OUTLINE OF RESEARCH IN THE APPLIED PROTEOMICS & CHEMICAL BIOLOGY GROUP

As an academic, Jonathan applies the expertise he has gained in both academia and in a UK start-up biotech environment to drive new independent and collaborative translational research programs aimed at providing a new level of understanding and prediction concerning the individual nature of disease progression and of drug response.

Jonathan's research strategy is to leverage the Blackburn Group's established technological platforms, technical expertise, and clinical collaborations to create internationally competitive, interdisciplinary clinical proteomics research programs that have a strong translational emphasis. His overarching goal is to combine basic and clinical research in order to create a pipeline for each lead program from discovery through to quantitation and ultimately to application in the human health sector.

Jonathan's academic expertise ranges from mechanistic enzymology, protein biochemistry, molecular biology and proteomics, to the creation of novel biomolecules by *in vitro* evolution. He is currently particularly interested in the translational applications of custom protein microarrays, mass spectrometry-based proteomics & metabolomics, and genomics in diagnostic marker discovery and validation, in unravelling molecular mechanisms of disease, and in the high throughput study of protein-drug interactions, as well as in studying the effects of polymorphic variation on protein function.

CURRENT RESEARCH PROGRAMS IN THE APPLIED PROTEOMICS & CHEMICAL BIOLOGY GROUP

The Blackburn Group currently has multi-disciplinary lead clinical proteomics research programs in the following four disease or application areas: **Tuberculosis research; HIV research; Cancer research; & Diagnostics research**. These research programs can be grouped in the following main areas:

Mass spectrometry-based differential proteomics and lipidomics research for diagnostic/prognostic biomarker discovery & validation*, e.g.*

- Identification of markers of active *vs* latent tuberculosis, as well as of drug response, in patient-derived biological specimens
- Understanding the correlation between variation in mycobacterial strain proteomes & clinical phenotypes
- Developing and validating an *ex vivo* model of TB-immune reconstitution inflammatory syndrome (TB-IRIS), as a route to understand the aberrant immune response in TB-IRIS patients, as well as to explore potential means to modulate this syndrome *via* selective drug treatment
- Understanding the molecular mechanisms that underpin the development of HIV-associated neurocognitive disorders and building an *in vitro* model to explore potential means of modulating these neurodegenerative disorders *via* selective drug treatment
- Determining the signalling pathways involved in human neurogenesis
- Identifying biomarkers of drug response and drug resistance in colorectal cancer
- Understanding the mechanistic link between bacterial infection and tumourogenesis in colorectal cancer

Understanding the quantitative effects of polymorphic variation and mutation on protein function and on protein-drug interactions, *e.g.*

- Developing and using protein microarrays to quantify mutational effects on CYP450 drug metabolizing enzymes
- Developing and using protein microarrays to quantify mutational effects on protein kinase enzymes
- Developing and using novel computational approaches to model mutational effects on CYP450 and protein kinase enzymes

Developing and using protein array-based tools for biomarker discovery & validation, *e.g.*

- Identifying autoimmune-based correlates of disease progression and of response to therapy in colorectal cancer & melanoma

Development of new diagnostic devices and assays for use at point of care, *e.g.*

- Exploring the application of surface enhanced Raman scattering (SERS) spectroscopy in a next generation point of care diagnostic device
- Exploring the application of DNA aptamer technology to create programmed capture reagents

REPRESENTATIVE CURRENT RESEARCH PROJECTS IN THE BLACKBURN GROUP ARE OUTLINED BRIEFLY BELOW:

Tuberculosis Biomarker Discovery
The tuberculosis (TB) field is in desperate need of new biomarkers in a number of areas, including markers of susceptibility to disease, of innate resistance to disease, of effective vaccination and of cure after therapy. In collaboration with a leading clinical groups in Cape Town, the Blackburn group is carrying out a mass-spec-based differential proteomic study of the emergent HIV-associated TB immune reconstitution inflammatory syndrome, TB-IRIS, a life-threatening syndrome that afflicts ca. 20% of patients who are dual infected with HIV and TB and on front line anti-TB and antiretroviral therapies. Through this project, we aim to identify and validate biomarkers that can be used to differentiate between the patients who will develop TB-IRIS and those who won't, as well as on selective routes to modulate this systemic syndrome.

Molecular mechanisms of HIV-associated neurocognitive disorders (HAND)
In South Africa, up to ~45% of HIV patients on long term antiretroviral therapy develop neurocognitive impairments. Neurons are not themselves thought to be susceptible to HIV infection so the molecular basis of HAND remains uncertain. A number of etiological agents seem plausible, including: HIV proteins such as Tat; CNS-penetrating anti-retroviral drugs; & inflammatory cytokines secreted by HIV-infected monocytes that have pemeated the blood-brain barrier. In collaboration with leading clinical groups in Cape Town and with Smith stem cell group in Cambridge, UK, the Blackburn group is building in vitro models of HAND using non-transformed human neural stem cells. We then use mass spectrometry-based proteomic analysis coupled with cell biology-based functional readouts to begin to unravel the possible molecular mechanisms that drive development of HAND, as well as to identify possible targets for selective therapeutic intervention.

Colorectal Cancer Biomarker Discovery
Colorectal cancer remains one of the most difficult cancers to accurately diagnose and stage, largely due to the strongly invasive nature of current tests. The Blackburn group is carrying out both genomic and proteomic studies on a family of candidate genes/proteins identified through bioinformatics methods, with the aim of identifying patterns of variation in the expression of this specific set of genes and/or proteins that might correlate with disease. Our work is based on access to well characterised tissue and blood samples from a cohort of colorectal cancer patients, rather than on immortalised cancer cell lines, and our goal is to identify a 'biosignature' that can provide both diagnostic and prognostic information in the pre-symptomatic stages of disease.

Effect of Polymorphic Variation on P450-Mediated Drug Metabolism
Genetic variation between patients lies at the heart of the observation that the efficacy of drugs varies widely between individual patients and also directly affects susceptibility to adverse drug-drug reactions. Currently however no accurate, quantitative and genuine comparative methods exist for assessing the effect of single nucleotide polymorphisms on the activity of P450 enzymes, the enzymes typically responsible for the first steps in drug metabolism. The Blackburn group is therefore developing and validating novel protein function microarrays - P450 biochips – as well as novel computational approaches that we can use, amongst others, to assess the effect of genetic variation on the primary metabolism of clinically-prescribed drugs, as well as on propensity to adverse drug reactions.

FACILITIES & INFRASTRUCTURE

The Blackburn group is housed within the Institute of Infectious Disease & Molecular Medicine, UCT, in BSL1 and BSL2 laboratories that are well equipped for research & training. Our state-of-the-art facilities include:

- High performance mass spectrometry (Thermo Q Exactive quadrupole-orbitrap MS and Thermo TSQ Vantage triple quadrupole MS; plus local access to Agilent 6530 qTOF MS and AB4800 MALDI-TOF/TOF MS)
- Nano-LC (Dionex NCS-3500RSnano UPLC and Proxeon Easy nLC)
- Bioinformatic analysis of data, including cluster-based data storage and processing
- Surface enhanced Raman spectroscopy
- UV/VIS and fluorescence spectroscopy
- Noble metal nanoparticle synthesis
- Gene cloning & mutagenesis
- Library creation and *in vitro* selection of biomolecules (including aptamers) based on function
- Protein expression in bacterial and and insect cell cultures
- Protein purification and enzymology
- Laminar flow hoods and incubators for tissue culture

In addition, the group has access via the Centre for Proteomic & Genomic Research and other local labs to:

- Custom protein microarraying (Genetix QArray2)
- Automated microarray hybridisation (Tecan HS 4800) and microarray scanning (Tecan LS ReLoaded)
- Bead array-based assays (BioRad Bioplex system)
- High throughput genomics, including Affymetrix DNA microarrays(Affymetrix GS3000 and GeneTitan systems), high density qPCR & next generation sequencing (MiSeq & IonTorrent)
- Confocal fluorescence microscopy
- Flow cytometry and mass cytometry
- Biosafety level 3 laboratories