Department of Biochemistry Stellenbosch University

Project portfolio 2018-2020

1918 · 2018

SCIENCE
NATUURWETENSKAPPE
EYOBUNZULULWAZI

ENZYME KINETICS

FOR SYSTEMS BIOLOGY

Project leader
Prof Johann Rohwer
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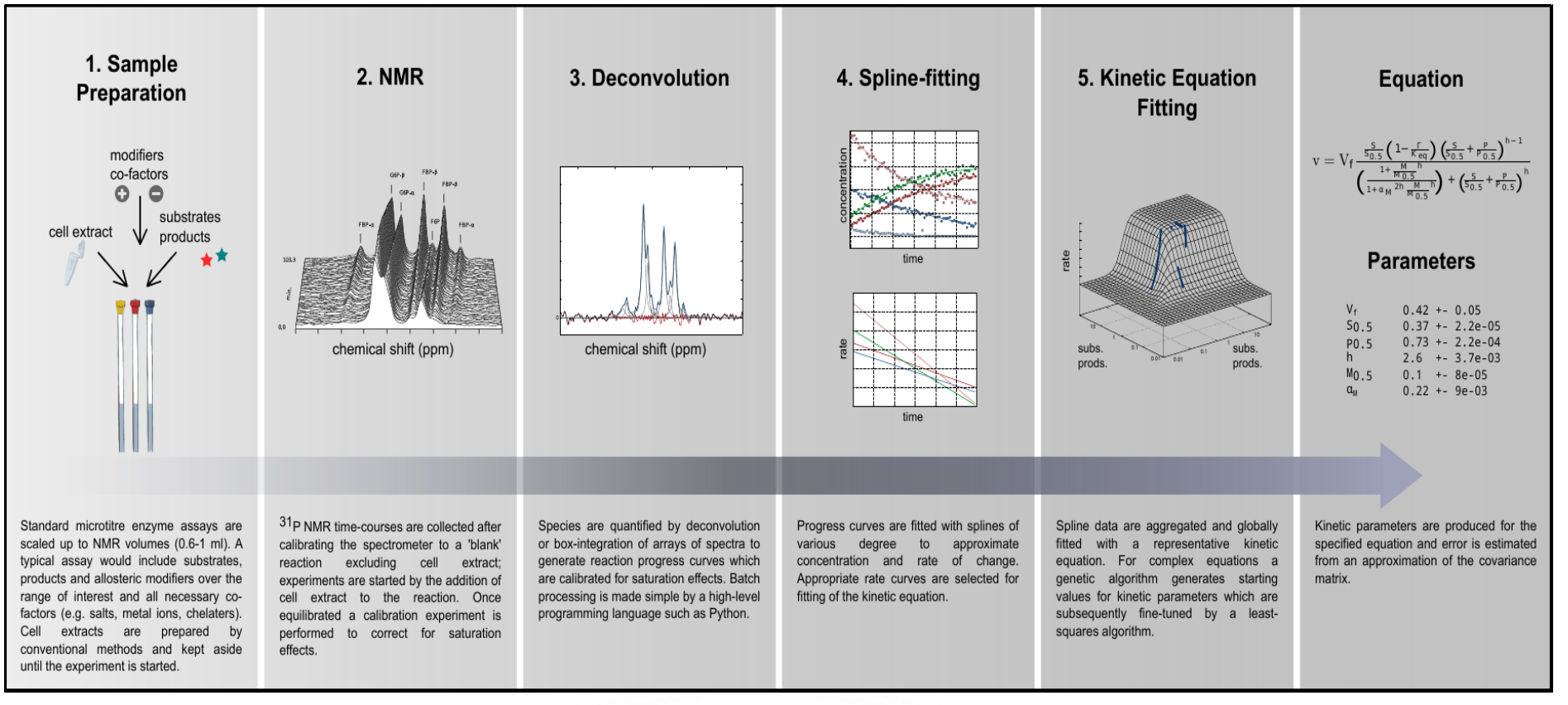
HIGHLIGHTS

- We construct kinetic models of metabolic pathways
- This requires enzyme kinetic parameters determined under realistic cellular conditions
- We have developed a high throughput method using NMR spectroscopy
- Using this, we can easily investigate effects of e.g. pH or macromolecular crowding

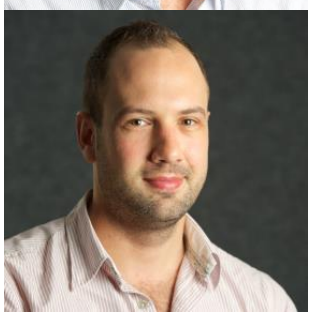


APPLICATION OF RESEARCH

- Kinetic models help us better understand emergent properties of complex cellular systems
- They can assist in drug discovery, biotechnology, crop improvement

MATHEMATICAL MODELLING TOOLS

JWS ONLINE



Project leaders
Prof Jacky Snoep &
Dr Dawie van Niekerk
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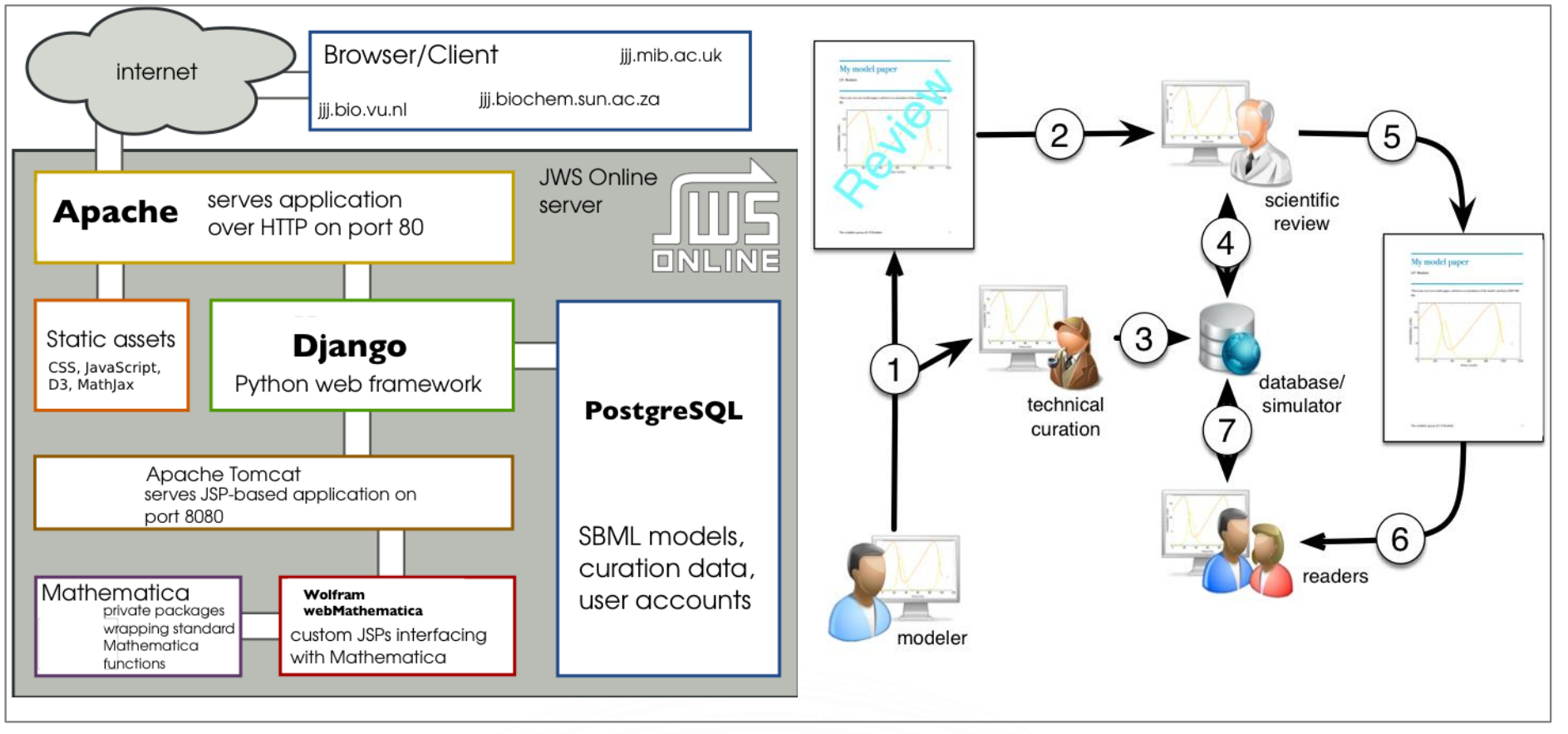
HIGHLIGHTS

- Curated open-access database of published models of metabolism, signal transduction, disease, pharmacology and epidemiology
- Browser-based simulation and manipulation of models
- International collaboration with journals on published models
- Part of FAIRDOM Association for Findable, Accessible, Interoperable, and Re-usable research (fair-dom.org)

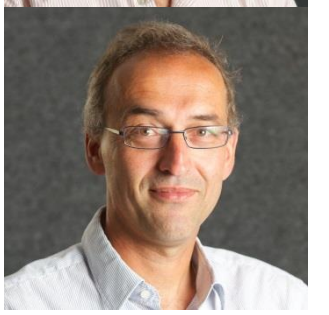


APPLICATION OF RESEARCH

- Model builder and simulation tools for research and teaching
- Simulation database for one-click figure reproduction
- Technical curation of models during peer review process

METABOLIC MODELLING

YEAST GLYCOLYTIC OSCILLATIONS



Project leaders
Dr Dawie van Niekerk &
Prof Jacky Snoep
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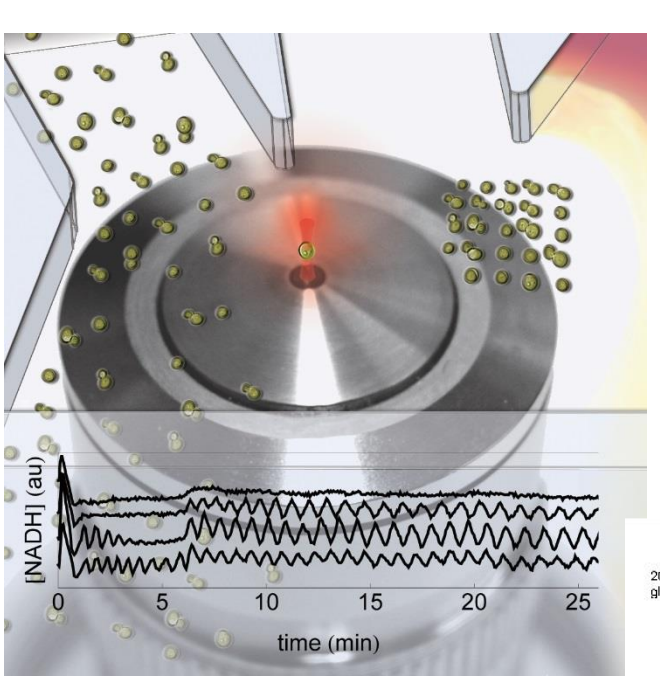
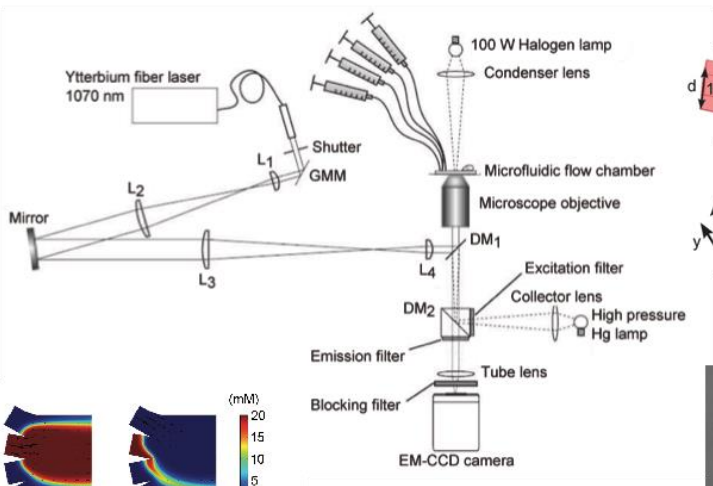
HIGHLIGHTS

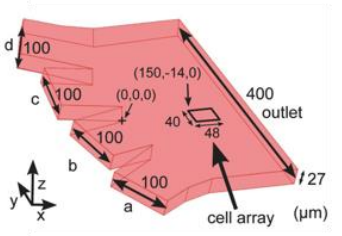
- **Constructed and validated detailed mathematical models of glycolysis during oscillations**
- **Predicted and confirmed oscillation-features in single cells**
- **Gaining a mechanistic understanding of phase shifts and synchronization in populations of cells**

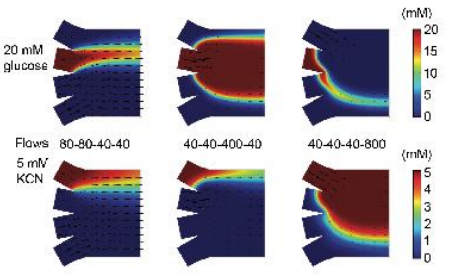


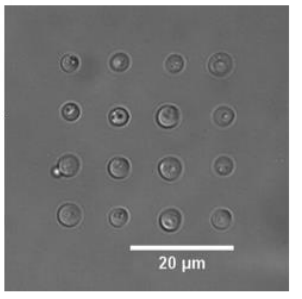
Gustavsson, van Niekerk et al. FEBS Journal 279, 2012.

Experimental setup with optical tweezers and microfluidic flow chamber.









APPLICATION OF RESEARCH

- **Broadened understanding of oscillation characteristics and communication and synchronization phenomena in biological oscillators**

MODELLING CELLULAR NETWORKS

REDOXIN

Project leader
Prof Johann Rohwer
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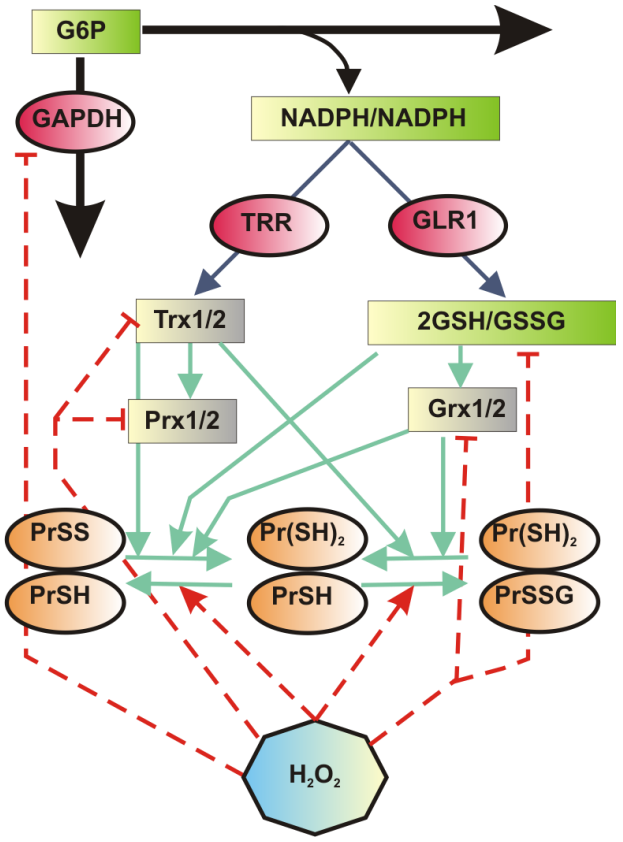
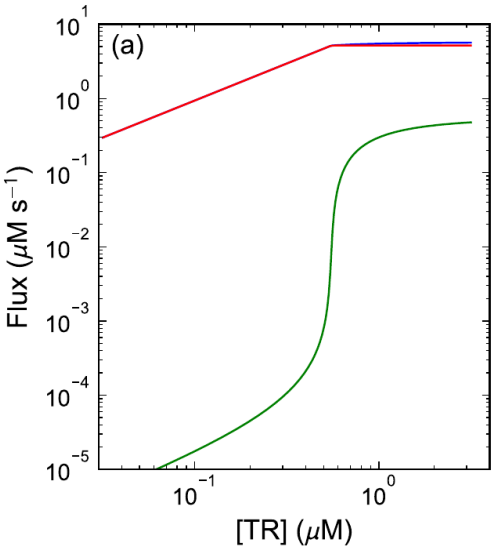
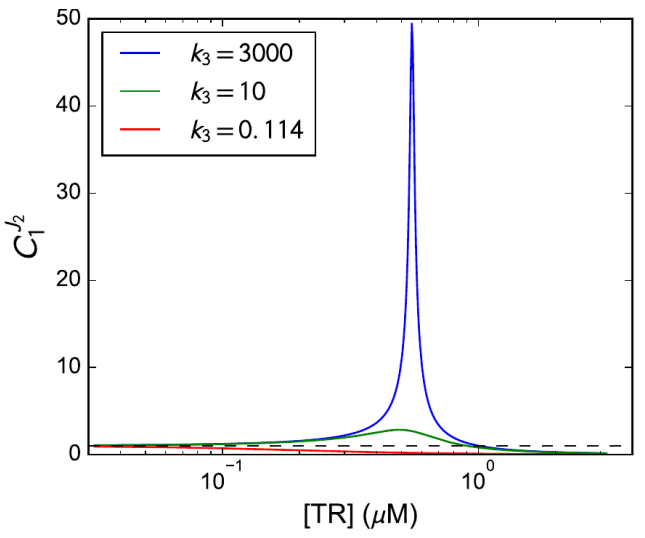
HIGHLIGHTS

- **Studying the importance of redoxin networks in regulation, antioxidant defence and signalling**
- **Developing quantitative measures for describing these networks in computational models**
- **Constructing new models, e.g. for *E. coli* and *M. tuberculosis***
- **Comparing these networks, e.g. between host and pathogen**

Redoxin networks display complex inter-connectivity with multiple interacting loops







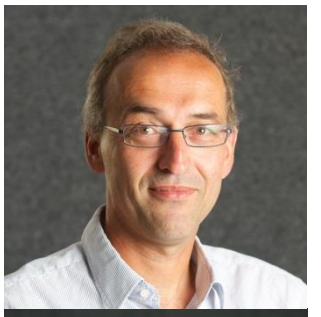
They can show ultrasensitive responses, which can be quantified and understood with metabolic control analysis

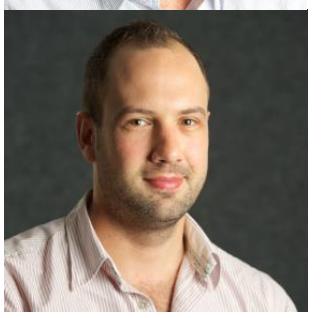
APPLICATION OF RESEARCH

- **Redoxin networks contribute to health and disease**
- **A thorough quantitative description of their regulation may assist the development of intervention strategies for combatting disease**

MECHANISTIC MODELLING

THE BIOCHEMISTRY OF DISEASE





Project leaders
Prof Jacky Snoep &
Dr Dawie van Niekerk
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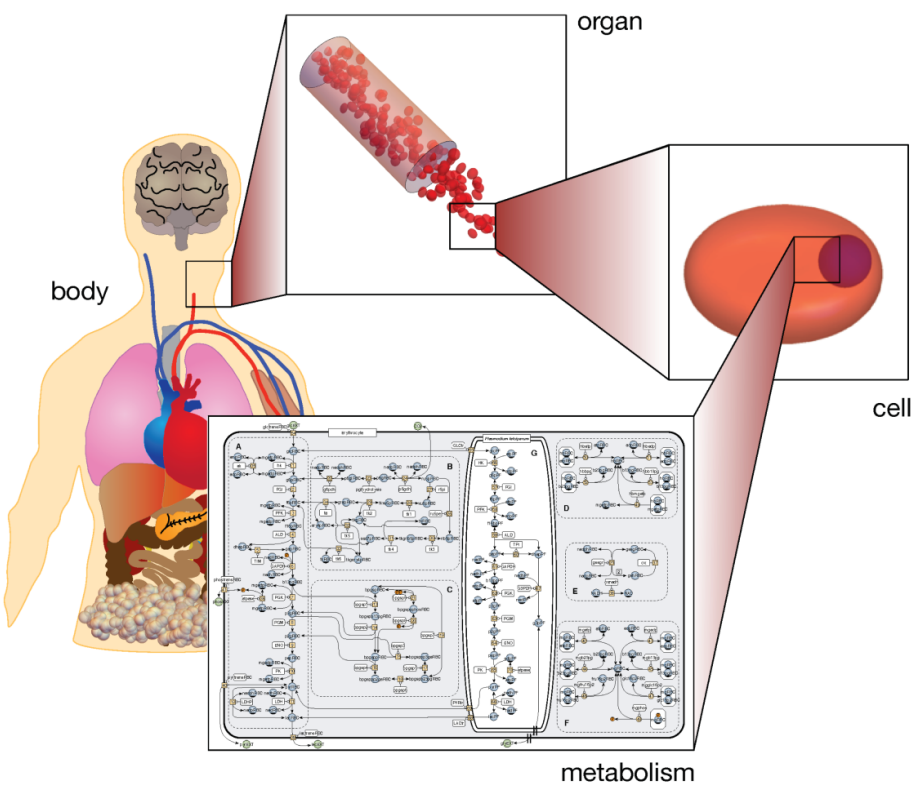
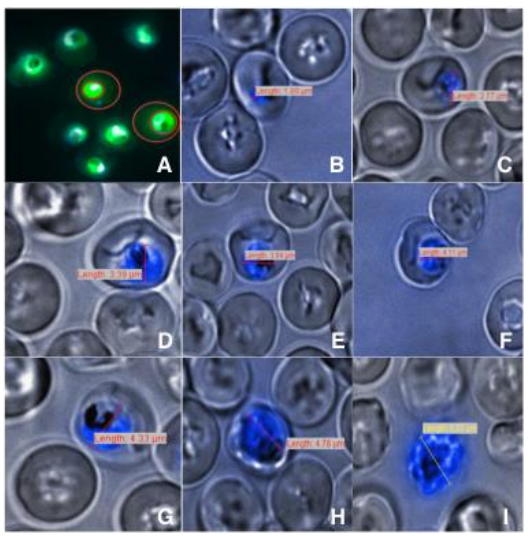
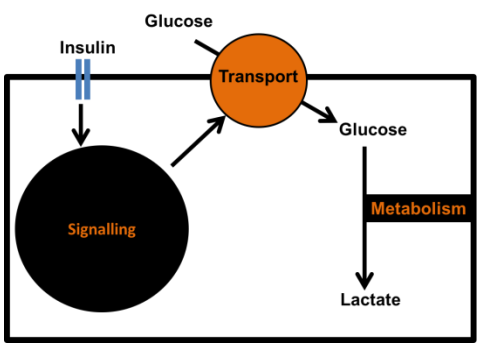
HIGHLIGHTS

- **Constructed a detailed model of glycolysis in the malaria parasite and glucose homeostasis in the malaria-infected human**
- **Characterising kinetics of insulin signalling and glucose metabolism in type 2 diabetes**
- **Elucidating cholesterol metabolism in *Mycobacterium tuberculosis***
- **Investigating dynamics in models of HIV disease progression**



Snoep et al, Biochem. Soc. Trans. 43, 2015.





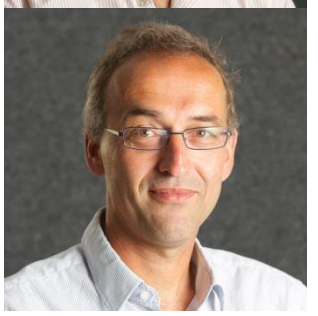
APPLICATION OF RESEARCH

- **Drug target identification in malaria and TB**
- **Mechanistic understanding of disease pathophysiology and progression**

SACEMA

DISEASE MODELLING

LINKING DISEASE TO PHARMACOLOGY AND EPIDEMIOLOGY



Project leaders
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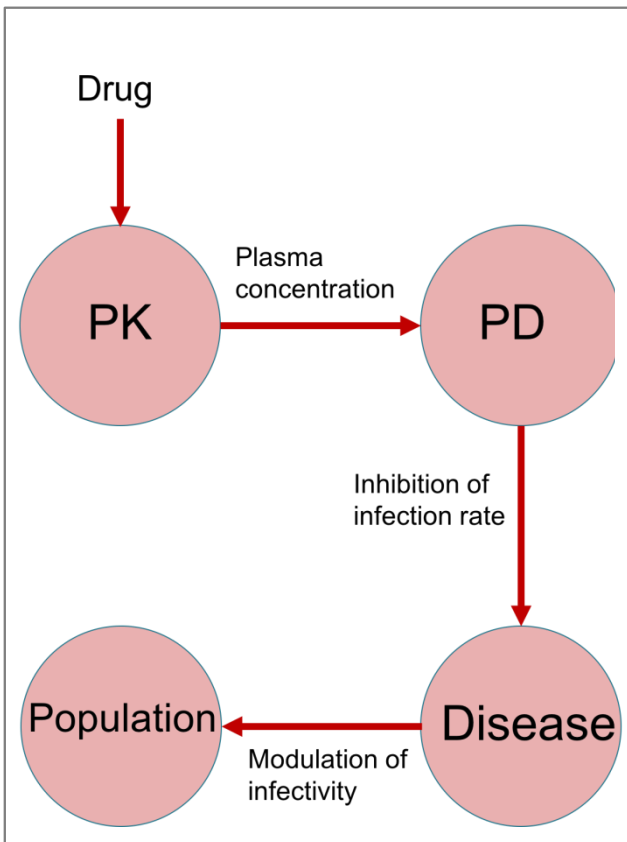
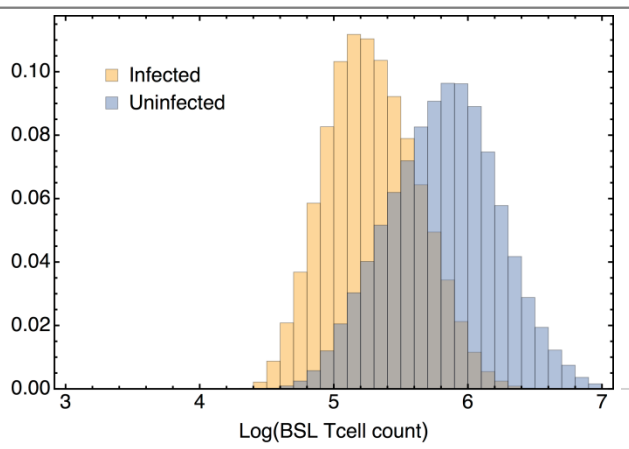
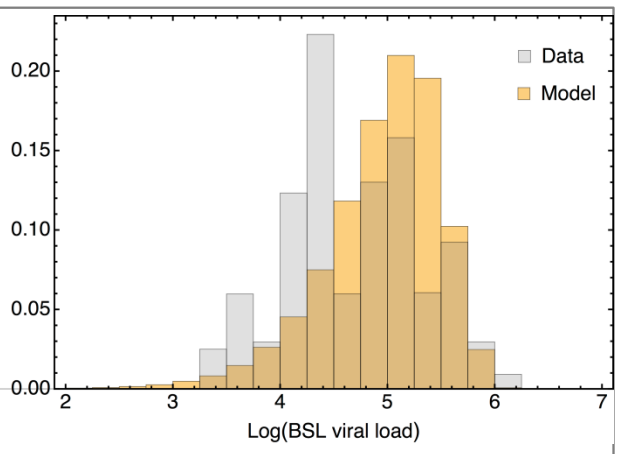
HIGHLIGHTS

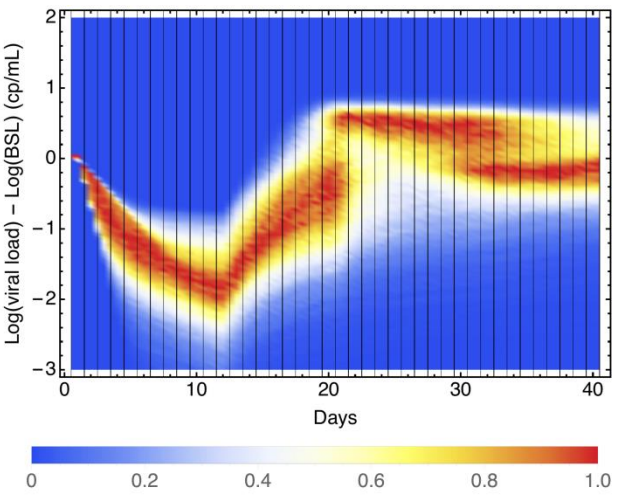
- **Linking models of personal health and pharmacology to epidemiology (public health)**
- **Prediction of disease progression upon drug intervention**
- **Investigating clinical features and epidemiology as a result of patient heterogeneity and drug regimens**



Adapted from Rosario et al., Clin. Pharm. & Ther. 78, 2005.







Effect of patient heterogeneity on disease biomarkers and progression when treated with an ARV for 10 days.

APPLICATION OF RESEARCH

- **Mechanistic understanding of drug side effects and efficacy**
- **Informing strategies for public health**

SACEMA

PROGESTINS AND CANCER

ROLE OF STEROID RECEPTOR CROSSTALK

Project Leader
Prof. Donita Africander
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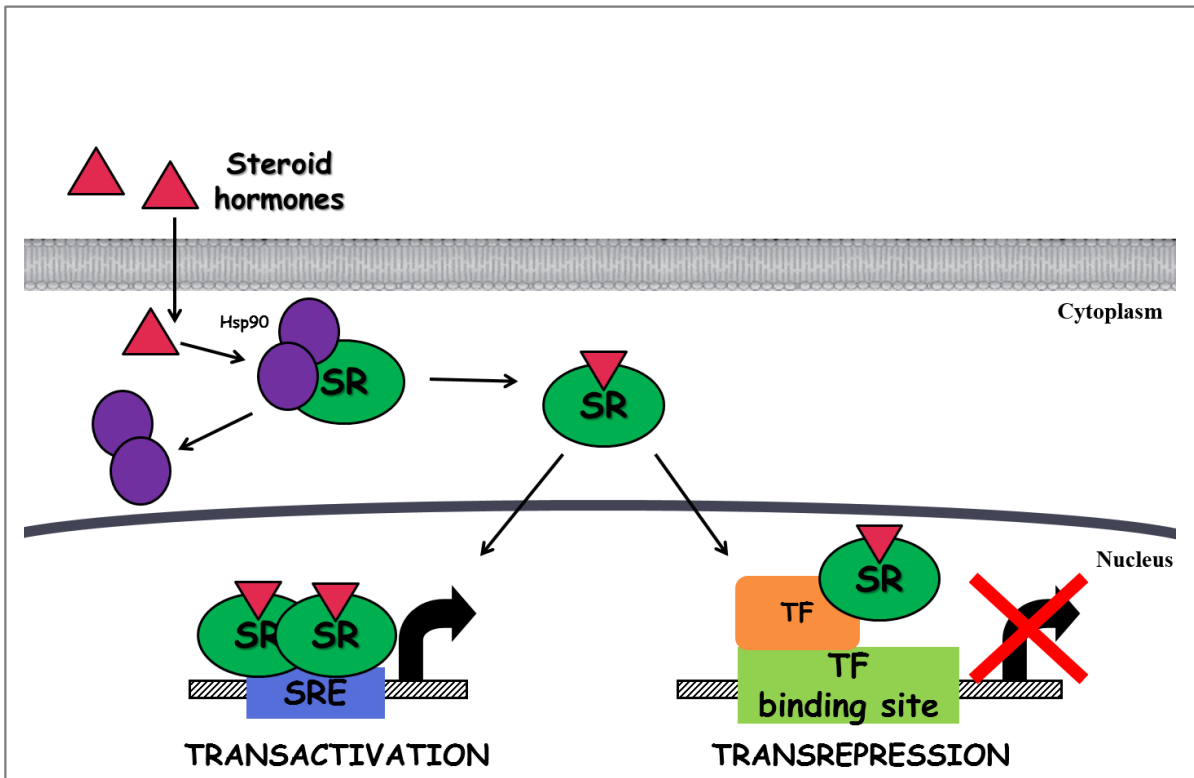
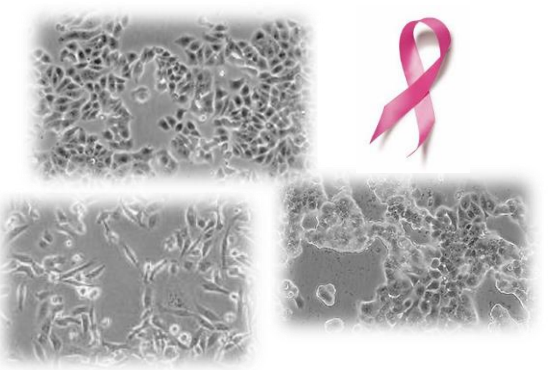
HIGHLIGHTS

- Hormone therapy (HT) is prescribed to relieve symptoms of menopause.
- Estrogens and progestins used in HT have been associated with increased breast cancer risk.
- Interplay between several steroid receptors (SRs), to which these hormones bind, have been implicated in breast cancer pathogenesis.
- We showed that the SR mediated effects of progestins often differ from one another and natural progesterone, suggesting that not all hormones used in HT may increase breast cancer development.



Steroid hormones used in HT elicit their effects by binding to intracellular SRs. The ligand activated SR translocates to the nucleus where it activates (transactivation) or repress (transrepression) transcription of target genes. Regulation of gene expression affects various physiological processes known to play a role in breast cancer biology. SRE – steroid response element; TF – transcription factor



APPLICATION OF RESEARCH

- Aid in the design of new HTs that would alleviate menopausal symptoms without increasing breast cancer risk.
- The outcome of this study may influence the current implementation of health practices in South Africa.

ADRENAL SEX STEROIDS

THE IDENTIFICATION IN DISEASE STATES

Project leader
Prof Amanda C Swart
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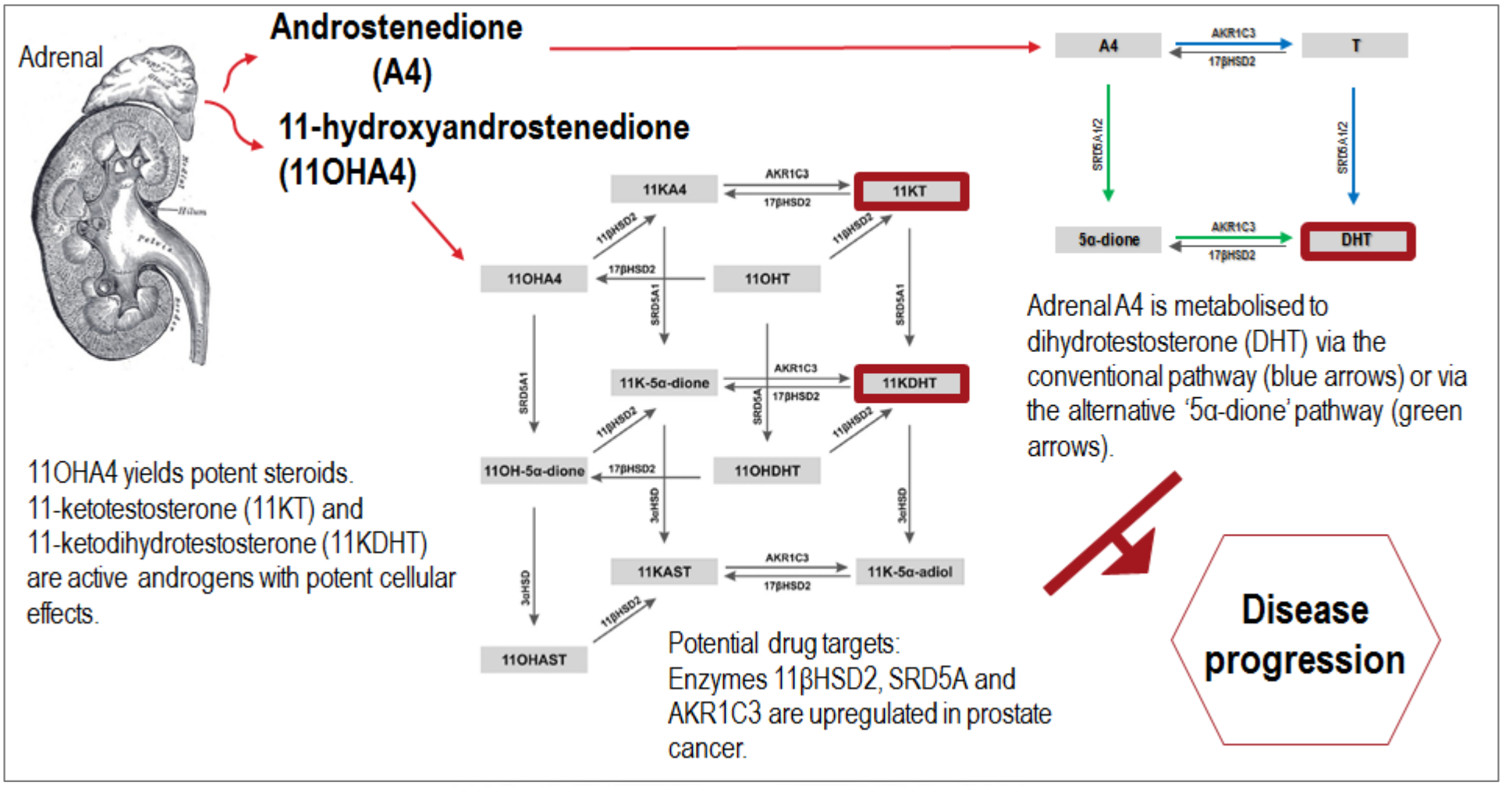
HIGHLIGHTS

- C11-oxy adrenal steroids drive disease development/progression.
- C11-oxy androgens and progesterones contribute to adrenal hyperplasia, polycystic ovary syndrome, prostate cancer, etc.
- *In vitro* cell models are utilized to investigate integrated steroid production and metabolism.
- UPC^2-MS/MS enables comprehensive & accurate steroid evaluation in tissue and blood establishing steroid fingerprint profiles.



APPLICATION OF RESEARCH

- Steroid fingerprinting – identifying steroids as biomarkers in various disease states
- Drug targets – enzymes in metabolic pathways can be identified as drug targets

11-OXYGENATED ANDROGENS

IN HEALTH AND DISEASE

Project leader
Dr Karl Storbeck
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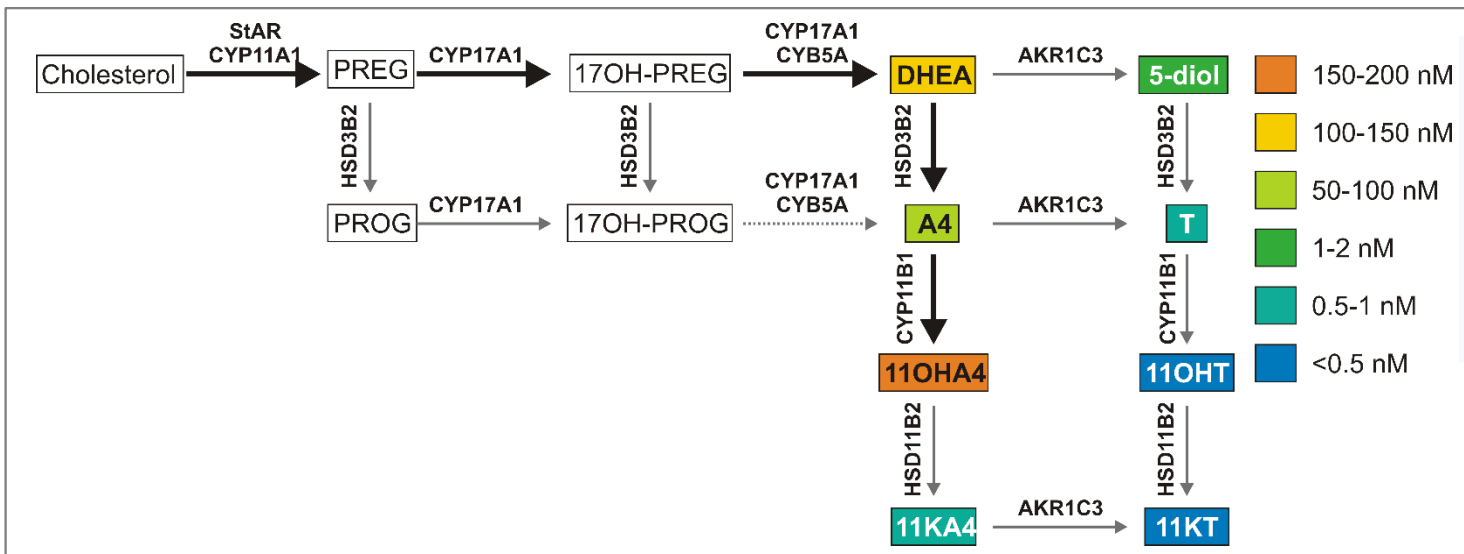
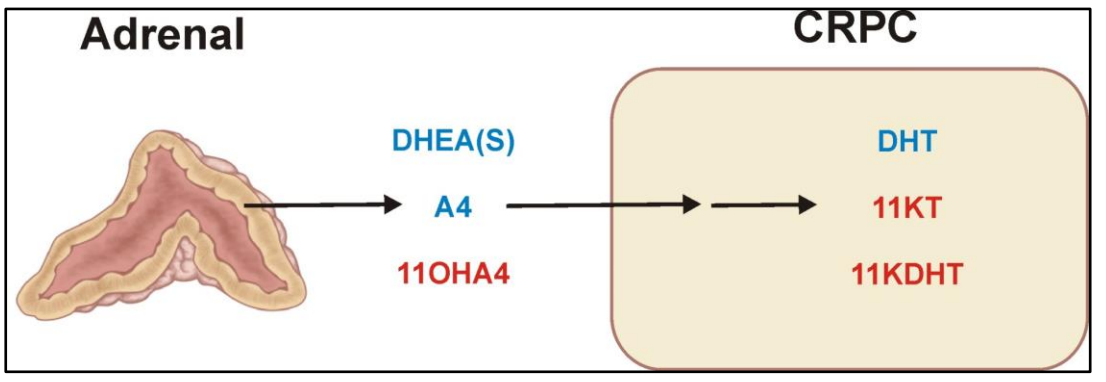
HIGHLIGHTS

- **Adrenal 11β-hydroxyandrostenedione (11OHA4) was previously written off as a dead end product.**
- **Recent studies have shown that 11OHA4 is the precursor to novel 11-oxygenated androgens (e.g. 11-ketodihydrotestosterone)**
- **11-oxygenated androgens are as potent as classical androgens.**



11OHA4 is an abundant product of the human adrenal

11OHA4 is the precursor for the production of potent novel androgens in target tissue



APPLICATION OF RESEARCH

- **11-oxygenated androgens have recently been implicated in disease states including Castration Resistant Prostate Cancer (CRPC), Polycystic Ovary Syndrome (PCOS) and Congenital Adrenal Hyperplasia (CAH).**

GLUCOCORTICOIDS AND CYTOKINES

IMPLICATIONS IN INSULIN RESISTANCE

Project leader
Dr Nicky JD Verhoog
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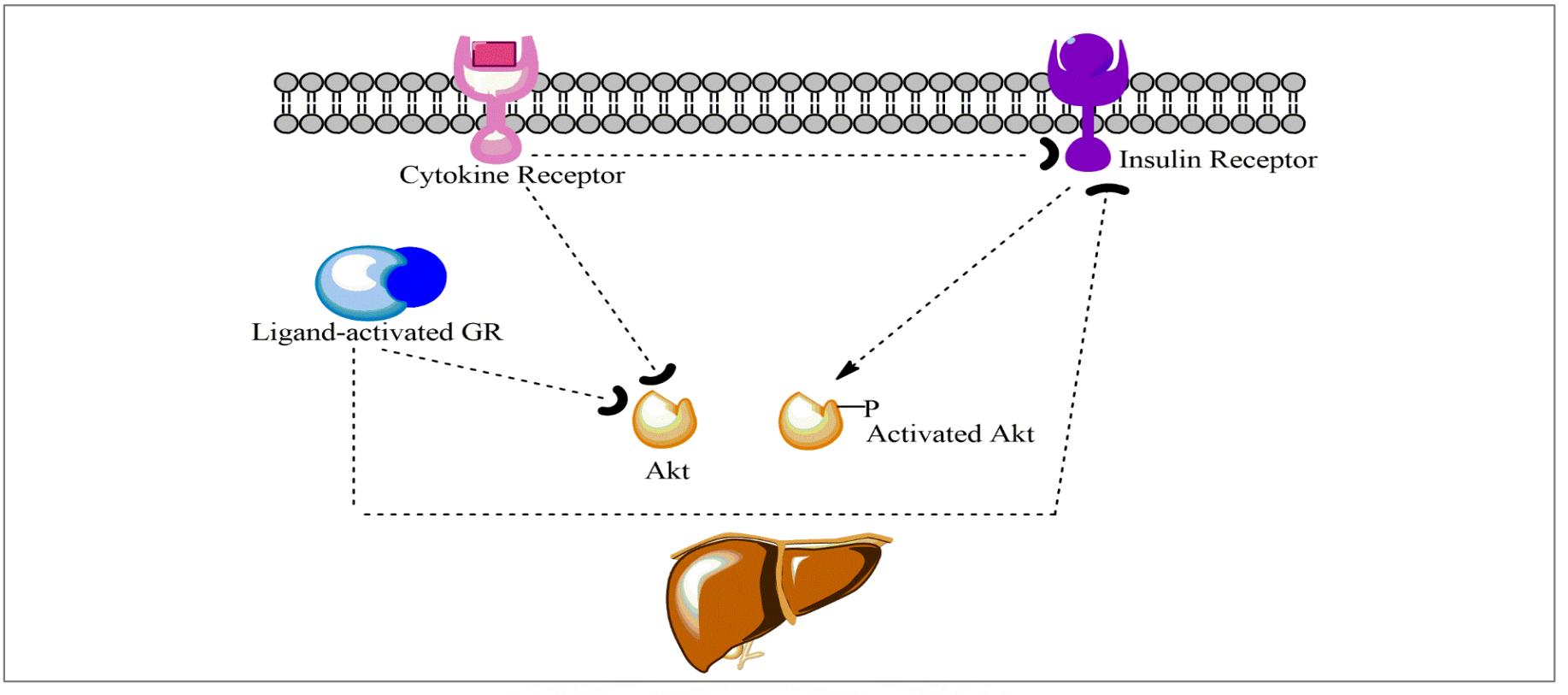
HIGHLIGHTS

- Investigating the role glucocorticoids together with cytokines (inflammation) have on developing insulin resistance
- These classic antagonists (GCs & inflammatory mediators) both negatively affect insulin signalling
- This project identified a possible role PAI-1, a Type 2 diabetes bio-marker and upregulated by both GCs & inflammatory mediators, in insulin signalling



APPLICATION OF RESEARCH

- This project aims to better understand the non-communicable disease Type 2 diabetes as a result of insulin resistance
- This research could contribute to designing better drugs for treating Type 2 diabetes or insulin resistance

GLUCOCORTICOID RECEPTOR

THE IMPORTANCE OF BEING DIMERIZED

Project leader
Prof Ann Louw
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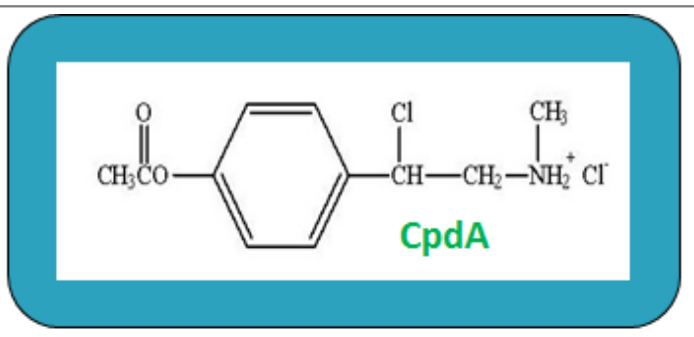
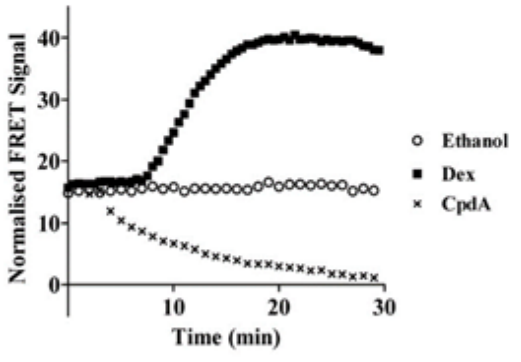
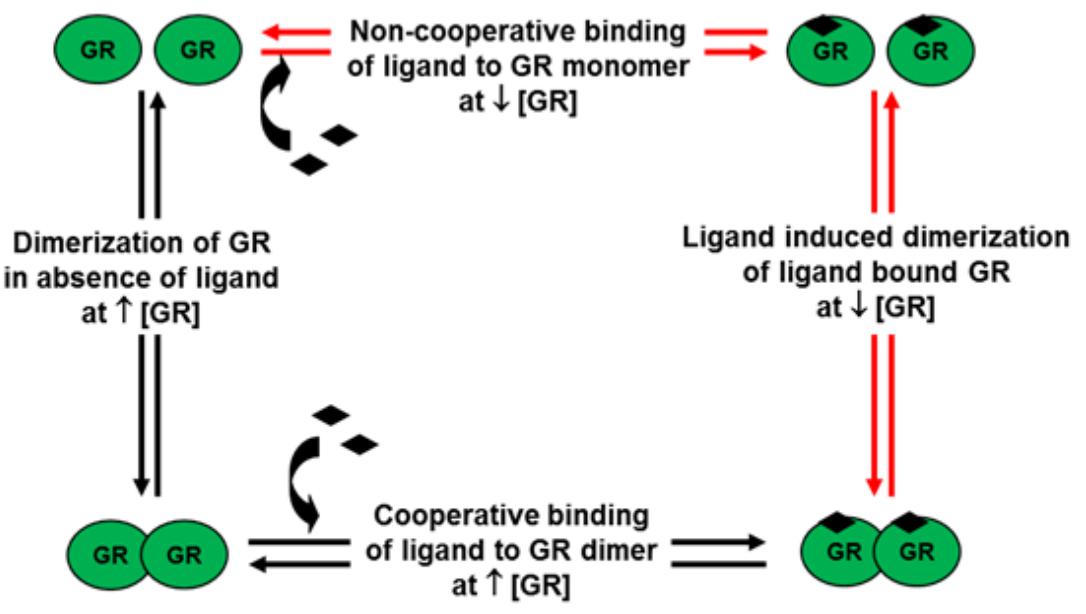
HIGHLIGHTS

- **CpdA, unlike other glucocorticoid drugs, abrogates GR dimerization**
- **Loss of dimerization:**
  - **Correlates with SEGRM activity which maintains anti-inflammatory activity while reducing side-effects**
  - **Correlates with loss of autologous down-regulation which maintains glucocorticoid sensitivity**







CpdA, unlike dexamethasone, abrogates GR dimerization

Robertson et al. (2013) *PLoS ONE* 8(5): e64831.

APPLICATION OF RESEARCH

- **Understanding of the implications GR dimerization could aid the search for anti-inflammatory drugs that**
  - **display less side-effects**
  - **have a reduced risk for glucocorticoid resistance**

UPSFC-MS/MS

ULTRA-PERFORMANCE SUPERCRITICAL FLUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY OF STEROIDS

Project leader
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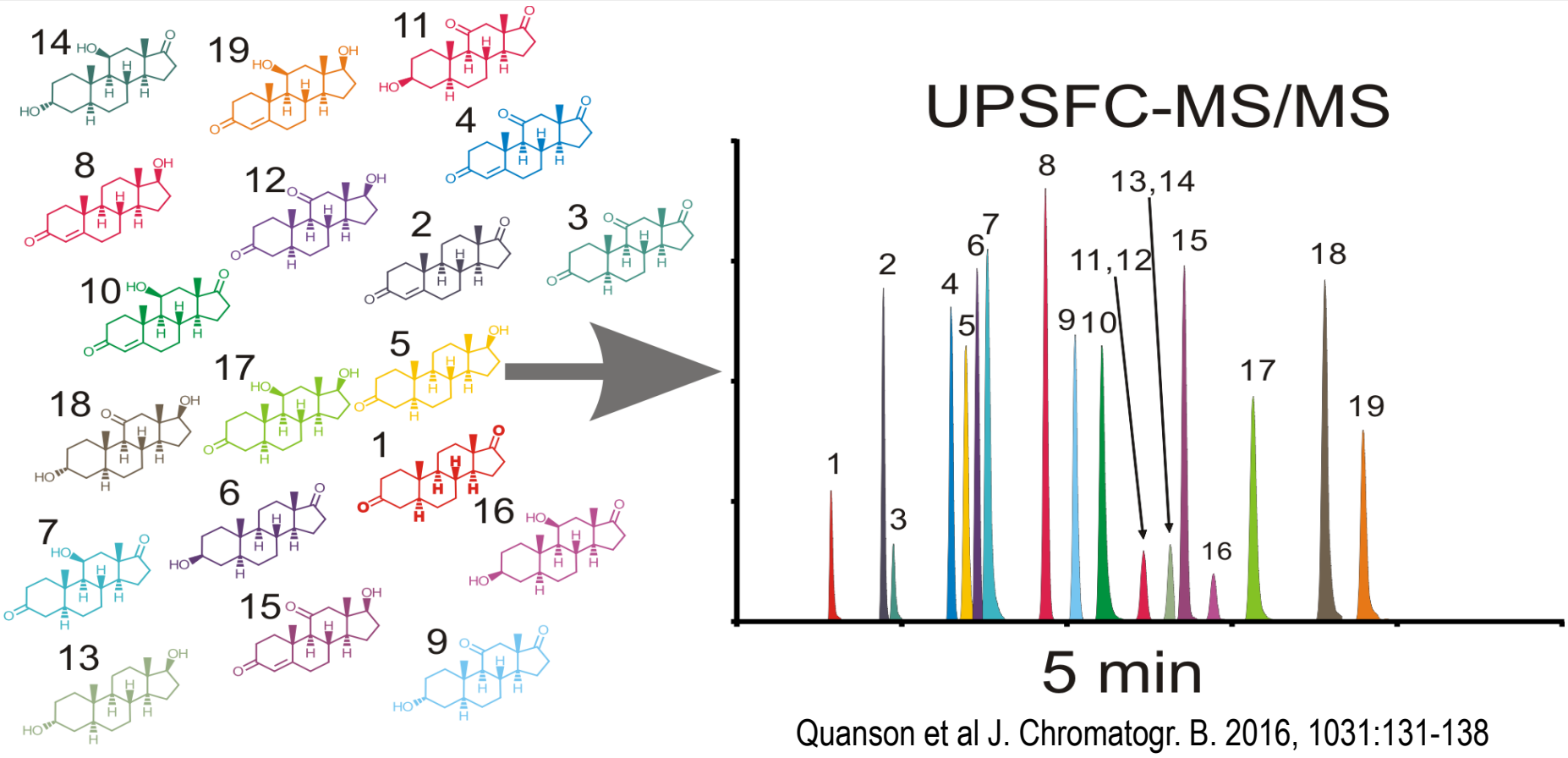
HIGHLIGHTS

- **UPSFC-MS/MS combines the strengths of both GC-MS and UHPLC-MS/MS.**
- **UPSFC-MS/MS offers orthogonal selectivity and increased chromatographic efficiency.**
- **High throughput methods for the separation and quantification of multiple structurally similar steroids have been developed.**



Quanson et al J. Chromatogr. B. 2016, 1031:131-138

APPLICATION OF RESEARCH

- **UPSFC-MS/MS has broad application in analysis of pesticides, environmental toxins and pharmaceuticals**
- **Ideal for the separation and quantification of steroids for**
  - **application in steroid-dependent disease diagnosis**
  - **identifying anabolic steroid abuse in sport**

ROOIBOS TEA

A FUNCTIONAL FOOD

Project leader
Prof Amanda C Swart
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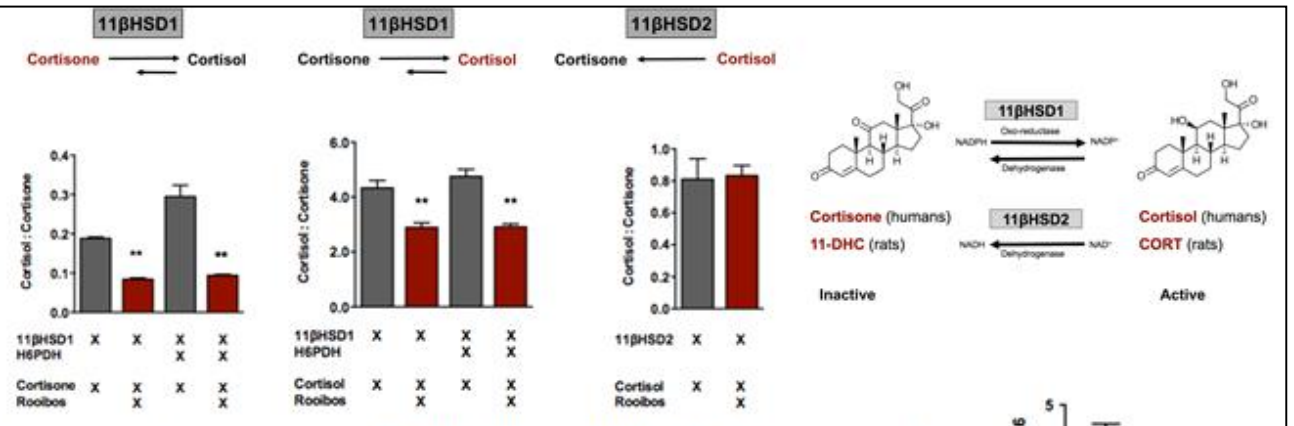
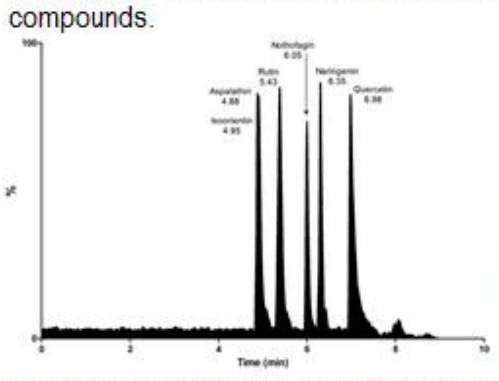
HIGHLIGHTS

- In-laboratory extraction and UPLC-MS/MS analysis of Rooibos polyphenols.
- In cell models: Rooibos modulates steroidogenic enzymes, reducing the stress hormone, cortisol.
- Aqueous Rooibos extracts influences the immune response.
- Rooibos reduces pro-inflammatory cytokines, while increasing anti-inflammatory cytokines.

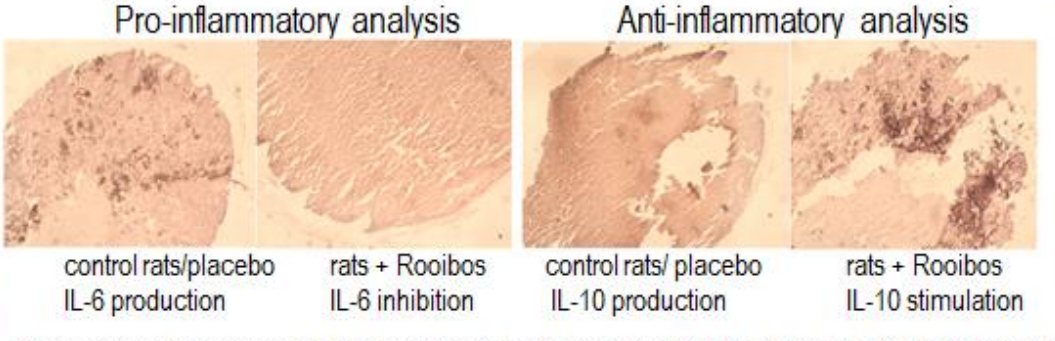
In-laboratory extraction of Rooibos bioactive compounds.

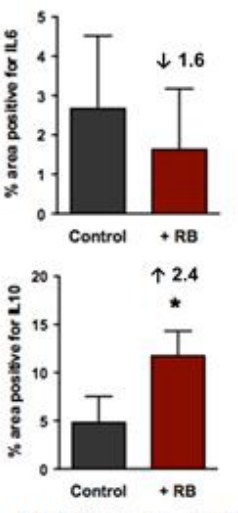


Cortisone and cortisol conversion by 11βHSD1 and 11βHSD2, without and in the presence of Rooibos methanolic extracts in the CHO-K1 cell model system. Steroid substrate, 1 µM; extract, 1 mg/mL; after 24 hours.



Analysis of flavonoids extracted from Rooibos leaves using ultra-performance liquid chromatography tandem mass spectrometry at the Central Analytical Facility, Stellenbosch University.





Immunohistochemical analysis of rat adrenal tissue: rats fed Rooibos had 1.6 fold lower IL-6 levels compared to control rats, while IL-10 levels were significantly higher (2.4 fold).

APPLICATION OF RESEARCH

- Rooibos may act as therapeutic agent for patients suffering from anxiety & the metabolic syndrome.
- Rooibos is a natural food additive with nutraceutical aspects.

HONEYBUSH TEA

MORE THAN A CUP OF TEA

Project leader
Prof Ann Louw
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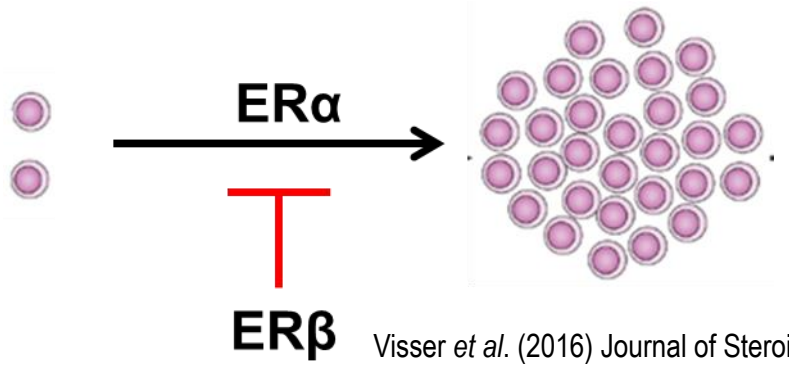
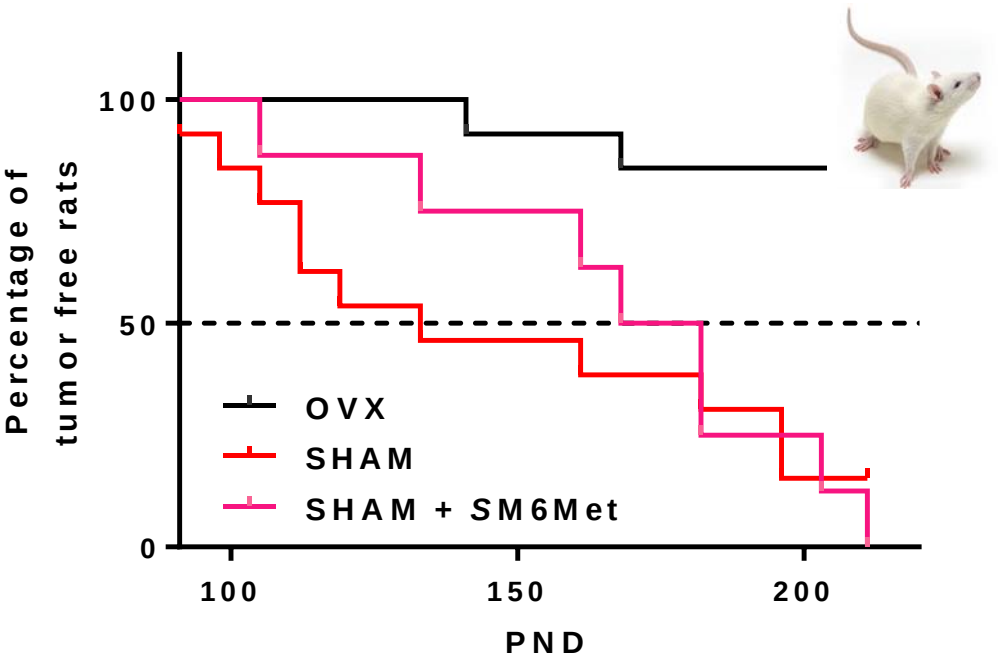
HIGHLIGHTS

- Honeybush tea (*Cyclopia*) extracts have phytoestrogenic potential
- Benchmarking suggests comparable potency and efficacy to commercial phytoestrogen extracts for menopausal use
- ER subtype specific effects reduces risk profile in rats
- Breast cancer chemo-preventative properties in rats

Photo courtesy Lizette Joubert, ARC Inruitec-Nietvoorbij





- Latency extended by 7 days
- Median latency extended by 42 days

Visser *et al*. (2016) Journal of Steroid Biochemistry and Molecular Biology 163, 129-135

APPLICATION OF RESEARCH

- *Cyclopia* extracts have potential as marketable phytoestrogenic nutraceuticals for women's health:
  - Relief of Menopausal symptoms
  - Chemo-prevention of breast cancer

NATURE'S ANTIBIOTICS

BIOMINING IN THE ENVIRONMENTAL PHARMACY

Project leader
Prof Marina Rautenbach
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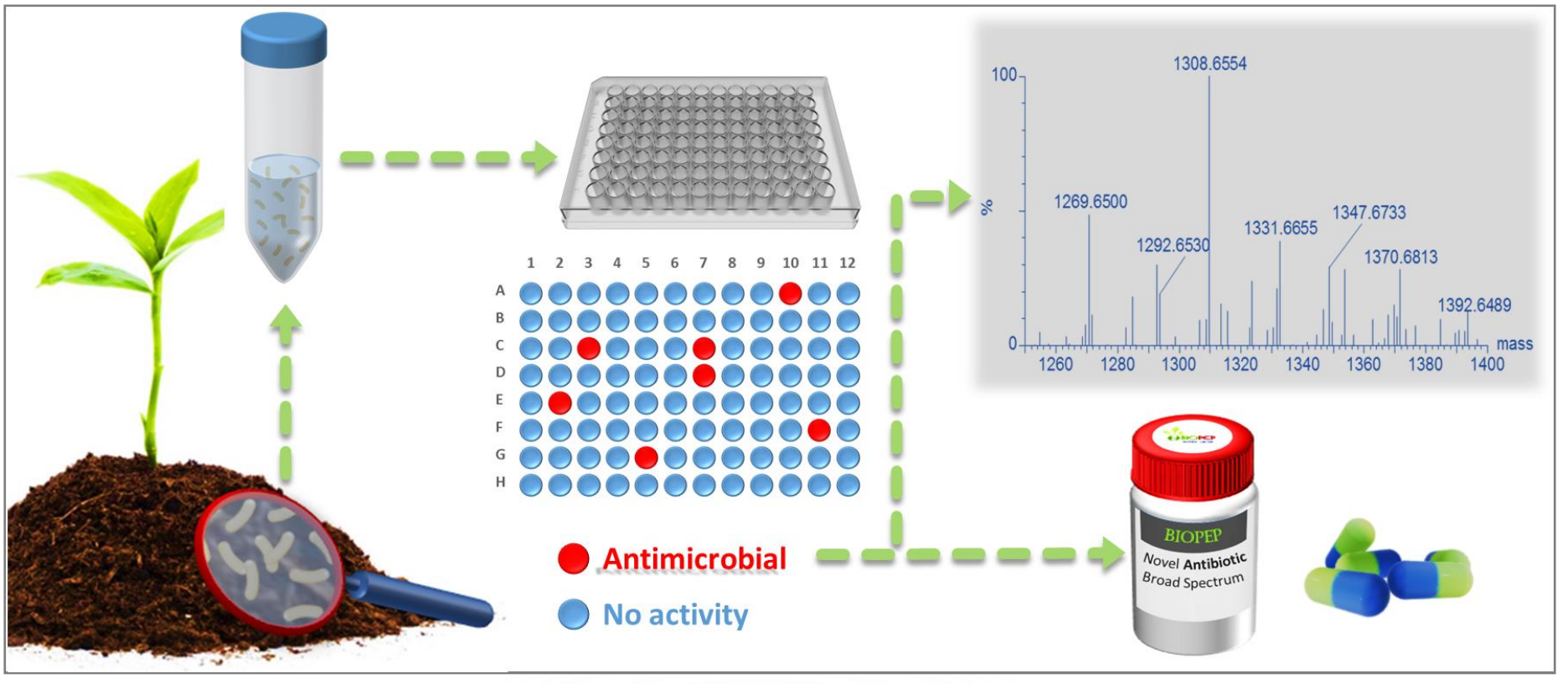
HIGHLIGHTS

- The environmental microbiome holds a pharmacy of novel antibiotics that can to combat the disastrous rise in antibiotic resistance
- High throughput assays combining antimicrobial activity and advanced mass spectrometry are used to identify bacterial producers and their products with anti-biofilm, -fungal and -bacterial activity
- Optimised microbial production and isolation procedures are used to obtain enough of the antibiotic compound for characterisation and development.



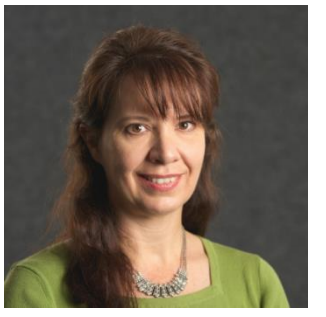
APPLICATION OF RESEARCH

- New antimicrobial compounds can be developed into:
  - anti-fungal/anti-bacterial creams and dressings
  - antibiotics and last resort drugs for systemic infections
  - preservatives and biocides for industrial use

BIOPEP
PEPTIDE GROUP

TYROCIDINES

POTENTIAL ANTIBIOTICS AND DRUGS



Project leader
Prof Marina Rautenbach
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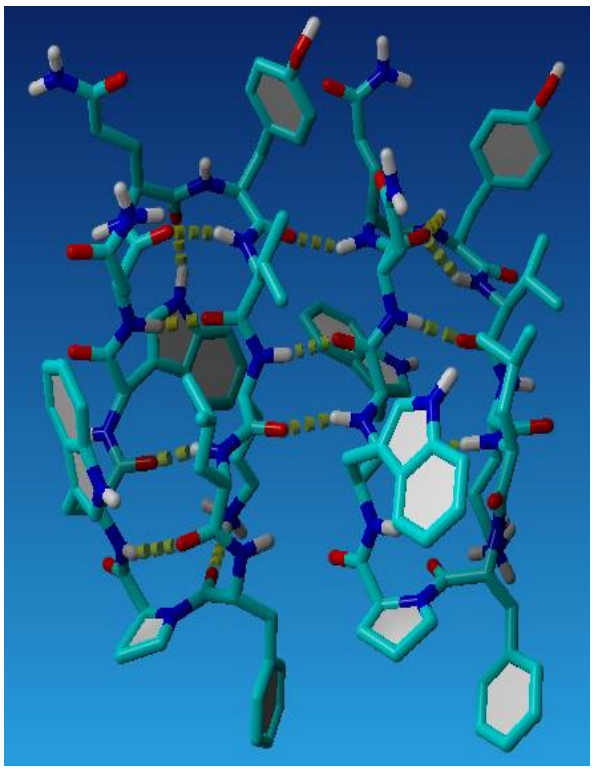
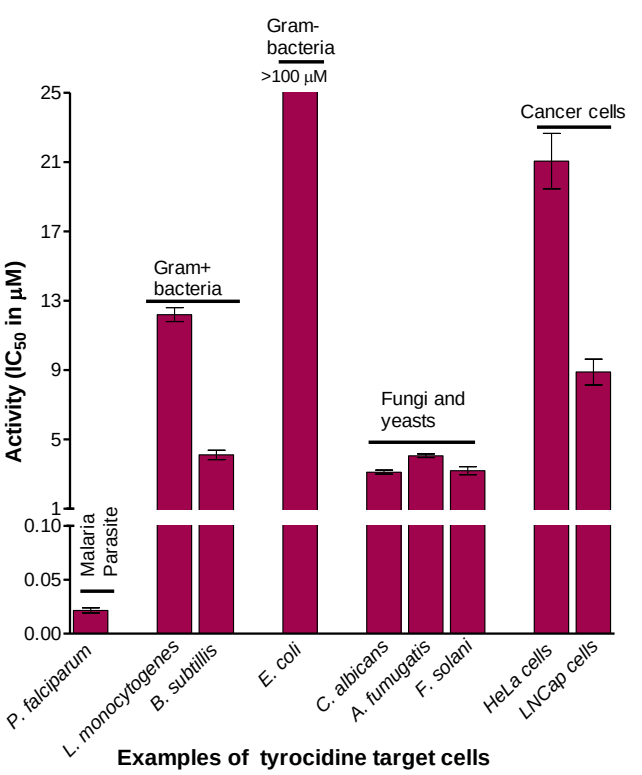
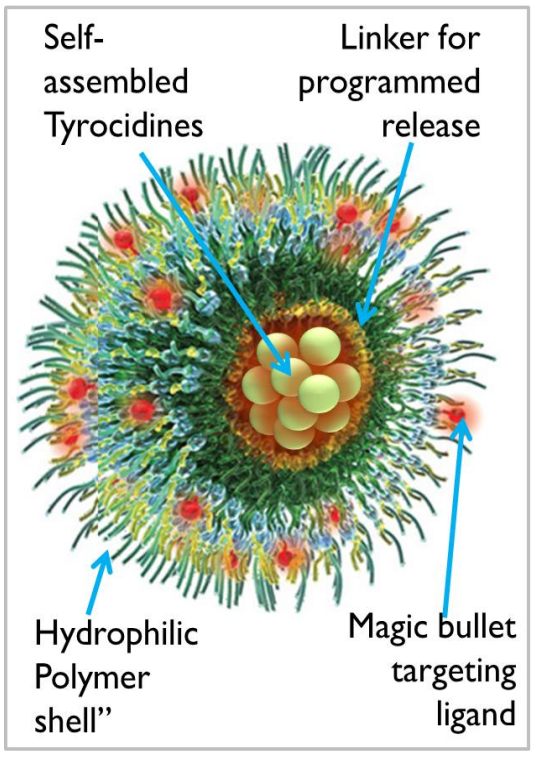
HIGHLIGHTS

- Cyclic non-ribosomal antimicrobial peptides are regarded as a new antibiotic class with a very low potential for resistance
- The cyclic tyrocidines (Trcs) from *Brevibacillus parabrevis* have a broad spectrum of anti-bacterial, -fungal, -malarial and -cancer activity
- Selective activity against fungi is significantly improved in dextran formulations of Trcs
- Designer nanoparticles and micelles with Trcs have high selective activity against malaria parasites and certain cancers







APPLICATION OF RESEARCH

- The Trcs can be developed into health products:
  - anti-fungal/anti-bacterial creams and dressings
  - selective chemotherapeutic drug against cancers
  - selective last resort drug against malaria

BIOPEP
PEPTIDE GROUP

PANTOTHENATE ANALOGUES

AS NOVEL ANTIMALARIALS

Project leader
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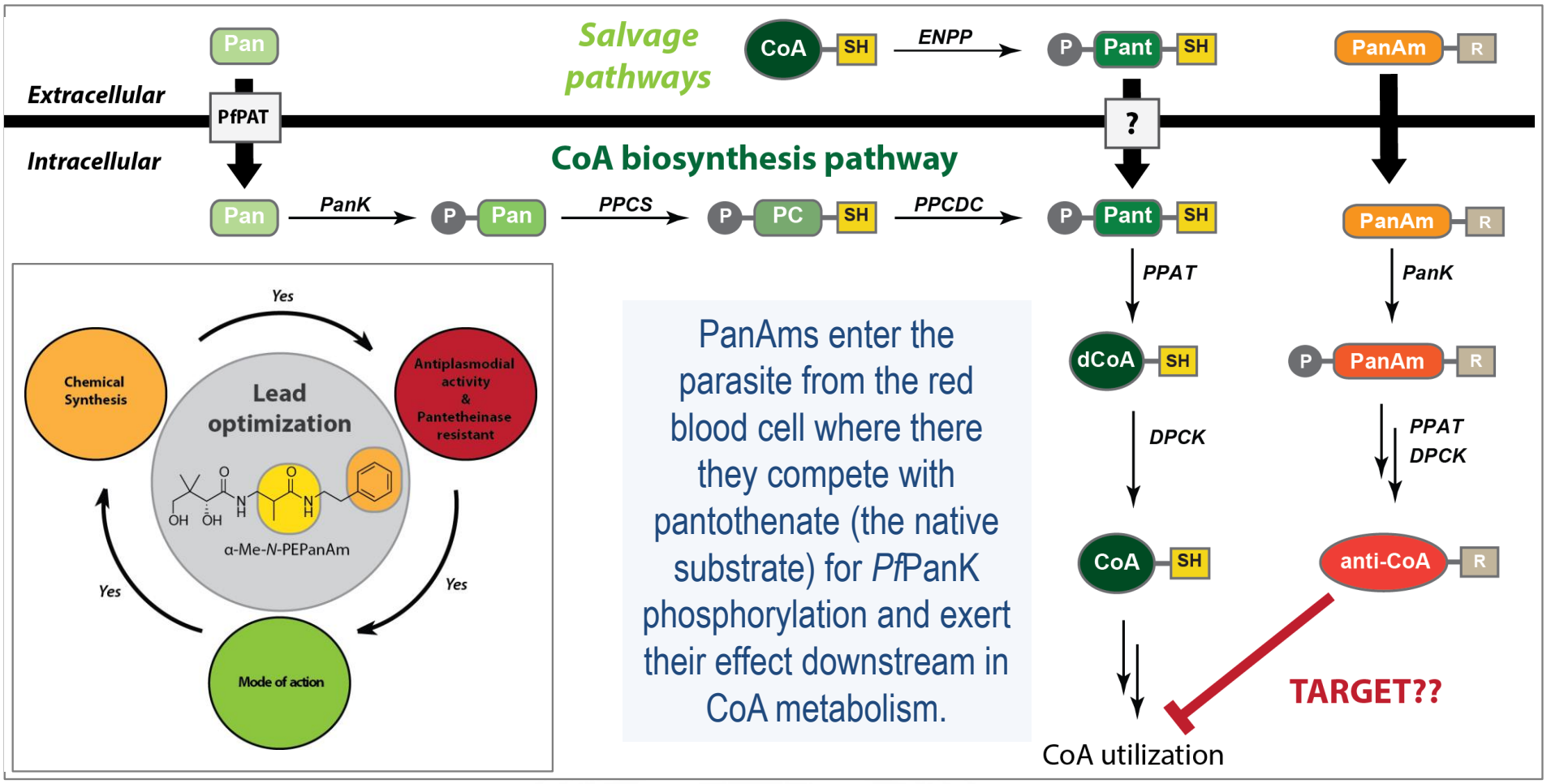
HIGHLIGHTS

- Pantothenamides (PanAms) have potent antiplasmodial activity *in vivo* with similar activity as the known antimalarial chloroquine
- PanAms target CoA metabolism overall; the specific point of inhibition is still unknown
- Mode of action studies are currently conducted in malaria causing parasites like *Plasmodium falciparum*



APPLICATION OF RESEARCH

- PanAms are being investigated as possible new antimalarials by MMV as part of a MMV-supported projects
- These scaffolds from these compounds might be useful as agents against other diseases

ANTIMICROBIAL DRUGS

TARGETING COA BIOSYNTHESIS

Project leader
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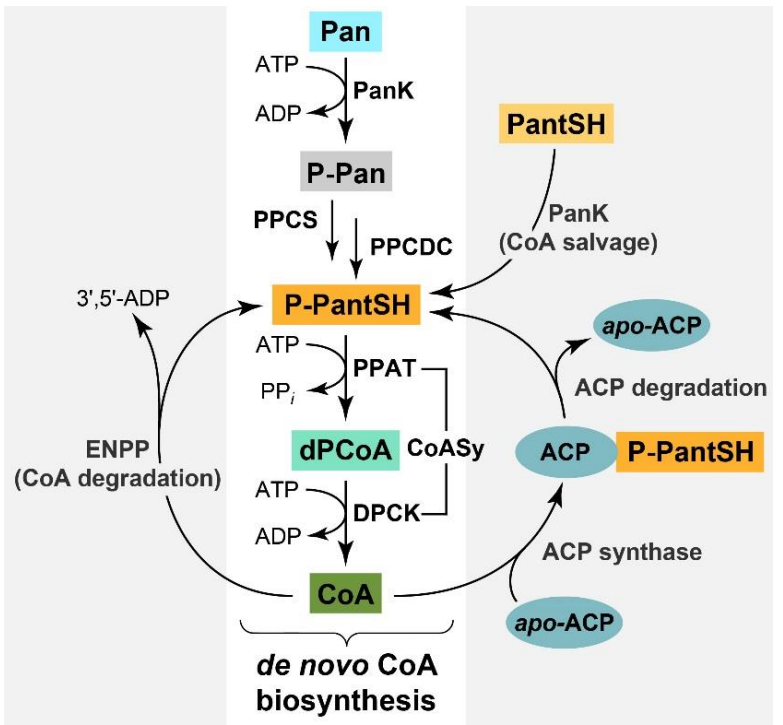
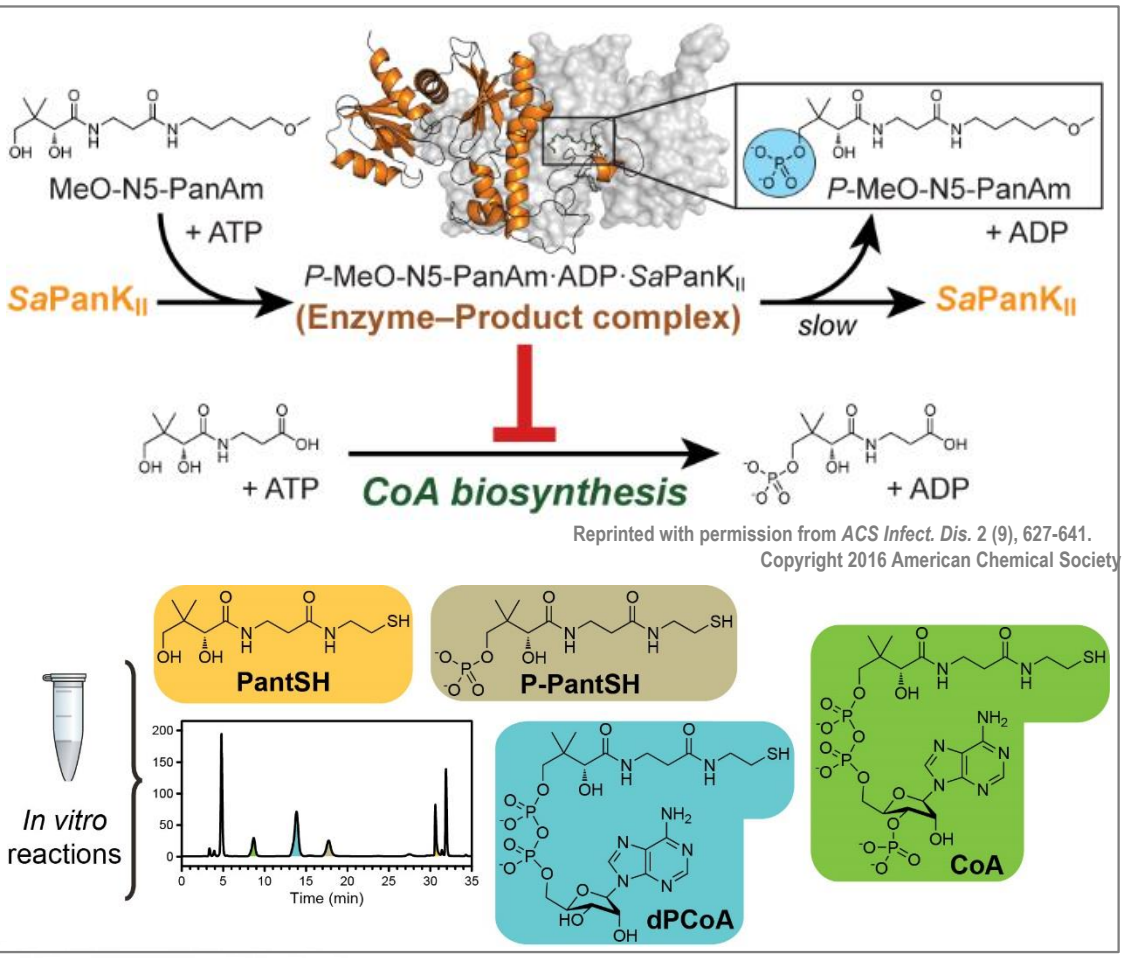
HIGHLIGHTS

- Developing new antistaphylococcal agents
- Discovery of new compounds that can be used as tools for the elucidation of key biological processes
- Developing new analytical tools to study CoA-dependent biology
- Discovering new was to improve cell-permeability



Coenzyme A biosynthesis and utilization

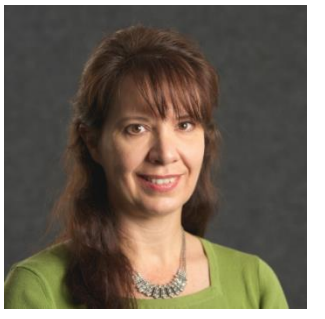


APPLICATION OF RESEARCH

- New antimicrobials for treatment of hospital-acquired infections caused by *Staphylococcus aureus*
- Furthering our understanding of the role of important cofactors in bacterial physiology

BACK TO NATURE

ANTIMICROBIAL PEPTIDES FROM SOIL BACTERIA



Project leader
Prof Marina Rautenbach
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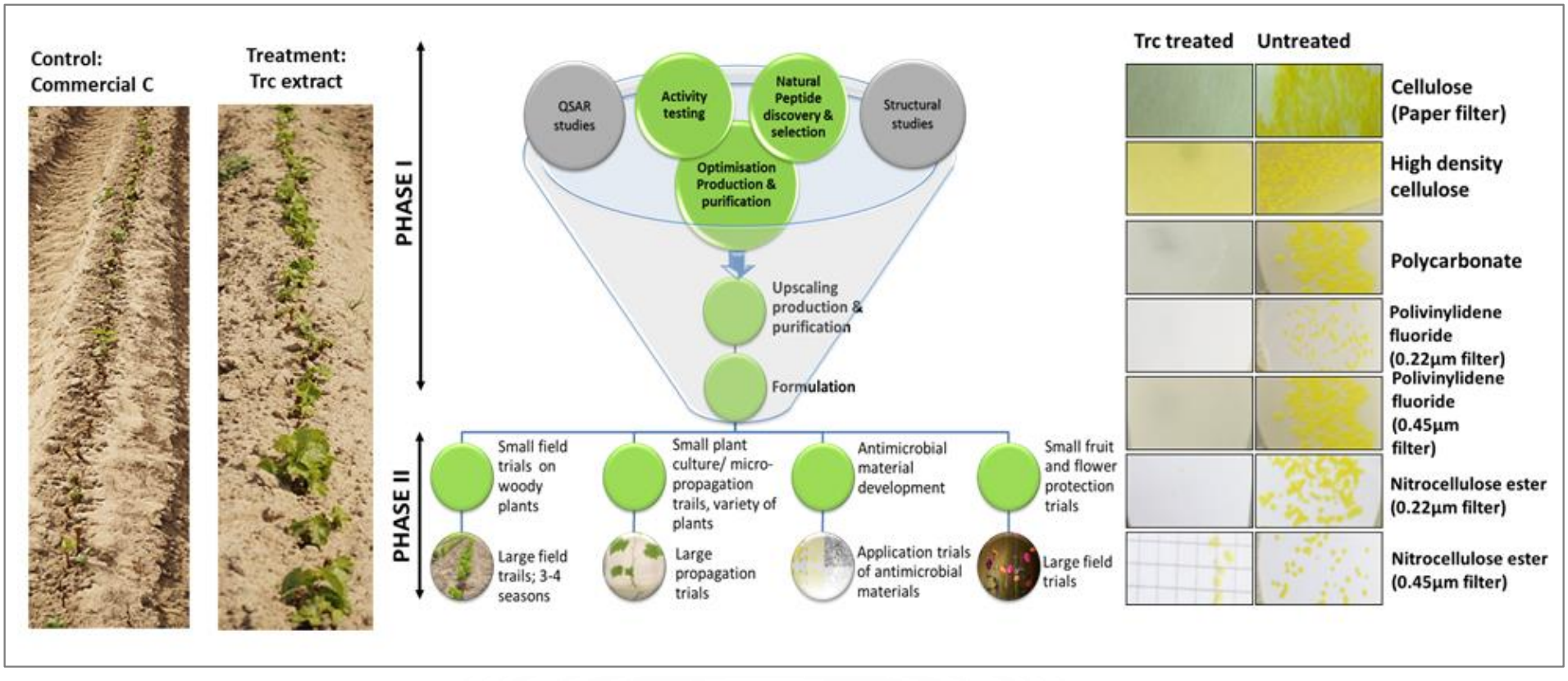
HIGHLIGHTS

- Non-ribosomal antimicrobial peptides (AMPs) from soil bacteria can be readily identified, produced and isolated at low cost
- The cyclic tyrocidines (Trcs) from *Brevibacillus parabrevis* have a broad spectrum of antibacterial and antifungal activity
- Trcs protect an stimulate growth of micro-propagated plants from grafts, cuttings and seeds (Patent registered: WO/2013/150394)
- Trcs have potent sterilisation activity in/on paper and a variety of polymers (Patent: PCT/IB2015/054166, WO registration pending)



APPLICATION OF RESEARCH

- Biodegradable cyclic AMPs can be utilised as
  - as green biocides in agriculture
  - as preservatives in food, beverages, cosmetics etc.
  - for producing sterilising biodegradable packaging and filters

BIOPEP PEPTIDE GROUP

OSTRICH PATHOGENS

DETECTION & CONTROL

Project leader
Dr Annelise Botes
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HIGHLIGHTS

- **Focus on disease causing organisms that impact production**
- **Determine the development of gut microbiome in chicks**
- **Development of tools for pathogen detection**
- **DNA-vaccines development and evaluation in field trials**

Intensive farming conditions

Samples are taken from GIT sections

Gastrointestinal tract infections

- Cause of deaths amongst intensively farmed chicks receiving high-concentrate feed
- Treated using antibiotics
- Determine change in GIT microbiome using next generation sequencing

Mycoplasma

- Bacterial infections
- 3 ostrich-specific species identified
- Causes reduced production and downgrading of carcasses
- Treated using antibiotics
- DNA vaccine development to control infections - requires genomic information

Tool development

- ELISA
- Secondary anti-ostrich antibodies
- Recombinant production of proteins
- PCR assays

Mycoplasma associated with respiratory symptoms

Foam in eye followed by infection

Swollen sinuses

Yellow lesions on carcass

APPLICATION OF RESEARCH

- **Establish dietary intervention and husbandry practices to control and prevent infections**
- **Optimal disease management, reduced antibiotic use and subsequent improved food safety**

RNA VIRUS DETECTION

POTATO PATHOGENS

Project leader
Prof Dirk Bellstedt
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HIGHLIGHTS

- **Methods were developed for detection of *Ralstonia solanacearum*, Potato Virus Y (PVY) and Potato Leaf Roll Virus (PLRV)**
- **Unique variants of PVY and PLRV documented in South Africa**
- **Book chapters published in world authoritative books**

Symptoms of Potato Leaf Roll Virus infection

Dramatic yield reduction due to infection

Determine RNA sequences of whole viruses

↓

Identify conserved regions to develop specific primers

↓

Develop realtime PCR methods for detection

APPLICATION OF RESEARCH

- **ELISA method for *Ralstonia* detection used for testing of all South Africa seed potatoes since 1991**
- **More pathogenic PVY and PLRV variants identified, PCR detection methods developed for use in Sandveld Region, South Africa**

PLANT MOLECULAR SYSTEMATICS

GENUS ERICA

Project leader
Prof Dirk Bellstedt
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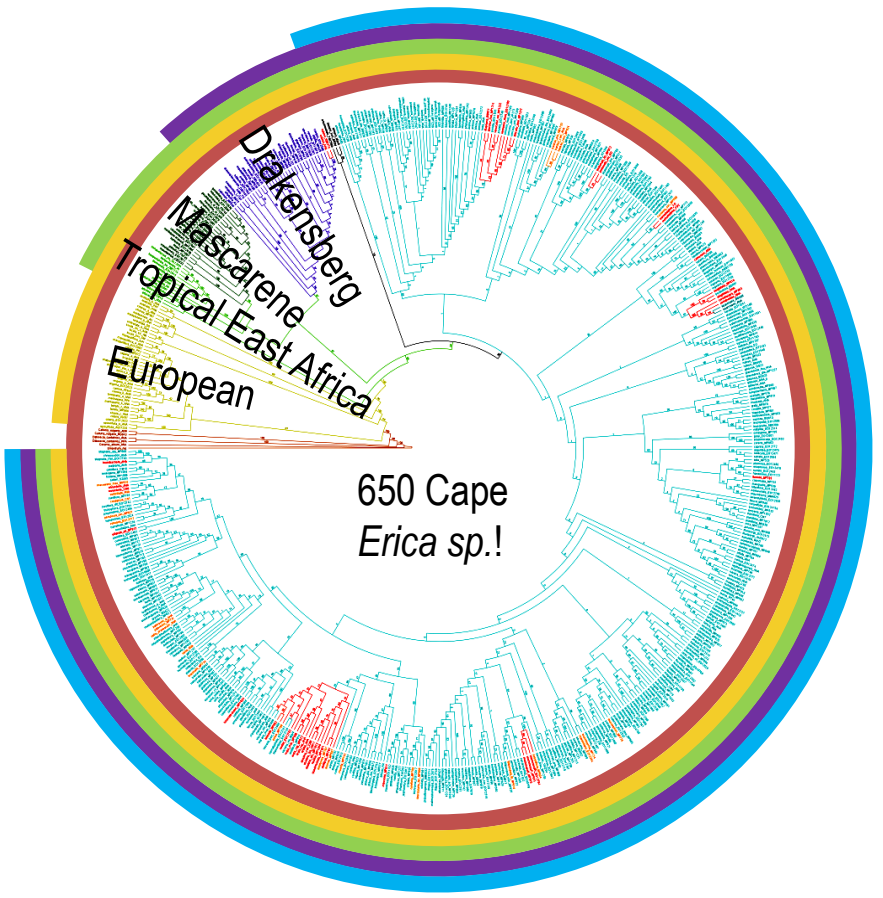
HIGHLIGHTS

- **Major advances in DNA sequencing technology has enabled us to determine phylogenetic relationships using bioinformatic methods**
- **This has enabled us to make major advances in plant classification and Biogeography in Cape plant groups**

Most recent project: Largest Cape plant genus *Erica*



APPLICATION OF RESEARCH

- **Documents South African plant diversity in detail, when it diversified, how many species, where did it come from and when, important for conservation**

EPIGENOMIC LANDSCAPE

Trypanosoma brucei

Project leader
Prof Hugh Patterton
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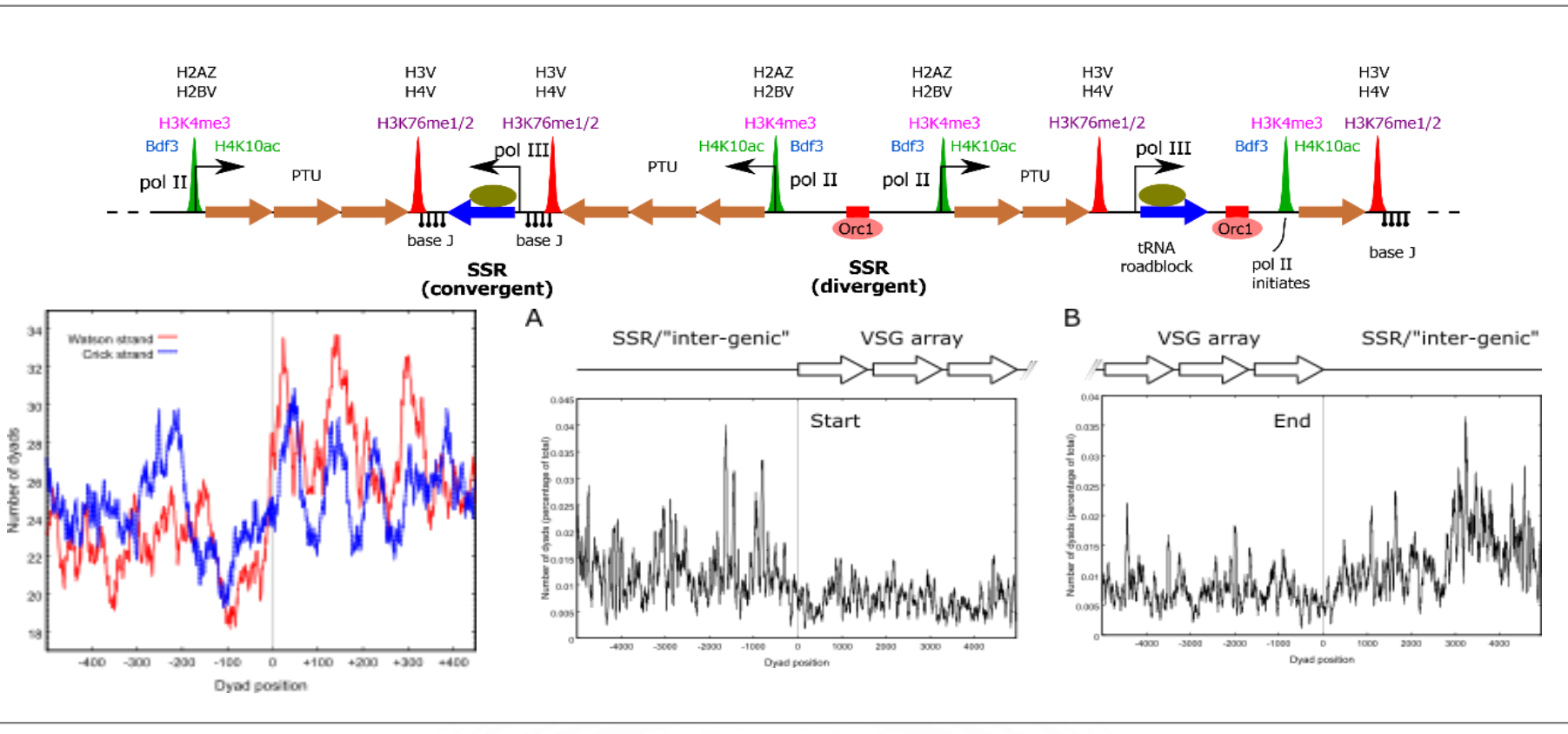
HIGHLIGHTS

- **We have performed MNase-seq to identify the positions of all nucleosomes in the genomes of bloodstream and insect life stage forms of *T. brucei***
- **Specialized software developed to analyse average structures at specified genomic loci**
- **Differences identified in the organization of chromatin in *T. brucei* and higher eukaryotes**
- **Specialized chromatin structures identified bordering the silent VSG cassettes, the source of sequences that allow *T. brucei* to escape immune clearance in humans**



APPLICATION OF RESEARCH

- **Novel software suitable for analysis of epigenetic data**
- **Identification of targets for development of epigenetic therapies to African sleeping sickness**

EPIGENETIC SIGNALS

IMPLICATION IN LIFESPAN EXTENSION

Project leader
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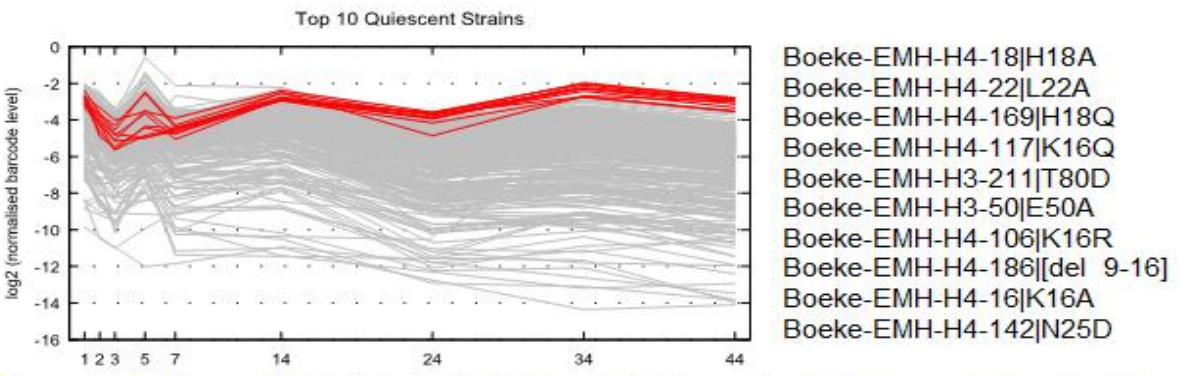
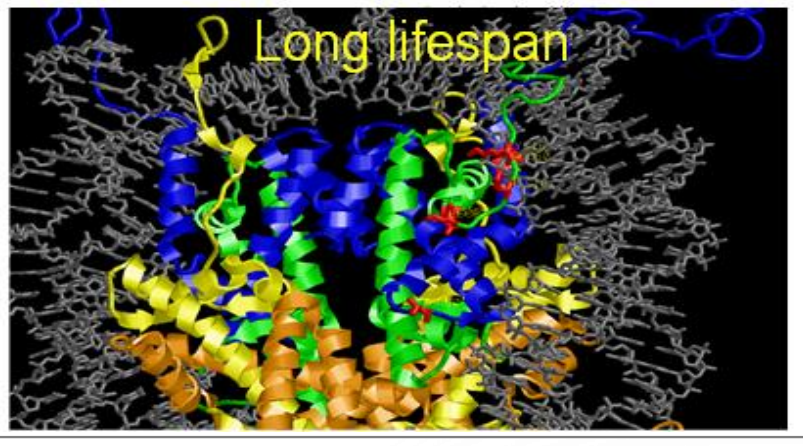
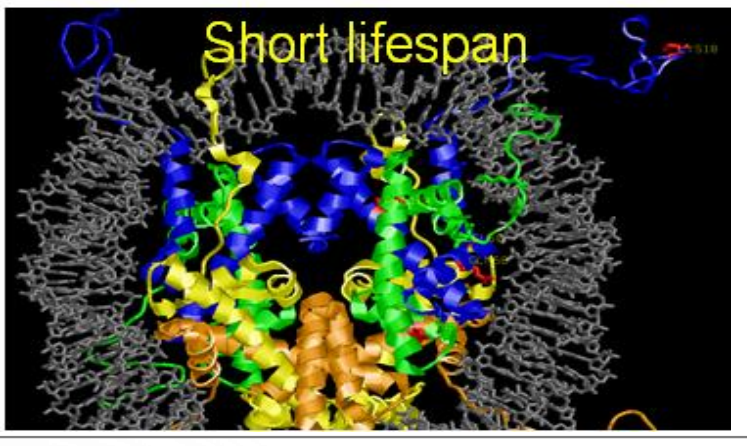
HIGHLIGHTS

- We have cultured a mixture of strains from a bar-coded histone mutant library of *Saccharomyces cerevisiae*, and identified long and short lifespan mutants
- Mutants that lived longer invariably exhibited mutations of residues on the solvent-exposed disk of the nucleosome
- Mutants that exhibit shortened lifespans exhibited mutations in the histone-fold region of the protein, possibly involved in destabilization of the nucleosome structure
- The transcriptomic and proteomic profiles of lifespan mutants were investigated







APPLICATION OF RESEARCH

- Links epigenetic signals to lifespan
- Proteome and transcriptome changes provides further insight into diseases of aging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