BioSciences in South Africa

Wolf-Dieter Schubert

Department of Biochemistry, Genetics and Microbiology

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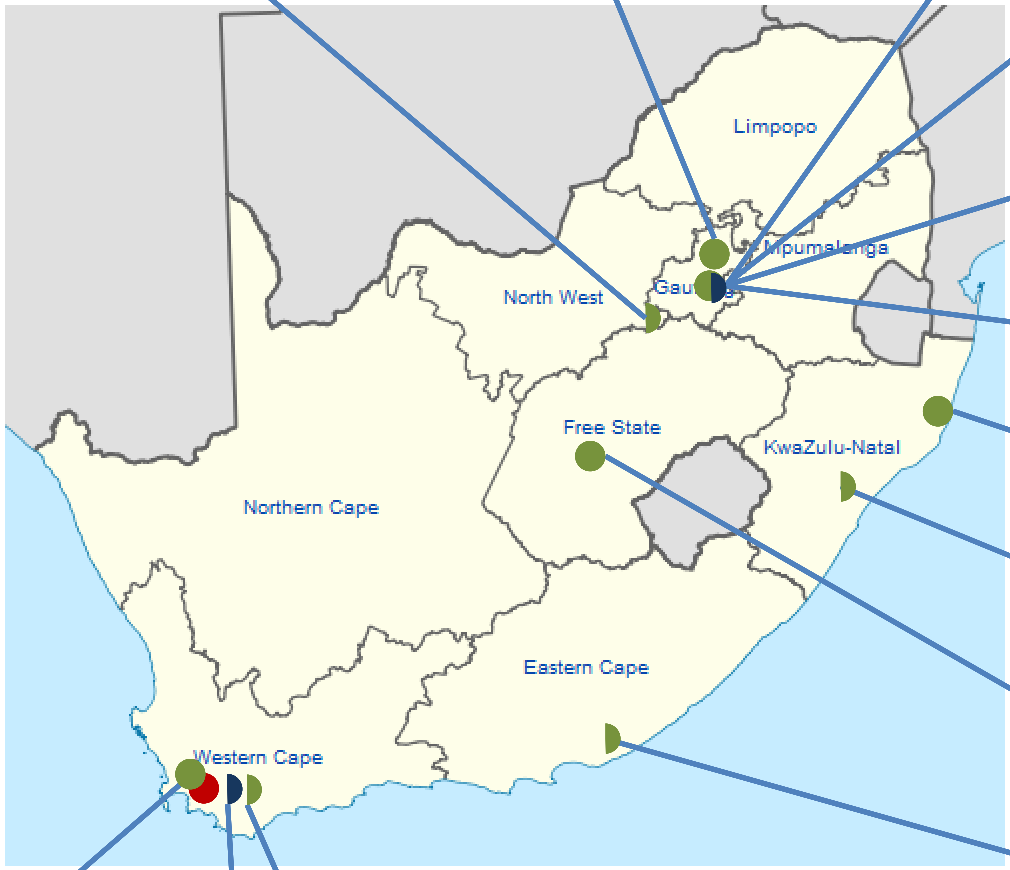
Denkleiers • Leading Minds • Dikgopolo tša Dihlalefi

Structural Biology in South Africa

● EM

● X-ray

● NMR



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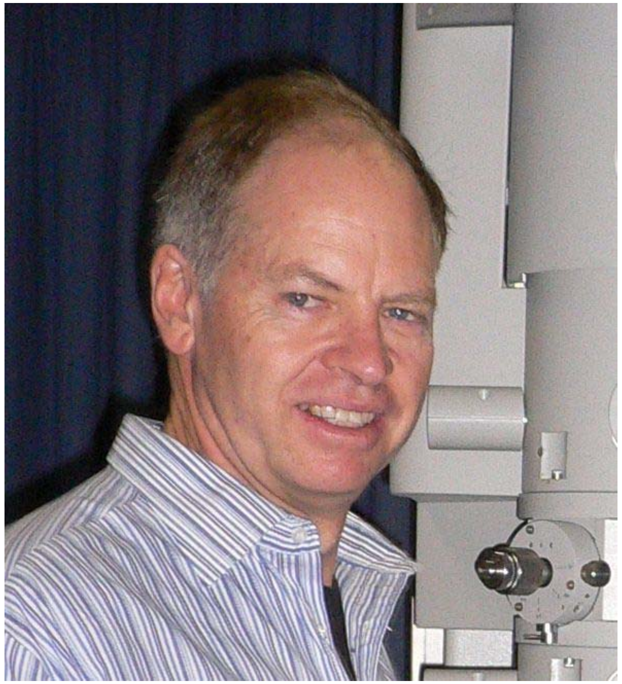
Prof Trevor Sewell



University of Cape Town

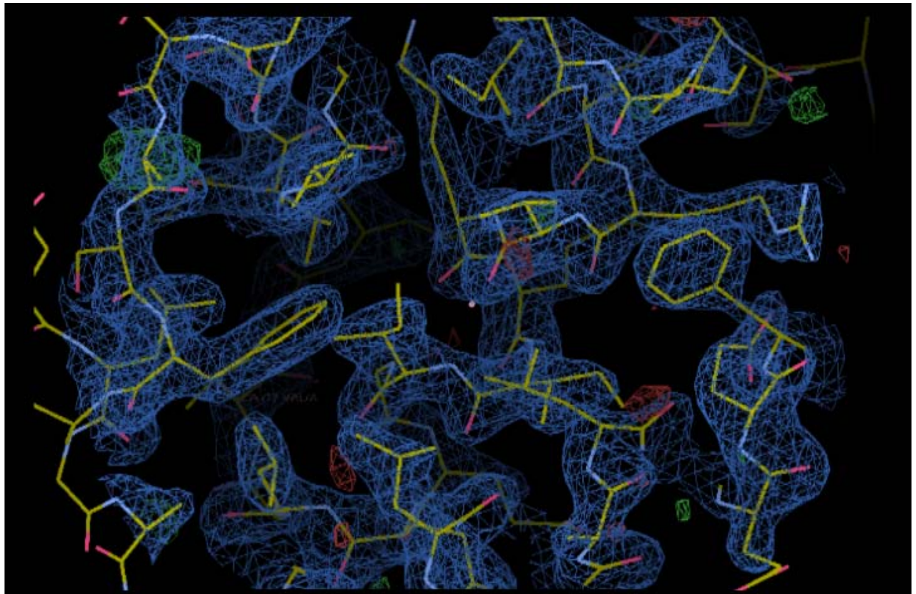
Director: Electron Microscope Unit, Division of Medical Biochemistry & Structural Biology, Department of Integrative Biomedical Sciences; Member, Institute of Infectious Disease and Molecular Medicine (IDM), Faculty of Health Sciences, UCT

Main interest: Nitrilases, but more generally proteins of industrial importance or related to human health.

Structural elucidation of a novel OlsB protein

START funding lead to:

- Student’s training in structural biology by sponsoring the Biophysics & Structural Biology at Synchrotrons workshop 17-24 January 2019.
- The structural elucidation of acyl carrier protein from Pseudomonas at the Biophysics workshop through X-ray crystallography.
- The initial crystal hits of OlsB protein.
- The opportunity to send crystals to Diamond Light Source to collect structural data of OlsB.
- Provision of all consumables related to this endeavor.



Structural model of acyl carrier protein from Pseudomonas

Structural Biology Research Unit

Supervisor: Prof. Trevor Sewell
Co-supervisor: Prof. Marla Trindade
Student: Mr. Phillip Venter

University of Cape Town – Trevor Sewell

Aaron Klug Centre for Imaging and Analysis

Purpose: To provide the instrumental resources and expertise for structural analysis in all disciplines including structural biology for all as part of the National Research Infrastructure

Macromolecular Structure Determination Service

A proposal to build a National facility around existing infrastructure at UCT

Structural
Biology
Research
Unit



Main Interests and Infrastructure

- Nitrilases
- Amidases
- Industrial enzymes
- Health and disease

- Rigaku Rotating Anode
- Various Electron Microscopes
- Protein production and purification
- Isothermal titration calorimeter

Structural Biology in South Africa

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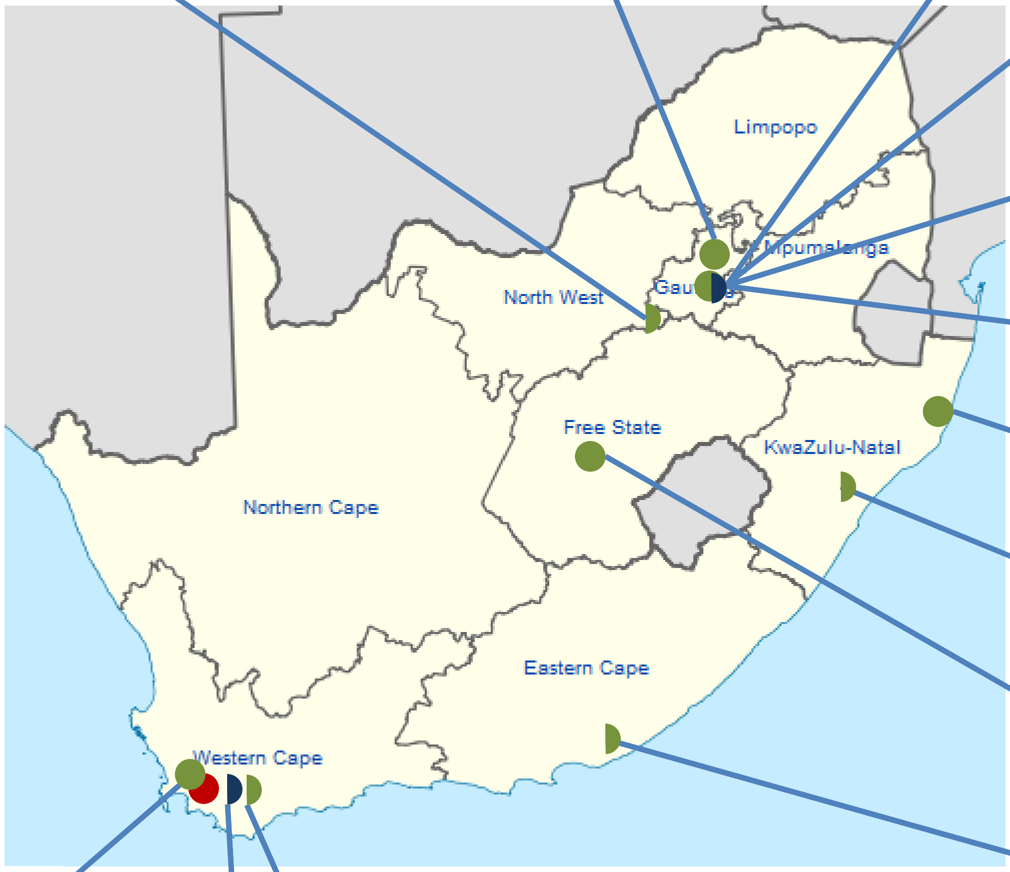

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Prof Heini Dirr

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

NRF/DST SARChI Chair in Protein Biochemistry and Structural Biology Director: URC Protein Structure-Function Research Unit

The Sydney Brenner Institute for Molecular Bioscience (SBIMB).

Correlating structure, energetics and dynamics is key to obtaining a coherent picture of the biological functions of proteins.

Research interests:
- Folding mechanisms of multidomain and oligomeric proteins.
- Mechanisms of molecular recognition and biological functions.
- Protein dynamics and function.

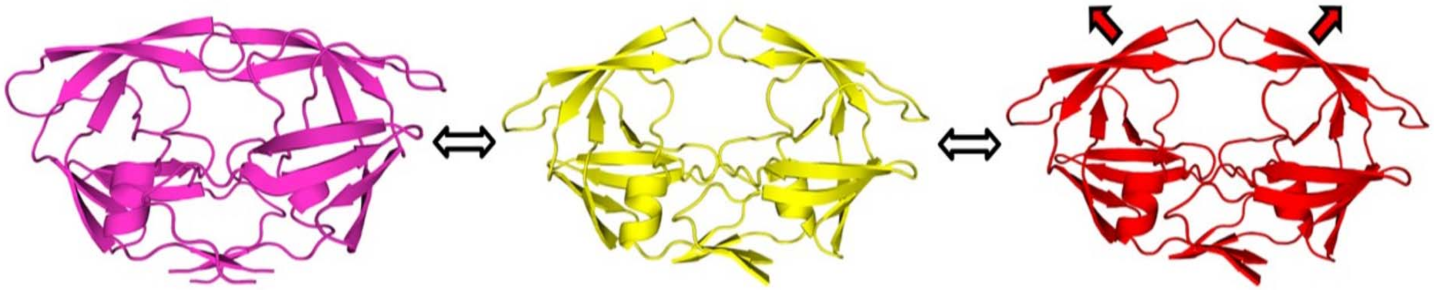
UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Prof Yasien Sayed

- structural and thermodynamic basis of binding of protease inhibitors to the South African HIV-1 subtype C protease.
- binding of non-nucleoside reverse transcriptase inhibitors to the reverse transcriptase

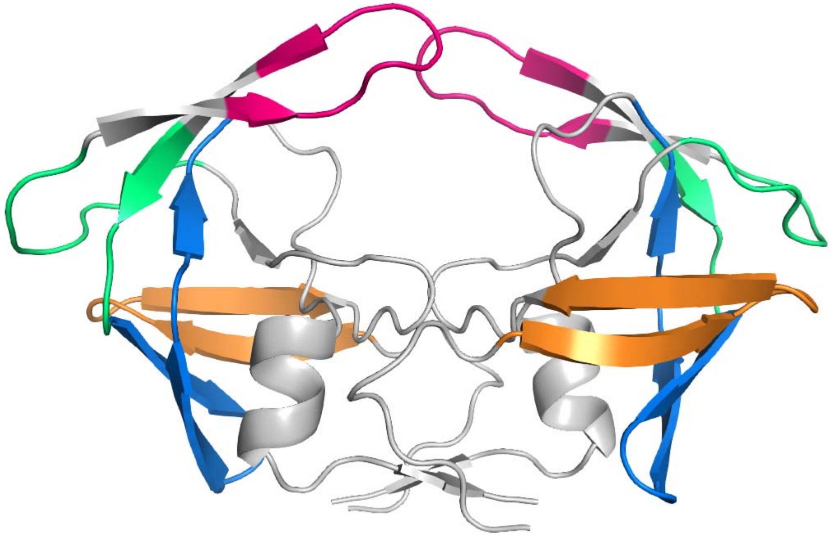
HIV-1 subtype C protease – Yasien Sayed

- AIDS remains a world-wide health problem
- Global research focuses on HIV-1 **subtype B**
- HIV-1 subtype C is most prevalent globally
- HIV-1 subtype C protease structures:
  - Very few in PDB (<5)
  - Only 1 South African subtype C protease structure in PDB: resolution ~ 2.7 Å
  - Require high resolution structures
  - Next generation protease inhibitors require detailed knowledge of structures of non-B subtype proteases



Potential for solving **many** subtype C protease structures

- **Wild type South African HIV-1 subtype C protease**
    - Apo
    - Drug complexed to all 9 FDA-approved protease inhibitors
- **Polymorphism-containing** variants
    - F99A
    - I13A
    - I13V/I62V/V77I (solved to 1.8Å; 6I45.pdb; Diamond Light Source)
- Variants with **insertions** in hinge region
    - L38↑N↑L → Apo, LPV, ATZ, DRV
    - N37T↑V → Apo, LPV, ATZ, DRV
    - I36T↑T
    - E35D↑G↑S



(Sherry, D *et al.*, 2018; unpublished work; 6I45.pdb)

> 20 potential structures must be solved at the highest resolution possible

SYNCHROTRON RADIATON LIGHT SOURCE

Sylvia Fanucchi

- Mechanism and structures of protein interactions.
- Structural biology (x-ray crystallography), biophysical chemistry and thermodynamics
- Ion channel protein, CLIC1 (cell cycle regulation)
- FOXP2, transcription factor (acquisition of language).

Infrastructure

X-ray diffractometer (Bruker, rotating anode)
2 x Isothermal titration calorimeter
CD-spectrometer
Surface plasmon resonance spectroscopy

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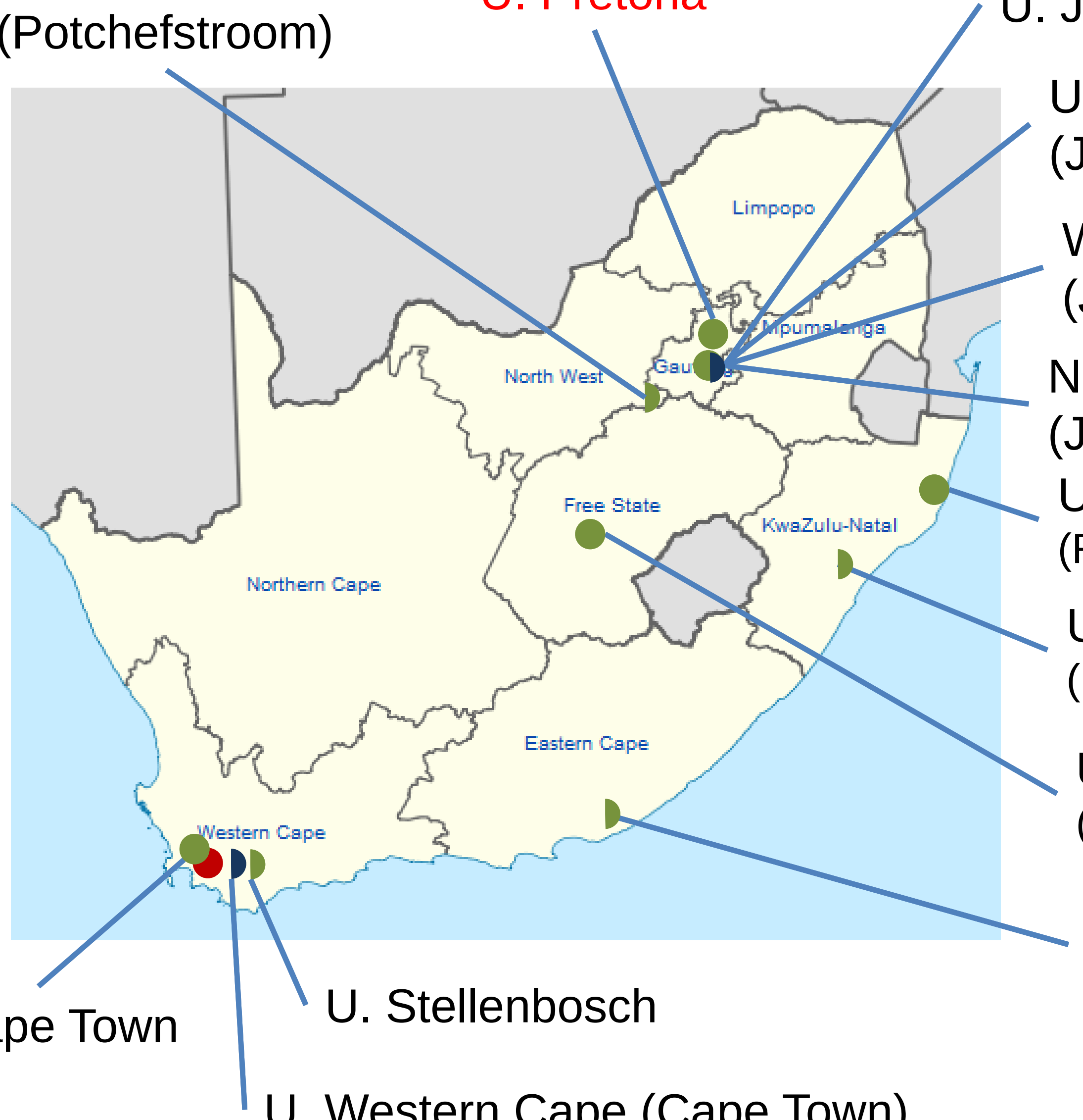

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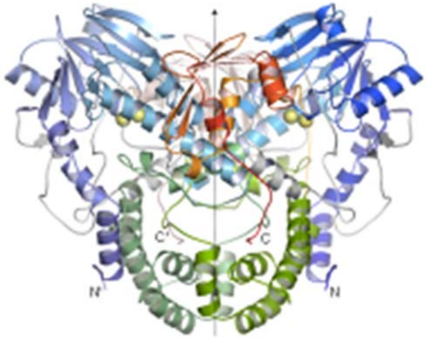
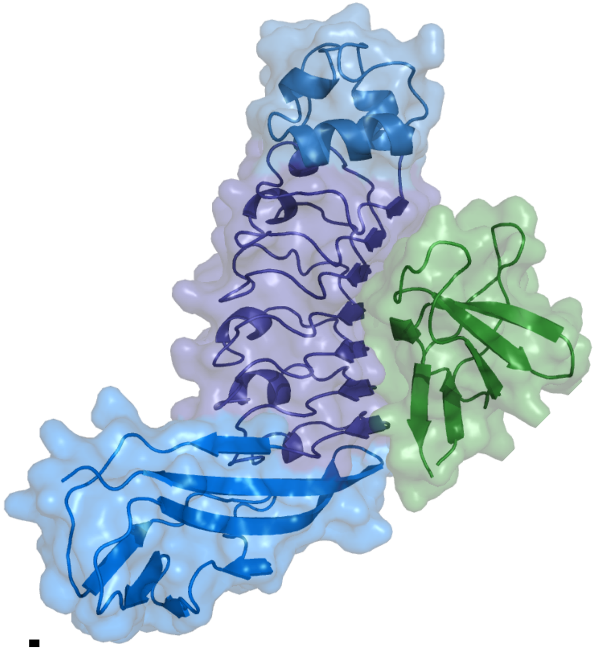
Wolf-Dieter Schubert

Structural Biology of Infectious Diseases

Molecular Host-Pathogen Interactions





University of Pretoria

Department of Biochemistry, Genetics and Microbiology

Projects

- Bacteria:

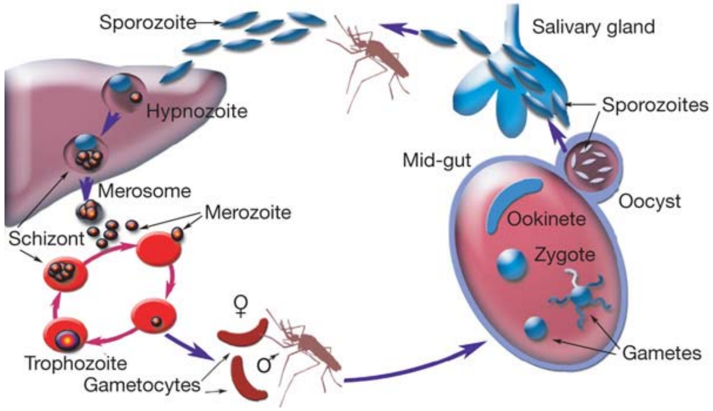
Listeria monocytogenes – Listeriosis (Keith Ireton)

Enterotoxigenic *Escherichia coli* (ETEC) – Travelers diarrhoea (James Fleckenstein)

Mycobacterium tuberculosis – Tuberculosis

- Parasites:

Plasmodium falciparum – Malaria (Lyn-Marie Birholtz)



- Viruses:

Norovirus (Janet Mans, UP Med. Virology)

African Horse-Sickness Virus (Vida van Staden)

- Biofuels:

Biomass degrading enzymes (Don Cowan and others)

- DNA repair

Human and trypanosomal primases-polymerases (primpols)

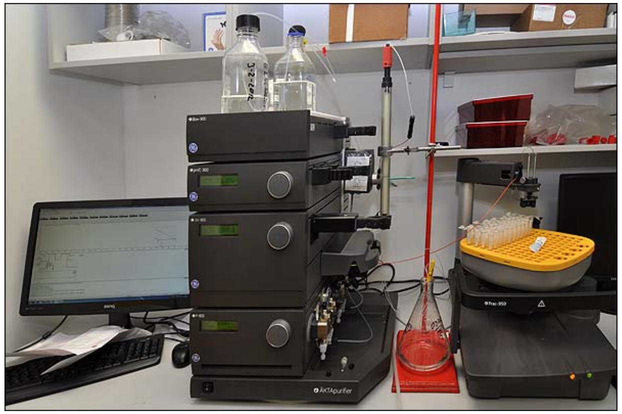
Equipment

Protein production

Protein purification

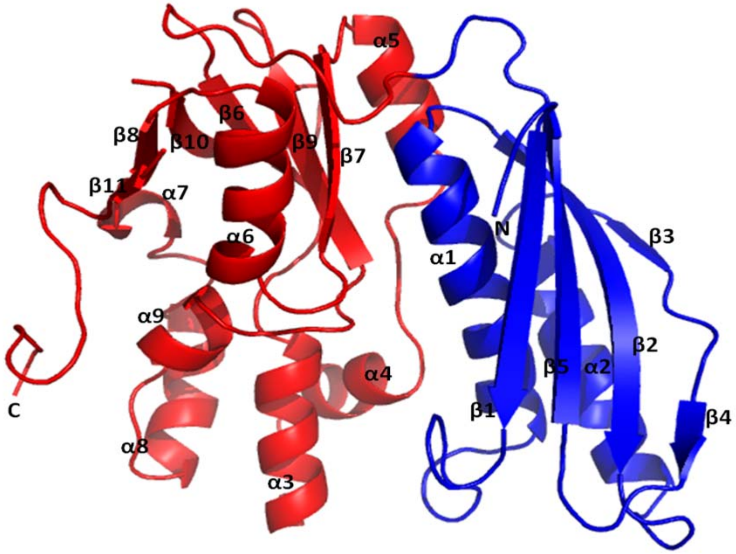
Rigaku rotating anode

- Dynamic light scattering (Eng.)
- CD spectroscopy (CSIR)
- Mass spectrometry (CSIR)
- Isothermal TC (ARC Onderstepoort)



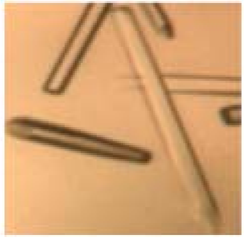
Protein crystallization

Mycobacterium tuberculosis



Thiamine Phosphate Kinase

- Only have the apo-structure at present
- Soaking not successful so back to co-crystallization



Homoserine Kinase

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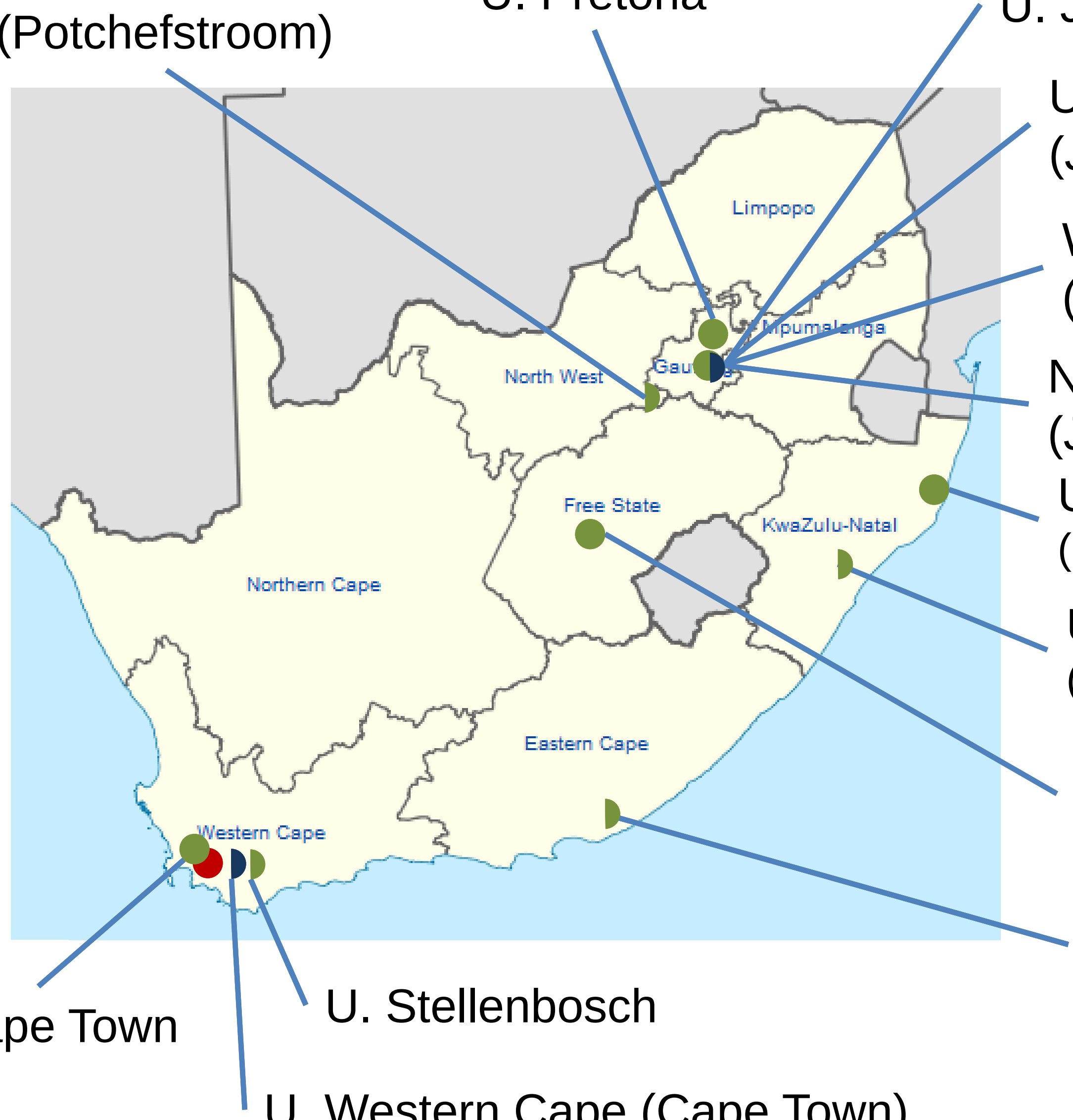

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University of the Free State

Biocatalysis: Oxidoreductases from Bacteria and Fungi

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BIOCATALYSIS


Dirk Opperman

Fundamental research / proof-of-principle vs commercially driven / industrially feasible reactions

- The discovery and characterization of new biocatalysts
- Their structural and mechanistic characterization
- Investigation as to their biocatalytic potential for biotechnology
- Their improvement through evolution

Advantages over chemical catalysis

- Cheap and easy to make
- Mild reaction conditions
- Selectivity and specificity


Carmien Tolmie


Martie Smit

Cytochrome P450 monooxygenases

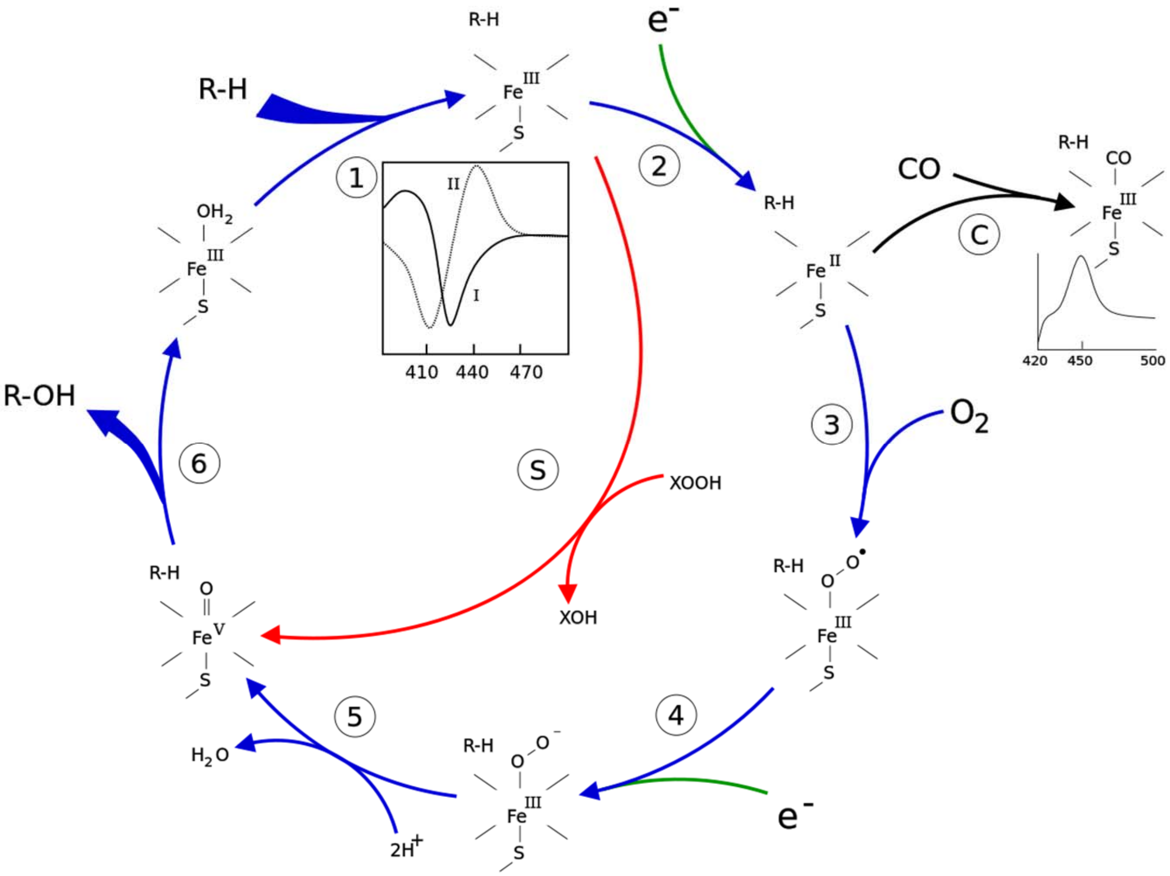
- Heme-thiolate proteins

Jacqui Van Marwijk

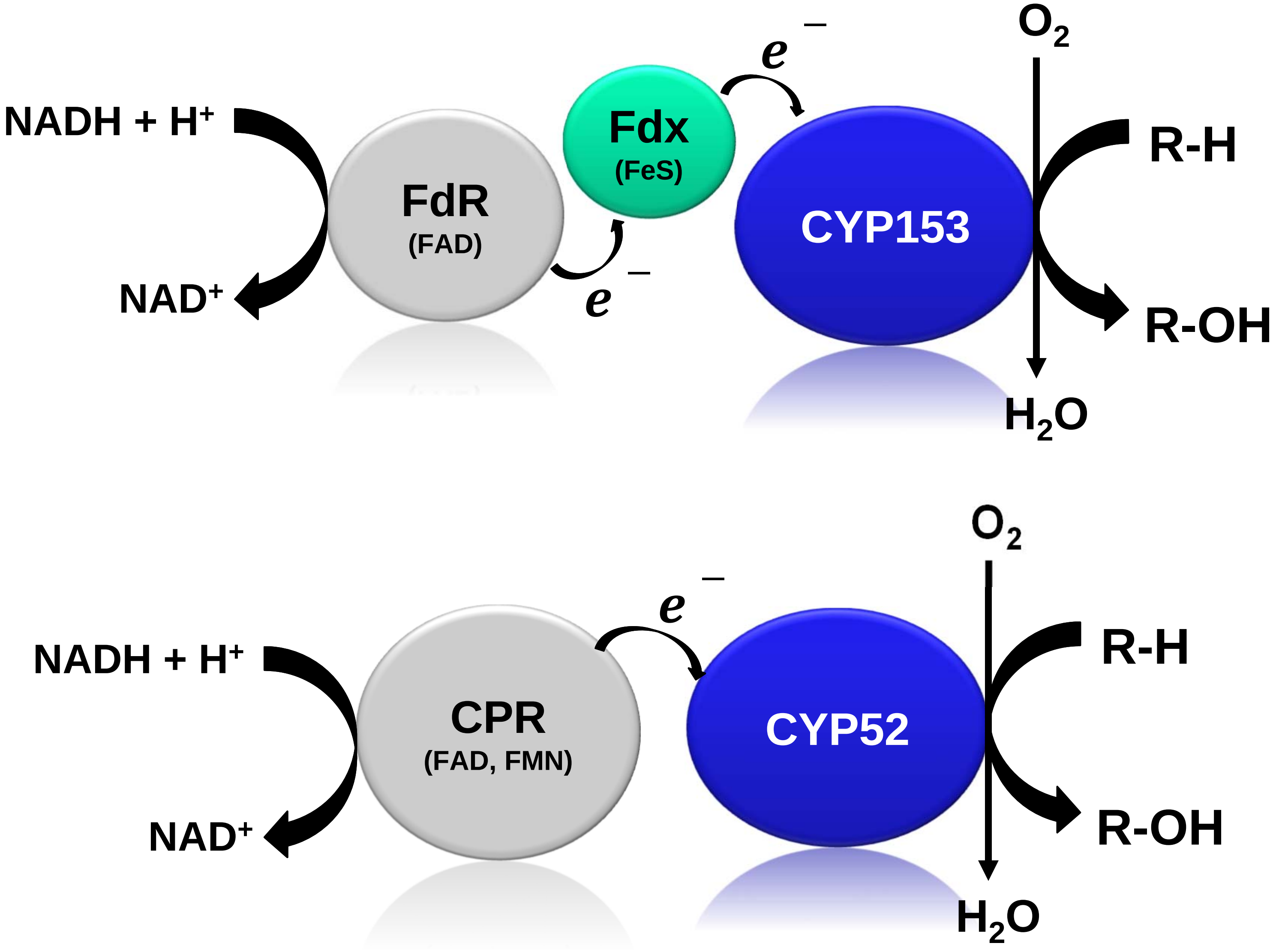
Cheri Jacobs

Alize Pennec

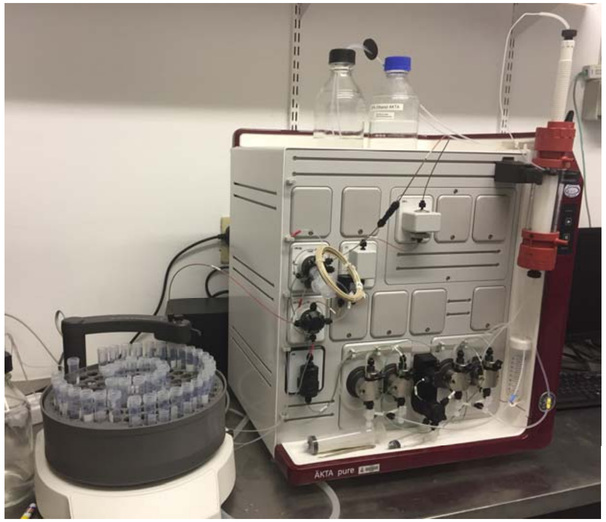
Thabang Maseme

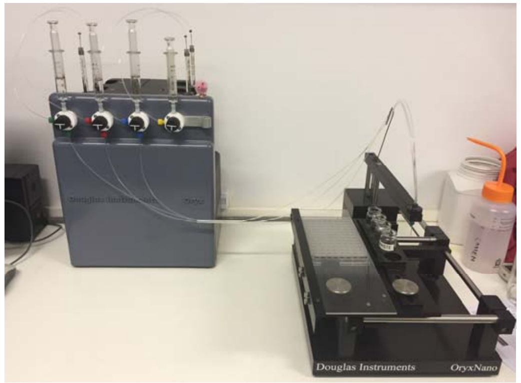


Cytochrome P450 monooxygenases



Infrastructure





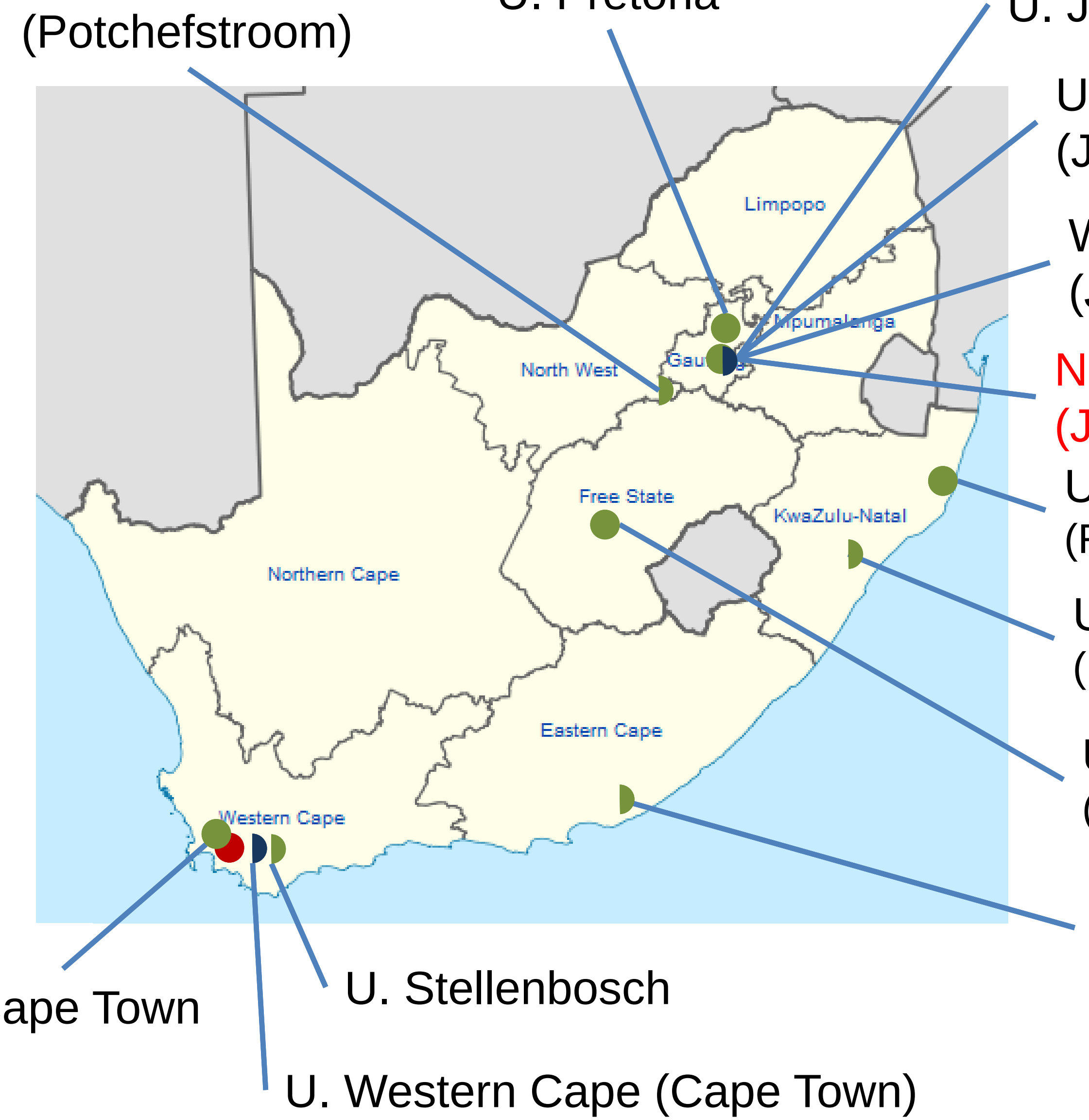
IµS microfocus - Diamond Cu
Department of Chemistry,
University of the Free State

Structural Biology in South Africa

● EM

● X-ray

● NMR



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Structural Biology of Neutralizing Antibodies

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

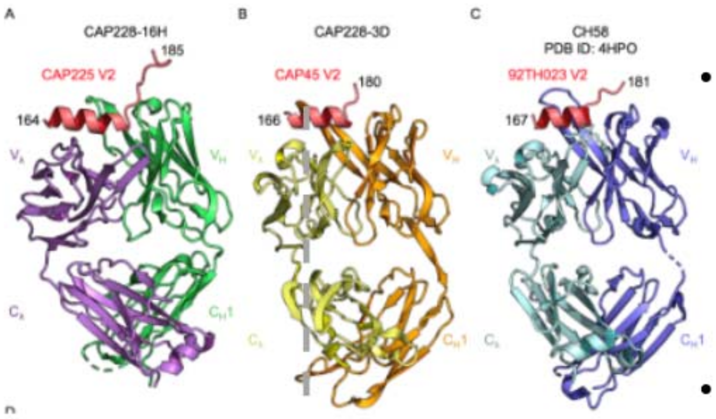

Lynn Morris


Penny Moore


Thandeka Moyo

Investigation of interactions between antibodies and their HIV envelope targets

- -define novel epitopes targeted by bNAbs
- -discover key residues important for the neutralization of HIV by bNAbs
- -define the differences in binding of different antibody isotypes
- -inform design of immunogens which will elicit bNAbs



V1/V2 scaffold subdomain

2.3 Å

V2 peptide

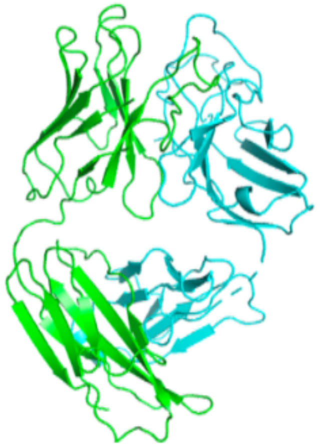
2.6 Å

- Co-crystal structures of two antibodies in complex with V1/V2 scaffold and V2 peptide

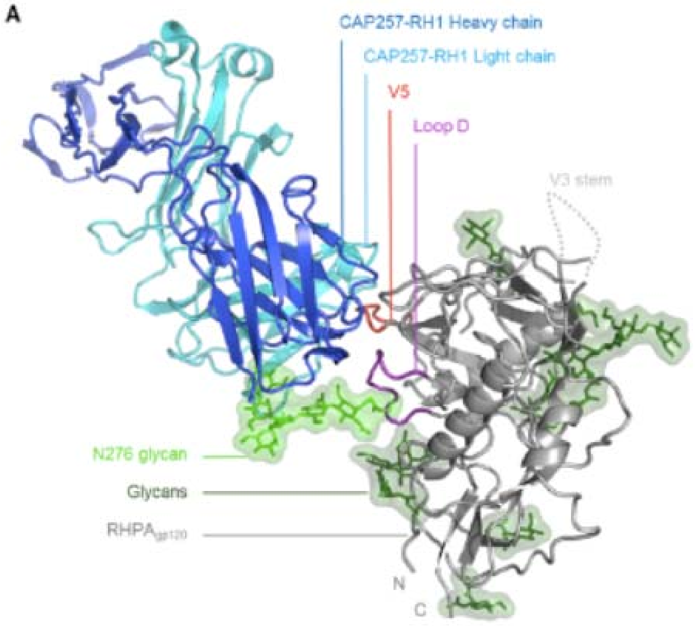
- These antibodies have similar epitope to a vaccine elicited antibody, CH58

van Eeden et al., 2018, Cell Reports

Crystal Structure of mAb 52



Crystal structure of an HIV antibody which helped drive the emergence of a broadly neutralizing lineage *Moyo, unpublished data*



Co-crystallization with HIV antigen gp120 revealed binding angle of this antibody was incompatible with glycosylated V5 loops present in almost all HIV strains; making it less broadly neutralizing

Wibmer et al., 2016, Journal of Virology

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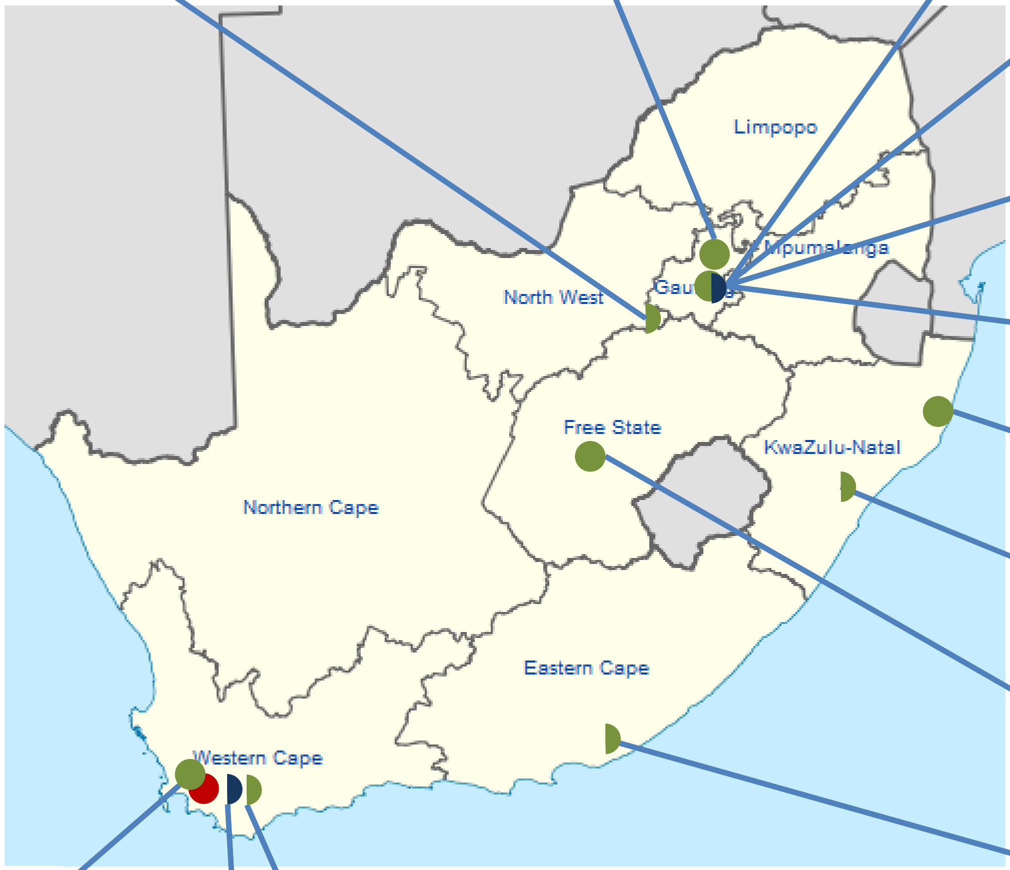

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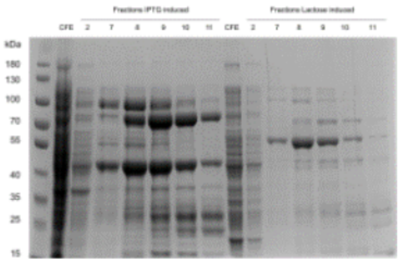
North West University

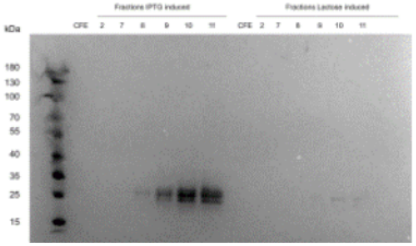
North-West University (NWU), Potchefstroom – Postdoc Dr Ana Ebrecht

1. Structure of Glycine N-acyltransferase (GLYAT)

SDS-PAGE of GLYAT expression

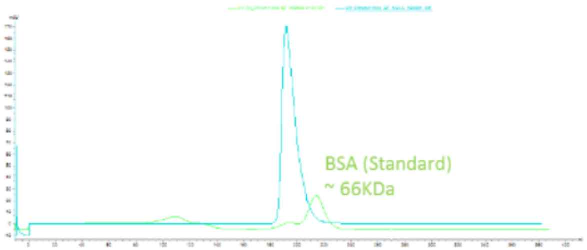


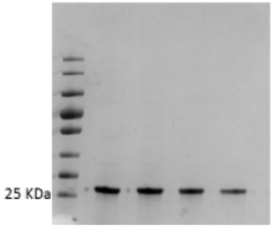
western blot confirms expression



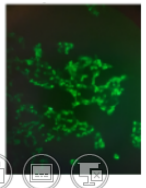
2. Structure of African horsesickness virus non-structural protein 4

Gel filtration of bacterial expressed AHSV NS4-L and SDS-PAGE of peak fractions

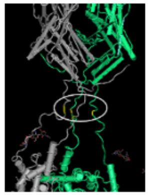




3. Structure of reverse genetics modified SA11 rotaviruses



Immunostaining of rescued rotavirus



In silico design: Location of first and last foreign amino acid for rotavirus VP4 chimera

Dr Ana Ebrecht

Prof Albie van Dijk

Luan Theart MSc student

Prof Christiaan Potgieter: Deltamune

Pierre le Roux MSc student

Martin Visser MSc student

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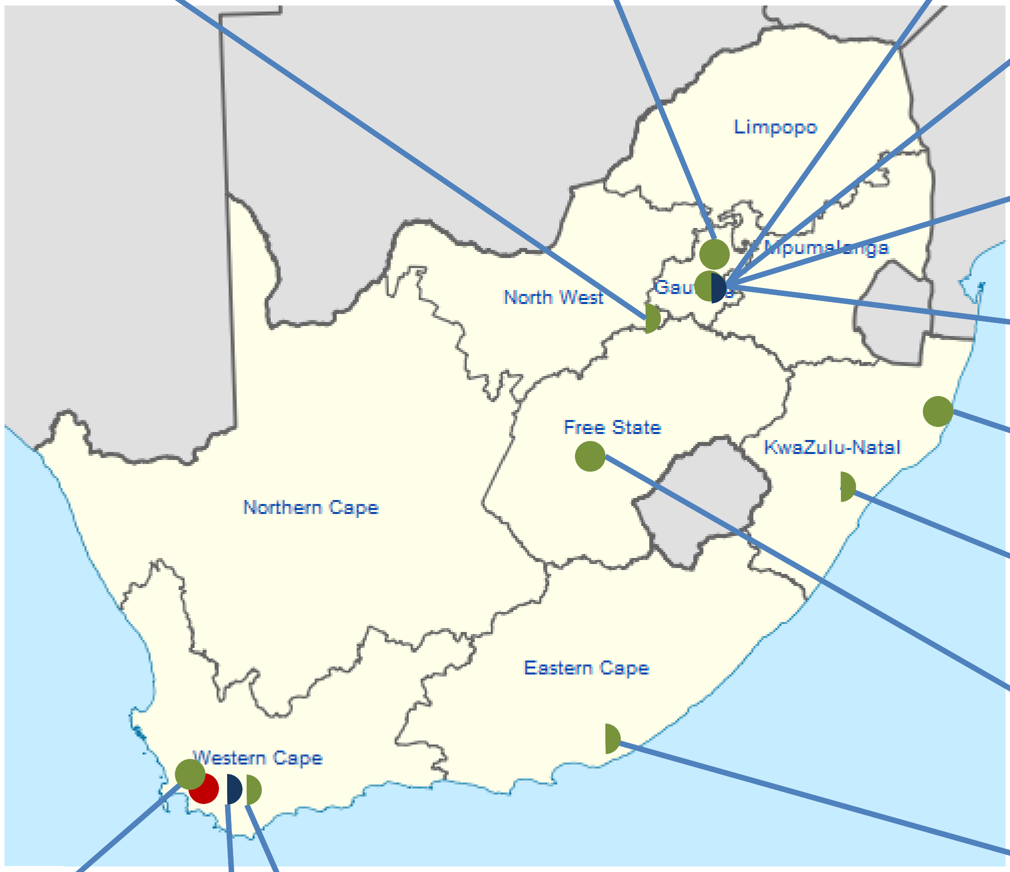

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University of Stellenbosch

Department of Biochemistry

Erick Strauss

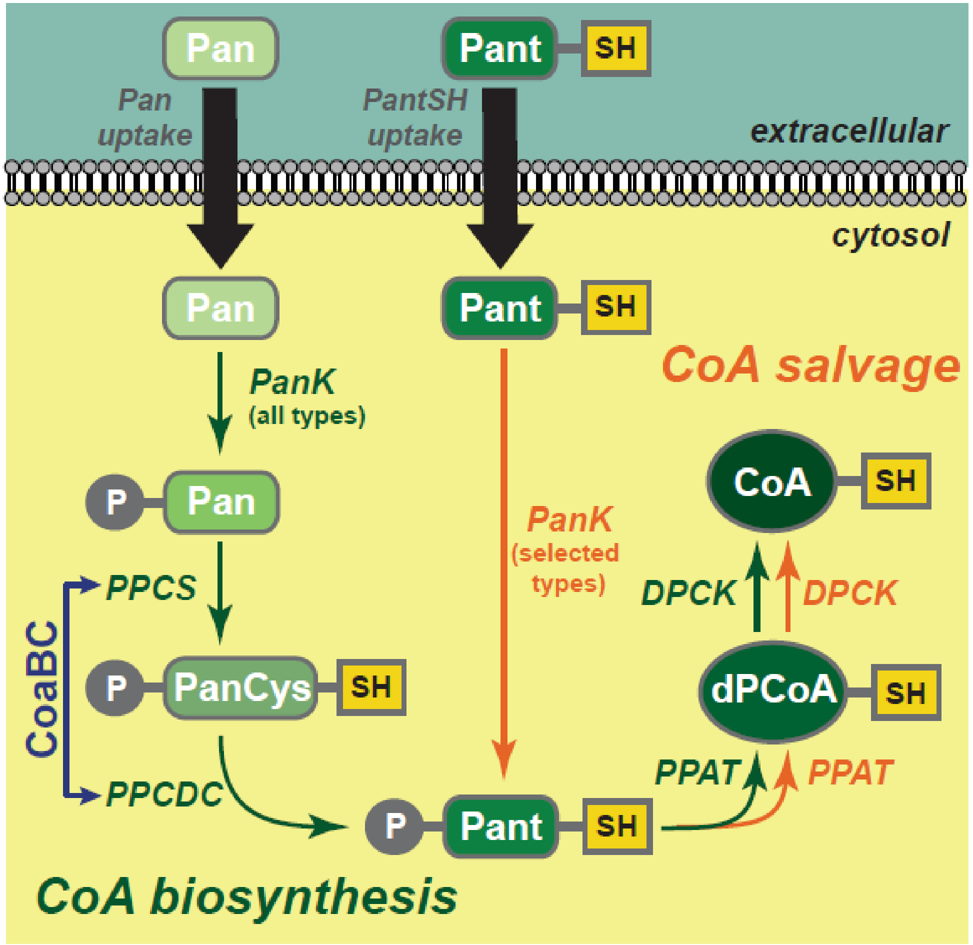
Anton Hamann

Blake Balcomb

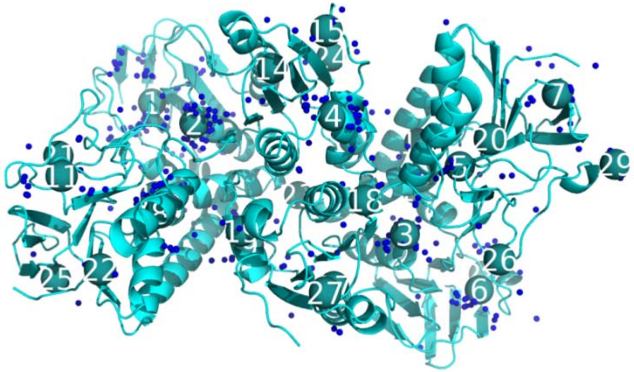
Chemical Biology:

- Chemistry and biology of the ubiquitous metabolic cofactor Coenzyme A.
- Inhibition of coenzyme A biosynthesis in *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Plasmodium falciparum.*
- Fragment screening @ Xchem, Diamond.

Structural analysis mostly done in collaboration with colleagues in the USA and South Africa.



X-ray crystallography based fragment screening (Xchem @ Diamond)



Med. Chem follow up & assays

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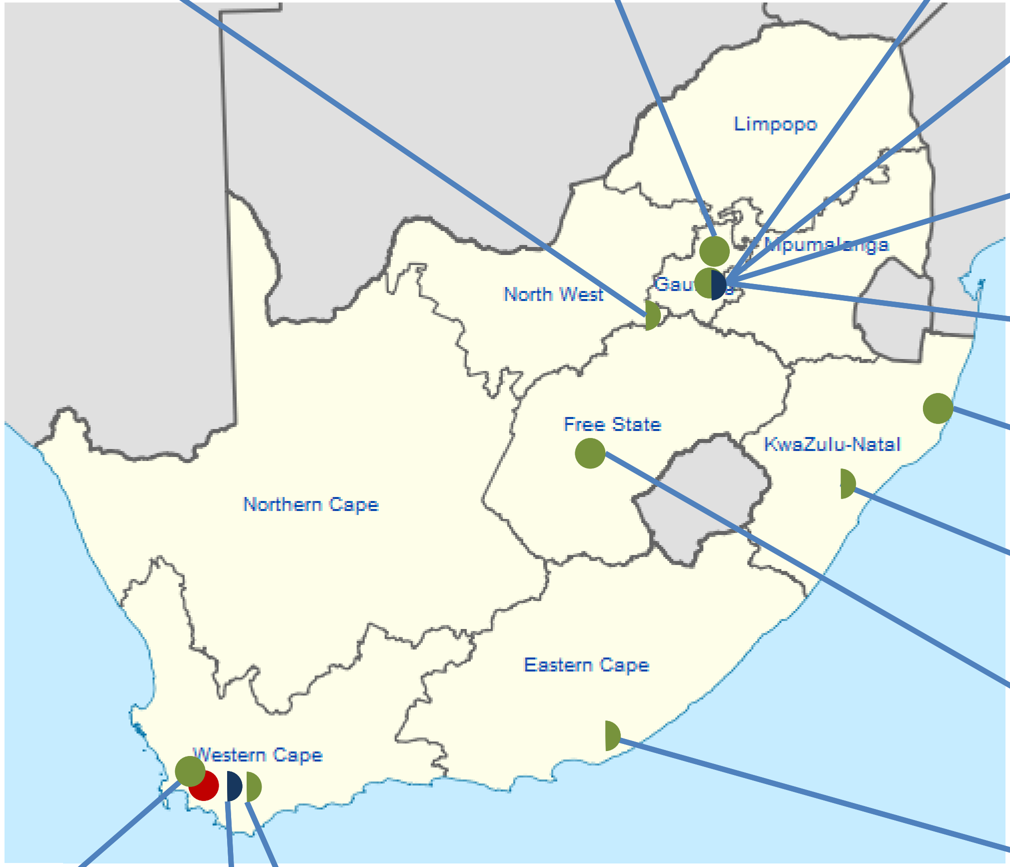

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Molecular Biophysics and Structural Biology (MBSB) Group

Group Leader: Prof Abidemi Paul Kappo

Research Group composition:
Research Fellow = 1
PhD candidates = 5
MSc candidates = 5
BSc (Hons) students = 3

Funding: SAMRC, NRF and UNIZULU

Molecular biophysics in studying protein-protein and protein-ligand interactions

Interaction between Hsp70.14 and RING finger domain of RBBP6 towards cancer drug discovery

Structural determination of proteins using macromolecular X-ray crystallography

Structural determination of S. mansoni Universal stress G4LZI3 protein towards the design of new anti-schistosomicidal agents

Development of point-of-care aptamer-based diagnostic biomedical device

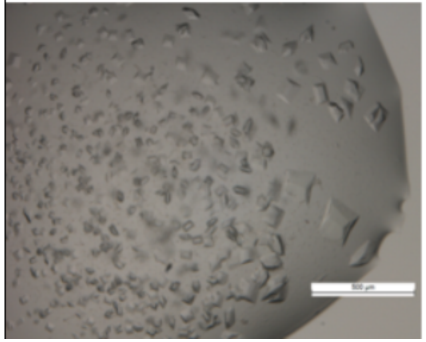
Early detection for Cancer and HIV infection in non-clinical settings almost completed
Studies on Schistosomiasis and Zika are currently ongoing

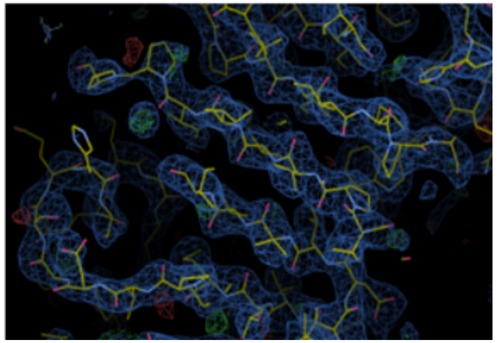
Priscilla Masamba

Molecular Biophysics and Structural Biology (MBSB) Group
University of Zululand, South Africa
Aaron Klug Centre for Imaging and Analysis, University of Cape Town, South Africa

My project is based on structure determination of the Universal Stress G4LZI3 Protein which has been labeled as a potential drug target for the development of novel treatment against schistosomiasis, a chronic and debilitating disease that affects an estimated 4.5million people in South Africa while causing the deaths of 200 000 people worldwide. So far, crystal data has been acquired and the structure is currently undergoing refinement.

Working under the START program in collaboration with UCT has allowed UNIZULU, a black and disadvantaged institution access to resources and expertise that we lacked before. Moreover, it has given me the opportunity to possesses scarce skills which will be transferred via training to fellow postgraduate students and emerging researchers at the institution.





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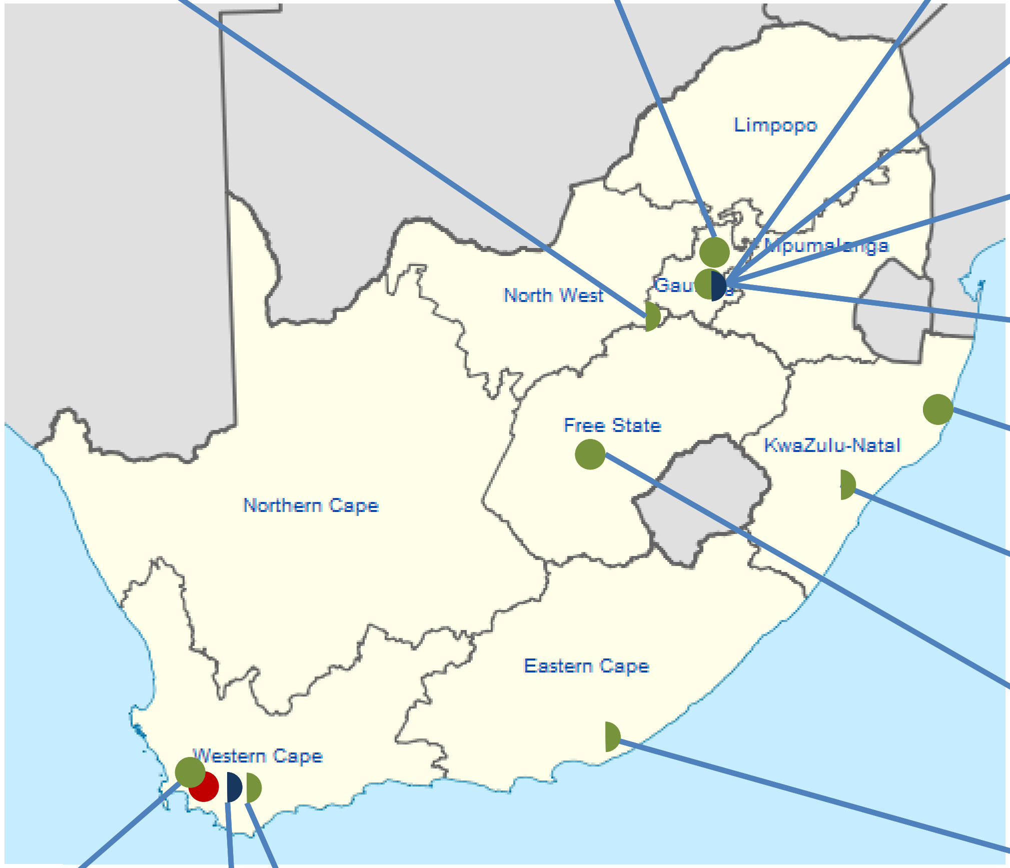

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(Pietermaritzburg)

U Free State
(Bloemfontein)

Rhodes U
(Makhandla)

U Cape Town

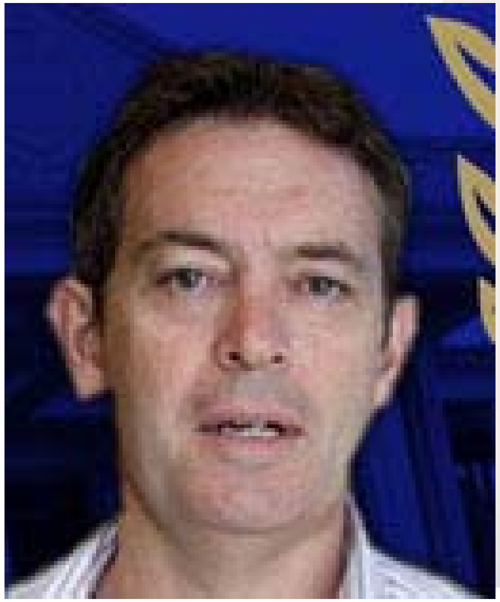
U. Stellenbosch

U. Western Cape (Cape Town)

David Pugh

Department of Biotechnology

University of the Western Cape



NMR spectroscopy
600 MHz NMR spectrometer in Stellenbosch

Cancer research

South Africa – associate of the European Synchrotron Radiation Facility (ESRF)

European Synchrotron Radiation Facility (ESRF), Grenoble, France

SA Structural Biology community part of international BAG (Germany, Russia, SA). Beam time: 1 day once a month

SYNCHROTRON TECHNIQUES
FOR AFRICAN RESEARCH
AND TECHNOLOGY

START

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 funds:

- Extended training in Synchrotron techniques - The nexus of START is Diamond Light Source, UK.
- Emerging and neglected diseases relevant to the African continent.

START (Bio) - SA co-investigators

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

Remote X-ray Crystallography

- Joint Bulk Allocation Group (BAG)
- ~1 8h shift per month.
- Divided into northern and southern clubs with 3 research groups each.
- Shipping of Dewars via DHL – Diamond contract, significant reduction in cost.
- Sufficient Dewars to ship crystals with a lead time of 7 to 10 days.

Remote data collection

- Most time spent on finding and optimally positioning crystals.
- All data automatically processed and solved if sequence provided.
- Data mostly remains and is archived at Diamond
- Remote re-evaluation.
- Only download structure factor file and coordinate file.

Remaining issues:

- Problems with DHL
- Coordination between groups
- Spotty internet connection

Diamond Light Source, Oxfordshire, UK