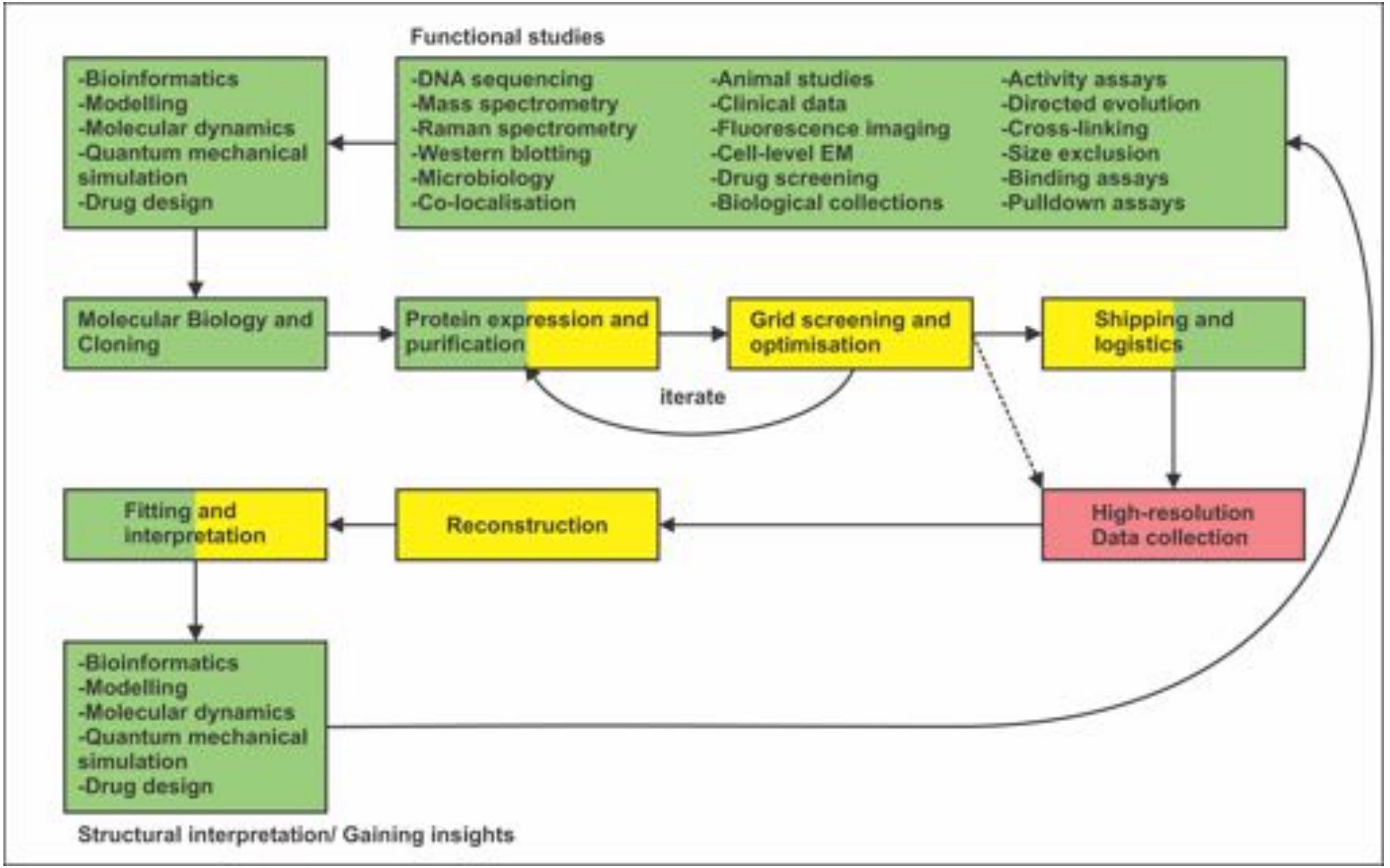
The new coronavirus spike protein pre-fusion conformation:

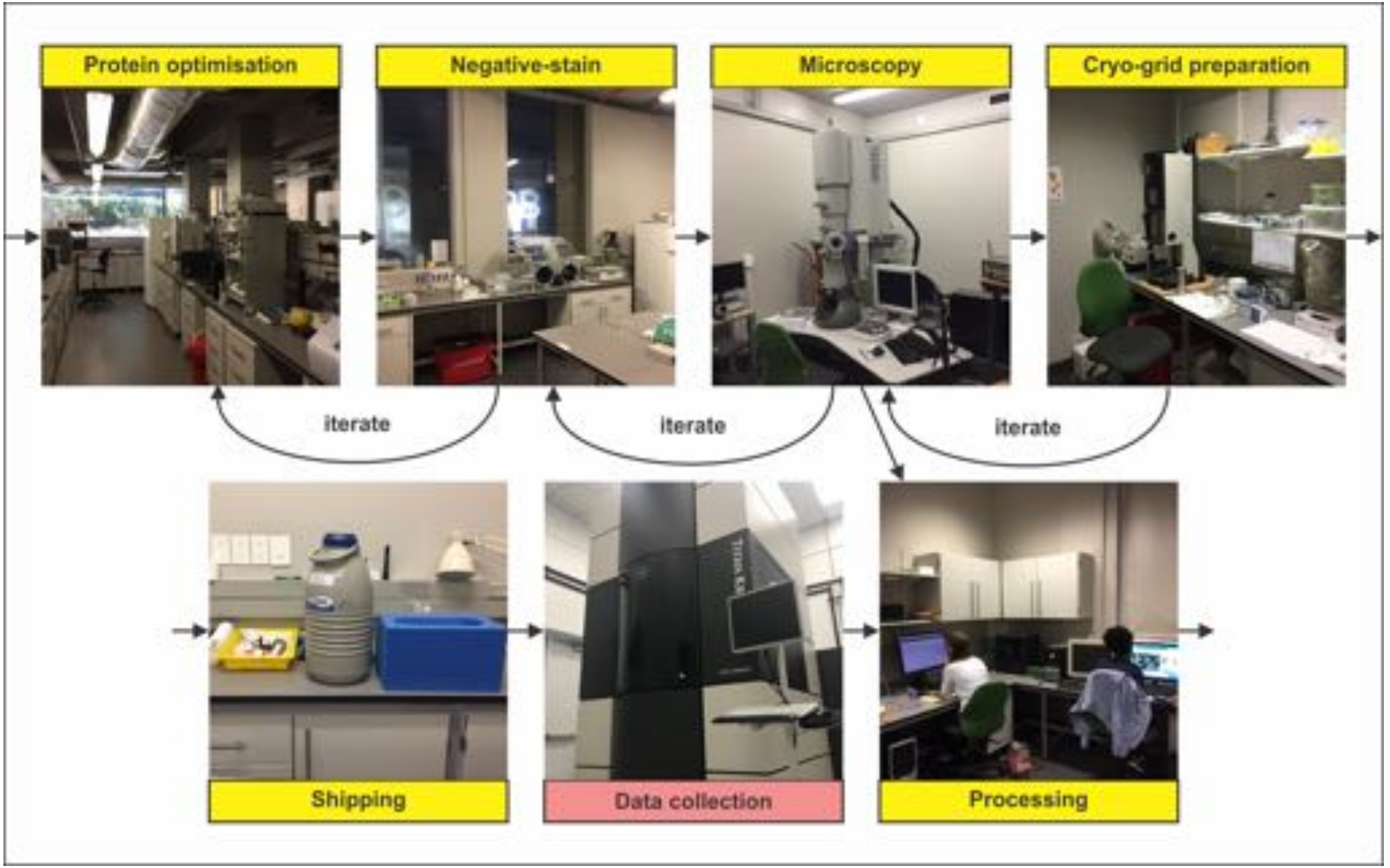


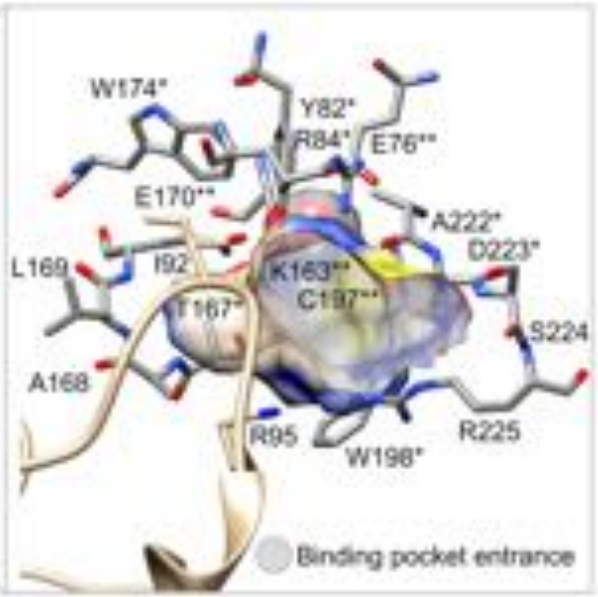
Yan et al., March 04 2020 **Science**

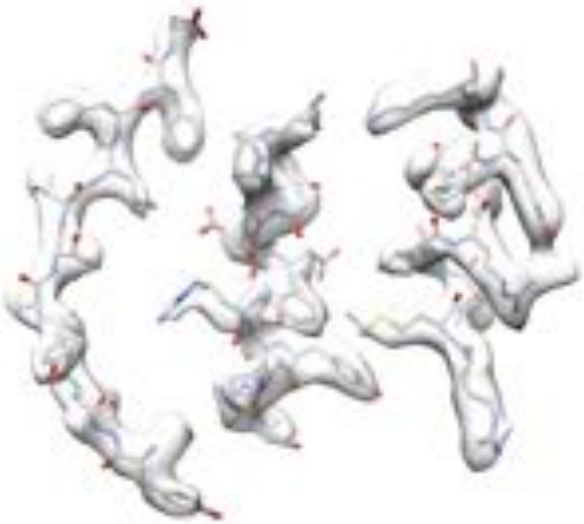
Wrapp et al., March 13 2020 **Science**

An electron microscope among the synchrotron beamlines at the Diamond Light Source


Functional studies
-Bioinformatics
-Modelling
-Molecular dynamics
-Quantum mechanical simulation
-Drug design
-DNA sequencing
-Mass spectrometry
-Raman spectrometry
-Western blotting
-Microbiology
-Co-localisation
-Animal studies
-Clinical data
-Fluorescence imaging
-Cell-level EM
-Drug screening
-Biological collections
-Activity assays
-Directed evolution
-Cross-linking
-Size exclusion
-Binding assays
-Pulldown assays
Molecular Biology and Cloning
Protein expression and purification
Grid screening and optimisation
Shipping and logistics
iterate
Fitting and interpretation
Reconstruction
High-resolution Data collection
-Bioinformatics
-Modelling
-Molecular dynamics
-Quantum mechanical simulation
-Drug design
Structural interpretation/ Gaining insights


Protein optimisation
Negative-stain
Microscopy
Cryo-grid preparation
iterate
iterate
iterate
Shipping
Data collection
Processing



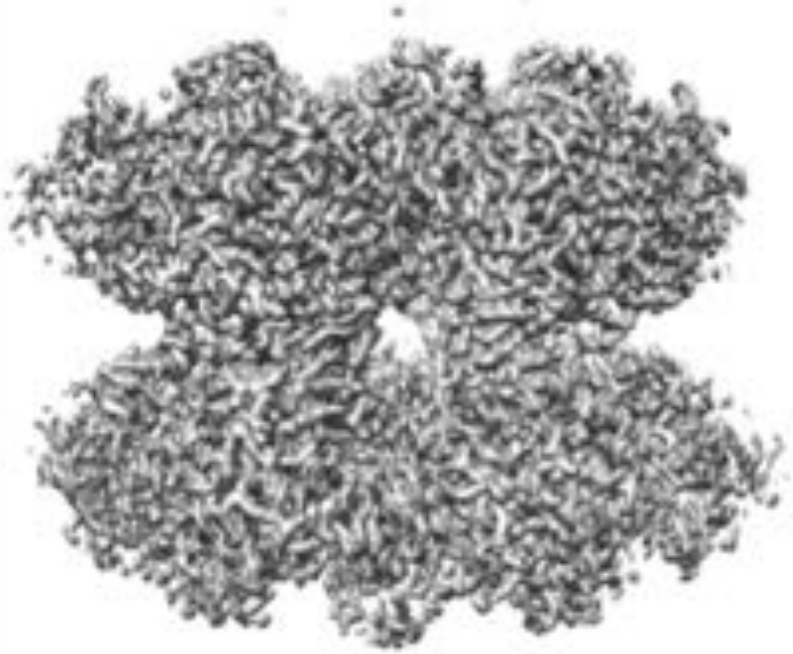


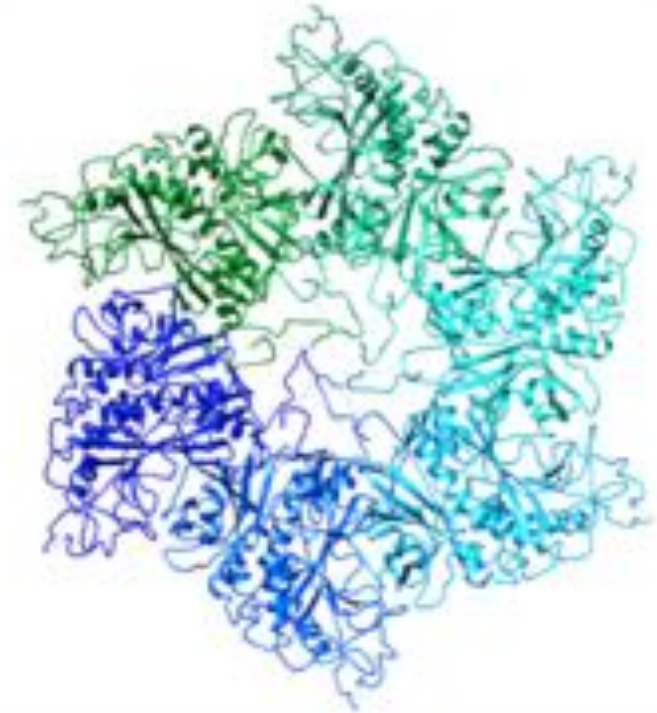
Nitrilase 4

First ever structure of an active nitrilase!

First close-to-atomic resolution cryo-EM structure published by an African team!

Andani Mulelu, 2019



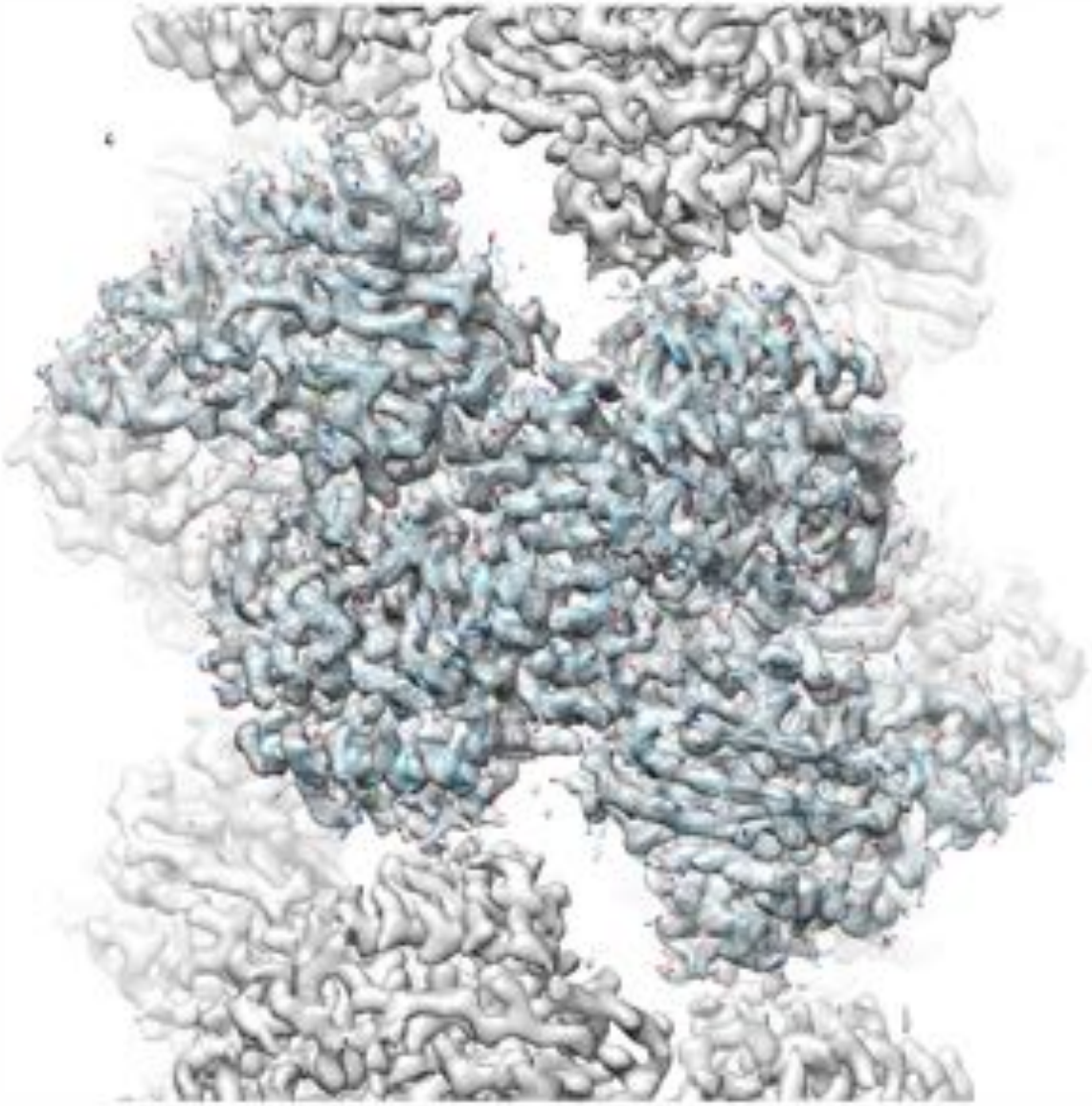


Glutamine synthetase from *Plasmodium falciparum*

Potential malaria drug target

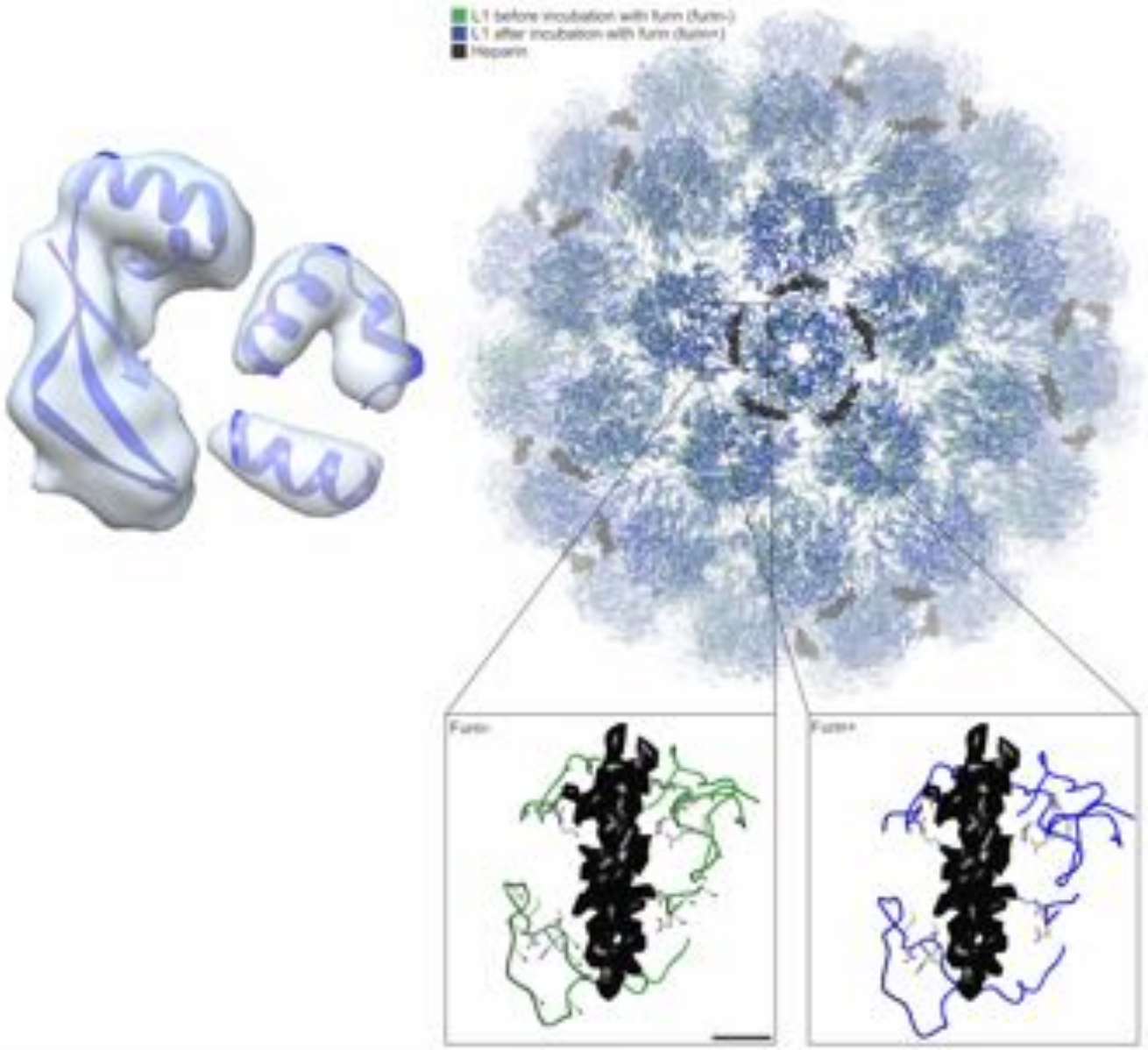
Stanley Makumire, 2021

Sinalo Gqunu, 2019



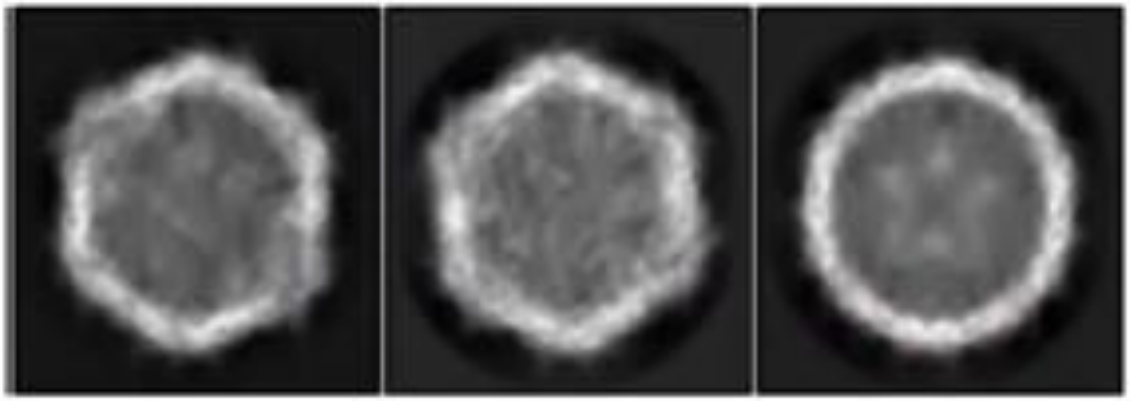
Cyanide Dihydratase

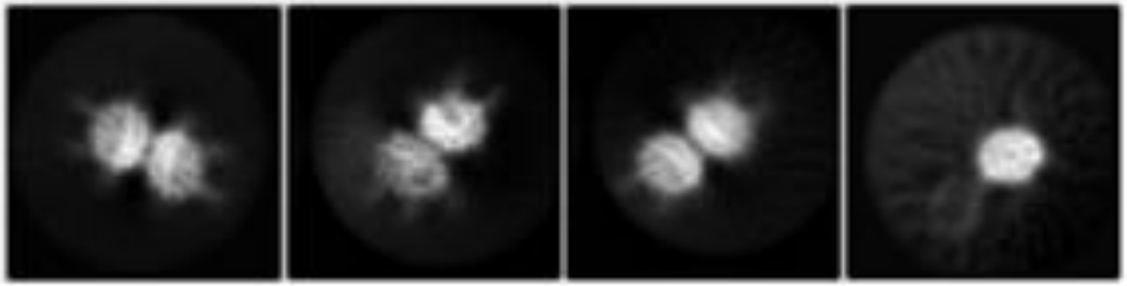
Various, 2018

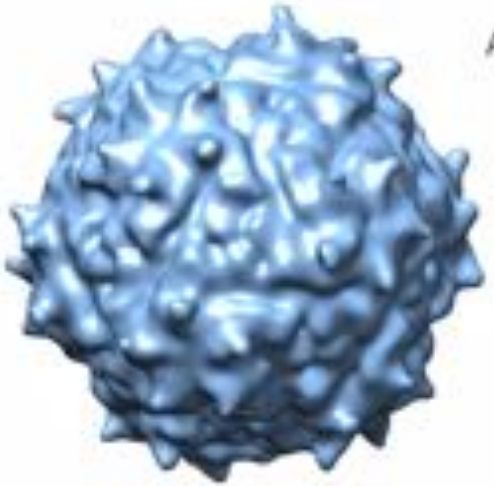


Human papillomavirus
Potential inhibitor target

Melissa Marx, 2019





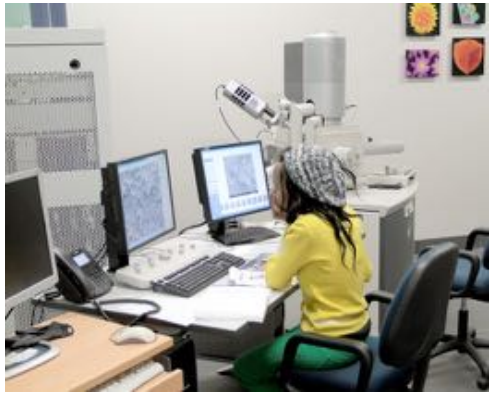


Encapsulin
Arthur Sarron

Human angiotensin-converting enzyme
Lizelle Lubbe

Aaron Klug Centre for Imaging and Analysis

Purpose: To provide the instrumental resources and expertise for structural analysis in all disciplines including structural biology



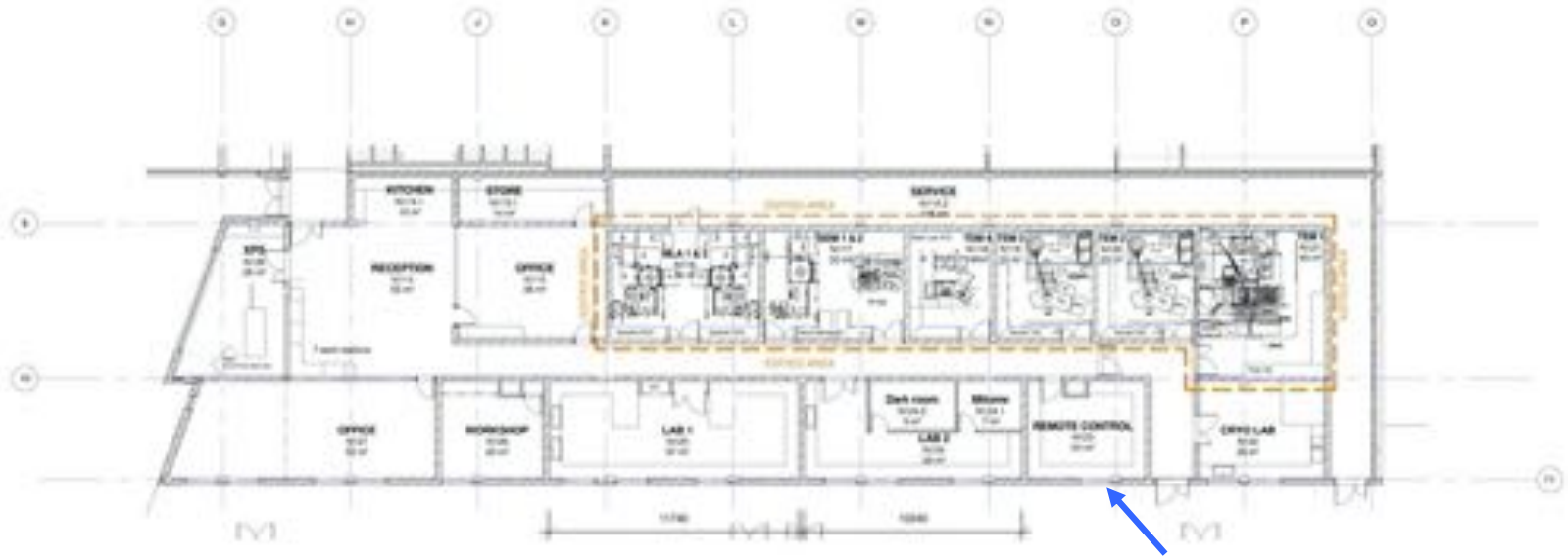
Staff: Director, 4 scientific officers, 2 technical officers employed by UCT

.

Space: 660m^{2,}, vibration free, magnetic field compensated, temperature controlled, humidity controlled

Equipment: 3 TEMs, 4 SEMs, Protein Diffractometer, Preparative equipment, computer infrastructure

Grant Applications for a Direct Electron Detector to NRF in process, high-end TEM (e.g. TITAN Krios) - pending



- **Automated acquisition systems**
- **Web-based image processing interfaces**
- **Integration with UCT HPC**

Chromatography

Crystal trials

Crystals

Data collection

Structural Biology Research Unit

The Structural Biology Research Unit

Purpose: To promote Structural Biology at UCT. To formulate and pose questions in structural terms. To plan and execute experiments that produce structural insights. To prepare material for structural analysis. To support the preparation of grants for work in structural biology. To raise grants to work with a substantial structural component.

Resources: 80m^2 lab space, molecular biology, cell culture, chromatography and biochemical assay facilities. Computers.

Status: Approved UCT research unit within the Department of Integrative Biomedical Sciences

Members:

PI Woodward

Sturrock

Sewell

The Problems facing Structural Biology in South Africa

1. Lack of infrastructure for:
    a. Educating people in the discipline
    b. Preparing proteins
    c. Characterizing proteins
    d. Determining structures
    e. Big Data / Computing
2. Funding
    a. Early Career Positions
    b. Students
    c. Projects
    d. Equipment
3. National Science Infrastructure
    a. Lack of transparency
    b. Failure to engage with scientists
    c. Poor planning
4. Credibility

JOURNAL OF SYNCHROTRON RADIATION

ISSN: 1600-5775

Volume 26 | **Part 5** | September 2019 | Pages 1843-1850
https://doi.org/10.1107/S1600577519008981
Viewed by 1742

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

SpringerLink

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

CCP4 Crystallographic School in South Africa

COVID-19 Corona Virus South African Resource Portal

Data Collection to Structure Refinement and Beyond

University of Cape Town, South Africa

~~18 – 26 November 2020~~ TBD in 2021

SYNCHROTRON TECHNIQUES FOR AFRICAN RESEARCH AND TECHNOLOGY
START

GCRF START

Synchrotron Techniques for African Research and Technology

The success of START

All the structural biology groups in South Africa are working together
In particular we have the crystallography BAG and a cryo EM BAG

We have established a mechanism to enable more people to become involved by giving people free access to the resources of the Aaron Klug Centre and by holding workshops conducted by leading experts.

The next event will be a virtual CCP4 workshop 22 Feb – 2 Mar 2021

Threats

South African Funding remains very limited, but it has
helped in critical areas

The largest threat we face is the loss of members of our very small community. This means that it is imperative to find sustainable funding to retain the people that we train.

Important messages

The START programme has shown that the SA bioscience community can and will adopt and use new technology – But

They need international support
There needs to be an established local resource to support them
Local funders have remained totally recalcitrant!

Acknowledgements

SYNCHROTRON TECHNIQUES FOR AFRICAN RESEARCH AND TECHNOLOGY
START

UKRI Science and Technology Facilities Council

GCRF Global Challenges Research Fund

diamond

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

University of Cape Town · iYunivesithi yaseKapa · Universiteit van Kaapstad

The University Of Sheffield.

UNIVERSITY OF LIMPOPO
Finding solutions for Africa

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

ADDIS ABABA UNIVERSITY

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

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

UNIVERSITEIT iYUNIVESITHI STELLENBOSCH UNIVERSITY

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

UNIVERSITY OF Southampton

WITS HEALTH CONSORTIUM

UNIVERSITY OF OXFORD

Faculty of Engineering · Ain Shams University · 1839

NATIONAL UNIVERSITY OF LESOTHO

cchange
DST-NRF Centre of Excellence in Catalysis

ANSOLE

SMART VILLAGES
New thinking for off-grid communities worldwide

ANSDAC

Thank you

To all the people involved with the programme, in particular:

At Diamond Light Source

Chris Nicklin
Gwyndaf Evans
Frank von Delft

In South Africa

All involved in the programme – but in particular:
Carmien Tolmie
Thandeka Moyo
Wolf-Dieter Schubert
Jeremy Woodward
Kelly Chibale