Amyloglucosidase for the bio-economy from an ericoid associated fungus

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Microbial enzymes have brought about dramatic changes in food, brewing, detergent, pharmaceutical, biofuel and related industries. The enzyme industry is among the major industries of the world, and novel enzyme sources are continually being investigated. Mycorrhizal fungi are known to produce some compounds that enhance the survival of their host plant in natural environment, among which are hydrolytic enzymes. Leohumicola incrustata is an ericoid mycorrhizal fungus used in this study to examine amyloglucosidase (AMG) activity.
A genus of Leohumicola (Genbank accession number MF374380) isolated from Erica chamissonis roots and was grown in a modified Melin-Norkrans (MMN) liquid medium for AMG production and analyzed for AMG activity. The molecular mass of the AMG was estimated to be 101 kDa by combined results of Sephadex G-100 gel filtration, SDS-PAGE and Zymography. The Km and Vmax were 0.38 mg/ml and 22.56 micromole/ml/min, respectively, against soluble starch as substrate. The AMG optimum activity of 39.2 U/mg protein was recorded with pH of 4.0 while the activity of this enzyme was stable at 45oC (pH 5.0) with activity value of 36.8 U/mg protein.
Mycorrhizal enzymatic capabilities underpin their role in the soil environments as well as opens new opportunities for exploring these novel sources of enzymes such as amylases for use in the bioeconomy.

Comparative Analysis of the Molecular Responses to Drought in Maize and Sorghum

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Sorghum and maize are used as food as well as livestock feed and biofuel. These cereal crops play a significant role in the diet of many people in Africa; they act as a major calorie source in both human and livestock nutrition. Drought is a major abiotic stress causing not only differences between the mean yield and the potential yield but also causing yield variation from year to year. Although selection for genotypes with improved productivity under drought environments has been a central goal of numerous plant breeding programs, the biological basis for plant tolerance to drought stress is still poorly understood. Exposure of plants to drought triggers excessive formation of reactive oxygen species (ROS), which induces cell death and reduces growth. Part of the mechanism of plant responses to drought involves alterations in the expression of antioxidant enzymes and biosynthesis of different compatible solutes such as proline. Sorghum is regarded as generally more drought tolerant than maize and it is a potential key model system for investigating the physiological and molecular mechanisms conferring drought tolerance due to its full genome sequence availability. Comparative studies in crop plants are essential for crop improvement to sustain production for food security under climate change in which drought becomes more severe. On this basis, the aim of this study is to determine molecular differences between maize and sorghum in response to drought stress in an attempt to identify novel biomarkers for drought tolerance.
The physiological and molecular responses of maize and sorghum were studied. Specifically, changes in growth, chlorophyll content, relative water content, ROS content, lipid peroxidation level, proline content and antioxidant enzyme activities. In this study, water deficit triggered overproduction of ROS in both maize and sorghum. However, sorghum showed less water loss and cell damage under water stress compared to maize. We identified differences in the antioxidant enzyme activities and proline content between maize and sorghum in response to water deficit conditions. The findings obtained through this study provide insight towards understanding the mechanisms that determine plant tolerance to drought.

Evaluation of genotoxic and oxidative stress potential of Ajakanga landfill leachate in rats

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Introduction: An understanding of the toxicity of landfill leachate is necessary in order to understand the potential effects of leachate upon nearby populations and the environment. Untreated wastes are dumped in Ajakanga landfill site in Oluyole local government area in Ibadan, Oyo State, Nigeria. This makes ground water sources in this area prone to contamination by leachate generated from the landfill posing environmental risks and endangering the lives of inhabitants close to the landfill. Therefore the genotoxicity and oxidative stress potential of Ajakanga landfill leachate was investigated using albino rats.
Method: Fourty two male albino rats (100g-160g) were divided into six groups with seven animals in each group. Group A animals served as the control, and they were given distilled water as drinking water for forty five days; while groups B-F animals were exposed to 12.5%, 25%, 50%, 75% and 100% leachate respectively via drinking water for forty five days. The effect of the leachate was assessed on markers of oxidative stress in the liver, kidney and testes of rats and the genotoxic effect of the leachate was investigated using micronucleus assay. Physicochemical and heavy metal analysis were also carried out on the leachate sample.
Results: There was decrease in protein concentration in the liver, kidney and testes of rats exposed to the leachate and increase in the activity of liver enzymes- aspartate amino transferase (AST) and alanine aminotansferase (ALT). There was induction of lipid peroxidation in the liver and kidney, as well as alterations in the antioxidant status in the liver, kidney and testes of the rats. There was also increase in micronuclei formation in the bone marrow of rats exposed to the leachate when compared with the control animals. The physicochemical and heavy metal analysis of the leachate revealed the presence of some heavy metals and other toxic constituents.
Conclusion: Ajakanga landfill leachate induced oxidative stress and genotoxicity in rats. This suggests that the leachate may be toxic to humans if exposure occurs.
Key words: Leachate, oxidative stress, genotoxicity, lipid peroxidation, antioxidant.

Discovery of potent Schistosome protein inhibitors: hidden Markov model, site-directed mutagenesis and molecular docking studies

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Schistosomiasis is a debilitating disease caused by a parasitic flatworm found in freshwater. After malaria, this disease is the second most prevalent disease in Africa and is endemic in both tropical and sub-tropical regions of the world. Morbidity and mortality attributed to this disease are very high with about 240 million people infected, 800 million persons at risk of the infection and an approximately 280,000 deaths occurring annually. With the exponential increase in morbidity and mortality resulting from Schistosomiasis, there is an urgent need for the development of new drug since studies have shown that schistosomes are becoming resistant to the widely accepted first-line drug-of-choice Praziquantel. Therefore, the present study describes the exploration of broad-spectrum therapeutic potentials of Antimicrobial peptides (AMPs) in the design of alternative anti-schistosomal treatment regimen. AMPs are natural antibiotics produced by all living species; they have multifunctional properties and are currently explored as a vital source for the development of new drugs. In this study, six putative AMPs (TAK1-TAK6) were identified to possess very strong anti-schistosomal capabilities using Hidden Markov Model. Added to this, glycosyltransferase and axonemal dynein intermediate chain schistosomal proteins were identified using in silico methods as vital proteins for the survival of the parasite in the host. Site-directed mutagenesis studies based on the putative anti-schistosomal AMPs was carried out to increase their biological activities; homology modelling of the mutated AMPs using I-TASSER showed they are identical to the parental AMPs. More so, results from molecular docking using PatchDock showed that these mutated AMPs are capable of interacting with the schistosome proteins. In conclusion, based on the strong interactions between the mutated AMPs and the schistosomal proteins, we propose that these peptides maybe potential "drug leads" in the design and development of alternative schistosomal therapy and could as well prove effective against PZQ-resistant schistosome strains.

Introducing cell-free DNA: Its origins, characteristics, applications and implications

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Cell-free DNA (cfDNA) research is a fast growing field due to its potential as a non-invasive means of studying physiological processes, pathophysiology and prenatal health and development. A brief review regarding the definition, origins and applications of cfDNA is provided in order to introduce and promote this research field. Despite decades of research the actual clinical application of cfDNA in diagnostics and prognostics remains significantly limited. This is due to the predominant, and somewhat premature, focus of current research on the clinical application of cfDNA before the understanding of the biological characteristics thereof. The complexity and shortfalls of cfDNA research will, therefore, be discussed along with the possible solution of utilising in vitro models to simplify cfDNA analysis. The fragment lengths of human plasma cfDNA and the cfDNA derived from two- and three-dimensional cell cultures are similar, indicating that in vitro results may sufficiently represent in vivo cfDNA characteristics. The utilisation of cell cultures to elucidate tissue-specific DNA release patterns and the relationship between cell metabolism and cfDNA release is illustrated. The ability of cfDNA to laterally transfer information in the form of pharmaceutically-induced effects from treated to untreated cells and the implications thereof are also discussed.

Potentials of S-allyl-cysteine on the modulation of inflammatory responses in high fructose-induced metabolic dysfunction in growing male Wistar rats

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Excessive fructose intake induces the features of metabolic syndrome, a combination of risk factors for developing diabetes mellitus, obesity and heart disease. S-allyl-cysteine (SAC) is known to be a natural constituent of Garlic (*Allium sativum*), a commonly consumed spice crop that has a great therapeutic potentials. This study was aimed at investigating the potentials of orally administered S-allyl-cysteine on inflammation in high fructose-induced metabolic dysfunction in growing Wistar rats. The rats were divided into three groups namely; Normal Control, High Fructose Diet (HFD) and High Fructose Diet + S-allyl-cysteine (HFD+SAC). The levels of inflammatory biomarkers such as interleukins (IL-1β, IL-4, IL-5, IL-10), vascular endothelial growth factor (VEGF) and monocyte chemotactic protein-1 (MCP-1) were measured in the blood using Bio-Plex Pro magnetic bead-based assays on the Bio-Plex platform. There were no significant differences in the levels of IL-1β and IL-4 in all the groups. The level of IL-5 was significantly increased in HFD group when compared with the normal control group. However, administration of S-allyl-cysteine (HFD+SAC) reduced the level of IL-5 but not significantly. Treatment with S-allyl-cysteine (HFD+SAC) significantly decreased the levels of MCP-1 and VEGF when compared with HFD group. The results showed that S-allyl-cysteine has the ability to modulate inflammatory responses in metabolic syndrome.

Investigating Telomere Dynamics in Oesophageal Squamous Carcinoma Cells using Standard and Nanoparticle-based Assays

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Oesophageal cancer is the 8th most common cancer in the world with an estimated 400,000 deaths reported in 2012 with the highest incidences of oesophageal cancer reported in Southern Africa. Up to 90% of all cancer show increased telomerase activity to overcome the "end-replication problem" which eventually leads to cellular senescence. Telomeres are located at the ends of chromosomes, consist of 10-15kbp of TTAGGG DNA repeats in humans and are maintained by the enzyme telomerase. Telomerase has been found to not only maintain and elongate telomeres but has been found to play many additional roles within the cell, such as protecting mitochondrial DNA from damage caused by reactive oxygen species. Due to the high number of cancers relying on increased telomerase activity to bypass senescence, telomerase could be a viable target for anti-cancer therapies.
This project assessed the role of telomere length, telomerase activity and the mRNA expression levels of the telomerase reverse transcriptase component (hTERT) in metformin treated oesophageal squamous cell carcinoma cell lines.
Metformin, a drug used for the treatment of type-2 diabetes, has been shown to reduce proliferation of cancer cells; however, the mechanism of action is not yet well understood. Since it has been found that both hTERT and metformin act in the mitochondria, the drug may influence hTERT and potentially telomerase activity. This makes metformin a potential anticancer candidate to be used in conjunction with traditional anticancer therapies.
After oesophageal cell lines were treated with 5 and 10 mM metformin, a significant decrease in telomerase activity was observed after 48 and 72h. A telomerase activity assay was then generated where gold nanoparticles were synthesised, characterised and functionalised with thiolated-DNA (telomerase substrate). To assess telomerase activity, the change in absorption spectra of the nanoparticle solution was recorded as the extracted enzyme elongated the telomerase substrate. This nanoparticle-based assay was then compared to traditional qPCR-based methods, to generate a more accurate, cheaper and easier to use alternative telomerase activity assay. The nanoparticle assay successfully detected telomerase activity, however further optimisation is required to increase the sensitivity of the assay.

Investigating the role of hsp90 and lrp1 in fn matrix dynamics and its implications in cancer cell migration

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Fibronectin (FN), a matrix protein responsible for regulating processes including migration and differentiation, is secreted as a soluble dimer which is assembled into an insoluble extracellular matrix. The dynamics of FN matrix assembly and degradation play a large role in cell migration and invasion contributing to the metastatic potential of cancer cells. Previous studies from our group have shown the direct binding of Heat Shock Protein 90 kDa (Hsp90) and FN in vitro, and that inhibition of Hsp90 with novobiocin (NOV) caused internalisation of the FN matrix. We tested several other Hsp90 inhibitors and found C-terminal Hsp90 inhibition necessary to induce FN turnover. Low density lipoprotein receptor-related protein 1 (LRP1) is a ubiquitous receptor known to bind both Hsp90 and FN. Using isogenic wild type and LRP1 null cell lines to study the endocytosis of FN, we demonstrate that this receptor is largely involved in NOV-induced turnover of FN. Interestingly, levels of the main FN receptor responsible for its export from cells, β1-integrin, did not change in response to NOV. The exact mechanism of how FN remodelling may be regulated by Hsp90 and LRP1 is unclear and is currently under investigation. Using wound healing assays we identified increased migration to be one of the consequences associated with loss of extracellular FN by Hsp90 inhibition, but only in cells containing LRP1. To look more closely at how NOV may be altering the FN matrix we generated cell derived matrices from Hs578T breast cancer cells and, using confocal and scanning electron microscopy, we identified NOV treated matrices to contain thicker, rope-like FN fibres compared to untreated matrices which presented as thinner, more delicate fibrils. We then determined (using adhesion and proliferation assays) whether these morphological differences in the matrices translated into any significant physiological consequences when either normal or cancerous cells were reseeded on each of these matrices. In summary, this study provides new insights into physiological consequences of Hsp90-LRP1 mediated loss of FN matrix; the regulation of which may be relevant to the future treatment of cancer metastasis.

Role of HOP in HSF1-mediated stress response in mammalian cells

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Our project aims to understand roles of the Hsp70-Hsp90 Organizing Protein (HOP)beyond the classical co-chaperone function in the survival of stressed cells via the master stress responsive transcription factor,heat shock factor 1 (HSF1).HOP expressing and depleted HEK293T cells had levels of HSF1 protein that were significantly reduced by Western blot and mass spectrometry.
The q-RT-PCR analysis of HSF1 mRNA under basal and stress conditions revealed no significant change between control and HOP depletedHEK293T cells ,suggesting HSF1 is regulated at the protein level in response to HOP depletion. However, there was no difference in short term or long term survival between the HOP depleted or control cells under either basal or stress conditions, despite the reduced HSF1 levels. Luciferase reporter assaysmeasuredHSF1 activity from the HSP70 promoter and under basal conditions there was a significantly lower luciferase activity in HOP depleted cells, which was consistent with the reduced levels of HSF1. Under stress, HOP depleted HEK293T cells had significantly higher promoter activity compared to the control, accompanied by an increase in HSF1. In basal conditionsHSP70 protein level were reduced in HOP depleted cells, with no change in HSP40 or HSP90α but a significant increase in HSP27 expression relative to controls. Under stress conditions, there was no significant difference in the chaperone levels in control or HOP depleted cells. There was also no difference in survival of control or HOP depletedHEK293T cells to the HSF1 inhibitor KRIBB11 by MTT assay under basal or stress conditions.

Nuclear fractionation and confocal microscopy showed an accumulation of HSF1 in the nucleus in HOP depleted cells compared to controls under basal and stress conditions. Western blot analysis of chaperone levels in stress recovery experiments showed a possible delayed but prolonged stress response in the HOP depleted HEK293T cells.
Taken together, these data suggest that HOP regulates HSF1 activity through regulating HSF1 protein levels by a currently undefined mechanism.
Key Words: HSF1,Chaperones, Cell Stress and Survival

Redox homeostasis as a novel drug target in asexual Plasmodium falciparum parasites and sexual gametocytes
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Malaria remains the most important parasitic disease, due to a high disease incidence and mortality associated with a remarkable loss in annual GDP. Chemotherapy, coupled with vector control, has reduced malaria disease mortality remarkably. However, emerging drug resistance towards currently used Artemisinin combination therapies, warrants the need for novel antimalarial therapies, which not only target the proliferative asexual stages, but also the transmissible gametocyte stages of P. falciparum parasites. Artemisinins are redox-active drugs through their bio-reduction and release of labile oxygen, oxidising reduced flavin cofactors of flavin disulphide reductases, which are essential for maintenance of redox-homeostasis in P. falciparum parasites.
Using novel 10-amino artemisinin derivatives, artemisone and artemiside, we show nM activity against asexual proliferative and sexual transmissible gametocyte stages of the parasite. Albeit with slower kill-kinetics against mature stage gametocytes compared to the asexual stages, this pan-reactivity makes these prerequisite drugs for blocking transmission of the parasite to the mosquito. Furthermore, co-treatment of these compounds with a pro-oxidant redox partner drug, methylene blue (MB), showed notable synergism particularly against mature gametocyte stages. Therefore, the induction of oxidative stress by artemisone and artemiside is sustained and even enhanced by the redox cycling action of MB in mature gametocytes. Thus we show that redox-homeostasis is essential to maintain gametocyte viability prior to transmission, making it an ideal drug target. Through utilizing redox-active coupled partner drugs, that are dual-active against the proliferative and gametocyte stages, in addition to a complementary third partner drug with a different mode-of-action, we aim to develop a novel triple drug combination strategy targeting multiple stages of the parasite's life-cycle for effective disease treatment, blocking of parasite transmission and limiting drug resistance formation.

Metabolomics investigation of Ndufs4 knockout mouse brain regions: a step closer to understanding the regional neurodegeneration in mitochondrial Complex I deficiencies
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The whole-body Ndufs4 knockout (KO) mouse is used as a model of mitochondrial Complex I deficiency which is the primary cause of the most common infantile mitochondrial disorder known as Leigh syndrome (LS). In Ndufs4 KO mice, all brain cells lack the NDUFS4 subunit of Complex I, the first and most important enzyme of the oxidative phosphorylation system, but only specific brain regions exhibit degeneration. The mechanisms underlying the region-specific neurodegeneration, also a characteristic feature of LS, is unclear and requires further investigation. Metabolomics together with the Ndufs4 KO mouse model provide an opportunity to study the effect of a Complex I deficiency on the metabolome of different brain regions to explore the nature of the underlying mechanisms.
In this study, a combination of untargeted- (GC-TOF/MS) and semi-targeted (LC-MS/MS) metabolomics approaches were used to investigate the differences in the metabolome of selected brain regions of Ndufs4 KO and wild-type (WT) mice. Samples were extracted using a modified Bligh-Dyer protocol, randomised and analysed intermittently with quality control samples. Metabolites were identified through either spectral matching using the NIST 2011 library and an in-house library or by using pre-optimised MRM transitions for metabolites in metabolic pathways frequently affected in mitochondrial disease (amino acids and acylcarnitines). The extracted data was cleaned using standard metabolomics procedures and then subjected to univariate statistical analysis to determine metabolic differences between the same brain region in KO vs WT mice and metabolic changes unique to the brain regions exhibiting neurodegeneration.
Complex I deficiency in the different brain regions was confirmed with a Complex I enzyme activity assay and high-resolution respirometry analysis of isolated brain mitochondria using the SeahorseTM Extracellular Flux Analyser. The results confirmed a significant decrease in Complex I activity and Complex I-driven mitochondrial respiration in the brain regions of KO mice. Metabolic profiling indicated significant differences between KO and WT brain regions and presented metabolite profiles unique to the brain regions exhibiting neurodegeneration.
Conclusively, the results bring us one step closer to understanding the regional neurodegeneration in mitochondrial Complex I deficiencies.

Proteome Analysis of Isonitrosoacetophenone-treated Plant Systems
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Proteins play a quintessential role in the immune response of host plants following elicitation by either biotic or abiotic stresses. Isonitrosoacetophenone (INAP), a novel oxime-containing stress metabolite/phytoalexin, has previously been scrutinised as a chemical inducer of plant defence responses. Both transcriptomic and metabolomic studies of various INAP-treated plant systems have provided substantial insight into INAP's defence-inducing and priming capabilities. The proteomic aspect had, however, yet to be examined in its entirety in order to complement the prior "omics" approaches. Thus, the proteomic analysis of INAP-treated A. thaliana plants and N. tabacum cell suspensions will provide a greater understanding of the physiological state of the plants and, in conjunction with the identification of the protein profiles, determine the validity of INAP as a priming agent.

In order to ascertain this, A. thaliana plants and N. tabacum cell suspensions were induced with INAP for 0, 8, 16 and 24 h, at the end of which the proteome was isolated before being subjected to various gel-based and gel-free/shotgun proteomics approaches.
The total protein analysis of the isolated proteomes of the N. tabacum and A. thaliana plant systems, through application of the gel-free 8-plex iTRAQ approach, identified 1530 and 1687 proteins respectively. Of these proteins, 127 (N. tabacum) and 305 (A. thaliana) were determined to be of significance. Analysis of these differentially regulated proteins emphasised pathways associated with a triggered defence response, and included ROS production, defence compound biosynthesis, and cell wall modification at various time points for both plant systems. Furthermore it was ascertained that defence-related proteomic activity was triggered at 8 h and deactivated at 16 h for N. tabacum, while up-regulation of the A. thaliana proteins was mostly at 24 h. The occurrence of an INAP-triggered defence was further implicated through the down-regulation of proteins linked to growth and photosynthesis. Lastly, proteins identified within the study allowed for correlation of INAP-associated responses with previously conducted studies, and suggested that several responses are affiliated with a priming process involving abscisic acid (ABA).

Investigating novel inhibitors for triple negative breast cancer

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Breast cancer is the most frequently diagnosed cancer in women worldwide and a recent study of 26 countries indicates that the same is true for Africa. Furthermore, it has been demonstrated that the aggressive and treatment-resistant triple negative or ER-PR-HER-2- subtype is more common among Black women, necessitating the search for novel chemotherapies for this form of the disease. The secondary metabolites produced by marine algae represent a rich source of structurally unique compounds with chemotherapeutic potential. In this study, a library of twenty-two novel marine algal compounds were screened for the ability to selectively inhibit MDA-MB-231 and Hs578T triple negative breast cancer (TNBC) cells, while having no adverse effects on non-cancerous MCF12A breast epithelial cells. From these, only the polyhalogenated monoterpenes RU004 and RU007, and the tetraprenylated quinone sargaquinoic acid (SQA) were identified as hit compounds since their low micromolar cytotoxicity was specific to breast cancer and not healthy breast cells. The mode of action of RU004, RU007, RU015 and SQA was assessed in terms of the type of cell death induced and the effect on cell cycle distribution, revealing an induction of apoptosis by these compounds. The mechanism of action of SQA was further investigated by the identification of specific signal transducer molecules involved in mediating its anti-cancer activities. The investigation into their mechanisms of action, represent one of the few reports in which characterization of algal metabolites goes beyond the initial cytotoxicity assays. We went further to assess the potential anti-cancer stem cell (CSC) activity of the compounds. TNBCs are associated with high levels of CSCs, a sub-population of cells resistant to traditional chemotherapeutics. Using the novel mammosphere assay, the halogenated monoterpene stereoisomers RU017 and RU018 were demonstrated to possess putative anti-CSC activity as evidenced by their ability to completely eliminate mammosphere formation, while having no adverse effects on either breast cancer or healthy breast cells. These results represent the first report of selective anti-CSC activity of a natural product.

Galactose-1-phosphate uridyltransferase (GALT) deficiency: Redefined as a secondary congenital disorder of glycosylation and the importance of galactitol measurement

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Introduction: Galactose-1-phosphate uridyltransferase (GALT) deficiency is commonly associated with jaundice, vomiting, poor feeding, poor weight gain, lethargy, irritability and cataracts. Significant prevalence with common mutations have been identified in the South African population. The vast accumulation of systemic galactose, result in several biochemical implications in the cell, including formation of galactitol and hypoglycosylation of structural/functional proteins. The latter result in a secondary congenital disorder of glycosylation (CDG).
Methods: We developed a dedicated sugar alcohol gas chromatography-mass spectrometry (GC-MS) application on urine, consisting of solid-phase extraction sample clean-up, the addition of an internal standard (Manitol-1-C13) and subsequent derivatization with TMSI:BSA:TMCS at 75^{o}C for 45 minutes. Chromatographic separation was achieved through an Agilent GC mass spectrometer installed with a DB-1MS column. The analysis was performed in the event of a strong clinical suspicion of galactosemia, where a lactose free diet was followed, sampling was performed after a blood transfusion and/or negative reducing substances were observed. The sugar alcohol analysis was also considered in the event of a positive CDG screening.
Results: The above approach was successfully utilized in the identification of GALT deficient patients on a lactose free diet. Increased galactitol (normal ref range: 2-5 mmol/mol creat) was also observed in patients whom underwent blood transfusions. Both groups had galactitol levels above 30 mmol/mol creat. One patient, with a primary neurological and secondary hepatic phenotype, had a relatively normal routine metabolic screen but a positive CDG result. Genetic testing revealed the common homozygous c.404>T (p.S135L) mutation in the GALT gene, resulting in GALT deficiency. Retrospective analysis indicated increased galactitol in a urine sample of the patient, correlating with the genetic findings.
Conclusion: We proposed broadening the GALT diagnostic protocol with the inclusion of urinary galactitol measurement, based on above findings. GALT deficiency has been redefined as a secondary glycosylation disorder and a positive CDG screen should be considered in the differential diagnosis of GALT deficiency

Molecular chaperone complexes regulate important cellular processes and represent putative drug targets in cancer

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The ability to overcome the effects of stress is an important component of cellular homeostasis. At a cellular level, the effects of stress predominantly lead to perturbations in protein homeostasis. The cell responds through activation of the conserved stress response and increasing the levels of a cohort of groups of proteins from the heat shock protein (HSP) family. HSPs subsequently function either as molecular chaperones or proteases to regularise cellular proteostasis. Together with their physiological role, HSP are implicated in the development of human disease, and as a consequence, may represent putative drug targets, particularly for cancer.

The most widely studied HSP cancer drug target is HSP90, with a number of HSP90 inhibitors currently in clinical trials. HSP90 is a molecular chaperone that maintains the correct conformation of a range of cellular 'client' proteins. HSP90 maintains its client proteins in an inactive, but easily inducible state, thereby promoting stability of labile intermediate conformations. Inhibition of HSP90 is considered a promising therapeutic approach for cancer treatment because HSP90 client proteins are signalling intermediates and transcription factors that control fundamental cellular processes, and are deregulated in cancer. However, although inhibitors of HSP90 have been developed, their application in the clinic has been limited by negative side effects. Therefore, novel approaches to inhibiting this complex are required. We have characterised the role of the HSP90 co-chaperone, HOP/STIP1, as a putative drug target. Our data demonstrate that HOP expression was part of a cancer gene signature regulated by p53 and Ras and that a novel isoform of the gene is differentially expressed between normal and cancer cells. HOP regulated a number of fundamental cellular processes via regulation of key cellular proteins, while depletion of HOP was sufficient to alter cell migration, proliferation and chemosensitivity of cancer cell lines. Interestingly, HOP mediated these effects both in conjunction with Hsp90, and independently of the chaperone, which suggests that HOP may play a wider cellular role than previously appreciated.
Taken together, our data suggest that HOP may represent an alternative mechanism by which to disrupt both HSP90 dependent and independent chaperone functions in cancer cells.

Oligopeptidase B-specific antibody fragments: possible diagnostic tool for animal infective trypanosomosis
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African trypanosomosis (AT) is a major obstacle in the establishment of agriculture and economic sustainability in Africa. Animal AT (AAT) is responsible for large numbers of livestock succumbing to the tsetse transmitted kinetoplastid parasites, Trypanosoma congolense and T. vivax, and as a result, losses in further downstream agriculture sectors are experienced. Due to the ability of the trypanosomes to undergo antigenic variation, vaccine candidates are highly unlikely. Peptidases, such as oligopeptidase B (OPB), have been identified as virulence factors and are the focus of the development of novel chemotherapies and diagnostics. Current diagnostics are based on antibody detection, but an antigen detection format is preferable as it could differentiate between active and cured infections since anti-trypanosome antibodies can persist for years. OPB could be the ideal diagnostic antigen since it is released into the host bloodstream by dying trypanosomes. Given the rural, resource-poor locations in the areas of AT incidence, the ideal rapid diagnostic test (RDT) or dipstick test would be robust, affordable, sensitive and specific and require minimal user training.
Here we report on the identification of OPB-specific single chain variable fragment (scFv) antibodies from the Nkuku® phagemid library. These scFv antibodies will be used for the possible diagnosis of current AAT infections by the detection of the OPB antigen in blood samples from infected cattle. This OPB-specific scFv recognised a conserved peptide in the T. congolense and T. vivax OPB homologs, and detected native OPB in a western blot. Molecular modeling predicted that the scFv interacts with OPB in such a way that it would restrict the hinge motion between the C-terminal catalytic and N-terminal regulatory domains of the peptidase and limits access to the active site pocket. An antigen detection ELISA using this scFv as capture antibody and rabbit-anti-OPB IgG-HRPO antibody showed that OPB levels fluctuated with parasitaemia in sera from T. congolense infected cattle.

Biochemical and Molecular Characterization of a Unique DyP-type Peroxidase from Raoultella ornithinolytica OKOH-1
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The increase in industrial demand for peroxidases has necessitated the search for novel peroxidase with versatility and catalytic efficiency. Peroxidase produced by Raoultella ornithinolytica OKOH- 1 (KX640917) was therefore characterized using biochemical and molecular approaches. Subsequently, the enzyme was evaluated for its dye decolourization potential. R. ornithinolytica OKOH- 1 peroxidase (RaoPrx) was capable of oxidizing various substrates with pyrogallol giving the optimum activity. RaoPrx had an optimum activity at pH 6 and was stable over a pH range of 5.0-7.0 with residual activity of above 40 % after 120 min of incubation. The enzyme showed an optimum activity at 50 oC and was very stable at higher temperatures (50 – 70 oC) with residual activity of above 70 % after 120 min. The activity of the enzyme was remarkably stable at 50 oC as it retained over 90 % of its original activity after 120 min. Moreover, the peroxidase activity was significantly enhanced by Ag+, Cu2+, Zn2+and Fe2+ while it was inhibited by Ca2+, Mg2+, Ba2+, Al3+, Co2+, NaN3 and EDTA. Furthermore, characterization of the peroxidase gene suggests it encodes a novel DyP-type peroxidase with molecular weight of 17.587 kDa and isoelectric point of 4.51. RaoPrx exhibited a remarkable dye-decolourizing activity on congo red (65.03%) and melanin (47.96 %) within 30 min. This indicates the potentiality of RaoPrx for applications in dye decolourization and development of cosmetic agent.
Keywords: DyP-type peroxidase, enzyme characterization, thermostability, polymerase chain reaction, peroxidase gene.

LRP/LR specific antibody IgG1-iS18 enhances mTERT levels and impedes neurodegeneration in Alzheimer's disease transgenic mice.
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Alzheimer's disease (AD) is a devastating neurodegenerative disease affecting the memory and cognitive abilities of elderly individuals. It is caused by accumulation of amyloid beta (Aβ) plaque and neurofibrillary tangle formation in the brain. We have shown, *in vitro*, that blockade of the 37kDa/67kDa laminin Receptor (LRP/LR) with a specific antibody (IgG1-iS18) resulted in a significant reduction in Aβ production and rescued cells from Aβ induced neurotoxicity. In order to test whether targeting LRP/LR using IgG1-iS18 antibody *in vivo* would produce similar effects on Aβ generation and alleviate disease associated symptoms, AD transgenic mice received intranasal administration of IgG1-iS18 for 8 weeks. Various memory tests were performed to determine if treatment with IgG1-iS18 improved the cognitive abilities of the AD mice.

Aβ plaque formation was investigated via histological analysis and to further discern the effects that the IgG1-iS18 antibody has on the molecular mechanisms underlying AD pathology, the levels of various AD related proteins were studied. We observed that this treatment resulted in an improvement in recognition, learning and short term memory as well as decreased Aβ plaque formation and Aβ42 protein expression. Moreover, a significant increase in amyloid precursor protein (APP) and telomerase reverse transcriptase (mTERT) levels was observed. Therefore, we recommend the anti-LRP/LR specific antibody, IgG1-iS18, as a novel and powerful potential therapeutic strategy for treatment of AD (Ferreira et al., 2008, Oncotarget).
Keywords: Alzheimer's disease, amyloid beta, LRP/LR, transgenic mice, mTERT.
Funding: SAMRC, NRF, Claude Leon Foundation.

Identification of differentially expressed proteins in drought stressed sorghum plants
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Drought is a major threat to global food security due to its detrimental effects on plant growth, productivity and yield quality. During periods of water deficit, plants attempt to maintain essential processes at the expense of non-life threatening ones. Sorghum shows excellent maintenance of normal growth and development even under severe drought stress. This makes the crop a suitable model plant for studying the adaptive responses to drought. In this study, we carry out a comparative proteomic study of two sorghum varieties with contrasting responses to drought stress. We mimic drought stress responses in the sorghum plants by withholding water at the V3 growth stage for 12 days. We then analyse the root and leaf tissue using isobaric tags for relative and absolute quantitation technology. The drought stress induced differential expression of at least 200 proteins in each of the two sorghum varieties. The putative functions of the identified proteins will be discussed. The identified proteins will be the initial stage of further gene expression analysis between the two sorghum varieties. The data generated in this study will provide potential gene targets for functional analysis study with the aim of identifying drought tolerance genes for plant breeding programs.

Does exposure to road-dust pollution have effect on the quality of phytochemicals in the leaves and roots of Barleria dinteri?
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The aim of the study was to undertake qualitative phytochemical analysis of the samples of an African traditional herb, Barleria dinteri, with varying exposure to road-dust pollution. Both leaves and roots samples of B. dinteri were collected both near to (test sample) and away from a dusty road (control sample) in the Limpopo province of South Africa. The plant samples were extracted with solvents of different polarities through a cold maceration method in a serial sequential extraction procedure. The resultant extracts were subjected to qualitative phytochemical analysis through chemical tests, thin layer chromatography and ultraviolet-visible spectrophotometry. Subsequently, the qualitative phytochemical profile of the plant samples collected at the two locations were compared for similarities or differences. TLC profiles of the plant samples showed moderate differences, with the differences mostly seen in leaves. UV-Vis profiles of the plant samples were mostly similar, except with the acetone leaves and roots extracts, as well as the methanol roots extracts. The screened phytochemical compositions of the samples were mostly similar, with generally all detected phytochemicals present in one or more extracts of the two samples. The results demonstrate that exposure to road-dust pollution have no substantial effect on the quality of phytochemicals possessed by the leaves and roots of B. dinteri. The findings of the study suggest that the leaves and roots of B. dinteri may be collected either near to or far away from dusty roads with no substantive disparity to the benefits emanating from its usage in traditional medicine.

Three-dimensional cell culture models: physiologically relevant *in vitro* research
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In vitro pharmaceutical and biological evaluations are traditionally performed in two-dimensional (2D) or "flat" mammalian cell cultures. These 2D models, however, have been shown to have extensive shortcomings and reduced physiological relevance. Three dimensional (3D) cell culture systems have been proposed as an alternative to mimic *in vivo* conditions more closely.
Cells grown in 2D or in 3D represent two 'extremes' of cellular programming. At one extreme is exponential growth with diminished functionality (as seen in traditional 2D cultures, similar to that of wound healing or cancer) and at the other extreme is a dynamic equilibrium with very slowly proliferating cells with a highly specialized functionality (as observed for cells grown as active 3D spheroids and in tissues). Cells grown as active 3D spheroid cultures therefore mimic human tissues better than cells grown in 2D. This ensures higher physiological relevance of experiments.
3D cell culture systems can refer to a wide variety of cell culture approaches, and these complex and advanced models have numerous applications in basic research, but also in applied research such as drug development, cancer research and toxicity studies. The dynamic clinostat based spheroid 3D system exhibits the ability to overcome many of the 2D culture shortcomings, and by implementing this system, we aim to establish specific spheroid models and platforms for health research.
The immortal hepatocellular carcinoma cell line HepG2/C3A grown as 3D spheroids was used to perform herbal toxicity assessment. Cell viability was assessed following treatment with a crude *Xysmalobium undulatum* (Uzara) aqueous extract for 21 days, evaluating basic physiological parameters, namely cell proliferation, glucose uptake, intracellular adenosine triphosphate (ATP) levels and adenylate kinase (AK) release. The results were compared with studies performed in a 2D culture of HepG2/C3A cells and the Sprague Dawley rat model.

The HepG2/C3A active 3D spheroids have been shown to exhibit physiological performances that mimic those seen in human tissues, and we have shown hepatotoxicity in this model to correspond much better with results from an *in vivo* model than their 2D counterpart. Other cell lines are expected to respond similarly to 3D growth.

Cholesteryl Ester Transfer Protein as a Possible Molecular Target for Reducing Drug-resistance in Breast Cancer

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Cancer is defined as an abnormal growth of cells due to an unrestricted cell proliferation. Breast cancer (BC) is the second leading cause of cancer deaths in South African women. Majority of BC cases are hormone induced (75%) therefore hormone dependent for cell survival. These hormones are derived from cholesterol thus BC cells are high in cholesterol. Treatment for hormone induced BC include endocrine therapy targeting hormone receptors (hormone antagonists) for example; Tamoxifen, the gold standard treatment for estrogen receptor positive (ER+) BC cells. However, following long term therapy of Tamoxifen may cause adverse side effects and relapses due to a build-up of drug resistance. Therefore, further research is warranted to reduce the toxicity and resistance to Tamoxifen. Due to the relationship between cholesterol and estrogen, we investigated the effect of CETP (involved in the cholesterol signalling pathway) knock-down on drug efficacy and resistance. The cytotoxicity of various drugs was tested using MTT and APOPercentage assays and an increase in growth inhibition and cell death (10-30%) was observed in transfected MCF-7 cells (knock-down confirmed by qPCR and western blotting) when compared to non-transfected MCF-7 cells. In addition, there was approximately a 70% decrease in cholesteryl esters in transfected MCF-7 cells compared to non-transfected MCF-7 cells. A combination treatment of Tamoxifen and Acetyl-Plumbagin (AP – a cholesterol-depleting agent) was tested and an increase in growth inhibition and cell death (by ±3 fold) was observed, even at lower concentrations, in transfected MCF-7 cells when compared to the single treatment in transfected cells. Therefore, CETP knock-down and treatment with Tamoxifen in combination with AP increases drug efficacy and reduces resistance to tamoxifen. We anticipate that this may possibly lead to reduction in possible side effects during long-term use of tamoxifen in breast cancer patients. This hypothesis will also be tested in an in-vivo study.

Local implementation of a plasmid only rotavirus reverse genetics system

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Reverse genetics (RG) has revolutionized virology, allowing the rationally guided generation of recombinant viruses from cDNA. To date, it remains one of the most definitive ways to study the function of various viral genome elements and has various other applications such as viral vaccine development and viral modification. For the segmented dsRNA Reoviridae family RGs has proved to be quite a bit more challenging than that of non-segmented, and DNA viruses. The first member of the Reoviridae family to be rescued through a RG system was mammalian orthoreovirus (MRV), followed by bluetongue virus (BTV) and African horsesickness virus (AHSV).
For rotaviruses (RV) only single genome segment replacements were done through the use of a plasmid and helper-virus system that relies on the ability of rotavirus to undergo reassortment. Although recombinant viruses can be, and have been rescued this way, the success of each system relies almost solely on the selection system to distinguish between unaltered helper-virus and recombinant viruses carrying the gene of interest, mostly reserved to outer capsid proteins VP4 (genome segment 4) and VP7 (genome segment 9). RV was only rescued from an entirely plasmid based system last year (Kanai, Y., et al. 2017. PNAS, 114:2349-2354). However, to date mixed results have been obtained in recreating the Japanese plasmid-based viral rescue across various institutions, showing that this RG system still needs further development and optimization.
We purchased the RV plasmid based RG system and are implementing it locally. We succeeded to rescue recombinant rotavirus after introducing several modifications to the system based on experience with BTV and AHSV RG systems. These results will be presented. This lays the foundation for further research into many of the outstanding issues of the RV viral replication cycle and possible applications of the system for the rational design and development of regionally specific vaccine candidates.

Biochemical and Physiological responses of selected cowpea (Vigna unguiculata) genotypes to elevated iron levels in a ferruginous ultisol

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Crop production in subtropical Africa is hampered by a number of environmental factors including elevated soil iron levels. Being a region where production of cowpea is highest, the screening of iron-tolerant cultivar is imperative in order to sustain or better improve overall cowpea production. The present therefore study investigated the growth and antioxidative responses of selected cowpea (*Vigna unguiculata*) accessions to iron toxicity in a ferruginous ultisol.
Top soil (a ferruginous ultisol) was obtained from an undisturbed garden, and sun-dried to constant weight. The soils were divided into 2 groups. One group was the selected ferruginous garden ultisol, whereas the iron level in the other group was elevated by twice the ecological screening benchmark of iron in agricultural soils (400 mg/kg). One week later, 15 accessions of cowpea, *Vigna unguiculata* (TVu-3742, TVu-3769, TVu-5348, TVu-5760, TVu-5768, TVu-5782, TVu-5883, TVu-6102, TVu-6193, TVu-6219, TVu-6290, TVu-10600, TVu-10881, TVu-11114, and TVu-11214) were sown in both iron-amended and control soils.
Twenty weeks later, results showed differential responses of the accessions to elevated iron levels. There was general morphological growth suppression in Fe-elevated soils ($p<0.05$), particularly in plant heights and main root lengths; TVu-3742, 3769, and 6290 were the worst hit. However, elevated Fe enhanced rooting parameters of TVu-3769 and 6219. Per plant yield in TVu-5768, 6102, 10600 and 11114 were significantly reduced by over 35% while the reduction in TVu-5760 and 11214 is over 50%. However, no significant yield changes were reported for TVu-3742, 5768, 5782, 5883, 6193, 6219, 6290 and 10881 under elevated oil iron condition.

Given the fact that the control soil was ferruginous, with iron levels higher than 1g/kg, the reported capacities for selected accessions to maintain yield levels under further elevated iron conditions suggest possible iron tolerance for those accessions.

Predictive interaction between Hsp70.14 and the RING finger domain of Retinoblastoma binding protein 6: an in silico study

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Over the years, heat shock proteins (Hsps) have become a subject of interest in the field of biomedicine due to their diverse functions in the pathology of many human diseases such as neurodegenerative and human inflammatory diseases, and cancer. Hsp70.14 like other molecular chaperones has been postulated to play a role in responses to stress signals such as heat, infections, and cancer. Human RING finger domain from RBBP6 is a small cysteine-rich domain that coordinates two zinc ions in a “cross-brace topology”.
This special conformation makes it suitable for the RING finger domain to perform many biological functions including oncogenesis, viral infections and apoptosis. A yeast-2-hybrid study hypothesized an interaction between Hsp70.14 and RING finger domain; this interaction is suggestive of possible roles in degradation of aggregated, denatured, and misfolded proteins through protein quality control and chaperone-mediated ubiquitination. In this study, several bioinformatics tools were employed to analyse the sequences of both proteins, which in turn was used to generate several antigenic epitopes on Hsp70.14, with an average antigenic propensity score of 1.0270. More so, three of the identified epitopes were within the transmembrane topology of the protein thus, are predicted to play a crucial role in eliciting immune response on the surface of cancer cells. In total, two linear B-cells and thirteen discontinuous B-cell epitopes were also predicted. The 3D structures of the proteins were modelled and the quality of these structures was validated using PROCHECK. Finally, PatchDock was used to investigate the interaction between the two proteins and it was shown that a strong interaction exists between Hsp70.14 and the human RING finger protein. Taken together, our results suggest the interaction between these proteins as a target in the discovery, design and development of diagnostics or therapeutics against cancer.

Cancer Cell Behaviour Following Parasite Exposure

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Infectious diseases, including helminth parasites, are estimated to cause 33% of the cancer cases in sub-Saharan Africa. While certain helminths are conclusive biological carcinogens, others have been shown to modulate inflammatory diseases, including cancer. It is currently unknown why differing helminth infections promote or prevent cancer development, or which cellular mechanisms are altered following exposure to helminths. Using cell proliferation assays, two-dimensional scratch motility assays, transwell migration assays, western blot analysis and in vivo models this study aimed to determine the effect that antigens derived from certain helminths have on cervical and colorectal cancer behaviour. Through these techniques, it was revealed that in vitro exposure to Nippostrongylus brasiliensis antigen significantly decreased cervical cancer cell migration and the expression of markers associated with cancer cell metastasis, vimentin and N-cadherin. Expression of vimentin was also significantly reduced in the murine female genital tract following N. brasiliensis infection in vivo. In addition N. brasiliensis antigen significantly decreased the expression of cell-surface vimentin, a known restriction factor for the cancer-causing virus Human Papillomavirus (HPV), while unexpectedly resulting in a significant decrease in HPV16 pseudovirion internalisation. Furthermore, in vitro exposure of a murine and human colorectal cancer cell line to Heligmosomoides polygyrus antigen and its excretory-secretory (ES) product significantly decreased cell proliferation with an accompanied increase in the expression of the cell-cycle regulator proteins p21 and p53. This work generates the novel and fascinating hypothesis that helminth products can inhibit cancer progression through regulation of cancer cell metastasis marker expression and cell-cycle regulator protein expression. These findings have important implications for cancer patients in helminth endemic areas.

Enhancing the detection of a persistent RNA virus infection in mammalian cells

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Providence virus (PrV) is a positive-sense single-stranded RNA virus that belongs to the Family Carmotetraviridae. While this virus was originally isolated from an insect cell line, it is capable of establishing persistent viral infections in mammalian cells. PrV infected of mammalian cells is evaluated by detecting the viral replication accessory protein (p40) as well as double-stranded RNA, which is indicative of a replicating single-stranded RNA virus infection, by confocal immunofluorescence microscopy. Persistently infected cells show low levels of both p40 as well as dsRNA, localized to punctate structures in the cytoplasm.
Treatment of persistently infected mammalian cells with Triton X-100 and NP-40, both non-ionic detergents, result in an increase in the level of detectable p40 and dsRNA. Treatment with the detergents saponin and digitonin show some increase in the levels of p40 and dsRNA. In contrast, treatment of mammalian cells with tween 20, sodium dodecyl sulphate or hexadecyltrimethylammonium bromide does not show any increase in the levels of p40 or dsRNA. We propose that the treatment of persistently infected mammalian cells with detergents results in the disruption of the cytosolic viral replication factories, exposing the p40 and dsRNA for enhanced detection of the persistent viral infection.

Mitochondrial adaptations to adipogenesis

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Mitochondria are at the centre of metabolism hence the cells' energy requirements and therefore their mitochondrial biogenesis are modified depending on whether they are proliferating or differentiating, and that mitochondria can be a major limiting factor in ensuring the efficacy and differentiation potential of mesenchymal stem cells (MSCs). The adipose tissue maintains metabolic homeostasis through insulin, which facilitates the uptake of glucose. However, excess energy is stored as fat culminating in an increase in the fat tissue as through addition of new fat cells and increase in the size of fat cells, leading to obesity related metabolic diseases. such as type 2 diabetes. Adaptation to excess nutrient environment interferes with mitochondrial quality control functions and, as a result, affects mitochondrial function. These changes in mitochondrial biogenesis affects the mitochondrial networks, structure, membrane potential, substrates and products. Therefore, this study aimed to investigate changes to mitochondrial mass, DNA, distribution, membrane potential and its networks changes during the adipogenic differentiation of adipose derived human MSCs. The results show that upon adipogenic differentiation of hMSCs, mtDNA, ROS and mitochondrial mass increased, the mitochondrial membrane potential became more polarised, its networks became more tortuous, while the branches decreased in thickness but increased in length and neutral lipids drastically increased. These results suggest that mitochondrial biogenesis increased, mitochondria became healthier and its networks became more fragmented when human mesenchymal stem cells were differentiated into white adipocytes.

Screening of new artemisinins as cytotoxic modulators of the stress response in triple-negative breast cancer (TNBC) cells

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Introduction: Triple-negative breast cancer (TNBC) is a global concern due to lack of targeted therapies as a consequence of undetectable expression of the key hormonal drug targets in breast tumours, namely estrogen receptor, progesterone receptor and human epidermal growth receptor 2 (HER2). TNBC is more prevalent among black women of African ancestry and has a high mortality rate. Identifying important tumour survival pathways and targeted chemotherapeutic agents is crucial for the treatment of this aggressive disease.
A major pro-survival mechanism is the heat shock response in which cells survive extreme temperatures, oxidative stress or genotoxic agents by upregulating heat shock proteins (HSPs) by activation of the master transcription factor, heat shock factor 1 (HSF1). Therefore, HSPs and HSF1 are important therapeutic targets.
Method: Using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assays, western blots, flow cytometry and qPCR, we explore the ability of novel artemisinin-derivatives to modulate the stress response as a strategy of inhibiting TNBC.
Result: A series of chemically modified artemisinin derivatives were screened for cytotoxicity against TNBC cell lines and showed half maximal effective concentration (EC50) values ranging from 0.19 µM to 255.0 µM. The most toxic compound, WHN-11, had an EC50 of ~1.0 µM across HCC1937, HCC70 and MDA-MB-231 TNBC cell lines. At increasing concentrations, WHN-11 mediates a steady decrease in Hsp70 protein levels without a significant effect on Hsp90α, Hsp40 or Hsp27 levels. WHN-11 actively inhibits the growth of cancer stem cell-enriched tumourspheres which are implicated in tumour resistance and metastasis. Importantly, WHN-11 increased protein levels of activated caspases and PARP1, verifying its pro-apoptotic nature.
Conclusion: Interestingly, in TNBC cell lines, it is not clear whether WHN-11 acts via induction of reactive oxygen species (ROS) like its parent compound, artemisinin. However, the promising EC50 value and in-vitro activities substantiate WHN-11 as a hit for further mechanistic studies to investigate the molecular targets as well as the mechanism(s) by which WHN-11 exhibits its chemotherapeutic effects.

High resolution mass spectrometry as a tool for mapping the reservoir of secondary metabolites in complex marine natural product chemical extracts

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Marine organisms, specifically benthic macrofauna and their associated microbiota, are amongst the most important sources of novel bioactive small molecules for the pharmaceutical industry. However, the challenge is to prioritise extracts with potentially novel chemistry and to de-replicate known compounds early in the process. Here we describe the use of high resolution tandem Mass Spectrometry (HR-LC-MS/MS) to characterise chemical extracts of marine sponges endemic to the South African coast. Latrunculid sponges are rich in pyrroloiminoquinone alkaloids, including discorhabdins, makaluvamines and tsitsikammamines that inhibit Topoisomerase activity and have potential in the development of anti-cancer therapies. We wanted to characterise the diversity of pyrroloiminoquinone compounds produced by related species of latrunculid sponge to isolate new compounds with potentially novel bioactivity. Organic crude extracts were generated from more than 60 sponge specimens representing four species, collected at two locations in Algoa Bay. The extracts were analysed by HR-LC-MS/MS and the data processed using the Global Natural Product Social (GNPS) Network workflow 2.0 to create a molecular network of compounds. Observed chemical profiles were species-specific with some overlap between species. Several known and potentially new pyrroloiminoquinone compounds were identified, purified and assayed for bioactivity. Interestingly, the chemical profiles revealed marked differences between taxonomically identical specimens that likely reflect environmental influences, which may contribute to broadening the diversity of secondary metabolites produced by these sponges and the potential for discovering new compounds.

The effect of DWNN of RBBP6 in prostate cancer

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Prostate cancer accounts for the second largest male cancer in the United States alone and because cancer has the ability to avoid apoptosis and continue proliferating, novel therapies that are able to induce and promote apoptosis are needed. The manipulation of genes in cancer therapy has proven to to better our understanding of cancer and its overall mechanisim. In the present study we elucidated the effect of DWNN of RBBP6 on prostate cancer cell line PC3. The gene Retinoblastoma Binding Protein 6 (RBBP6), has been overexpressed in other cancers making it a target in anticancer therapy; this gene encodes for three isoforms with the shortest being isoform 3 commonly known as the Domain With No Name (DWNN), DWNN has been reported to be involved in the induction of apoptosis. The cell lines were cultured and silenced using siRNA to get a better understanding of the genes relationship with each other and the cancer of the prostate. Gene expression of RBBP6 was measured using quantitative real time PCR.
Tumour suppressor p53, caspase 8 and the MDM2 were also used to run qPCR. Tumour suppressor p53, caspase 8 and the Mouse double minute 2 (MDM2) proved to have a higher fold increase when DWNN was silenced, this finding led to the conclusion that they might be inversely proportional to DWNN. DNA fragmentaion using agarose gel electrophoresis in the silenced PC3 cell line was also carried out and the results demonstrated the occurence of apoptosis.
The overall results attained from this study suggested that indeed DWNN has an effect on RBBP6, they further proposed the need for further studies on the use of the two genes and others like them in the fight against cancer.

Tbx3 directly represses the pro-cell death factor, Cers1,in Malignant Melanoma

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Introduction: The T-box transcription factor, TBX3, is overexpressed in malignant melanoma, the deadliest form of skin cancer, where it drives tumour formation and invasion. It is thus a potential therapeutic target to treat this aggressive cancer. Transcription factors are however notoriously difficult to target and we therefore propose that a more effective therapeutic approach would be to identify TBX3 target genes that mediate its role(s) in melanomagenesis. We thus performed microarray analyses and ceramide synthase 1 (CERS1), a key enzyme in C-18 ceramide synthesis, was identified as a putative target gene repressed by TBX3. C-18 ceramide is a lipid that promotes cell death and its induction sensitises cancer cells to therapeutics. We hypothesise that TBX3 contributes to melanomagenesis by repressing CERS1. Here we test if CERS1 is a bona fide TBX3 target and examine the functional significance of this regulation.
Materials and methods: We performed microarray analysis in control and TBX3 knock-down melanoma cells to identify TBX3 target genes; qRT-PCR and western blotting to determine the levels of TBX3 and CERS1 mRNA and protein in a panel of melanoma cells and TBX3 knock-down and overexpression melanoma cell culture models; chromatin immunoprecipitation and luciferase assays to test whether TBX3 directly binds and represses the CERS1 promoter; and bioinformatic analyses using TCGA database to compare the relationship between TBX3 and CERS1 mRNA and melanoma invasiveness in patient samples.
Results: We show that (1) CERS1 is upregulated 6-fold in TBX3 knock-down cells; (2) there is an anti-correlation between TBX3 and CERS1 mRNA and protein levels in a panel of melanoma cell lines and TBX3 knock-down and overexpression melanoma cell culture models; (3) TBX3 binds the CERS1 promoter in vivo and that it represses CERS1 through a mechanism requiring its DNA-binding and C-terminal repression domains; and (4) TBX3 and CERS1 mRNA levels correlate directly and inversely respectively with melanoma invasiveness in melanoma patient samples.
Conclusion: This study identifies CERS1 as a TBX3 target gene, with relevance to patient samples, and provides a novel mechanism by which TBX3 contributes to melanomagenesis.

Repurposing of commercial drugs that target the oncogenic TBX3 for anti-cancer activity in breast and cervical cancer

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Breast and cervical cancer have the highest and fourth highest incidence rates among females respectively. A disproportionate burden (70%) of these cancers fall on developing countries (550,000 annual deaths) and is expected to rise by 36% by 2030. This is partly due to limited access to healthcare and failure to implement national screening and/or vaccination programs which lead to late presentation of disease. Therefore, there is need for more effective strategies to treat late stage cancer.
The T-box transcription factor TBX3 is key to the invasive phenotype of several cancer types including breast cancer and here we show that it is also important for advanced stages of cervical cancer. TBX3 is therefore an ideal candidate to be targeted in the treatment of advanced cancers but the pipeline for developing targeted therapies is arduous and costly. To circumvent these challenges, we have adopted a high throughput drug repurposing strategy to identify FDA-approved drugs that target TBX3 and that can thus be repurposed to treat TBX3-driven cancers.
The status of TBX3 in patient derived cervical cancer tissue was determined by immunohistochemistry. To elucidate the role of TBX3 in cervical cancer, TBX3 was knocked-down by siRNA in HeLa and CaSki cells and the effect on proliferation and migration was measured using growth curves and scratch motility assays respectively. A target-based high throughput drug repurposing screen was performed and 13 'hit' drugs that inhibit TBX3 were identified. Of these, piroctone olamine and pyrvinium pamoate, were validated in breast (MCF7) and cervical (HeLa) cancer cells using western blotting, cell viability and migration assays.

This study shows for the first time that TBX3 is increasingly overexpressed in dysplastic and invasive squamous cell carcinoma cervical cancer tissues. Depleting TBX3 reduced cervical cancer cell proliferation and migration. Importantly, we show that piroctone olamine and pyrvinium pamoate inhibit TBX3 levels and cervical and breast cancer cell viability.
This study identifies two FDA-approved drugs that have therapeutic promise to be repurposed for the treatment of TBX3-driven cancers such as breast and cervical cancers which would contribute towards adding valuable years of life to affected women.

Anticancer activity and cell cycle response of novel synthetic High Mobility Group Box 1 inhibitors in colorectal cancer cells

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Introduction. Cancer recurrence and chemoresistance is an increasing challenge for its treatment and often results in patient relapse. In a process called autophagy, cancer cells acquire anoikis resistance and escapes chemotherapy. High mobility group box 1 (HMGB1) molecule is a key mediator of autophagy and can be exploited to develop effective targeted anticancer therapy. Gabexate mesilate (GM) is used in the treatment of pancreatitis and clotting. GM is a synthetic inhibitor of HMGB1 and inhibits metastasis. Structure mimetics of GM can hold promise to suppress HMGB1 to arrest cancer growth, recurrence and resistance mechanisms. We synthesized structure mimetics of GM and tested them for anticancer activity in colorectal cancer cells. As HMGB1 is critical for cell cycle, we analyzed cell cycle response to active GM mimetics in colorectal cancer cells. In silico docking studies were proposed to be evaluated for any possible physical interaction of HMGB1 and active mimetic. Methods. A total of thirteen GM mimetics were synthesized and their anticancer activity was performed against SW480, HT29 and DLD1 colorectal adenocarcinoma cells. Anticancer activity was determined in terms of IC80 using alamar blue screening and trypan blue exclusion assays, while cell cycle analysis was performed using a propidium iodide based staining assay in a Muse flow cell analyzer. Molecular docking studies were performed using AutoDock4.2. Results. Novel synthetic GM mimetics A1-A3 and A6 were found most active with an anticancer IC80 of 250-500µg/ml, however A4-A5 and A7 showed moderate anticancer activity (IC80 500-750µg/ml) and mimetics A8 and A9 showed weak anticancer activity (1000-1500µg/ml) against SW480, HT29 and DLD1. A10-A13 exerted no inhibitory activity against the tested cell lines. Mimetic A1, A2, A3 and A6 showed an altered cell cycle with the colorectal adenocarcinoma cells mainly arrested in G0/G1 and G2/M phase. Docking studies established that the active GMMs possessed specific binding affinity with the target, compared to the inactive GMM. Conclusions.
Quantitative structure-activity relationship of the synthetic GM mimetics reveals that the anticancer effects of GM mimetics were reduced when the complexity of attached side chain is increased.

Identification of the ergosterol-interacting proteome in the Arabidopsis thaliana plasma membrane

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The impact of fungal diseases to total crop production reflects on the economy, and results directly results from crop yield loss due to fungal diseases, reduced crop value and trade impact. Ergosterol is the major sterol component in fungal and yeast membranes. The perception mechanism in plants is not known but this microbe-associated molecular pattern (MAMP) molecule has been classified as a general elicitor due to plant responses, including changes in the membrane potential, accumulation of reactive oxygen species (ROS), increased intracellular Ca2+ levels and activation of defense genes. Here, Arabidopsis thaliana plasma membrane (PM) proteins were used to identify those differentially regulated by ergosterol treatment as well as subjected to affinity-based chromatography enrichment strategies to capture, identify and characterize ergosterol-interacting proteins using liquid chromatography with tandem mass spectrometry (LC-MS/MS). As such, mature Arabidopsis thaliana plants were induced with 250 nm ergosterol over a 24 h period.
The isolated microsomal and plasma membrane (PM) fractions were electrophoresed on 12% one dimensional (1D) SDS-PAGE, while an ATPase activity assay and Western blot (MAPK) was conducted to verify the success of the PM isolation. In addition, the differentially regulated PM proteins were observed using two dimensional (2D) SDS-PAGE. MAMP-interacting PM proteins were then enriched using ergosterol immobilized to epoxide microspheres and EAH Sepharose 4B, respectively, for affinity-based approaches.
Ergosterol evidently induces a defense response in higher plants and significant proteins identified subsequent to PM isolation, and 1D and 2DE gel-based analysis included the adenosine triphosphate (ATP)ase protein, glycosylphosphatidylinositol (GPI)-anchored protein, aquaporins, clathrin light- and heavy chain, leucine-rich repeat (LRR)-containing receptor-like kinases (RLKs), G-type lectin S-receptor-like serine/threonine-protein kinase and hypersensitive-induced response proteins. On the other hand, the affinity chromatography resulted in the identification of defense related proteins such as chitin elicitor receptor kinase, NDR1/HIN1-like protein, Ras-related proteins, aquaporins, remorin protein, LRR-RLKs and GPI-anchored protein.
Affinity-based enrichment of ergosterol-associated proteins was shown to be an effective technique with reproducible results. It is evident that ergosterol induces a basal defense response in higher plants and there are significant differences in the regulation of proteins involved in signaling, membrane trafficking, structure, metabolism, transport and defense response.

Antimicrobial Potential of Crude Extracts obtained from Marine Invertebrates Species collected from Phillip's Reef at Algoa Bay in Port Elizabeth in the Eastern Cape Province of South Africa

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The marine environment has been found to contain a greater biodiversity as compared to terrestrial environments. Without any doubt the marine environment has become the focal point of interest for scientists when it comes to novel bioactive compounds. Without any doubt the marine environment has become the focal point of interest for scientists when it comes to novel bioactive compounds.

Bioactive compounds which have shown activity against pathogenic strains of bacteria such MRSA have been successfully isolated from marine sponges. Inhibitors of the HIV-1 virus have also been discovered. Current studies are mainly focused on the identification and screening of microorganism living within the marine invertebrates for antimicrobial potentials as opposed to the actual animal. Selected marine invertebrates were collected at Phillip's Reef from Port Elizabeth in the Eastern Cape Province of South Africa. Samples were collected approximately 10m below the sea and physicochemical parameters of the immediate were recorded using a CTD device. Identification of the specimens was done using both molecular and morphological methods. Extraction solvents which were used include ethyl acetate, methanol, chloroform, hexane, dichloromethane: methanol (1:1), and water (freeze drying). Vacuum evaporation was done between 40°C-50°C depending on the solvent to be removed. Susceptibility tests using the crude extracts obtained from the different marine species was done against 5 human pathogens which are S.aureus (MRSA), C. difficile, P. eruginosa, C. albicans and A. fumigatus using the disc diffusion method at different concentrations (v:v). Thirty six marine specimens have been collected over two seasons. Zones of inhibition greater than commercial antibiotics were observed from 3/36 (8%) marine species. An amount of 47% of the ethyl acetate crude extracts showed bioactivity against the test. Only 1/36 (3%) marine specimens showed inhibition against all 5 of the test pathogens. The highest zone of inhibition obtained was 38 mm against Candida albicans. Significantly 25/36 (69%) different marine species showed bioactivity. In conclusion, the current research outcomes prove that specific marine invertebrate species located at Phillip's Reef do produce antimicrobial bioactive compounds. Such research is critical in identifying new sources of novel bioactive "future drugs".

Riboswitch regulation of methionine metabolism and vitamin B12 uptake in mycobacteria – a role in pathogenesis?

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Tuberculosis is caused by *Mycobacterium tuberculosis* (Mtb), which causes an estimated 10.4 million new infections and 1.5 million deaths annually. Despite the availability of antibiotics targeting various aspects of Mtb physiology and metabolism, emergence of drug resistance poses significant challenges, necessitating new strategies to exploit as yet unidentified Mtb vulnerabilities. Employing coordinated gene regulation via regulatory RNAs such as riboswitches, Mtb can tolerate various forms of host-induced stressors and become reactivated when host immunity is weakened. Two vitamin B_{12} (B_{12}) riboswitches have been identified upstream of *metE* – encoding a B_{12}-independent methionine synthase, and *ppe2* – the first gene in a putative operon with the B_{12} biosynthetic genes, *cobQ1* and *cobU*.
We showed previously that disruption of the alternative, B_{12}-dependent methionine synthase, MetH, rendered Mtb sensitive to B_{12}, due to methionine starvation caused by B_{12} riboswitch-dependent suppression of *metE*. Using the avirulent *M. smegmatis* (MSM) as a proxy for Mtb, we describe the riboswitch regulation of methionine and, by extension, the one-carbon metabolism by folate, which is linked to methionine biosynthesis by MetH activity. We make novel use of CRISPR interference, combined with genetic recombination, microbiology and analytical chemistry to demonstrate that unlike Mtb, MSM is a constitutive producer of B_{12} under standard conditions *in vitro*. Therefore, we hypothesized that *metH* would be essential in MSM, and that deletion of *metH* would be possible only when *metE* is released from B_{12}-mediated inactivation. As expected, targeted knock-out of *metH* proved facile in a MSM Δ*cobK* mutant in which *de novo* B_{12} biosynthesis is disrupted. Curiously, however, the double Δ*metH*/Δ*cobK* deletion mutant was not sensitive to exogenous B_{12}, although whole-genome sequencing of these strains failed to detect any mutations in the *metE* riboswitch. Comparative genomic analyses identified additional B_{12} riboswitches controlling two separate BtuFCD-type B_{12} transporters that are present in MSM but not Mtb. Taken together, these findings suggest that B_{12}-dependent metabolism in MSM is regulated both at the level of cofactor transport and enzymatic function, thus identifying B_{12} uptake as one potential branching point in the evolution of Mtb.

Establishment and characterisation of a mouse xenograft model of human ovarian cancer

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Background: To effectively study the tumour biology of ovarian cancer and to evaluate the efficacy of newly synthesised therapies prior to clinical trials, valid and true cancer models of the ovary should be used. For modelling human cancer in vivo, xenotransplantation of human cancer cells in immune-deficient mice is common. In this study, we aimed to develop a xenograft model of human ovarian cancer by subcutaneous transplantation of human ovarian adenocarcinoma (OVCAR-3) cells in athymic nude mice and optimising tumour growth condition by addition of Matrigel, a basement membrane matrix.
Methods: The human ovarian cancer model was established by subcutaneous injection of cultured OVCAR-3 cells (1×10^6 /mouse), in phosphate buffered saline (PBS) or Matrigel, into the hind flank of athymic nude female mice. Once the tumour was palpable, tumour progression was monitored 2-3 times a week by measuring tumour size using a digital calliper and determining the tumour volume.
The animals were humanely euthanised once tumour volumes of 300-400 mm^3 were attained. The tumours were then harvested and weighed to determine the tumour growth rate. Finally, tumour characterisation was done using haematoxylin and eosin (H & E) staining.
Results: Following implantation of OVCAR-3 cells in athymic nude mice, tumour take rates of 5/6 mice and 3/4 mice was reported for PBS and Matrigel inoculum, respectively. For the PBS inoculum, tumours were detected 66 days post transplantation with a tumour growth rate of 0.14 mg/day. In comparison to the PBS inoculum, a more rapid tumour onset of 33 days with 2.9 mg/day tumour growth rate were observed after inoculation of OVCAR-3 cells with Matrigel. Although the use of Matrigel resulted in higher tumour growth rate than PBS, the difference was not statistically significant ($p>0.05$).
For tumour characterisation, paraffin-embedded tumour tissue sections were prepared and stained using H & E staining. The analysis of the results by a pathologist are still underway and will be presented.
Conclusion: A human ovarian cancer mouse model was successfully established using athymic nude mice. However, to obtain earlier tumour onset and enhance tumour growth the use of an extracellular matrix, such as Matrigel, is necessary.

Understanding skeletal muscle from superior athletes – what makes them so different?

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The exact role nature or nurture plays in elite exercise performance is still unclear. Currently, no gene can predict who will win gold at the Olympic Games. Fortunately, the human body adapts well to exercise training, especially skeletal muscle. How factors like muscle fibre type, size, metabolism and contractility contributes to the success in specific sports is still not well understood. Specifically, what should the muscle profile of an elite sprint or endurance athlete be composed of?
Aim: This study therefore compared muscle morphology and metabolism of human and selected athletic mammal species (e.g. cheetahs, lions, horse, antelope, baboon) and related the findings to their performance ability.
Methods: Samples were analysed for skeletal muscle fibre type using antibodies directed against the myosin heavy chain isoforms, fibre size, enzymatic markers of the main metabolic pathways (citrate synthase (Kreb's cycle, CS), 3-hydroxyacetyl Co A dehydrogenase (beta oxidation, 3HAD), phosphofructokinase (glycolysis, PFK) and lactate dehydrogenase (LDH). Additionally, maximum force, shortening velocity and power of single muscle fibres were also determined. Where applicable, associations were determined to predict exercise performance.
Results: All animals had more fast twitch muscle fibres than humans, and correlated positively with their sprinting ability. The muscle of endurance athletes and animals of prey had significantly more mitochondria and high CS and 3HAD activities, whereas all animals had very high anaerobic capacities (i.e. LDH and PFK activities) than humans. Overall, wild animals (except baboons) had smaller muscle fibres compared to domestic animals and humans. Finally, muscle fibre contractility (force, shortening velocity and power) was better in some of the faster species compared to humans, but similar in primates fast species.
Conclusion: These findings echo the exercise abilities of each species. Humans would require more highly oxidative fast twitch fibres to reach the speeds of fast animals. To what extent these findings could be altered with exercise training interventions or genetic manipulation, is not yet clear

Comprehensive acquisition by mass spectrometry and comparison of omics data: progress towards personalized medicine

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The incredible progress in the life sciences in the last decades was mainly driven by advances in analytical technologies, including mass spectrometry. Recent advances in high resolution mass spectrometry, specifically QqTOF technology, have made it possible to acquire qualitative and quantitative information simultaneously from highly complex samples.
The extreme speed and sensitivity of current instrumentation allows in depth analysis in information dependent (IDA) experiments. However, the concept of data independent acquisition (DIA) can now also be realistically applied for the first time. DIA workflows avoid the bias introduced by precursor selection and thus increase the reproducibility and comprehensiveness of data collection. This, in turn, allows the direct comparison of very large datasets due to the near complete coverage of all analytes in different sample sets, derived from proteomics and metabolomics projects utilizing DIA. In addition, these datasets can be analyzed retrospectively, as indeed they can be conceptualized as permanent digital records of the samples.
The concept of DIA - in a workflow called SWATH™ - will be introduced. Published examples of the workflow will be discussed, including the identification of biomarkers for kidney disease as well as the analysis of histone modifications. Also, the OneOMICS™ cloud environment, that allows fast data processing and comparison of proteomics data with genomic and metabolomic datasets in the public domain, will be introduced. Finally, the concept of “industrialized proteomics”, will be introduced on the example of the ProCan Institute within the Children´s Medical Research Institute (CMRI) based in Sydney. The aim of ProCan is to provide individual, complete proteome analysis for large patient cohorts within very short time scales. This – in the near future – should provide personalized, proteomic-driven therapy that is driven by comprehensive data sets, not by a doctor´s experience.

Metabolomics reveals the depletion of intracellular metabolites in HepG2 cells after treatment with gold nanoparticles

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Studies on the safety of gold nanoparticles (GNPs) are plentiful due to their successful application in drug delivery and treatment of diseases in trials. GNP-induced cytotoxicity has been studied on the physiological and biochemical level; yet, the effect of GNPs (particularly gold nano-spheres) on the metabolome of living organisms remains understudied. In this investigation, metabolomics was used to study the metabolic alterations in HepG2 cells caused by GNPs; and to investigate the role of representative GNP coatings. GNPs were synthesized, coated and characterized before use on HepG2 cell cultures.
The morphology, size distribution and net surface charge of the GNPs were determined with UV–Vis spectrometry, agarose gel electrophoresis, dynamic light scattering (DLS) and transmission electron microscopy (TEM). Cells were treated for 3 hours with citrate-, poly-(sodiumsterene sulfunate)-, and poly-vinylpyrrolidone (PVP)-capped GNPs, respectively. The internalization of the different GNPs and their effect on mitochondrial respiration and the metabolome were studied. Results indicated that the PVP-capped GNPs internalized more and also caused a more observable effect on the metabolome. Conversely, it was the citrate- and poly-(sodiumsterene sulfunate) coated particles that influenced ATP production in addition to the metabolomic changes. A holistic depletion of intracellular metabolites was observed regardless of GNP coating, which hints to the binding of certain metabolites to the particles.

TB or not TB: New metabolomics biomarkers better characterizing and diagnosing tuberculosis.

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Statement of the Problem: Despite the fervent genomic and proteomic based research efforts to date, since its discovery in 1882, TB is still a major global problem, and hence new approaches are necessary to better characterize and diagnose this disease. One such a strategy would be to investigate this from a metabolomics research perspective, in order to identify new metabolite markers better characterizing the disease. Methodology & Theoretical Orientation: A typical metabolomics workflow, including using various semi-targeted and untargeted extraction procedures on cell cultures and patient collected sputum and urine, analysis on various LC-MS, GC-MS and NMR based approaches, followed by data clean-up and biomarker identification using various univariate and multivariate statistical approaches, was applied.
Findings: The new TB biomarkers identified in the different sample material shed light on new metabolic pathways and improved our understanding of the mechanisms related to Mycobacterium growth, virulence, drug resistance, host and microbe interactions/adaptations, and also the development of improved diagnostics and predicting treatment outcome. Conclusion & Significance: Over the past 10 years, metabolomics has led to an exponentially increased number of new biomarkers identified, and subsequently rapid expansion of new knowledge and our understanding of TB, which was utilized towards improved diagnostics and treatment approaches.

Comparative analyses of the antioxidant enzyme activities of two contrasting sorghum lines in response to drought and heat
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Drought is a major limiting factor in crop production, impacting food and nutrition security adversely. For summer crops such as sorghum, drought effects on their production can be exacerbated by heat waves that are caused by excessively high temperatures during the summer growing season. Part of the plant responses to these abiotic stresses includes alteration of the activity of antioxidant enzymes aimed at controlling the levels of reactive oxygen species in plant tissue, thought of as a mechanism to offer protection against the cellular damage cause by the reactive oxygen species. Despite this knowledge, very limited information exists on how a combination of the two abiotic stresses, namely simultaneous exposure of plants to drought and excessive heat, affects antioxidant enzyme activities in Sorghum bicolor (L.) Moench (sorghum). We thus investigated the effect of combined drought and heat stress on sorghum biomass, cell viability, superoxide content, hydrogen peroxide content, leaf chlorophyll content, superoxide dismutase, catalase, ascorbate peroxidase, dehydroascorbate reductase and glutathione reductase enzymatic activities.
Furthermore, we evaluated the effect of the two abiotic stresses on the levels of osmoprotectants proline and glycine betaine. Ours results show that drought affected these responses differently compared to heat and a combination of the two stresses lead to pronounced differentiation of the responses. We propose that the insight provided by these findings offers an opportunity to tailor plant responses for better adaptation to both stresses, providing an opportunity for improving drought and heat stress resilience in sorghum.

The role of the HOPco-chaperone in the formation of HSP90 complexes: Chaperone regulation of Glycolysis
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The Hsp90 chaperone machine stabilizes and activates more than 200 client proteins. In cancer, Hsp90 is overexpressed and present in an activated multi-chaperone complex, and co-chaperones such as the Hsp70/Hsp90 organizing protein or Hop, are an essential part of that complex. The Hsp90 complex was analysed in Hop-expressing and Hop-depleted HEK293T cells using immunoprecipitation coupled to mass spectrometry. Essential glycolysis proteins were found to interact to Hsp90 exclusively in the presence of Hop (phosphoglycerate kinase 1 and lactate dehydrogenase A), primarily in the absence of Hop (glycogen phosphorylase L and phosphofructokinase) and independently of Hop (aldolase A, enolase 1, lactate dehydrogenase B, pyruvate kinase M and triose phosphate isomerase). Altered metabolism is a hallmark of cancer and the link between chaperones and tumour metabolism is yet to be established. There was an increased level of glycolysis in the Hop depleted cell line measured via extracellular acidification, and conversely, a decrease in the uptake of glucose analogue 2-NBDG in the Hop depleted cell line. The Hop depleted cell line also a higher proliferative rate in the absence of glucose and pyruvate compared to the Hop expressing cell line. However, the proliferation rate of the Hop expressing and Hop depleted cell lines was significantly decreased when amino acids and glutamine removed from culture media. This suggests that the Hop depleted cells are sequestering glucose from glycogenic amino acids and are unable to proliferate when these amino acids are depleted. Taken together, our data provides a novel role for Hsp90 and its co-chaperone Hop as putative regulators of glycolysis and cancer metabolism.
Keywords: Chaperone, Hsp90, Hop, glycolysis, cancer metabolism, cell biology

Lithium attenuate viral load in Rift Valley Fever Virus infected Raw 264.7 macrophage through induction of programmed cell death
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Rift Valley fever virus (RVFV) is a mosquito based zoonotic viral infection that elicits a variety of symptoms including fibril illness and haemorrhagic fever. Survival and replication of RFVs into multiple copies relies on induction of anti-apoptosis processes to keep the infected host cells viable so as to allow progression of viral multiplication. Hence, this study investigates lithium as a potential drug to reduce RVFV viral load, beyond its traditional use in management of manic depression. This work has shown that lithium stimulate proliferation of immune Raw 264.7 macrophage cells as seen in MTT and Cyquant assay. Moreover, it induces drastic death of infected Raw 264.7 macrophage cells. The Annexin-V PI apoptosis assay has shown that this virus induces apoptosis in MNA and Raw 264.7 macrophage cells as opposed to necrosis. Furthermore, the expression of Bax and Bcl-2 ratio infer that RVFV induces apoptosis. More interestingly these two apoptosis assays show that lithium-treated and RVFV-inoculated cells have accelerated apoptosis than control RVFV.

This induced early apoptosis suggested lowed viral load from abortion of viral progeny replication. The viral titration showed that this drug lower viral load as cells are treated with various concentrations of lithium and infected with RVFV compared to RVFV infected cells without lithium. The qRT-PCR show that lithium treated RVFV infected cells produce similar or elevated viral RNA copies in comparison with the RVFV infected cells not treated with lithium. Since lithium is known to stimulate haematopoiesis in normal immune cells, the accelerated apoptosis mechanism induced by lithium is suggested to limit viral spread by eliminating virally infected cells at a high rate. This work is pointing to the use of lithium as the potential treatment for this detrimental viral infection by limiting viral replication in haemorrhagic fever-related infections.

Investigation of the effect of the GGMP repeat motif of Plasmodium falciparum Hsp70 on its chaperone function and its interaction with a co-chaperone, PfHop

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The main malaria agent, Plasmodium falciparum expresses an Hsp70 (PfHsp70-1) which is thought to play a significant role in the survival of the parasite. PfHsp70-1 is distinct in that it habours seven GGMP tetrapeptide repeats in its C-terminal domain. Initially thought to be immunogenic, it was recently shown that PfHsp70-1 lacks immunomodulatory activity. To date the GGMP motif of PfHsp70-1 has received no attention. The motif falls within the C-terminal region of PfHsp70-1 in the lid (SBDα); hence it will be interesting to elucidate how mutating this motif will impact on the structure and the chaperone activity of PfHsp70-1. The motif is also about 15 residues upstream the terminal EEVD residues responsible for the interaction of PfHsp70-1 with its functional regulators (co-chaperones). P. falciparum Hsp70/Hsp90 organizing protein (PfHop) constitutes one of the functional regulators of PfHsp70-1. PfHop allows PfHsp70-1 and its chaperone partner, PfHsp90 to form a functional partnership. Given the proximity of the GGMP repeats to the C-terminus of PfHsp70-1, it is possible that they may regulate attachment of PfHop to both PfHsp70-1 and PfHsp90. Hence the current study seeks to elucidate the effect of the GGMP motif of PfHsp70-1 on its interaction with PfHop. Furthermore, since the GGMP motif is located in the peptide binding domain of PfHsp70-1, the study further investigated the role of the of the GGMP motif on the chaperone function of PfHsp70-1. To investigate the chaperone function of PfHsp70-1 and its GGMP repeat mutants, we conducted a complementation assay in E. coli cells whose Hsp70 function is compromised.
We further expressed and purified recombinant PfHsp70-1 and its GGMP mutants. Using an ELISA approach, we confirmed that the GGMP mutations did not affect interaction of PfHsp70-1 with PfHop. We identified a GGMP repeat whose mutation appeared to affect the protein's chaperone function. Altogether, our findings suggest that the GGMP motif though not essential for PfHsp70's interaction with PfHop, may play a role in substrate binding (chaperone activity).

The effects of zinc and newly synthesized 6-nitro-2 (2- (thiophen-3-yl) ethyl) quinoxaline on apoptosis and proliferation of MCF-7 cells

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Breast cancer has become a world epidemic since it is the most diagnosed and the second leading cause of death in women world-wide with a lifetime risk of 1 in 26 women in South Africa. Recent investigations have preferred target specific treatment using quinoxaline derivatives for treatment of cancer. In this study, the effects of 6-nitro-2 (2- (thiophen-3-yl) ethyl) quinoxaline and zinc combination on proliferation and their ability to induce apoptosis in MCF-7 cells were investigated. Cytotoxic effect of 6-nitro-2 (2- (thiophen-3-yl) ethyl) quinoxaline and zinc on MCF-7 cells was analysed using the MTT assay and changes in cell morphology. The effect of 6-nitro-2 (2- (thiophen-3-yl) ethyl) quinoxaline and zinc on proliferation of cells was analysed using BrdU incorporation assay. Flow cytometry analysis of apoptosis (MuseTM Multicaspase and Annexin-V FITC/ PI assays) and fluorescence analysis of nuclear morphology alterations were carried out to evaluate the onset of apoptosis. The results show that combination treatment of 6-nitro-2 (2- (thiophen-3-yl) ethyl) quinoxaline and zinc significantly decreased viability, suppressed proliferation and induced apoptosis of MCF-7 cells when compared to single drug treatment.
Caspase activity also increased after exposure of MCF-7 cells to 6-nitro-2 (2- (thiophen-3-yl) ethyl) quinoxaline and zinc combination and this is an indication that activation of caspases is associated with apoptotic process induced in MCF-7 cells. The combination of the novel quinoxaline derivative, 6-nitro-2 (2- (thiophen-3-yl) ethyl) quinoxaline, and zinc could serve as potential therapeutic agent for breast cancer treatment.

The cooperation of EBV and HIV in the pathogenesis of HIV-associated Burkitt's lymphoma

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Infection with the human immunodeficiency virus (HIV) is associated with a high incidence of B cell lymphomas. Burkitt's lymphoma (BL) is one of the most common B cell malignancies among HIV-infected individuals and the incidence of this highly aggressive cancer continues to increase despite the use of highly active antiretroviral therapy.
BL is characterized by the translocation and overexpression of the c-MYC oncogene to the immunoglobulin locus.
This c-MYC-IGH translocation has been linked to overexpression of the DNA modifying enzyme activation induced cytidine deaminase (AID). Human B cells are the primary targets of Epstein Barr virus (EBV) infection. In most cases, EBV infection is asymptomatic because of an effective host immune system. However, immune-compromised individuals are susceptible to EBV-associated lymphoid malignancies. Unlike EBV, HIV is not classified as an oncogenic virus, but recent investigations have revealed that components of the virus can enhance the cancer phenotype. Coinfection with multiple agents is known to accelerate cancer development and this project aims to investigate the cooperation of EBV and HIV in the development of BL.

LMP1, a major EBV-encoded oncoprotein, has been shown to upregulate AID expression through binding to promoter elements found directly upstream of the transcription start site (TSS) of the gene. We have shown, in our laboratory, that LMP1 can significantly upregulate AID expression via other enhancer regions found further upstream of the TSS. We have also shown that the HIV trans-activator of transcription (Tat) protein is able to increase AID expression by enhancing its promoter activity. We now aim to determine the cooperative effect of LMP1 and HIV Tat on AID transcription. Similarly, we plan to investigate the cooperative effect of EBV encoded-proteins EBNAs and HIV Tat on c-Myc expression. Furthermore, we will measure the frequency of the c-MYC-IGH translocations in B cells, using 3D-FISH, in the presence of both HIV and EBV proteins.

In Silico Bio-Informatics analysis of cell-cycle related genes in response to Lopinavir/ritonavir (LPV/r) in in vitro human lung cancer models

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The Health-related quality of life (HRQoL) for HIV positive patients has improved since the introduction of the highly active antiretroviral treatment (HAART). However, in the present HAART era, HIV co-morbidities such as lung cancer have been documented to be on the rise. Lung cancer is a non-AIDS defining cancer and the association between the use of HAART, the development and progression of lung cancer is poorly understood. This study aimed at investigating the effects of Lopinavir/ritonavir (LPV/r) on adenocarcinoma (A549) and normal fibroblasts (MRC-5) lung cells. The differentially expressed genes from the human cell-cycle focused array panel were screened for the determination of (gene-gene)/ protein-protein interaction (PPI), and mapping the molecular pathways influenced by the identified targets. For this purpose, STRING, DAVID, Reactome and Ingenuity Pathway Analysis (IPA) databases were used. Direct PPIs were evident from STRING analyses, while results from DAVID analysis showed that the significantly up/down regulated targets were mostly associated with the cell-cycle checkpoints. In addition, MAD2L2, CASP3 and AURKB (members of the cell-cycle focused pathway) were illustrated to be of interest in this study due to their differential expression patterns in LPV/r treated vs vehicle control samples. Moreover, Reactome and IPA analysis indicated the activation of the DNA damage response pathways such as the ATM and the p53 signalling pathways in LPV/r treated cells. These findings show stress inducing effects of LPV/r on the DNA strands, suggesting its role in genome instability and lung carcinogenesis.

Protection of mitochondrial disease pathology by metallothionein over-expression: a phenotype study

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Leigh syndrome is a complex I deficiency with a prevalence of 1 in 5000. Most treatments involve the use of vitamins, cofactors and exercise therapy, which all have some value and may alleviate certain symptoms, but are mostly ineffective and none offer a cure.
Some of these treatments are based on stabilization of the reduction-oxidation (redox) state within mitochondria by performing an antioxidant function. Metallothioneins can protect cells in a similar way by exerting an antioxidant effect and stabilizing the redox state, with consequent protective effects, during mitochondrial respiratory chain dysfunction.
Many studies have shown the important neuroprotective role of MT1 and 2 in vivo in brain disorders. In this study, a complex I deficient mouse model (Kruse et al., 2008) was crossed with a metallothionein I (MT1) over-expressing mouse model (Palmiter et al., 1993) in order to investigate the whether or not metallothioneins protect against the severe cellular damaging consequences of a primary mitochondrial disorder.
Methods: To investigate the mitochondrial related phenotype in these mice at postnatal day 30 and postnatal day 50; survival rates, growth curves, locomotor activity, motor coordination and balance was evaluated .
Results and discussion: While the survival rate of complex I knockout (KO) mice with MT1 over-expression was higher than that of untreated complex I KO mice, this difference was not statistically significant. The growth curves, balance beam test, open field activity, rotarod and wire grid hang test all showed significant differences between complex I KO mice (with and without MT1 over-expression) and wild-type mice (with and without MT1 over-expression) at postnatal day 50, however, there were no statistically significant differences between complex I KO mice with MT1 over-expression and those without.
Conclusion: While metallothioneins may play some role in improving the longevity in complex I KO mice, this difference is not statistically significant and is not detected in the above mentioned phenotyping methods. Further biochemical analyses on tissues from these mice are required to determine whether or not the over-expression of MT1 truly does have a therapeutic effect in complex I KO mice.

Engineered Ancestral AAV (Anc80L65) vector carrying primary microRNA mimics effects long-term HBV inhibition

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Chronic hepatitis B virus (HBV) infection is a global pandemic affecting 240 million people world-wide. The shortcomings with nucleoside/nucleotide-analogues or interferon-α based drug therapy calls for different treatment approach such as gene therapy to treat chronic viral hepatitis. HBV's highly conserved and overlapping open-reading frames (ORFs) are vulnerable to viral protein production interruption. Recently, long-term RNA interference (RNAi) mediated inhibition of HBV replication was demonstrated using adeno-associated virus serotype 8 (AAV8) vector. However, prevalence of anti-AAV8 broadly neutralising antibodies in human population hinders effective therapeutic gene transfer. This study investigated transfer and liver-specific gene expression of therapeutic primary-microRNA (pri-miR) mimic (pri-miR 122/5) using engineered ancestral viral vector (Anc80L65) to activate RNAi against HBV. Anti-HBV Anc80L65 vector (Anc80L65-122/5) was produced and characterized in liver-derived Huh7 cells and in HBV transgenic mouse model.

For assessment of transduction efficiency and long term transgene expression by Anc80L65 in vivo, a nano-luciferase expressing vector (Anc80L65-NLuc) was also produced. Significant expression of nano-luciferase from Anc80L65-NLuc was observed in vitro. A high and sustained nano-luciferase expression was also observed in vivo. Following a single dose of Anc80L65-122/5 in mice, an impressive and sustained HBV gene expression knockdown of up to 70% was observed over four months. Safety of Anc80L65 was demonstrated by absence of harmful immune-stimulatory effects and resultant liver toxicity, a fact supported by normal levels of serum alanine transaminase enzyme. There were no mice fatalities related to Anc80L65 mediated RNAi stimulation.
The safety, high efficiency and the prolonged HBV inhibition by Anc80L65 makes it a useful therapeutic delivery tool and will significantly contribute to the progression of anti-HBV RNAi based therapy in clinical applications.

Adiponectin regulation of AMPK on oleanolic acid treated insulin resistance Sprague Dawley rats

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AMPK is known to control both glucose and lipid metabolism, two main candidates critical in the development of type-2 diabetes (T2D). Studies have shown that AMPK can be activated by adiponectin. Patients suffering from T2D are known to have low adiponectin concentration in their blood plasma. In this study we have assessed one of the anti-diabetic compound Oleanolic acid (OA) if it could produce desirable effect in upregulating adiponectin concentration and the subsequent regulation of AMPK. Sprague Dawley rats were fed with high fructose diet (HFD) to induce T2D, and the rats that developed insulin resistance were considered as diseased, they were then treated with OA. Analysis of adiponectin concentration in blood plasma was done, AMPK gene expression and subsequent genes that play vital role in glucose and lipid metabolism (GLUT-4 and CPT-1) in skeletal muscle tissue was also performed. The results showed 1.19 fold increase in blood plasma adiponectin concentration after OA administration. Furthermore AMPK gene expression showed 3.98 fold increase and GLUT-4 gene expression was increased with 1.5 fold whereas CTP-1 gene expression was increased with 1.59 fold. These results clearly indicate that OA produced good effects in ameliorating insulin resistance since it was able to upregulate all the genes and adiponectin concentration which are well known to be abnormally suppressed in a situation of T2D. In conclusion these study further confirms that OA can be used as an effective therapeutic agent to ameliorate T2D and these study also suggest that OA's mechanism of action it could be through AMPK pathway.
Keywords: Type-2 diabetes, Insulin resistance, AMPK, Adiponectin, Oleanolic acid

The potential anticancer activity of Commelina benghalensis against breast cancer MCF-7 cell line

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Commelina benghalensis is used by traditional healers to treat many ailments, including skin lumps or skin outgrowths and inflammation. *Commelina benghalensis* plant extracts have been shown to possess anticancer properties against the leukaemia Jurkats cancer cells. However, it is unclear on whether *Commelina benghalensis* has anticancer activities against solid cancers.
This study was aimed at determining the potential anticancer activity of *Commelina benghalensis* extract against breast MCF-7 cancer cells.
Cytotoxicity of both arsenic trioxide and *Commelina benghalensis* extract [which was fractionated into two, F1 (n-hexane) and F2 (dichloromethane)] was determined using the MTT Assay. Morphological changes were observed in *Commelina benghalensis*- and arsenic trioxide-treated MCF-7 cells. F1 was found to have no anti-proliferative effect on MCF-7 cells while both F2 and arsenic trioxide showed anti-proliferative effects. The effect of the arsenic trioxide and F2 *Commelina* plant extracts on cell cycle regulation and apoptosis was assessed using the MUSE Cell Cycle Assay, the MUSE Annexin V and Dead Cell Assay kits, MUSE MAPK Activation Dual Detection kit, MUSE MitoPotential kit respectively. The MUSE PI3K Dual Detection kit was used to assess if the F1 extract has an effect on the PI3K survival pathway in MCF-7 cells.
Commelina benghalensis extract and arsenic trioxide reduced MCF-7 cell viability in a dose- and time-dependent manner and have effects on several biological processes. These results suggest that the *Commelina benghalensis* F2 extract has anticancer activity against breast cancer cell line.

Antihyperglycemic, Antihyperlipidemic and Antioxidant Effect of White Butterfly (Clerodendrum volubile) leaves in Streptozotocin-induced diabetic rats

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Introduction: White Butterfly (Clerodendrum volubile) leaf is commonly used in traditional medicine for the management of various diseases including diabetes without the full understanding of the scientific basis for its use. This study seeks to evaluate the antihyperglycemic, antihyperlipidemic and antioxidant effect of Clerodendrum volubile leaves in streptozotocin (STZ)-induced diabetic rats. Methods: Aqueous extract of C. volubile was prepared and its involvement with key enzymes associated with diabetes was determined. Fifty Male Wistar rats were divided into 10 groups of 5 rats each. The induction of diabetes in rats was by a single intraperitoneal administration of STZ (65 mg/kg body weight) while C. volubile extract was administered orally to both diabetic and non-diabetic animals, at the doses of 50,100 and 200 mg/kg body weight for a period of 14 days. Metformin; a standard antidiabetic drug (100mg/kg body weight) served as a positive control. Results: C. volubile extract showed inhibitory activity against α-glucosidase (IC50 = 0.20 mg/ml) and α-amylase (IC50 = 0.58 mg/ml). Furthermore, administration of C. volubile extract significantly reduced elevated plasma glucose level and body weight as well as improved kidney functions in diabetic rats. Also, C. volubile extract attenuated oxidative stress by decreasing the level of MDA, superoxide dismutase, catalase and glutathione peroxidase activities, reinstated the lipid profile to nearly normal level and restored the histological integrity of rat pancreas in diabetic rats. Conclusion: The inhibitory potential of C. volubile extract on these enzymes suggests a positive and probable therapeutic role of this extract in diabetes mellitus management and treatment.

Also, the results reveal thatC. volubile could improve the glycemic control, modulate atherogenic indices and enhance antioxidant enzyme in diabetic rats. Thus,C. volubile could serve as a source of phytochemicals and antidiabetic agent for mitigating against free-radical mediated diabetes and its complications.

Identifying the binding partners of the scaffold protein hCNK1 IN the NF-κB PATHWAY

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The human Connector Enhancer of KSR1 (hCNK1) protein is involved in a number of key mammalian cellular pathways including the MAPK, Hippo and PI3K/AKT pathways. In addition, hCNK1 is a tumour promoter protein that is functional in breast cancer cells and plays a role in the NF-kB pathway. The NF-kB pathways are involved in cell proliferation, cell development, cell survival, inflammatory responses and immune responses. Our current understanding is that there are two distinct branches of the NF-kB pathways, termed the canonical and the non-canonical pathways, and while these two pathways are induced by different ligands, there is cross talk between the pathways. In a study by Fritz and Radziwill (2010), the authors demonstrated that hCNK1 plays a role in the NF-kB pathway. In this report, we show that hCNK1 interacts with IKKα in the non-canonical branch of the NF-kB pathway.

Host immune responses to Pneumocystis murina

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Background: Pneumocystis pneumonia (PCP) remains a major cause of mortality and morbidity in AIDS and non-AIDS immunosuppressed individuals. This severe form of pneumonia is caused by an opportunistic fungal pathogen, Pneumocystis jirovecii, and is responsible for an estimate of 200 000 and 50 000 deaths worldwide annually, in both AIDS and non-AIDS individuals, respectively. The risk of infection is correlated with a significant decrease in CD4+ T cells in AIDS patients. However, studies have shown the role of the innate immunity during infection by which the host clears Pneumocystis and maintains lung functionality. C-type lectin receptors (CLRs) are Pattern Recognition Receptors (PRRs) critical in the resolution of fungal infections mostly affecting immunocompromised patients (such Candida albicans and Cryptococcus neoformans).
The aims of this study is to determine the role of a newly described CLR, dendritic cell immunoactivating receptor (DCAR), in Pneumocystis (PC) infection. We also would like to shed light on the role of alternatively activated macrophages (M2) during PC infection due existing conflicting evidence on the role of these cells in PC clearance and immune response control.
Methods: Using the previously established Pneumocystis mouse model we aim to interrogate the role of DCAR in mice during Pneumocystis infection. We will also determine the role of M2 cells in Pneumocystis. Mice are infected with Pneumocystis cysts isolated from immunocompromised Rag1-/- mice (T and B cell deficient) lungs. Disease progression is measured at week 1, 2, and 3 post infection using qPCR. Host immune responses are determined using different parameter such as, serum antibody levels, cellular inflammation, cytokine production, and Pneumocystis burden by histology.
Results: Our preliminary data suggests that DCAR-deficient mice clear infection more efficiently compared to wild type mice, which correlated with a significant increase in dendritic cells and alveolar macrophages. Further analysis are needed to confirm these findings.
Conclusion: DCAR maybe an interesting CLR as it seems to delay PC clearance in contrast to Mincle and other CLRs.

Antimicrobial Activity as a New Frontier in the Biological Activities of the Triterpenoids Tormentic Acid and Betulinic Acid Isolated from Callistemon citrinus

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The traditional use of herbal medicines frorm ancient times has influenced investigations of plant antibacterial activity. Tormentic acid and betulinic acid are triterpenes isolated from many plants including the leaves of *Callistemon citrinus* and have been reported to exhibit anticancer, antiviral, anti-inflammatory and anti atherogenic properties. Whist many studies have focused its biological activity as an anticancer agent, few studies have been carried out on their antimicrobial activity. The antimicrobial activity of tormentic acid and betulinic acid obtained from *Callistemon citrinus* leaves was evaluated. The antibacterial and antimycobacterial activities of tormentic acid were determined using the following microbial species *Mycobacterium aurum*, *Mycobacterium smegmatis*, *Staphylococcus aureus* and *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The minimal inhibitory concentrations (MIC) were determined using the microbroth dilution method. For antimycobacterial activity, the minimum inhibitory concentration (MIC) coincided with the minimum bactericidal concentrations for all the pure phytochemicals. The leaf extract was active against both bacterial species at MIC 50 µg/ml. Tormentic acid was the most potent compound which was bactericidal at the MIC of 25 µg/ml to both organisms. Betullinic acid inhibited *M. smegmatis* growth by 50% at 100 µg/ml while an MIC of 100 µg/ml was obtained with *M. aurum*. Tormentic acid was the more potent of the two compounds against *S. aureus* with an MIC of 25 µg/ml and MBC of 50 µg/ml. The crude leaf extracts displayed antibacterial activity and the MIC and MBC for both species coincided at 50 µg/ml and 100 µg/ml. Protein leakage assay was carried out for *S. aureus* using tormentic acid.

At high concentrations the compound was able to lyse the bacterial cell and cause protein leakage. Tormentic acid was also shown to inhibit the growth and biofilm formation in *Klebisiella pneumoniae, Candida tropicalis* and *Pseudomonas aeruginosa*.
These results show that antimicrobial activity of the triterpenoids is, thus, a new frontier in their biological activities. Further studies need to be carried out on structure activity relationships of the derivatives of these compounds since they are already on the market as lead compounds for the treatment of other diseases.

Recombinant expression of cytochrome P450-2D6 and its application in tamoxifen metabolism

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Introduction: Breast cancer is the leading cause of most fatal incidences in women worldwide. It is mostly induced by hormone oestrogen which stimulates the DNA to proliferate cells into cancerous cells. Tamoxifen is administered as a pro-drug for both treatment and prevention of breast cancer in women and men who are oestrogen positive. Tamoxifen metabolites functions by binding and competing with oestrogen for binding to oestrogen receptors thus block the development of cancerous cells. Cytochrome p450-2D6 (CYP2D6) is one of the main enzymes in the tamoxifen metabolism. Due to inter-individual differences caused by polymorphism in this enzyme, there is a need to assay patients's tamoxifen metabolic status. The currently used assays for patient's tamoxifen metabolism have limitations including non-specific, time consuming and cost effective. This study aimed to develop a CYP2D6 based electrochemical biosensor which will test the metabolism of tamoxifen in breast cancer patients.
Methods: Physico-chemical parameters of CYP2D6 were determined to pave way for CYP2D6 gene amplification, cloning into pTrcHis TOPO vector, over-expression in E.coli followed by purification, in order to obtain an active recombinant CYP2D6. Tamoxifen metabolism by CYP2D6 was assayed using UV-Visible and emission spectra and validated by electrochemical techniques using CYP2D6 based-biosensor.
Results and Discussion: CYP2D6 is a 50.05397 kDa insoluble trans-membrane protein that is slightly acidic in nature (pI = 6.21). The CYP2D6 gene was successfully amplified as characterised by a 1.375 bp band and subsequently cloned into pTrcHis TOPO vector.
The protein was overexpressed in TOP10 E. coli cells, extracted and purified under denatured condition since it was expressed in the inclusion bodies. The protein was successfully refolded to its active form as determined by the P450 GLO CYP2D6 assay. CYP2D6 is characterised by a soret band at 215 nm (UV-Vis) and 425 nm (emission spectra). Electrochemical assays indicated that the enzyme is active and has the ability to metabolise tamoxifen into its active forms at a potential of 0.6 V. Therefore, these results are adequate to be applied in the development of CYP2D6 based sensor for tamoxifen metabolism during breast cancer therapy.
Keywords: Breast cancer, Tamoxifen, CYP2D6, electrochemistry, Biosensor, Recombinant expression.

Targeting the oncogenic TBX3 and its co-factors in anti-cancer drug development

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Introduction: Sarcomas are heterogeneous neoplasms of mesenchymal origin whose clinical management is compromised due to inadequate diagnostic markers and limited therapeutic options. While the incidence rates for sarcomas in Africa remain uncertain, there is a widely held view that they are common in black African children and adolescents. The transcription factor, TBX3, is overexpressed in a diverse range of sarcomas and carcinomas where it plays a direct oncogenic role and it has been proposed as a novel target for their treatments. Direct targeting of transcription factors for therapies however continues to represent a serious challenge and therefore the molecular mechanisms that mediate its biological activity may be more amenable as anti-cancer drug targets.
Methods: To address this, affinity purifications coupled with mass spectrometry was performed to identify TBX3 protein co-factors that regulate its oncogenic activity in sarcomas. Here we describe the validation and characterisation of nucleolin as a bona fide TBX3 co-factor by affinity pulldown assays and confocal microscopy/co-localisation analysis. The effect of nucleolin on TBX3 function was determined using RNAi rescue experiments coupled with growth curve and scratch motility assays to assess cell proliferation and migration respectively. Finally, the effect of the nucleolin targeting aptamer, AS1411, as a therapy for sarcomas was tested.
Results: Results show that the overexpression of TBX3 and nucleolin are common in a number of sarcoma subtypes and breast cancer.
We provide compelling evidence that nucleolin directly interacts with TBX3 through the T-box DNA binding domain and co-operates with TBX3 to promote proliferation and migration. Furthermore, AS1411 exhibits potent and selective anti-cancer activity in multiple sarcoma cell types and breast cancer. This anti-cancer activity is in part, due to the mislocalisation of TBX3 and nucleolin to the cytoplasm and the re-expression of TBX3 tumour suppressor target genes.
Conclusion: We propose that TBX3 and nucleolin can be used in combination as biomarkers for the diagnosis of a diverse range of sarcoma subtypes and targetting the TBX3: nucleolin complex with a readily available nucleolin inhibitor may be a promising novel therapeutic approach for cancers where this complex plays an oncogenic role.

Characterization of human KIAA0513 protein that is upregulated in invasive glioblastoma multiforme

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Glioblastoma (GBM) is a grade IV primary malignant tumour known to be very aggressive and accounts for 78 % of all brain tumours. Various diagnostic and therapeutic GBM biomarkers have been identified using gene expression profiling. The KIAA0513 gene is amongst those that have been shown to be upregulated in invasive GBM, however, it has never been characterized. Due to limited homology of "uncharacterized protein KIAA0513", prediction of its function remains challenging. KIAA0513 is ubiquitously expressed and enriched in the cerebral area of the brain.

It is thought to be involved in signalling pathways including neuroplasticity, cytoskeletal regulation and apoptosis. In the current study, we sought to infer structure and functional features of KIAA0513 using in silico analysis.
Through various bioinformatics analyses we identified a conserved domain, the DENN (differentially expressed in normal and neoplastic cells) domain belonging to the myotubularin-related protein 13 (MTMR13). The DENN domain (DENNd) proteins function as nucleotide exchange factors of Rab GTPases. Phylogenetic analyses further confirmed conservation of the DENN domain within KIAA0513 through clustering of KIAA0513 with the human DENNd2 protein family. We mapped the network partners of KIAA0513 using STRING analyses, which predicted interaction with numerous GTPase proteins. Based on these predictive results, we therefore hypothesize that KIAA0513 functions as a nucleotide exchange factor of GTPase proteins. We thus set out to biochemically characterize KIAA0513. The data generated from the full characterization of KIAA0513 may lead to understanding of its role in GBM towards identifying a successful biomarker.

Expression and kinetic characterization of novel enzymes (Catalase and alpha-galactosidase) in Red Algae from the Algoa Bay region
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Red algae are beneficial for industrial applications, as they are readily accessible, easy to harvest, have a lower cost of production, and biosynthesize commercially important molecules. However, enzymology studies on Red Algae are still relatively limited compared to higher plants. Therefore this study aims to identify and characterize novel enzymes from Red Algae, in order to understand their biological functions and potential uses in the food or health industries. Bioinformatics tools were used to identify and retrieve all information required for the prediction of a protein function. The functions of the identified novel proteins were predicted using homology-based methods which is prediction based on amino acid sequence and structure similarity with known proteins. The genes encoding catalase and alpha-galactosidase from red seaweed were cloned and analyzed for the first time. The catalase gene comprises 1602 nucleotide base pairs and encodes 494 amino acids. Alpha-galactosidase however, comprises of 4258 nucleotide base pairs and encodes 992 amino acids. The identified genes were amplified using the designed primers; for amplification of alpha-galactosidase, primer pair: CcAGAL-FWD 5' ATGAATGGTCACAGCAAGGC 3' and CcAGAL-REV 5' TTATATCGTGATTTCGGCCATG 3' was used; Catalase was amplified using the primer pair CcCAT-FWD: 5' ATGGACACTGAAAAACTGCAAC 3' and CcCAT-REV 5' TTACAAGCTGCTCATCTTAACT 3'. The recombinant genes were inserted into the pET-28a (+) vector system and expressed in E. coli BL21(DE3) cells as a soluble protein.

The proteins were purified by affinity chromatography using a Ni-NTA agarose matrix and shown on SDS-PAGE. kinetic parameters such optimum pH, optimum temperature, thermal stability, Km, and Vmax were determined for catalase and alpha-galactosidase using hydrogen peroxide and PNP a-D-Galactopyranoside as substrates for the purified enzymes, respectively. The high yield of both the catalase and alpha-galactosidase enzymes is a valuable feature for their application in industries.

The biochemical functions of the Retinoblastoma binding protein 6 (RBBP6) isoforms in metabolic reprogramming occurring during carcinogenesis
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Introduction: Cancer cells reprogram their metabolism and prefer to metabolize glucose via the glycolysis pathway regardless of the ample yield of ATPs during Oxidative phosphorylation. This preference is known as the Warburg effect. The tumour suppressor p53 is mutated in 50% of all human cancers. During normal conditions, p53 levels are maintained by a host of mechanisms including negative regulation by Mdm2. The Retinoblastoma binding protein 6 (RBBP6) is a 250 kDa protein that negatively regulates p53 and is highly expressed in most cancers and as such can be used as a diagnostic marker as well as a potential target for cancer therapy. RBBP6 homologs exist in most eukaryotes and not prokaryotes and possess different isoforms. For example, Humans possess four isoforms while Drosophila melanogaster has two isoforms; A and B. This study aimed to elucidate the biochemical role of RBBP6 isoforms in metabolic reprogramming associated with carcinogenesis.
Methods: Drosophila melanogaster wild type and p53 null mutants were treated with drug permutations of irinotecan (a DNA damaging agent) and exogenous pyruvate to perturb metabolism. Moreover, using RT-PCR and Western blot, expression profiles of SNAMA (Drosophila Orthologue of RBBP6) isoforms were shown followed by survival studies to investigate the effects of these drugs. Furthermore, using bioinformatics the domains of RBBP6 isoforms in various species were shown.
Results: Results indicate that RBBP6 isoforms show contrasting expression patterns. Furthermore, exogenous pyruvate protects the wild type flies from irinotecan toxicity while killing p53 null mutants. All eukaryotic species share a common N-terminal DWNN domain which in polyadenylation antagonizes larger isoforms by competitive binding.
Conclusion: Isoforms have contrasting roles during carcinogenesis and RBBP6 proves to be a potential druggable target for cancer therapy.

Antidiabetic effects of methanolic extract of bitter kola (Garcinia kola) leaves on streptozotocin-induced diabetic rats
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Introduction: Diabetic mellitus, (DM), is a non-communicable disease with increasing incidence and prevalence in Africa. This chronic metabolic disease can either be inherited or acquired. DM is characterized primarily by hyperglycemia. Secondary complications of DM include liver damage, dyslipidemia, retinopathy and cardiovascular diseases. Presently, there is no cure for DM. The exacerbating side effects of diabetes medications justify research on medicinal plants as safer alternative.

The leaves of *Garcinia kola* plant has been reported to possess antioxidant activities. Antihyperglycemic effect of extract of *G. kola* leaves (GKL) was assessed. Effects of the extract on serum lipid profile, liver carbohydrate metabolizing enzymes and antioxidant activities in streptozotocin-induced diabetic rats were also evaluated.
Methods: Forty eight male albino rats (200 + 10 g) were grouped randomly into seven: control (distilled water), GKL100 mg/kg, GKL200 mg/kg, DM, DM + GKL100 mg/kg, DM + GKL200 mg/kg, DM + Glibenclaimide, 0.5 mg/kg). After 14 days of treatment the animals were sacrificed and biochemical analysis carried out.
Results: Oral administration of the extract reversed hyperglycemia and lipid peroxidation significantly ($p < 0.05$) to normal. Increased reduced glutathione (GSH), catalase and glutathione peroxidase activities were observed in the liver after oral administration of the extract. A significant decrease in lactate dehydrogenase activity and a significant increase in glucose-6-phosphatate dehydrogenase activity were also observed in the liver after oral administration of the extract. Furthermore, a significant decrease in serum total cholesterol, triglyceride, low density lipoprotein-cholesterol and a significant increase in high density lipoprotein-cholesterol were observed after oral administration of the extract compared with the untreated group. Histopathological evaluations revealed regeneration of the pancreas and liver of the diabetic rats after oral administration of the extract as against necrosis, degeneration and inflammation observed in the untreated group. The results observed after treatment with GKL were more statistically significant when compared with the results obtained in the group of diabetic rats treated with the standard drug, Glibenclaimide. Phytochemical screening showed the presence of flavonoids, tanins, saponins, alkaloids, anthraquinones and terpenoids.
Conclusion: These results suggest that *Garcinia kola* leaves have antidiabetic properties which may have implications for drug discovery.

Biodegradation of chloroethenes (CEs) of a South African CEs-contaminated water using bioreactors
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Introduction: Chloroethenes (CEs), which are prevalent groundwater contaminants worldwide, were initially considered recalcitrant to biodegradation. However, under anaerobic conditions, CEs can be completely biodegraded to ethene, which is non-toxic. However, the redox cycling during the CEs biotransformation in a continuous system is still unclear. Here we used a consortium of mixed dechlorinating bacteria grown in bioreactors to biotransform CEs from a plume of contamination.
Methods: A commercial mixed dechlorinating bacterial consortium (106 cells/ml) was inoculated into two anaerobic bioreactor columns filled with dolomite matrix, anaerobic minimal medium containing 30 mM tetrachloroethene (PCE) and 35 mM lactate. After acclimation for 30 days the columns were supplemented with lactate 8.5 mM and 19 mM, respectively. The columns were then fed with CEs contaminated groundwater spiked with 0.6 mM and 6 mM of PCE, respectively, at a flow rate of 28 ml/h for 90 days. The biodegradation process of CEs was monitored using gas chromatography (GC) analysis and bacterial diversity was analyzed using denaturing gradient gel electrophoresis (DGGE).
Results: GC analysis showed biotransformation of PCE and trichloroethene (TCE) to Dichloroethene (DCE) isomers, vinyl chloride (VC) and ethene in the bioreactor with 0.6 mM of PCE at the beginning of the experiment. In contrast, only cis-1,2-DCE isomer, VC, and ethene were detected during biotransformation of PCE and TCE in the bioreactor with 6 mM of PCE. At the end of the experiments, no considerable CEs were detected in both columns as 99.9% of CEs biotransformation was achieved.
The detection of 0.6029 mM and 6.0023 mM ethene in the bioreactors, confirms that CEs were completely biotransformed after 90 days. DGGE analysis showed that Dehaloccocoides, Clostridium, Desulfovibrio and Geobacter species were the most abundant bacteria at the end of the experiment.
Conclusion: The column experiments showed that the remediation of South African CEs-contaminated water can be achieved through bioaugmentation processes.

Molecular detection and characterization of maize rhizobacteria for plant growth promoting traits
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Maize rhizobacteria isolated from dry rhizosphere soil were identified, characterized and their plant growth promoting abilities determined. Fourteen rhizobacteria isolates were screened for the ability to produce plant growth promoting properties such as Indole-3-acetic acid, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, phosphate solubilization, as well as ammonia, siderophore and exopolysaccharide productions, which stimulate plant growth under abiotic stress. Isolates were evaluated for stress tolerance at 7 % NaCl, 20 % PEG 8000, 405 mgl^{-1} D-Sorbitol (0.919 Aw at 40 ^{o}C) and the ability to grow at 50 ^{o}C. 16S rRNA gene sequencing based on universal primers 341F (5'-CCTACGGGAGGCAGCAG-3') and 907R (5'-CCCCGTCAATTCCTTTGAGTTT-3') identified three genera of rhizobacteria; *Bacillus* (57.14 %), *Pseudomonas* (35.71 %) and *Stenotrophomonas* (7.14 %). Siderophore (*Sid*) and ACC deaminase (*accd*) genes linked to plant growth promotion in each bacterial isolate were also screened and confirmed by PCR amplification and gel electrophoresis. *Pseudomonas* sp. strain MRBP 13 (MG953568.1) produced significant amount of IAA (20.46 μgml^{-1}), while *Bacillus* sp. strain MRBP10 and *Bacillus cereus* strain MRBP2 had the best production of exopolysaccharide (0.87 ± 0.03mm & 0.80 ± 0.06mm) respectively. *Bacillus* sp. strain MRBP10 also had the highest growth at 50 ^{o}C (0.26±0.05). Hydrogen cyanide production was only observed in *Pseudomonas* sp. strain MRBP13 (MG953568.1) which was also the isolate with the highest siderophore production qualitatively with a phosphate solubilizing efficiency of 178 ± 6.89. *Pseudomonas* sp. strain MRBP 4 and *Pseudomonas* sp. strain MRBP 13 which grew at low water activity (0.919 Aw), significantly enhanced maize growth when co-inoculated in a greenhouse experiment for drought tolerance compared to sole inoculation. These isolates are effective bioinoculants for enhancement of maize growth *under* water deficit conditions.

Heavy Metals Mediated Oxidative Stress in Artisanal Tin Miners in Barkin-Ladi Area of Plateau State, Nigeria

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Background: Depleted tin mines in Jos, Nigeria are usually taken over by artisanal miners who eke out a living by extracting metal ores therefrom. Heavy metal toxicity results as these miners usually work without adequate personal protection. This study investigated several oxidative stress biomarkers (Thiobarbituric acid reactive substances - TBARS, nitric oxide (NO3-, reduced glutathione - GSH, and of glutathione-s-transferase - GST, glutathione peroxidase - GPx, catalase – CAT and superoxide dismutase - SOD) in the plasma of subjects involved in artisanal mining or residents living close to the mines. Methods: Three different groups (n=93 each) were exposed to heavy metal contaminations associated with tin mining activities; underground miners, surface sorters/washers, residents of adjoining settlements and a control (a fourth group; n=93) unexposed group (mainly sex and age-matched students of Plateau State University) took part in the study following ethical approval and informed consent. Standardized questionnaire was administered on the respondents while serum and plasma samples were used to determine the level of oxidative stress following standard protocols. Results: The level of TBARS was significantly elevated in the underground workers (3.64±0.85 µmol/l) compared to the control group (1.26±0.32 µmol/l) and washers (1.34±0.22 µmol/l) (p0.05). Glutathione-s-transferase activity was however significantly reduced in underground workers, washers and residents (8.80±0.20 µmol/min/mL, 8.73±0.30 µmol/min/mL, and 8.66±0.12 µmol/min/mL respectively) compared to the control group (10.44±0.60 µmol/min/mL). In this study also, GPx similarly showed statistically significant reduction in underground workers compared to the control, residents and sorters/washers (11.77±0.59 µmol/min/mL, 12.64±0.99 µmol/min/mL, and 14.67±1.19 µmol/min/mL respectively), (p 0.05). Concentrations of GSH, SOD, CAT, and NO3 - levels however, showed no statistical significance at p 0.05 in all the groups studied. Conclusion: This research findings reveals some level of oxidative stress in exposed groups. This study found significant evidence of increased oxidative stress in the underground miners, sorters/washers and even residents living close to the mines when compared with the control. It is therefore timely to regulate artisanal mining, provide adequate habitation and encourage the use of personal protective equipment among these miners.

***Ecklonia maxima* and *Gelidium pristoides* exhibit antioxidant activity and inhibit beta-amyloid aggregation, Cholinesterases and Beta-secretase activities: Potential implication for neuroprotection against Alzheimer's disease**

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Beta-amyloid aggregation, upregulation of beta-secretase and cholinesterase activities in neuronal cells have been hypothesized to be involved in the etiology of Alzheimer's disease (AD). Current research is geared towards the discovery of new compounds with strong neuroprotective potentials for the treatment and management of AD. Experimental investigations were carried out in this study to determine the neuroprotective potentials of *Ecklonia maxima* (ECK) and *Gelidium pristoides* (GLD) via their modulatory effect on biomolecules linked to the pathogenesis of AD. Anti-aggregation and disaggregation effects of aqueous-ethanol extracts of GLD and ECK on beta-amyloid ($A\beta_{1-42}$) protein fibrils were determined in three different phases via the Thioflavin-T assay and electron microscopic study. The modulatory effects of the macroalgal extracts on beta-secretase (BACE-1) and cholinesterase activities, as well as their hydroxyl radical scavenging and metal chelating activities were also assessed *in vitro*. Gas Chromatography-Mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MC) analysis of the samples were carried out to determine their chemical constituents. Results from the Thioflavin-T experiment revealed an increase in fluorescence signals in the control ($A\beta_{1-42}$ alone) at different intervals (0-96 h) compared to the test samples which were significantly ($P < 0.05$) lower. Electron micrographs from the transmission electron microscope revealed that GLD and ECK incubated with $A\beta_{1-42}$ at different intervals (0-96 h) were devoid of protein fibrils or showed very low levels of fibrils compared to the control. Furthermore, GLD and ECK inhibited BACE-1, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities in a dose-dependent manner. Furthermore, GLD and ECK scavenged hydroxyl radicals and were able to chelate Fe^{2+} in a dose dependent manner. Some of the active principles identified via GC-MS analysis include alpha- tocopherol (vitamin E), alpha-amyrin, beta-amyrone, stigmasterol and beta-sitosterol. The LC-MS analysis of the samples also revealed the presence of some phenolic compounds. The observed anti-aggregation and disaggregation effects as well as antioxidant, cholinesterase and beta-secretase inhibitory activities of the aqueous-ethanol extracts of the macroalgae could be linked to their active constituents. This study provides insights on the search for new biologically active compounds from marine algae for AD therapeutics

Contractile Force is Crucial for Microchaete Patterning on Drosophila melanogaster Notum

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Drosophila melanogaster notum microchaete arrangement occurs mostly by lateral inhibition. Microchaete precursor cells (SOPs) use notch signaling to induce lateral inhibition in neighbouring cells, thereby restricting them from developing into microchaete. Prior to this study, a set of cells called 'SOP-like cells' were observed to initially start their development like SOP cells. Eventually, these SOP-like cells take up an epithelial fate –either undergoing apoptosis or cell division. This study was aimed at characterizing SOP-like cell occurrence by quantifying their expression of pro-neural genes in *zipper* and *squash* mutant flies.

We also investigated how SOP-like cells distant from SOP cells have their cell fate restricted and the effect of *zipper* and *squash* mutations on the final alignment/ refinement of SOPs at late stages of notum development.Temperature-sensitive Gal80 and Gal4-UAS system were used to induce a spatio-temporal expression of genes. *Zipper* mutant, *squash* mutant and control flies were used. In all flies, the pro-neural gene, *neuralized* was labeled with GFP. *Shotgun* was also labeled with GFP to aid visualization of cell-cell junctions. Live cell imaging was carried out on the notum of young pupae 12 hours APF with a confocal microscope. Images were analyzed with Fiji and GraphPad Prism.
Squash mutation reduced the occurrence of SOP-like cells. *Zipper* mutation increased the percentage of SOP-like cells undergoing apoptosis. Both mutations reduced the percentage of SOP-like cells undergoing cell division. The distance between SOP-like cells and the nearest SOP cells did not affect the speed with which SOP-like cells underwent their developmental fate. The mutations significantly affected the expression of the pro-neural gene, *neuralized* at time points preceding their developmental fate change. SOP-like cells undergoing cell division were located adjacent to one another and underwent cell division simultaneously, as opposed to those having apoptotic fates. The mutations caused significant misalignment of SOPs. In conclusion, the mutations altered the occurrence/fates of SOP-like cells and final alignment of SOPs. This shows that the ability to generate contractile force is crucial for microchaete patterning in *Drosophila melanogaster* notum.

37 kDa LRP::FLAG enhances telomerase activity and reduces ageing markers in vitro and in vivo

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Introduction: Ageing is a degenerative process characterized by the accumulation of detrimental changes that cause deterioration of the physiological functioning of the organism. One of the core regulators of cellular ageing are telomeres, repetitive DNA sequences at the ends of chromosomes that are maintained by the ribonucleoprotein DNA polymerase complex, telomerase. Recently, we demonstrated that knockdown of the 37kDa/ 67kDa laminin receptor (LRP/LR), a protein that promotes cell viability in tumourigenic and normal cells, reduces telomerase activity. We therefore hypothesized that upregulating LRP/LR might increase telomerase activity and impede the ageing process.
Methods: We have successfully developed a cell line that stably overexpresses LRP::FLAG and have overexpressed LRP::FLAG in pilot study mice. We used western blotting, immunochemistry, confocal microscopy and qPCR to assess the effects of overexpression of LRP::FLAG on ageing markers as well as telomere dynamics in both cell lines and the pilot study mice.
Results: Here we show that overexpression of LRP::FLAG resulted in significantly elevated hTERT levels, telomerase activity and telomere length, respectively, with concomitantly reduced levels of senescence/ageing markers β-galactosidase and λH2AX in HEK293 and MRC 5 cells, respectively. In addition, LRP::FLAG overexpressing mice displayed less hair graying and balding as well as significantly increased levels of telomerase activity in various organs.
Conclusion: These data suggest a novel function of LRP/LR hampering the onset of senescence through elevating hTERT levels and telomerase activity, respectively. LRP::FLAG might therefore act as a potential novel anti-ageing drug through the impediment of the cellular ageing process.
Keywords: Ageing, telomerase, LRP/LR, LRP::FLAG, senescent markers
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SNAP-Tag Technology Mediates Site Specific Conjugation of Mesothelin Antibody Fragments with a fluorophore to improve target specific cancer therapy.

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The treatment of central nervous system (CNS) disorders is inhibited by the blood brain barrier (BBB), which prevents entry of many therapeutic agents into the brain. Nanoparticles have gained much attention for neurogenetic therapy, as they have the potential to transport therapeutic agents across the BBB. Gold nanoparticles (AuNPs) are popular vectors, due to their biocompatibility and low toxicity, as well as their ease of synthesis and functionalization with targeting proteins and other ligands.
Treatment for brain disorders often target the transferrin (Tf) receptor due to its over-expression on the blood brain barrier, enabling efficient passage of the bound therapeutic. This project aimed to evaluate the characteristics, DNA binding ability, and in vitro cytotoxicity and transfection abilities of Tf-targeted AuNPs (TfAuNPs) and untargeted AuNPs. AuNPs were synthesized by citrate reduction, and functionalised with chitosan, polyethylene glycol (PEG) and Tf. The functionalized gold nanoparticles (FAuNPs) were characterized using UV spectroscopy, Transmission Electron Microscopy (TEM) and Nanoparticle Tracking Analysis (NTA). Gel retardation and ethidium bromide intercalation studies were used to assess the DNA binding potential of FAuNPs, while their protective capabilities were assessed using the nuclease protection assay. Cytotoxicity and transfection was evaluated in vitro in the HEK293, Caco-2 and HeLa cell lines using the MTT and Luciferase reporter gene assays respectively. The AuNPs appeared spherical, with hydrodynamic diameters ranging from 64.8 to 174.9 nm. Binding studies showed that FAuNPs were able to efficiently complex and condense pCMV-luc DNA, and partially protect the DNA from degradation by serum nucleases. FAuNPs exhibited low cytotoxicity profiles, with TfAuNPs generally exhibiting higher cell viabilities than untargeted AuNPs. The TfAuNPs displayed favourable transfection activities in the Tf receptor-positive HeLa cells, suggesting cellular uptake via receptor mediated endocytosis. The promising results obtained for these transferrin-targeted nanocomplexes augur well for their future use in gene delivery investigations and further investigation in an in vivo system is warranted.

A novel metallo-endopeptidase required for peptidoglycan remodelling during osmolarity changes in *M. tuberculosis*

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Mycobacterium tuberculosis persists in the human population, causing a diverse spectrum of TB disease. Novel TB therapeutics are required, given the global rising incidence of multiple (MDR) and extensively drug resistant (XDR) strains. In South Africa, TB is the leading cause of death, particularly common in the HIV-positive population. An intuitive approach is to target bacterial cell division to prevent replication and disease in the host. The LytM family of metallo-endopeptidases regulate cell division in model organisms such as *Escherichia coli, Vibrio cholerae* and the non-pathogenic relative of M. *tuberculosis, Mycobacterium smegmatis* by activating amidases which degrade the peptidoglycan cell wall permitting cell separation. Deletion of lytM genes is expected to lead to cell separation defects. The aim of this study was to characterise the lytM genes of *M. tuberculosis* to validate them as novel drug targets for TB treatment. Two highly conserved lytM genes *mepA* and *nlpAL* were identified and characterised using various strains of *M. tuberculosis* including knock-out mutants as well as overexpression and genetically complemented strains, bearing various domains of *mepA*. Unexpectedly, deletion of the *M. tuberculosis* lytM genes did not cause a cell separation defect as seen in *E. coli* and *M. smegmatis* lytM deletion strains. Instead, heterologous expression of *M. tuberculosis mepA* in *M. smegmatis* resulted in reduced colony formation in the presence of high concentrations of salt. The severity of colony toxicity was further dependent on the relative *mepA* domain expressed: the most severe effect in the presence of full length *mepA*, followed by the predicted C-terminal metallo-endopeptidase domain alone, whereas presence of the uncharacterised N-terminus alone was negligible.
This suggests a role for *mepA* in osmolarity sensing. A recent study revealed that *M. tuberculosis* responds to an increase in salt by remodelling peptidoglycan coinciding with increased *mepA* expression.
Therefore, *mepA* is likely required for degrading *M. tuberculosis* peptidoglycan during osmolarity changes via the predicted, C-terminal metallo-endopeptidase domain. This is being investigated further in the *M. tuberculosis mepA*-knockout. Collectively, our data highlight a role for *MepA* in adapting to stress conditions, suggesting that this enzyme may be a useful new drug target for TB.

Genomics of Oesophageal Cancer in Africa
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The human genome has increasingly been shown to exhibit a vast degree of invasion by viral genomes. During the process of evolution several RNA and DNA viruses have become integrated into the vertebrate genome and there is no reason to believe that the process has ended. Integrated viral DNA may account for as much as 10% of the human genome and these “Trojan horses” could be hidden carcinogens within the human genome. In this study we have compared the viral DNA sequences present in normal and tumour OSCC samples. DNA from normal and tumour biopsies were subjected to whole genome sequencing and subtracted from the reference human HG20 sequence to identify all non-human DNA sequences. All sequences were aligned to the reference human genome HG20 using the ELAND and CASAVA software packages. The unmapped reads were then aligned against a complete set of the NCBI RefSeq Viral Genomes (build 64) using the Burrows Wheeler Aligner. Using this approach, a large number of viral DNA sequences were identified; these included HPVs, Herpes simplex viruses, adenoviruses and Hepatitis C viruses. Amplification and rearrangements of endogenous retroviruses such as the Human Endogenous Retrovirus K113 were also observed. Endogenous retroviruses (ERV) can serve as a long-lasting viral reservoirs and provide evidence for the coexistence between retroviruses and their hosts over many generations. Defective ERVs can also recombine to affect the expression of adjacent genes. Targeted studies of specific viral sequences may elucidate the mechanisms of gene disruption and may ultimately lead to the development of more effective therapeutic interventions.

Characterizing cell-free DNA – An in vitro study on human cells
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The biological origin of cell-free DNA (cfDNA) is still under scrutiny. If we are to fully utilize it as a screening tool, we need to know how and why it is produced. Metabolic DNA, the precursor of cfDNA, is newly synthesized and this fraction of DNA is not involved in processes of DNA damage repair or normal replication prior to mitosis. In vivo settings are too complex to interpret. We investigate cfDNA release in an in vitro setting.
Cell-free DNA was extracted from the growth media of cells grown in culture at consecutive periods of time in order to obtain a real-time view of cfDNA release following media renewal. The extracted cfDNA was quantified by Qubit High Sensitivity assay, while the sizes of DNA were determined by use of the Bioanalyser. Next-gen sequencing was implemented in the study of the 143B cell line to determine cfDNA’s representation with regard to expected ratios of coverage with regard to both the human genome and cellular DNA content of 143B cells. Bioenergetics were analysed by Seahorse. Aphidicolin treatment was used to inhibit DNA polymerase and thus replication foregoing mitosis.
Size analyses of cfDNA released from various cell lines shows a peak at 2000 bp, while peaks representing breakdown was also present in some cultures. In 143B Osteosarcoma cells, next-gen sequencing revealed that transposable elements that are currently active in the human genome, satellite DNA and microsatellite DNA are overrepresented with regard to expected ratios. There is a correlation between cfDNA levels and glycolysis in some cell lines. Lowered levels of cfDNA were released following Aphidicolin treatment.
The in vitro model shows that a large fraction of cfDNA is actively released. Levels and sizes of cfDNA variate in cell culture over periods of time following media renewal. Various patterns of release are associated with different cell lines. The overrepresented amount of active transposable elements associates cfDNA with genetic rearrangement. CfDNA levels correlate with certain bioenergetic processes. We argue that metabolic DNA is involved in interphase differentiation and must be skimmed prior to mitotic replication to avoid unnecessary obstructions.

Transcription analysis of an immune factor in a main African malaria vector Anopheles funestus.

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Anopheles funestus is one of the vectors that plays a major role in the transmission of malaria in sub-Saharan Africa. The transmission of the malaria parasite to a human host depends on the successful completion of the parasites sexual development in the Anopheles vector. The parasites life cycle is initiated immediately after ingestion of an infected blood meal, with the resulting invasive stage of the malaria parasite (ookinetes) traversing through the hostile gut epithelium to develop into oocysts. The malaria parasite is most vulnerable in the midgut, due to the activation of the Anopheles innate immune system that leads to major parasite loses. One immune pathway present in the Anopheles mosquito is melanization, a unique mechanism that results in encapsulating and destroying the parasite. C-type lectins are part of the mosquito's immune system and have been identified to inhibit melanization by preventing spontaneous proteolytic reactions that may be detrimental to the mosquito, thereby supporting the survival of ookinetes to develop into oocysts. Here, we show the successful infection of a laboratory An. funestus colony with Plasmodium falciparum (NF54 strain) as well as the isolation of the C-type lectin gene. Infection by P. falciparum was confirmed by the presence of oocysts in the midgut and the effect of the C-type lectin transcription was quantified. Considering the significant role of the Anopheles mosquito in the transmission of the malaria parasite, interruption of this transmission cycle is a key target as an intervention strategy. Preliminary studies using the Plasmodium lactate dehydrogenase assay have shown a dose-dependent inhibition of P. falciparum (NF54) growth when incubated with immunomodulators, such as chloroquine. Moreover, modulating the immune system of An. funestus with chloroquine may lead to transcription changes of C-type lectins, leading to increased melanization of P. falciparum, which might result in the subsequent increased mortality of An. funestus mosquito.

Role of the Asp632 loop of cytochrome P450 reductase in control of NADPH binding and hydride transfer

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Conformational changes of NADPH-cytochrome P450 oxidoreductase (CYPOR) associated with electron transfer from NADPH to electron acceptors via FAD and FMN have been investigated through structural studies of the 4-electron-reduced NADP+-bound enzyme and kinetic and structural studies of mutants affecting the conformation of the mobile Gly631-Asn635 loop (Asp632 loop). The structure of 4-electron-reduced, NADP+-bound wild type CYPOR shows the plane of the nicotinamide ring positioned perpendicular to the FAD isoalloxazine with its carboxamide group forming H-bonds with N1 of the flavin ring and the Thr535 hydroxyl group. In the reduced enzyme, the C8-C8 atoms of the two flavin rings are ~1 Å closer compared to the fully oxidized and 1-electron-reduced structures, which suggests that flavin reduction facilitates interflavin electron transfer. Structural and kinetic studies of mutants, Asp632Ala, Asp632Phe, Asp632Asn, and Asp632Glu demonstrate that the carboxyl group of Asp632 is important for stabilizing the Asp632 loop in a retracted position that is required for the binding of the NADPH ribityl-nicotinamide in a hydride-transfer-competent conformation.
Structures of the mutants and reduced wild type CYPOR permit us to identify a possible pathway for NADP(H) binding/release to/from CYPOR. Asp632 mutants unable to form stable H-bonds with the backbone amides of Arg634, Asn635, and Met636, exhibit decreased catalytic activity and severely impaired hydride transfer from NADPH to FAD, but leave interflavin electron transfer intact. Intriguingly, the Arg634Ala mutation slightly increases the cytochrome P450 2B4 activity. We propose that Asp632 loop movement, in addition to facilitating NADP(H) binding and release, participates in domain movements modulating interflavin electron transfer.

Sterols in milk samples of ruminant and non-ruminant animals

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Plant sterols are structurally related to cholesterol, but with obvious differences in the side chain structure. They fulfil an analogous role in plants to that of cholesterol in animals. They are implicated in the reduction of plasma total and low density lipoprotein cholesterol in humans and studies have shown correlation between their presence in blood and milk samples of animals. The present study analysed the phytosterol constituents in milk of selected ruminant and non-ruminant animals using gas chromatography-flame ionisation detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The results obtained revealed that, as expected, the cholesterol content (mg/mL) in ruminant and non-ruminant species were significantly higher while phytosterols occurred in low concentrations (µg/mL), with some, particularly in the non-ruminant animals, not even quantifiably detected by the GC-FID or GC-MS. Also, while the concentration of cholesterol (mg/mL) in ruminant animals ranged between 2.9±1.5 (Reedbuck) to 5.6±3.9 (Impala), it was between 3.7±3.3 (horse) to 12.7±5.6 (Jackal) for the non-ruminant species. Lathosterol, a validated marker for endogenous synthesis of cholesterol in humans, was also detected in the range 5.0 – 23.0 µg/mL and 6.0 – 15.0 µg/mL for the ruminant and non-ruminant animals, respectively. Furthermore, normalised to cholesterol (ratio phytosterol: cholesterol) the values were generally about $5.0x10^{-3}$:1.0 for the non-ruminant candidates but ranged from $5.0x10^{-3}$:1.0 (sheep, reedbuck and red hartebeest) to $1.0x10^{-2}$:1.0 (sable antelope and impala) for the ruminant species. Compared with reported studies, a significant level of correlation existed between the phytosterol constituents in milk and blood samples for most of the studied animals.

Fractions of Azadirachta indica ethanol stem bark extract scavenges free radicals, inhibits key enzyme linked to type 2 diabetes in vitro, abates Fe2+ induced oxidative stress in hepatic tissue and enhances muscle glucose uptake ex vivo

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The progression of secondary complications in type 2 diabetes has been linked to oxidative stress caused by hyperglycemia. Therefore, the control of hyperglycemia is the main target in the treatment of diabetes. Present study investigated the scavenging and ameliorative potentials of different fractions of Azadirachta indica ethanol stem bark extract in Fe2+-induced oxidative injury in hepatic tissue as well as their ability to inhibit enzymes linked to diabetes and in enhancing muscle glucose uptake via some in vitro and ex vivo experimental models. The results revealed that the butanol fraction of the extract showed a significantly ($p<0.05$) higher DPPH scavenging activity than all other fractions (IC50 0.0154 µg/ml) while the aqueous fraction showed the highest FRAP activity (IC50 25.32 µg/ml). Although all the fractions ameliorated Fe2+-induced oxidative injury in hepatic tissue by significantly reducing the MDA concentration in dose dependent manner, the butanol fraction showed the highest activity in these regards. In addition, the activity of catalase and SOD were significantly improved by the butanol and dichloromethane fractions compared to other fractions. Butanol and Ethyl acetate fractions showed the highest antidiabetic activity in term of α-glucosidase (IC50 0.23 µg/ml) and α-amylase (IC50 14.79 µg/ml) inhibitory activity, respectively. Although the fractions significantly improved glucose uptake in psoas muscle with or without insulin, the butanol fraction showed the highest activity (GU50 6.22 µg/ml) in this regard. The GC-MS of showed the presence of a number of bioactive compounds. The results of this study suggest that the butanol and ethylacetate fraction of the ethanol stem bark extract may have antidiabetic potentials

Mitochondrial disease in South Africa: clinical, biochemical and genetic outcomes in paediatric patients

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The molecular genetics of an ethnically diverse South African paediatric cohort with clinical suspected mitochondrial disease (MD), and biochemically confirmed respiratory chain enzyme deficiency, was genetically investigated in a retrospective manner. Muscle involvement was present in 80% of patients and muscle respiratory chain enzyme analyses were performed on all 212 patients from the original cohort. A respiratory chain deficiency (RCD) were confirmed in 127 (60%) patients, of which complex I deficiency (59%) was the most prevalent. In these stratified RCD patients, we used a prudent first tier investigative approach by doing whole mitochondrial DNA sequencing and panel-based nuclear gene next-generation sequencing. In 23 (18%) of these patients, a novel or reported variant of interested was detected. Upon further scrutiny, only four patients presented with disease-causing variants based on ACMG guidelines. The poor diagnostic outcome of 3% supported the notion for whole exome sequencing in this population. From the cohort, a subset of patients was selected and diagnostic yield increased tremendously to 50%. We conclude that panel sequencing was not an effective approach for identifying disease-causing variants and exome sequencing should be considered a first option in paediatric patients, especially in heterogeneous population groups. Furthermore, we conclude that effective clinical, biochemical, and genetic evaluations, as a diagnostic strategy and improvement on the knowledge for genotype-phenotype correlations, should be handled in unison when investigating MD aetiology in South Africa.

Interdomain interactions within a multimodularxylanase Xyl

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The need for a sustainable source of biofuels and other environmentally friendly products has focused recent research towards the utilization of plant-based biomass. Hemicellulose, the second most abundant component of plant-based biomass is made up of pentose and hexose sugars linked by β-(1–4) bonds to create a polysaccharide chain. The degradation of hemicellulose is a vital step in the efficient utilisation of plant biomass. Xylan, the most prevalent hemicellulose, is digested by xylanases and many attempts have been made to improve the efficiency and stability of this enzyme for specific processes. Xyl, a multimodular xylanase was isolated from the hindgut metagenome of the snouted harvester termite (*Trinervitermes trinervoides*). Xyl may be an industrially important enzyme by generating xylobiose and xylotriose from various xylan sources, including beech and birch wood, as well as wheat arabinoxylan. Xyl consists of four domains connected by flexible linkers. These are an N-terminal xylanase domain (GH11), two carbohydrate binding modules (CBM1 and 2) and a C-terminal esterase domain (CE4). Our aim was to biochemically, structurally and biophysically characterizing this enzyme and to investigate the possibility of interdomain interaction in this xylanase. We could show that 1) deleting CBM1 slightly increases the enzyme activity at lower pH, however, deleting both CBMs decreased the enzyme activity at 60°C from 90% (SD = 1.3) to 56% (SD = 2.9); 2) The crystal structures of the GH11 and CE4 domains show a good match to similar domain structures from related enzymes, though some difference are noticeable; 3) in the crystal structure of the GH11-CBM1 two-domain construct, the CBM1 domain is not visible, implying little to no mutual recognition of these domains; 4) the GH11-CBM1-CBM2 protein construct is highly stable yet fails to crystalize, potentially confirming that domains to not interact. Currently analyses by isothermal titration calorimetry are under way to determine whether or not these domains interact.

The structure of the cyanide dihydratase (CynD) from Bacillus pumilus

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We present the first structure of an intact, helical nitrilase. It was determined at a resolution of 3.2Å by cryo-electron microscopy. The locations of residues 3-319 (of a total of 330) were clearly interpretable in the electric potential maps. The enzyme was engineered for increased thermostability by means of the following mutations: Q86R+H305K+H308K+H323K. This combination resulted in stable active fibres that were ideal for image processing.
We have visualized the carboxy-terminal "tail" (residues 278-319) that was absent in the structure of a fragment of a homologous nitrilase from Synechocystis sp. PCC6803 (Zhang et al, 2014). This plays an important role in stabilizing the helical structure through multiple interactions, both at the interfaces between the monomers and with the "tails" of other monomers on the inside of the helix.
The active site is different to that of the homologous amidases and these differences may ultimately give insight into the mechanisms of both nitrilases and amidases.

Evaluating the role of Nitric oxide on myoblast proliferation, migration and differentiation

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Satellite cells are quiescent myogenic precursor cells, present between the basal lamina and sarcolemma of skeletal muscle. They are activated in response to skeletal muscle injury for muscle regeneration. Nitric oxide is a gaseous free radical that is known to stimulate satellite cell activation to myoblasts; NO production is facilitated by nitric oxide synthase (NOS). The role of NO as a potential mediator of myoblast proliferation, migration or differentiation is unclear. The current study, therefore, aimed to firstly establish the level of nitric oxide released by myoblasts during their proliferation, migration and differentiation. Secondly, it aimed to assess the effect L-NAME (a NOS inhibitor) on these processes. C2C12 cells were cultured in standard growth media and subsequently plated for analysis in serum-free media. Proliferation or differentiation was induced via the addition of either 2 ng/ml HGF or 2% horse serum respectively, while migration was analyzed using the standard in vitro wound healing assay. L-NAME was utilized at a concentration of 100 µM and 200 µM. NO levels were assessed using a colorimetric assay.

Proliferation was assessed via cell counts, while migration was determined by assessing the percentage wound closure. Differentiation was determined by assessing myoblast alignment and subsequent fusion into multinucleated myotubes. HGF (2 ng/ml) stimulated myoblast proliferation, however levels of NO were only found to be 0.58 nmol at 1-hour post-HGF stimulated. Similarly, NO following myoblast wounding was 0.31 nmol at 1h and increased to 0.56 nmol over 16 h. In response to differentiation cues, NO levels rose sharply to 6 nmol; these levels dropped as differentiation progressed over five days. Addition of L-NAME (200 µM) only resulted in a small (43% and 10%), but significant decrease in proliferation and migration. Addition of L-NAME (200 µM) to differentiating cells significantly reduced myoblast alignment and fusion by 18% and 6% at day five of differentiation. Results suggest that nitric oxide play a significant role during myoblast differentiation, making NO crucial for skeletal muscle regeneration.

Construction of Heat Shock Factor Double-stranded RNA for prospective understanding of Anopheles funestus male genitalia rotation

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Malaria is an acute haematological disease that mainly affects the children of Africa and the Anopheles (An.) funestus mosquito acts as a vector for the transmission of the disease in this region. The An. funestus male mosquito needs to undergo a 180° rotation of its terminal genitalia for sexual maturation. Increasing temperature increases the rate of genitalia rotation, posing threats to humanity since an increase in global temperature would result in an increased propensity for wild male mosquitoes to breed sooner post-emergence from their pupal casing. The heat shock transcription factor is temperature-dependent transcription factor that has a high degree of functional heterogeneity. This study aimed to design heat shock factor double-stranded RNA. Anopheles mosquitoes used in this study were from the FUMOZ colony, which were successfully identified as the An. funestus species using a multiplex polymerase chain reaction assay. The expression of the heat shock factor gene in An. funestus pupae and adults was confirmed by reverse transcription polymerase chain reaction. Subsequently, double-stranded RNA targeting the heat shock factor gene in An. funestusmales was successfully synthesised. The heat shock factor double-stranded RNA in a feeding solution was confirmed to be stable under insectary conditions after 24 hours, meaning that it would be appropriate to feed to newly emerged An. funestus males without the risk of degradation. For prospective studies, heat shock factor double-stranded RNA can be used to knockdown heat shock factor messenger RNA in male An. funestus mosquitoes to elucidate the role that heat shock factor may play in genitalia rotation.

The Cathepsin L of Toxoplasma Chronic infection and its endogenous inhibitor, Iso-mukaadial acetate

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Toxoplasmosis is a parasitic disease caused by Toxoplasma gondii. Chronic infection resulting from T. gondii is one of the most common parasitic infections in humans. About 200,000 cases of congenital toxoplasmosis occur a year globally. People with weakened immune systems are likely to suffer from mental illnesses and cognitive impairment. There are currently no options for curing this infection.

The lack of effective therapeutics is due partly to a poor understanding of the essential pathways that maintain long-term infection. Recently, cysteine proteinase Cathepsin L (TgCPL) has been found to be a key enzyme in the chronic infection. TgCPL is associated with a discrete vesicular structure in the apical region of extracellular parasites but is found in multiple puncta throughout the cytoplasm of intracellular replicating parasites. It is important to find new compounds able to inhibit parasite invasion or proliferation. In this study, we isolated a new drimane sesquiterpenoids (Iso-mukaadial acetate) and was screened for its anti-Toxoplasma gondii activity. A preliminary in vitro screening performed over 5 day's growth assay by an enzyme β-galactosidase revealed that the compound was effective against T. gondii at 6.59 µM. Its cytotoxicity was estimated on HFF cells, and their 50% inhibitory concentrations (IC50) was 36.01 µM with the therapeutic index of 5.46. We have also found that M. acetate inhibits TgCPL, this was done by monitoring the human cathepsin and TgCPL catalysed hydrolysis of Z-Phe-Arg-AMC in the presence of inhibitors M. acetate and LHVS. We further showed that disrupting the vacuolar compartment (VAC) or VAC-localized cysteine protease compromised VAC digestive function and remarkably reduced chronic infection. These findings provide important insights into the proteolytic cascades of T. gondii and their endogenous control.
Key words: Toxoplasma gondii, Iso-mukaadial acetate, Cathepsin L

Compounding evidence for the role of overlooked 11-oxygenated androgens in castration resistant prostate cancer
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Castration resistant prostate cancer (CRPC) is an androgen-dependent disease driven by the intratumoral conversion of adrenal androgen precursors to potent androgens. While most studies focus on the classic adrenal androgen precursors, dehydroepiandrosterone (DHEA) and androstenedione (A4), evidence for the contribution of adrenal derived 11-oxygenated androgen precursors is mounting. We have previously shown that 11β-hydroxyandrostenedione (11OHA4) is converted to 11-ketotestosterone (11KT) and 11-keto-5α-dihydrotestosterone (11KDHT), which bind and activate the human androgen receptor (AR) with affinities and potencies similar to that of testosterone (T) and 5α-dihydrotestosterone (DHT), respectively. Moreover, both 11KT and 11KDHT upregulate AR-regulated gene and protein expression, and induce cell growth in androgen-dependent prostate cancer cell lines. We have subsequently measured the circulating levels of adrenal derived 11-oxygenated androgens and their precursors in patients undergoing treatment for advanced prostate cancer and show that the precursor, 11OHA4, circulates at levels comparable to, or higher than, those of DHEA and A4, and that 11KT circulates at higher levels that T in castrated men. Moreover, in vivo studies revealed that 11KT induces the growth of three xenograft models in castrated mice to the same extent as T. Interestingly, characterisation of AKR1C3, a key enzyme essential for the intratumoral activation of both classic and 11-oxygenated androgen precursors, revealed an 8- to 22-fold catalytic preference for 11-oxygenated androgen substrates over classic substrates.
Conversely, 17βHSD2, the enzyme catalysing the reverse inactivation demonstrated little to no substrate preference. Significantly, three independent experimental systems and a validated computational model revealed that increased AKR1C3:17βHSD2 ratios, as measured in CRPC tissue, significantly favoured the activation of 11-oxygenated androgens. These results, coupled to our previous findings that 11KT and 11KDHT are metabolised at a significantly lower rate than T and DHT, strongly suggest that 11-oxygenated androgens may accumulate within CRPC tumours. Taken together our findings challenge the paradigm that DHEA and A4 are the only adrenal androgen precursors involved in the development of CRPC and clearly show that the contribution of 11-oxygenated androgens should no longer be overlooked.

Antimicrobial drug development by systems-driven target assessment: the case of Coenzyme A-directed inhibitors
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The potential of the coenzyme A (CoA) biosynthetic enzymes as targets for especially antimicrobial drug development has solidified over the past few years, with several compounds acting on or through the pathway being reported. However, the decision-making process whereby the most appropriate target for inhibition in a specific organism is selected largely remains a guessing game, directed more by the outcome of actual inhibitor screens than by a model that can provide clear guidance in this regard. The discovery that many of the CoA-directed inhibitors also only functions once they are metabolically activated by one or more of the CoA biosynthetic enzymes adds additional complexity to the analysis.
In this talk the need for such models will be discussed using recent examples that described the failed translation of potent inhibitors of CoA biosynthetic enzymes into compounds that show whole cell inhibition. Using experimental results from our own studies we will demonstrate how such failures could have been predicted if a systems-driven approach was used to perform target assessment, and show how this approach has been applied in our studies to guide the organism-specific selection of targets in the CoA biosynthetic pathway and beyond. This work highlights the utility and importance of systems-driven target assessment in antimicrobial drug development studies.

The development of selective, dual angiotensin-converting enzyme C-domain/neprilysin inhibitors
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Angiotensin-converting enzyme (ACE) inhibition has been successful in treating hypertension. Pre-clinical and clinical studies suggested that in combination with neutral endopeptidase or neprilysin (NEP) inhibition, protective cardiovascular effects would be greater than with ACE alone. However, major side effects were observed, such as persistent cough and angioedema, due to bradykinin accumulation. Somatic ACE comprises two homologous domains (N- and C-domains), the differing substrate preferences of which present a new avenue for domain-selective inhibitor design. A set of mercaptoacyl and carboxyl dipeptides, based on the crystal structures of omapatrilat, LisW and previously reported dual ACE/NEP dipeptide inhibitors, were synthesized to probe the S1'/S2' pocket requirements for C-domain selectivity and NEP potency. Purified recombinant ACE N-domain, ACE C-domain and NEP proteins were used for in vitro inhibition assays. End-point ACE assays were carried out using the Cbz-Phe-His-Leu peptide substrate (Z-FHL).

The bradykinin based FRET peptide, MCA-RPPGFSAFK(Dnp)-OH substrate was used for NEP inhibition assays. The carboxyl dipeptides 3a, 4a and 4b displayed classical competitive inhibition and Ki values of 3a and 4a were in the nanomolar range for NEP and C-domain. Inhibition assays for the mercaptoacyl compounds were more challenging due to the instability of the compounds, the tight binding nature of the carbonyl-thiol zinc binding group (ZBG), and the slow off rates observed, particularly for the C-domain. The most promising mercaptoacyl compounds were: i) the 5-phenyl proline 2b with IC50 values of 0,8 and 9 nM for C-domain and NEP, respectively, and ii) the Lis-Trp derivative 3b with IC50 values of 7 nM and 1.2 µM. Compound 2b showed the greatest C-domain selectivity (25-fold). Crystal structure determination of these compounds in complex with ACE and NEP is currently underway and will provide new data to drive the next round of structure-based drug design.

Neurobiochemical roles of vitamin C and Water melon juice on elctrolytes, antioxidant enzymes and oxidative stress biomarkers in the treatment of ischemic stroke in wistar rats.

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Oxidative stress is a major participant that contributes to brain reperfusion injury following ischemic stroke (IS). Excessive generation of reactive oxygen species (ROS) and impairment of endogenous antioxidant defense mechanism begins immediately after the onset of IS, resulting in secondary events leading to neuronal dysfunction and cell death. This study reports the role of vitamin C and water melon juice extract in the treatment of induced ischemic stroke in Wistar rats. Twenty five wistar rats were subdivided into five groups of five rats each. Ischemic stroke was induced in wistar rats using middle cerebral artery occlusion (MCAO) method, 47.5mg/kg body weight of Vitamin C and water melon juice was orally administered to the rats for two weeks. Antioxidant enzyme biomarkers (catalase (CAT) superoxide dismutase (SOD) and glutathione peroxide (GPX) activities and there gene expressions, oxidative stress biomarkers (malondialdehyde (MDA) concentration, were assessed. Electrolytes (Sodium, potassium, calcium, magnesium, phosphorus) and glucose levels were also assessed. Ischemic stroke caused significant ($p<0.05$) decrease in the activities of the enzymes and significant increase ($P>0.05$) in the concentration of MDA. IS also caused significant ($p>0.05$) increase in electrolytes and glucose levels in stroke induced non treated rats (SINT). Treatment with the 47.5mg/kg BW of the vitamin C and water melon juice resulted in the significant increase ($P>0.05$) of the activities of CAT, SOD and GPX and there gene expressions.
Also, there was significant ($p<0.05$) decrease in the concentration of MDA. There was significant ($P>0.05$) in the expression of BCL2 in vitamin C group and significant decrease in SINT group. There was significant ($p<0.05$) decrease in the expression of Bax in vitamin C treated group and a significant increase in SINT group. After the treatment, there was significant ($p<0.05$) decrease in the electrolytes and glucose levels in the stroke induced treated rats. The study concluded that antioxidants and water melon juice reduces oxidative stress and its biomarkers, so also, it balances the level of electrolytes in ischemic rats and underscores relevance of antioxidants and water melon juice in the management of IS, this might open a new therapeutic possibilities for stroke treatment.
Key words: Ischemic stroke, Antioxidants, water melon juice extract, Oxidative stress, Wistar rats

SWATH analysis of differentially expressed proteins in resistant (SST374) and susceptible (SST317) wheat cultivars, infested with Russian Wheat Aphid-SA3 under varying atmospheric CO2 levels

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South African wheat farmers spend millions of Rands to produce about 2 million tons of wheat annually. RWA is a serious pest of important agricultural crops such as wheat and it can cause up to 60% and 90% yield loss. Increase in atmospheric CO2 has been shown to increase aphid population and this will affect food security. Thus it is crucial to develop new aphid-resistant cultivars using proteomic approach. The aim of this study is to identify and compare differentially expressed proteins and understand signalling pathways in wheat cultivars SST317 and SST374 in response to RWA SA3 infestation under ambient and elevated CO2 using SWATH-MS. For this study, the proteins that are differentially expressed in cultivars SST317 (susceptible) SST374 (resistant) due to the feeding of RWA SA 3 were evaluated using SWATH-MS (Sequential Window Acquisition of All Theoretical Mass Spectra-Mass Spectrometry) which is based on quantitative proteomics approach in combination with bioinformatics analysis. Leaves from SST 317 (susceptible) and SST 374 (resistant) cultivars were harvested according to the time frame and homogenised using pestle and motor. The homogenate was resuspended in an extraction buffer (2% SDS, 1% PVPP, 50mM Tris-HCl pH 8.8, Protease Inhibitor Cocktail, 10mM β-Mercaptoethanol) at 4^{o}C. Pellets were resuspended in minimum volumes of PBS. Protein quantification was done using the RC DC Protein Assay. The samples were analysed by SWATH-MS analysis. The results of this study demonstrate that under ambient and elevated CO2 SST 317 and SST374 showed visible feeding symptoms associated with RWA SA3 infestation. These symptoms include leaf chlorosis, necrosis, longitudinal streaks on leaves, as well as leaf rolling. Under elevated CO2 conditions, there are more proteins expressed in SST 317 than in SST 374, because RWA SA3 infested on SST317 grew exponentially but on SST374 RWA SA3 growth was suppressed. This suggests that SST 374 has the potential to improve the negative effect of denser aphid infestations. SWATH-MS based proteomic study can and will provide innovative understandings into the control mechanism of wheat cultivars at protein levels in order to find out essential regulators for potential application to increase wheat yield in future.

Development and validation of liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods for the quantification of two peptide drugs in biological samples

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Introduction: Peptide-based drugs are known for being highly potent and selective within a broad range of therapeutic applications, and also relatively safe and well tolerated. Vancomycin and goserelin are peptide drugs employed as antibiotic and anti-cancer agents, respectively. Immunological methods have traditionally been used to quantify peptide-drugs.
However, these methods are insensitive, non-selective and expensive. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) with its inherent advantages of high speed, sensitivity, and selectivity is well suited for rapid quantification of peptides in complex samples. This study aimed to develop and validate rapid, selective and sensitive LC-MS/MS methods for the quantification of vancomycin and goserelin in human plasma and gastrointestinal fluids to support clinical and preclinical studies, respectively.
Method and results: The physical chemical characteristics of goserelin and vancomycin were investigated in each study to develop their respective methods. In both methods, the analytes (vancomycin and goserelin) and their respective internal standards (ISTD) (norvancomycin and alarelin) were separated on a C18 column using a gradient program. Positive ion electrospray ionization was used to detect vancomycin, goserelin and their internal standards. The ion transitions monitored in multiple reaction monitoring (MRM) mode were m/z 725.0 →144.2/725.0 →100.2; 718.4 →144.1/718.4→100.2 for vancomycin and norvancomycin respectively, with a total run time of 3 min and retention time of 0.79 min and 0.77 min for vancomycin and its ISTD, respectively. Goserelin and alarelin, respectively, were monitored via the following MRM transitions: m/z 635.4→607.4/635.4→110.0; 584.5→110.1/584.5 →249.1.The total run time was 2 min and retention times were 0.77 and 0.73 min for goserelin and its ISTD, respectively.
Sample extraction of the peptide drugs from human plasma and gastrointestinal fluids involved protein precipitation, solid phase extraction and dilution. The methods were validated observing the parameters described in FDA Guidance for industry on Bioanalytical Method validation and ICH guideline on method validation.
Conclusion: The LC-MS/MS methods are rapid and sensitive and can be applied to vancomycin pharmacokinetics studies and goserelin enzymatic stability studies.

The effect of Psidium guajava aqueous leaf extract on glycogen enzymes and hormone sensitive lipase enzyme in liver and muscle of diabetic rats.

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Introduction: Diabetes mellitus is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism. Psidium guajava (PG) leaf is known to exhibit an antidiabetic effect. The aim of the study was to investigate the effect of PG leaf extract on i.) activity and expression of glycogen synthase (GS) and glycogen phosphorylase (GP), ii.) activity of hormone sensitive lipase (HSL), iii.) phytochemical study of PG leaf aqueous extract.
Methods: Diabetes was induced in male Sprague-Dawley rats by administering a single dose of 40 mg/kg body weight streptozotocin. The aqueous extract of PG leaves was used to treat both normal and diabetic animals (400 mg/kg body weight) for 14 days while control animals were treated with the vehicle. At the end of the experiment, liver and muscle samples were collected for subsequent analysis.
Results: PG restored glycogen synthase activity depressed by diabetes and this was accompanied by reduced glycogen phosphorylase and hormone sensitive lipase activities. PG also increased the amount of glycogen in the muscle. Phytochemical characterization of the aqueous extract of PG using Gas Chromatography-Mass Spectroscopy (GC-MS) revealed the presence of phenolic compounds and triterpenes.
Conclusion: We conclude that P. guajava has significant anti-diabetic effects, and the effects may be associated with increased GS activity and reduced GP and HSL activities.

***Ndufs4*$^{-/-}$ skeletal muscle reveals fiber type-specific bioenergetic and metabolic disturbances**

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Mitochondria produce the majority of adenosine triphosphate (ATP) via the oxidative phosphorylation (OXPHOS) system, which consists of four multi-subunit complexes (CI–CIV) and ATP synthase (CV). The dysfunction of complex I (CI), the first and largest enzyme in OXPHOS, is the most common defect in mitochondrial energy metabolism. CI dysfunction results in severe multisystem deterioration and currently lacks a cure as the underlying pathogenic mechanisms remain incompletely understood. Current studies on the *Ndufs4*$^{-/-}$mouse model of CI dysfunction, which presents with symptoms of Leigh Syndrome, attempt to better comprehend this disease. However, proper understanding of the metabolic changes in *Ndufs4*$^{-/-}$skeletal muscle is lacking.
We combined biochemical strategies and multi-platform metabolomics to gain insight into the energy metabolism of skeletal muscles from wild type (WT) and *Ndufs4*$^{-/-}$ (KO) mice. Since the metabolic properties and unique subpopulations of muscle mitochondria depend on muscle fiber type composition, both glycolytic (white quadriceps) and oxidative (soleus) muscles were investigated. Biochemical investigations included CI enzyme activity assays and Seahorse XFe 96 CI-driven respiratory assays.
Metabolomics investigations comprised of liquid chromatography-tandem mass spectrometry (LC-MS/MS), gas chromatography time-of-flight mass spectrometry (GC-TOF-MS), and nuclear magnetic resonance (NMR) spectroscopy. The biochemical investigations revealed a substantial reduction in CI activity as well as CI-driven respiration in *Ndufs4*$^{-/-}$white quadriceps and soleus skeletal muscle mitochondria, when compared to controls. These results confirmed the expected biochemical state in
Ndufs4$^{-/-}$skeletal muscle. Metabolomics investigations revealed numerous significant alterations in the metabolic profiles of *Ndufs4*$^{-/-}$white quadriceps and soleus skeletal muscles compared to controls. Among the metabolites discriminating KO and WT muscles, previously reported markers of mitochondrial metabolic dysfunction, along with metabolites not previously linked to mitochondrial disease, were uncovered. Furthermore, metabolic differences as well as similarities between white quadriceps and soleus muscles were also evident. Taken together, these results suggest muscle fiber type-specific metabolic alterations due to CI deficiency, thereby shedding light on the underlying pathogenic mechanisms of CI dysfunction.

Differences in microbiome of rat models of cardiovascular diseases

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Approximately 25% of the world's adult population has hypertension. It is a major risk factor for cardiovascular disease, stroke, and heart and kidney failure; however, its cause remains unknown. Gut microbiota have been shown to have a causal role in the development of hypertension. In animal studies, it has been shown that eradication of certain gut microbes leads to decreased blood pressure and that gut dysbiosis may cause an increase in blood pressure. Furthermore, there's a difference in microbial flora composition in hypertensive and normotensive rats. The aim of this study was to compare the gut composition of hypertensive and normotensive animal models. Stomach, intestinal and faecal samples were collected from spontaneously hypertensive rats (SHR), Dahl salt sensitive rats (SSR) and normotensive Dahl rats. The samples were cultured in microaerophilic conditions (5% O2–10% CO2–85% N2) and identified by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). Genomic DNA isolation, 16S rRNA gene sequencing, and analysis of microbial composition was done on the samples. Normotensive rats presented with species diversity, richness and a balanced gut microbiota. There was decrease in microbial species diversity, richness, and abundance in the hypertensive rat models. In addition, there was an increase in Firmicutes and Bacteriodetes ratio in the hypertensive rat models. The observed results demonstrate that a dysbiotic gut microbiota is associated with hypertension. Previous studies have shown that bacteria from Bacteriodetes and firmicutes phyla play a crucial role in development of hypertension and are needed for the maintenance of physiological homeostasis.

The central role of glycine in cheetah metabolism and health

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Introduction: Glycine forms an important structural component of collagen. It is also required for the synthesis of creatine, glutathione, porphyrins, bile and nucleic acids. Glycine also plays an important role as an inhibitory neurotransmitter and is required for the conjugation of potentially toxic aromatic acids. This amino acid has conventionally been considered to be non-essential in the diet since it can be synthesised from serine.
Recent studies, however, suggest that the endogenous synthesis of glycine is insufficient to meet metabolic requirements in humans. In captivity, cheetahs are normally fed diets that are rich in muscle meat that contains 3 to 4 times less glycine than collagen rich tissues such as skin, internal organs and bones. The reduced intake of glycine may result in insufficient glycine being available to meet their metabolic demands and this may contribute to their general poor health in captivity. Here I present evidence from previous metabolomics studies in cheetahs that support this glycine deficiency hypothesis and discuss how this may impact on their health.
Methods: Since serum glycine concentrations are unlikely to reflect total body glycine availability, I use urine organic acid and creatinine data as evidence for a glycine deficiency in captive cheetahs.
Results: Cheetahs appear to require large quantities of glycine for the detoxification of phenolic compounds produced by the intestinal fermentation of aromatic amino acids. The dramatic variation in creatinine excretion in their urine also suggests that the endogenous synthesis of creatine is potentially limited by glycine availability. Increased oxidised pyrimidine derivatives in the urine of captive cheetahs furthermore suggests that reduce glycine availability may limit glutathione production.
Conclusion: Taken together, this data suggest that glycine plays an important role in cheetah metabolism and health. Further studies to evaluate the physiological effects and potential benefits of glycine supplementation in captive cheetahs are thus warranted.

Delivery of antibodies targeting the V2 apex of HIV using adeno-associated virus (AAV) vectors

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HIV infection continues to be a major health challenge in South Africa. Anti-retroviral therapy has greatly improved life expectancy, but adjunctive therapies may further reduce infection and improve treatment. Broadly neutralising antibodies (bNAbs) that inhibit multiple HIV strains have been described but have been difficult to generate using conventional immunisation. Alternatively, DNA sequences for mature bNAbs can be delivered to cells in a vectored immunoprophylaxis (VIP) approach using adeno-associated virus (AAV) vectors to provide long-term antibody expression and protection against HIV infection. Here, we sought to generate AAV vectors for the delivery of potent antibodies targeting the V2 region of the HIV envelope protein. Sequences of previously characterised antibodies from donors of the CAPRISA cohort (CAP256) were inserted into an AAV-based expression plasmid using Gibson assembly and a novel cloning intermediate (pMin-delta). Corresponding AAV vectors were purified using an iodixanol gradient and quantified by droplet digital PCR. Functional CAP256-VRC26 antibodies were produced and secreted by human cells following AAV treatment. In vivo studies in mice showed that human IgG concentrations in the serum increased rapidly in the first 2 weeks after AAV administration before stabilising to 15 - 60 µg/mL by week 12. Mouse sera neutralized the autologous virus (CAP256_SU) at picomolar concentrations, thus confirming the functionality of AAV-expressed antibodies. Novel AAV vectors developed here are therefore successful vehicles for the delivery of potent antibodies against the HIV envelope protein in vivo. Further characterisation of these therapeutic vectors is important towards vectored immunoprophylaxis and immunotherapy against HIV subtypes prevalent in South Africa.

The application of biotechnology on genus Agapornis (lovebirds)

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The genus Agapornis consists of nine small African parrot species that are globally well known as pets, but are also found in their native habitat. Illegal trappings, poaching and habitat destruction are the main threats these birds face in the wild. In aviculture, Agapornis breeding is highly popular all across the globe. Birds are mainly selected based on their plumage colour variations but very little molecular research has been conducted on this topic. There are 30 known colour variations amongst the nine species and most of these are inherited as Mendelian traits. However, to date none of the genes or polymorphisms linked to these variations have been identified or verified. Due to unethical breeding practices the need for the development of molecular tests such as identification verification tests or speciation tests is growing. Future research is paramount to ensure the conservation of wild populations as well as aiding breeders in improving breeding strategies.

Variable selection and classification in the presence of observations below the detection limit

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We present here a novel approach to variable selection and classification considering two groups. The approach was purposefully modified to accommodate zero-valued observations, which often occurs in large proportions in metabolomics data.

The approach, known as XERp, makes use of classification error rates as test statistics. XERp accounts for the proportion of zero-valued observations by including a jump discontinuity when constructing null distributions for said test statistics.

The motivation behind the development of the approach in its current form, was two-fold:

1. In metabolomics, missing values are believed to primarily be the result of the limit of detection (LOD) and metabolites being absent from a given biological sample (truly zero). Missing values are often automatically replaced by zeroes by platform software. This implies zero-value processing is a much more involved process than most researchers currently consider. One mainstream approach to the zero-value problem involves the removal of variables with large proportions of zeros (not related to study design) and subsequently imputing those that remain. However, the impact of imputing a large proportion of data can be severe, while excluding variables may be detrimental to biological interpretation.
2. The statistical comparison of two groups occurs frequently metabolomics research, even when more complex designs are used two-group comparisons are still performed to aid biological interpretation. Comparisons are primarily performed to rank variables according to their discriminatory ability and select a subset of variables for metabolic mapping or to construct classification models.

XERp therefore strived to and succeeded in: (i) better accommodating zero-valued observations, without excluding variables when comparing two groups; (ii) retaining variable selection as a functionality; and (iii) the simultaneous construction of classification rules of new cases. In addition, XERp is almost completely non-parametric; can account for groups of different size; and can accommodate many times more variables than cases. XERp can adjust for the impact of misclassification, that is, the cost of misclassifying ill individual is often different from the cost of misclassifying healthy individuals. Importantly, XERp is easy to implement, understand and interpret, in turn making user interaction possible and robust. For example, users are able to rank variables in an interpretable order of importance and relax or make stricter the selection criteria of variables based on a sound understanding of the criteria itself and the underlying biology. Finally, XERp requires only validation, as with all statistical models, but no further development (e.g. software) to be implemented in a clinical setting.

Investigating the prevalence of mtDNA variants in Chronic Fatigue Syndrome patients

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Chronic Fatigue Syndrome (CFS), also known as myalgic encephalomyelitis (ME) is a prevalent debilitating condition that is poorly understood. The aetiology of this condition is likely multi-factorial and could include biological, genetic, infectious or psychological risk factors. There is growing interest in a possible role for mitochondrial function and, even further, mitochondrial DNA (mtDNA) variation in CFS. Supporting such a link, fatigue is common and often severe in patients with mitochondrial disease, irrespective of their age, gender or mtDNA genotype. Mitochondria produce the bulk of cellular ATP, the cell's main energy source, via oxidative phosphorylation (OXPHOS), as well as a significant proportion of reactive oxygen species (ROS), which are involved in several downstream cell-signalling processes. Mitochondrial DNA (mtDNA) is a circular, 16 569 bp molecule that encodes 13 catalytically important subunits of the OXPHOS system, as well as 22 tRNAs and two rRNAs. mtDNA is a viable target in the search for genetic markers that could be involved in common complex disease onset and progression, including CFS.

Here we investigate whether mildly deleterious mtDNA variants are more prevalent in CFS patients compared to matched controls. Two independent CFS/control cohorts were analysed, one from the UK (n = 181) and the other from South Africa (n = 241).

For both cohorts, CFS patients had an excess of individuals without a variant that was predicted to be mildly deleterious, i.e. controls had higher counts of mildly deleterious variants than CFS patients.

This result seems counter-intuitive and might reflect a physiological mechanism where mitochondrial function is involved in CFS aetiology. This indicates a need to investigate the biological impact of mtDNA variants on bioenergetic processes in CFS patients. Our study provides the important insight that CFS does not involve a simple aetiology and provides informed directions for subsequent analysis.

Functional genomics of male Anopheles funestus genitalia rotation.

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Anopheles funestus is recognised as a major malaria vector in Africa, however knowledge on many aspects of the biology of this species is still lacking. A key event for the An. funestus male to mate is genitalia rotation. This involves the 135° to 180° rotation of claspers, which are tipped with claws. This physical change then enables the male to grasp the female during copulation. In the An. funestus male it takes 36 hours for the genitalia to rotate through stages 0 to 4. Understanding the underlying events that occur during genitalia rotation in the An. funestus male could give us insight to possible avenues of control. The aim of this investigation was to compare and discover differentially expressed genes of Anopheles funestus between stage 0 and stage 3 of the genitalia rotation. Collections of stage 0 and stage 3 Anopheles funestus males were made from the Botha De Meillon insectary at the NHLS, Johannesburg. RNA was extracted from the different stages and RNA-sequencing was performed. Resulting sequencing reads were trimmed and mapped to the reference genome, VectorBase. The transcripts were assembled with aligned reads to form reads that contain paired-end information. Cufflinks calculated expression profiles of the assembled transcripts for each sample. Statistical hypothesis testing was performed on the differentially expressed transcripts. A total of 386 genes that had a fold change greater than two were discovered between the stages 0 and 3 of genitalia rotation. An average of 9-16% more genes were down regulated than up regulated between the stage 0 compared to 3. There were a number of cuticular protein and structural genes, such as actin, tubulin and fibrinogen, that were up regulated in stage 3. The most closely related genes to genitalia rotation would be genes coding for structural differences. A strong correlation of cuticle genes was investigated. The rest of the genes will require further examination to determine if they have an effect on genitalia rotation. Understanding the genitalia rotation of Anopheles funestus will provide valuable insight into their biology and might provide valuable information to identify novel control targets.

Plasmodial Hsp70s as antimalarial drug targets

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Heat shock proteins (Hsps), amongst them, Hsp70 and Hsp90 families, serve mainly as facilitators of protein folding (molecular chaperones) of the cell. The Hsp70 family of proteins represents one of the most important molecular chaperones in the cell. Plasmodium falciparum, the main agent of malaria, expresses six Hsp70 isoforms. Two (PfHsp70-1 and PfHsp70-z) of these localize to the parasite cytosol. PHsp70-1 is known to occur in a functional complex with another chaperone, PfHsp90 via a co-chaperone, P. falciparum Hsp70-Hsp90 organising protein (PfHop). (−)-Epigallocatechin-3-gallate (EGCG) is a green tea extract constituent that is thought to possess antiplasmodial activity. However, the mechanism by which EGCG exhibits antiplasmodial activity is not fully understood. A previous study proposed that EGCG binds to the N-terminal ATPase domain of Hsp70. In addition, another study suggested that a cyclic peptide antibiotic, polymyxin B (PMB) inhibits Hsp90. Since Hsp70 binds peptides, we hypothesized that PMB could inhibit Hsp70. To this end, we investigated inhibition of P. falciparum Hsp70 by PMB. To conduct the assays, we first expressed and purified recombinant forms of PfHsp70-1, PfHsp70-z, and PfHop. Using surface plasmon resonance (SPR), we demonstrated that both EGCG and PMB directly bind to the two Hsp70s. We further observed that binding of EGCG and PMB to the two proteins resulted in secondary and tertiary conformational changes. In addition, both PMB and EGCG inhibited the ATPase and chaperone function of the two proteins. Furthermore, both inhibitors abrogated association of the two Hsp70s with their functional partners. Using parasites cultured in vitro at the blood stages, we observed that EGCG suppressed parasite growth (IC50, 2.9 µM). On the other hand, PMB exhibited poor antimalarial activity (IC50, 190 µM). Our study constitutes the first direct evidence suggesting that both PMB and EGCG directly bind to parasite Hsp70. Furthermore, our findings demonstrate that inhibitors of parasite Hsp70s not only abrogate their chaperone activity but also tend to disrupt their association with network partners. Altogether our findings further demonstrate that EGCG is a promising antimalarial agent.