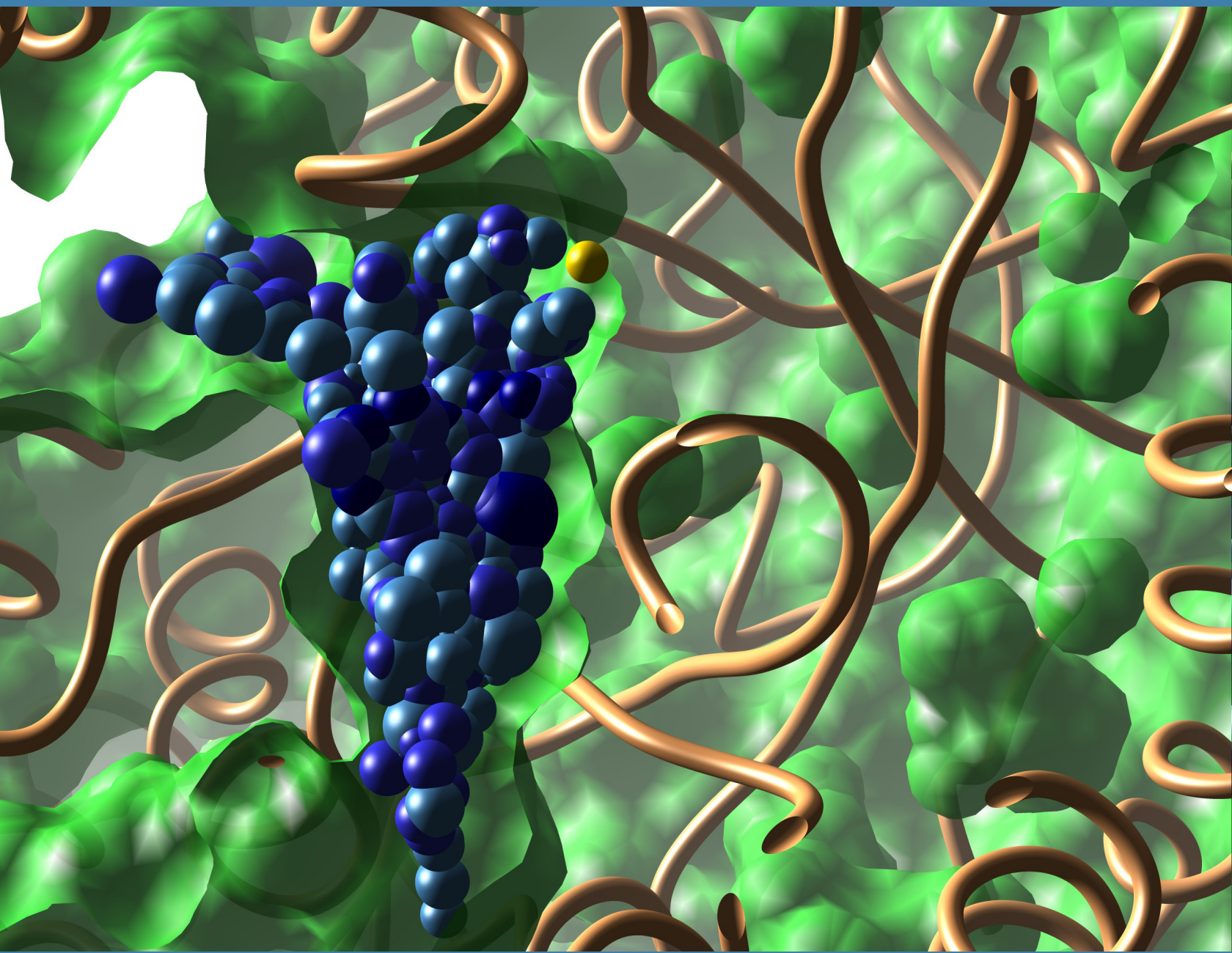
Biophysics in the Understanding, Diagnosis, and Treatment of Infectious Diseases

NOVEMBER 16–20, 2015 | STELLENBOSCH, SOUTH AFRICA

SPIER WINE ESTATE



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Organizing Committee

James Sacchettini, Texas A&M University

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November 2015

Dear Colleagues,

It is our great pleasure to welcome you all to the Biophysical Society Thematic Meeting on *Biophysics in the Understanding, Diagnosis, and Treatment of Infectious Diseases*. This meeting is the Biophysical Society's first venture onto the African continent, and the first thematic meeting to address an overtly medical topic. It is an extraordinary time in biophysics, as new technologies are being rapidly deployed that are changing the landscape of studies on infectious diseases in a manner that was previously inaccessible.

This week's conference brings together a diverse group of experts from many fields including biophysics, biochemistry, microbiology, microfluidics, and community health, to name a few. The 44 lectures and 47 posters at this week's meeting cover a broad range of topics, furthering our goal of providing you with a strong and varied program. Ultimately we hope that through this meeting we can all gain deeper insights into the challenging problems of infectious diseases. We are confident that this meeting will not only provide a venue for sharing our recent progress, findings, and ideas for the future but will also foster new collaborations.

We trust that you will enjoy Stellenbosch – it has a great deal to offer people with a wide variety of interests.

Thank you all for attending, we wish everyone a great meeting!

Best regards,

The Organizing Committee

Jim Sacchettini *Trevor Sewell*

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GENERAL INFORMATION

Registration Hours

The registration desk is located in the Auditorium Foyer of The Spier Wine Estate. Registration hours are as follows:

Sunday, November 15	17:00 – 19:00
Monday, November 16	8:00 – 17:00
Tuesday, November 17	8:00 – 17:00
Wednesday, November 18	8:00 – 12:00
Thursday, November 19	8:00 – 17:00
Friday, November 20	8:00 – 12:00

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in the Conference Center of The Spier Wine Estate. Speakers are required to bring their laptops. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) All poster sessions will held in the Old Wine Cellar of The Spier Wine Estate.

2) A display board measuring 2500mm high and 1000mm wide will be provided for each poster. Poster boards are numbered according to the same numbering scheme as in the online E-book.

3) There will be formal poster presentations on Monday, Tuesday, and Thursday, but all posters will be available for viewing during all three poster sessions.

4) During their poster session, presenters are requested to remain in front of their poster boards to meet with attendees.

5) All posters left uncollected at the end of the meeting will be discarded.

Internet

Wi-Fi access is available throughout The Spier Wine Estate. You will be provided a wifi code on site.

Smoking

Please be advised that smoking is not permitted inside The Spier Wine Estate.

Meals and Coffee Breaks

A reception Sunday evening, coffee breaks and luncheons Monday, Tuesday, Thursday, a picnic lunch on Wednesday, and a banquet Tuesday are included in the registration fee.

Name Badges

Name badges are required to enter all scientific sessions and poster sessions. Please wear your badge throughout the conference.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from November 16 - 20 during registration hours.

In case of an emergency, you may contact the following:

Dorothy Chaconas
dchaconas@biophysics.org

Biophysics in the Understanding, Diagnosis, and Treatment of Infectious Diseases

Stellenbosch, South Africa
November 16 - 20, 2015

PROGRAM

Scientific sessions will be held in the Auditorium and the poster sessions in the Old Wine Cellar of the Spier Wine Estate Conference Center unless otherwise noted.

Sunday, November 15, 2015

17:00 – 19:00	**Registration/Information**	**Auditorium Foyer**
18:00 – 19:00	**Reception**	**Auditorium Foyer and Lounge**

Monday, November 16, 2015

8:00 – 17:00	**Registration/Information**	**Auditorium Foyer**
	Opening Session	
9:00 – 9:05	Ed Egelman, Biophysical Society President	
9:05 – 9:10	Tjaart Kruger, Chairman of the Biophysics Committee of SAIP	
Session I	**Chair: Musa Mhlanga, CSIR Biosciences, South Africa**	
9:10 – 9:50	Sriram Subramaniam, NCI/NIH, USA ***The Cryo-EM Revolution: Applications to Biology and Medicine***	
9:50 – 10:30	Peijun Zhang, University of Pittsburgh School of Medicine, USA ***Structural Basis of HIV-1 and Host Cell Interactions***	
10:30 – 10:50	Gnana Gnanakaran, Los Alamos National Labs, USA* ***Characterization of Glycosylation Profiles of the HIV Envelope Protein***	
10:50 – 11:20	**Coffee Break**	
11:20 – 12:00	Erica Ollmann Saphire, The Scripps Research Institute, USA ***The Molecular Tool-Kit of the Filoviruses***	
12:00 – 12:40	Edward Egelman, University of Virginia, USA ***Cryo-EM of Helical Polymers***	
12:40 – 13:00	Caroline Ross, Rhodes University, South Africa* ***In Silico Analysis of Evolutionary Conserved Interacting Motifs within Picornavirus Capsids***	
13:00 – 15:00	**Lunch**	**Riverside Terrace**

**Short talks selected from among submitted abstracts*

Session II	**Chair: Edward Egelman, University of Virginia, USA**
15:00 – 15:20	Stefan Barth, University of Cape Town, South Africa ***Use of Supercomputational Simulation of Dynamic Protein Interaction to Generate Knowledge-driven Targeted Fusion Proteins for Treatment of Infectious Diseases***
15:20 – 15:40	Constantinos Kurt Wibmer, National Institute for Communicable Diseases, South Africa* ***Structural Characterisation of an HIV-1 Broadly Neutralising Antibody Epitope in the gp120-gp41 Interface***
15:40 – 16:20	Stefan Raunser, Max Planck Institute of Molecular Physiology, Germany ***How to Kill a Mocking Bug—Structural Insights into Tc Toxin Complex Action***
16:20 – 16:40	Brian Baker, University of Notre Dame, USA* ***High Resolution, High Throughput Structural Modeling of T Cell Receptor Specificity and Cross-Reactivity: Implications for Immunotherapy***
16:40 – 17:10	**Coffee Break**
17:30 – 19:30	**Poster Session I** **Old Wine Cellar**

Tuesday, November 17, 2015

8:00 – 17:00	**Registration/Information** **Auditorium Foyer**
Session III	**Chair: James Sacchettini, Texas A&M University, USA**
8:30 – 9:10	Tom Blundell, University of Cambridge, United Kingdom ***Biophysical Methods and Fragment-based Drug Discovery for Infectious Disease: Targeting Mycobacterium Tuberculosis and Mycobacterium Abscessus***
9:10 – 9:50	Robin Wood, Desmond Tutu HIV Centre (UCT), South Africa ***Novel Approaches to the Aerobiology of Tuberculosis Transmission***
9:50 – 10:10	Adam Yadon, Harvard University, and KwaZulu-Natal Research Institute for Tuberculosis and HIV, USA* ***Comprehensive Mutational Analysis of PncA SNPs Conferring in Vitro and in Vivo Pyrazinamide Resistance in M. Tuberculosis***
10:10 – 10:40	**Coffee Break**
10:40 – 11:20	Valerie Mizrahi, University of Cape Town, South Africa ***Identifying Vulnerable Steps in the CoA Biosynthesis Pathway of M. Tuberculosis***
11:20 – 12:00	Robert Stroud, University of California San Francisco, USA ***Targeting the Membrane Proteome of mTB for Structure based Approaches to Function***
12:00 – 12:20	Eric Galburt, Washington University School of Medicine, USA* ***Kinetic Regulation of Open Promoter Complexes by Mycobacterial Transcription Factors***

**Short talks selected from among submitted abstracts*

12:20 – 14:20	**Lunch**	**Riverside Terrace**
Session IV	**Chair: Eric Rubin, Harvard T.H. Chan School of Public Health, USA**	
14:20 – 15:00	Jonathan Blackburn, University of Cape Town, South Africa ***Raman Biosensing for TB Diagnosis***	
15:00 – 15:40	James Sacchettini, Texas A&M University, USA ***Combining Structural Genomics and Drug Discovery to Develop New TB Drugs***	
15:40 – 16:00	Ian Mbano, KwaZulu-Natal Research Institute for Tuberculosis and HIV, South Africa* ***Light Forge: A Microfluidic High Throughput Platform for Rapid and Affordable Detection of Drug Resistant Strains of Tuberculosis***	
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16:30 – 17:10	Adrie Steyn, KwaZulu-Natal Research Institute for Tuberculosis and HIV, South Africa ***Energy Metabolism in Mycobacterium Tuberculosis and the Infected Host Cell***	
17:10 – 17:30	Michael A. Reiche, University of Cape Town, South Africa* ***Visualizing the Mycobacterial Mutasome***	
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Wednesday, November 18, 2015

8:00 – 12:00	**Registration/Information**	**Auditorium Foyer**
Session V	**Chair: Stefan Raunser, Max Planck Institute of Molecular Physiology, Germany**	
8:30 – 9:10	Helen R. Saibil, Birkbeck College, University of London, United Kingdom *EMBO Keynote Lecturer* ***Actions of Plasmodium Falciparum on Its Human Erythrocyte Host Studied by Electron Tomography***	
9:10 – 9:50	Pradipsinh Rathod, University of Washington, USA ***Structure-inspired Disruption of Proper Folding of an Essential Malaria Parasite Protein***	
9:50 – 10:10	Jacky Snoep, Stellenbosch University, South Africa* ***Modelling Blood Glucose Concentration in Malaria Patients***	
10:10 – 10:40	**Coffee Break**	
10:40 – 11:20	David Stuart, University of Oxford, United Kingdom ***Structural Biology of Some Virus Pathogens***	
11:20 – 12:00	Mario Amzel, Johns Hopkins University School of Medicine, USA ***Inhibition of Parasitic Farnesyl Diphosphate Synthases (FPPS)***	

**Short talks selected from among submitted abstracts*

12:00 – 12:20	Ross Douglas, University of Heidelberg Medical School, Germany* ***Using Molecular Biophysics to Understand Plasmodium Actin Dynamics and Cell Motility***
13:00	**Excursions**

Thursday, November 19, 2015

8:00 – 17:00	**Registration/Information** **Auditorium Foyer**
Session VI	**Chair: Helen Saibil, Birkbeck College, University of London, United Kingdom**
8:30 – 9:10	Gabriel Waksman, University College London & Birkbeck, University of London, United Kingdom ***Structural and Molecular Biology of Type IV Secretion Systems***
9:10 – 9:50	Wolf-Dieter Schubert, University of Pretoria, South Africa ***Bacterial Infections at Atomic Resolution***
9:50 – 10:10	Tarakdas Basu, University of Kalyani, India* ***Calcium Phosphate Nanoparticle (CPNP)-entrapped Tetracycline: A Potential Drug against Diarrheal Diseases***
10:10 – 10:40	**Coffee Break**
10:40 – 11:20	Neeraj Dhar, École Polytechnique Fédérale de Lausanne, Switzerland ***Microengineering for Microbiology***
11:20 – 12:00	Frederick Balagaddé, KwaZulu-Natal Research Institute for Tuberculosis and HIV, South Africa ***Drug Tolerance in Mycobacteria Replicating in a Microdialyser Mediated by an Efflux Mechanism***
12:00 – 12:20	Lizbe Koekemoer, Stellenbosch University, South Africa* ***Elucidating the Differences between Eukaryotic and Prokaryotic Type II Pantothenate Kinases***
12:20 – 14:20	**Lunch** **Riverside Terrace**
Session VII	**Chair: Valerie Mizrahi, University of Cape Town, South Africa**
14:20 – 15:00	Sarah Fortune, Harvard T.H. Chan School of Public Health, USA ***Post-Translational Modification of a Nucleoid Associated Protein Regulates Cell State in Mycobacteria***
15:00 – 15:40	Frank von Delft, University of Oxford, United Kingdom ***XChem: From Crystals to Potent Molecules with X-Rays and Poised Synthesis***
15:40 – 16:00	Tom Solmajer, National Institute of Chemistry, Slovenia* ***Structure-based Discovery of Novel DNA Gyrase B Inhibitors***
16:00 – 16:30	**Coffee Break**

**Short talks selected from among submitted abstracts*

16:30 – 17:10	Alex Sigal, KwaZulu-Natal Research Institute for Tuberculosis and HIV, South Africa ***Mycobacterium Tuberculosis Growth in Dead Infected Cells Results in a Positive Feedback Loop Driving Additional Cycles of Host Cell Death***
17:10 – 17:30	Holger Gohlke, Heinrich-Heine-University Duesseldorf, Germany* ***Complex Long-Distance Effects of Mutations that Confer Linezolid Resistance in the Large Ribosomal Subunit***
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Friday, November 20, 2015

8:00 – 12:00	**Information** **Auditorium Foyer**
Session VIII	**Chair: Wolf-Dieter Schubert, University of Pretoria, South Africa**
8:30 – 9:10	Musa Mhlanga, CSIR Biosciences, South Africa ***Super Resolution Microscopy Reveals a Preformed NEMO Lattice Structure that is Collapsed in a Genetic Disease***
9:10 – 9:50	Cheryl Arrowsmith, University of Toronto, Canada ***Open Access Chemical Probes of Chromatin Regulators***
9:50 – 10:10	Mintu Chandra, Indian Institute of Science Education and Research, Bhopal, India* ***Insights into Molecular Switch: Crystal Structure Analysis of Wild Type and Fast Hydrolyzing Mutant of EhRabX3, a Tandem Ras Superfamily GTPase from Entamoeba Histolytica***
10:10 – 10:40	**Coffee Break**
10:40 – 11:20	Timothy Wells, Medicines for Malaria Venture, Switzerland ***Moving Forward New Medicines and New Targets in Malaria***
11:20 – 12:00	Michael Starnbach, Harvard Medical School, USA ***Bacterial Manipulations of the Host Cell Proteome***
12:00 – 12:40	Bryan Trevor Sewell, University of Cape Town, South Africa ***On the Mechanism of the Amidases – Consequences for Sulfhydryl Catalysis and Drug Design***
12:40 – 13:00	**Closing Remarks and *Biophysical Journal* Poster Awards**

**Short talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

The Cryo-EM Revolution: Applications to Biology and Medicine

Sriram Subramaniam.
NCI/NIH, Bethesda, USA.

Recent breakthroughs in the field of cryo-electron microscopy provide new prospects for determination of the structures of a variety of macromolecular assemblies and small dynamic protein complexes that are not amenable to analysis by X-ray crystallography or NMR spectroscopy. In addition, advances in technologies for imaging whole cells and tissues in 3D at high resolution have opened up new vistas for 3D structural imaging. I will review emerging opportunities in molecular and cellular imaging that are enabled with these developments, and discuss applications to cancer research and infectious disease in the coming decade.

Main points:

• Cryo-EM, electron tomography and related methods in 3D electron microscopy provide revolutionary new opportunities for bridging key imaging gaps in biology.
• Advances in correlative light and electron microscopic imaging enable simultaneous imaging of the “needle” and the “haystack” of cellular architecture.
• Advances in electron tomography and subvolume averaging are providing new and important insights into the structure and mechanism of neutralization of enveloped viruses such as HIV, influenza and Ebola.
• Advances in cryo-EM technology enable determination of structures of protein complexes and membrane proteins at near-atomic resolution, and offer unprecedented opportunities for accelerating drug discovery.

Structural Basis of HIV-1 Capsid Assembly and Host Cell Interactions

Peijun Zhang.
University of Pittsburgh School of Medicine, Pittsburgh, USA.

The mature HIV-1 capsid plays a major role in the early stages of HIV-1 replication by protecting the genome from innate immune sensing response and regulating infection by interacting with many host factors including CypA, CPSF6, MxB, TRIM5α and TRIM-Cyp. It contains two structural domains that are connected by a flexible linker and assembles into a distinct cone shaped capsid that encloses the viral genome. We have previously determined the CA tubular assembly to 8 Å using cryoEM and built an all-atom computer model of the complete capsid by large scale molecular dynamics (MD) simulations. Exploiting the recent advance in direct electron detection, we have now obtained the structure of HIV-1 capsid at near-atomic resolution, clearly resolving bulky side chain densities, helix grooves and connecting loops. For the first time, the flexible linker and the major homology region are clearly visualized in an assembly context, providing insights on their critical roles in capsid assembly and maturation. We have also determined the cryoEM structure of the host cell factor CypA in complex with HIV-1 capsid assembly. The density map unexpectedly displays a distinct non-random CypA binding pattern in which CypA bridges two adjacent CA hexamers and wraps selectively along the curved CA array. CryoEM structure-based modeling and large scale all-atoms MD simulations surprisingly reveal that the unique CypA pattern was achieved through an additional uncharacterized novel interface so that a single CypA molecule simultaneously interacts with two CA molecules, therefore, stabilizes and protects the capsid from premature uncoating. Our structure further highlights this novel CypA and CA interface as a potentially attractive therapeutic target for pharmacological intervention.

Characterization of Glycosylation Profiles of the HIV Envelope Protein

Cesar Lopez[1], Jianhui Tian[2], Cynthia Derdeyn[3], Abraham Pinter[4], Bette Korber[1], **Gnana Gnanakaran**[1].
[1]Los Alamos National Labs, Los Alamos, NM, USA, [2]Oakridge National Labs, Oakridge, TN, USA, [3]Emory University, Atlanta, GA, USA, [4]Rutgers University, Newark, NJ, USA.

Heavy glycosylation of the envelope (Env) surface subunit, gp120, is a key adaptation of HIV-1, however, the precise effects of glycosylation on the folding, conformation and dynamics of this protein are poorly understood. In general, glycosylation can stabilize protein conformation, accelerate protein folding, promote secondary structure formation, reduce protein aggregation, shield hydrophobic surfaces, promote disulfide pairing, and increase folding cooperativity. It is well known that gp120 can accommodate a remarkable heterogeneity in terms of the number and location of glycosylation sites. The network of glycans on gp120 is of particular interest with regards to vaccine design, because the glycans both serve as targets for many classes of broadly neutralizing antibodies, and contribute to patterns of immune evasion and escape during HIV-1 infection. We will present results from two separate computational studies. In the first study, large-scale all-atom and coarse-grained molecular dynamics simulations have been used to characterize the effect of glycosylation on the Env Trimer (SOSIP). We identify the key glycosylations that contribute to the stability of Env spike and quantify their energetic contributions. In the second study, we consider an antigenic peptide fragment from the disulfide bridge-bounded region spanning the V1-V2 hyper-variable domains of HIV-1 gp120. We used replica exchange molecular dynamics simulations to investigate how glycosylation influences its conformation and stability. We characterize how glycosylation can stabilize pre-existing conformations of this peptide construct, reduced its propensity to adopt other secondary structures, and provided resistance against thermal unfolding. These studies help to overcome the limited knowledge of how glycosylation and disulfide bonds affect the conformation and dynamics of short intrinsically disordered peptides complicates the design of immunogenic peptides. We will show how the sequence, structural and thermodynamic profiles of glycosylation of gp120 can aid in the design of glycopeptide-based immunogens.

The Molecular Tool-Kit of the Filoviruses

E O. Saphire[1], Z A. Bornholdt[1], M L. Fusco[1], D M. Abelson[1], R N. Kirchdoerfer[1], J E. Lee[1], T Hashiguchi[1], C R. Kimberlin[1], J M. Dias[1], J F. Bruhn[1], S Bale[1], A Zhang[1], P Halfmann[2], T Noda[3], Y Kawaoka[2,3], J M. Dye[4], K Chandran[5].
[1]The Scripps Research Institute, La Jolla, CA, USA, [2]University of Wisconsin, Madison, WI, USA, [3]University of Tokyo, Tokyo, Japan, [4]USA Army Medical Research Institute for Infectious Diseases, Frederick, MD, USA, [5]Albert Einstein College of Medicine, Bronx, NY, USA.

Viruses can be under tremendous pressure for economy of genomic information. As a result, evolution has compelled viral proteins to offer the most functional "bang" for the polypeptide "buck." The ability of viruses to maximize functionality from a limited genome, and to evolve their functionalities in real time offers us fundamental insights into the capabilities and plasticity of proteins in general.
Filoviruses have a compact genome of only 7 genes. Consequently, each protein is critical, many perform multiple functions, and some actually rearrange their structures to achieve those new functions. By employing a variety of structural and biophysical methods, we can illuminate this compact, but highly versatile tool-kit and gain fundamental insights into the biology of entry, immune evasion, replication and assembly. We use this information to decipher the collaborative roles of these proteins in pathogenesis and devise concrete strategies for medical defense.
Crystal structures are now available of the metastable, viral-surface glycoproteins of the Ebola, Sudan and Marburg viruses. These images illuminate how the receptor-binding sites become unsheathed in the host endosome, map the epitopes of neutralizing and non-neutralizing antibodies and provide the roadmap for medical defense against viral entry.
Proteins in the filovirus nucleocapsid complex play dual roles in viral replication and immunosuppression. Structure-function studies illuminate how these molecules control assembly and genome packaging while preventing cellular detection of the invading pathogen.
Other work, on the filovirus matrix protein VP40, challenges the pervading "one gene – one structure" hypothesis of molecular biology – by proving that a single polypeptide sequence can adopt different three-dimensional structures – each of which plays a completely separate and yet equally essential biological role. This discovery provides a novel mechanism to explain how RNA viruses can achieve multifarious functions using elementary genomes.

Cryo-EM of Helical Polymers

Edward Egelman.
University of Virginia, Charlottesville, VA, USA.

Cryo-EM has undergone a revolution, driven by direct electron detectors, and a near-atomic level of resolution can now be reached for many biological samples. While complexes such as the ribosome can be solved at higher resolution and more readily by cryo-EM than they can be by crystallography, they can still be crystallized. However, a vast number of complexes of biological interest are helical polymers, and most of these can never be crystallized. I will describe the application of cryo-EM to helical assemblies in four different areas: 1) *Vibrio cholera*, the organism responsible for cholera, uses a Type Six Secretion System in pathogenesis. We now understand in detail how parts of this system assemble and work. 2) Type IV pili are essential for the infectivity of bugs such as*Neisseria meningitidis*. We have shown for *Campylobacter jejuni* (responsible for most food-borne illnesses in the world) that the conserved flagellin protein can be assembled into different quaternary structures by small amino acid changes. We show the same thing for Type IV pilins. 3) Flexible filamentous plant viruses are responsible for half of the viral agricultural crop damage, but have resisted all attempts at structure determination since the studies of J.D. Bernal >75 years ago. We have solved the structure of two members of this family, bamboo mosaic virus (BaMV) and wheat streak mosaic virus (WSMV) and show how, because they are completely non-toxic, they can be used in biotechnology, in everything from medical imaging to serving as platforms for vaccines. 4) Viruses that infect hyperthermophilic archaea can survive in nearly boiling acid or organic solvents. We now understand how the stability of DNA in SIRV2 and AFV1 is achieved. AFV1, like Ebola, is a filamentous membrane-enveloped virus, and we present the first atomic structure of such a virus.

In Silico Analysis of Evolutionary Conserved Interacting Motifs within Picornavirus Capsids

Caroline Ross, Caroline Knox, Özlem Tastan Bishop.
Rhodes University, Grahamstown, South Africa.

The Picornaviridae family contains a number of pathogens with economic and clinical importance. Recent reports have indicated the emergence of novel picornaviruses associated with gastrointestinal, neurological and respiratory diseases in humans. Currently there are no antivirals available for the treatment of picornavirus infections and the application of effective vaccines has only been successful for certain viruses. Picornavirus capsids are icosahedral, comprising of 60 protomer structures each assembled through the interaction of four subunit proteins: VP1, VP2, VP3 and VP4. However, the protein-protein interactions that facilitate protomer assembly are poorly understood. An investigation into the role of conserved individual subunit residues in such interactions will broaden the understanding of picornavirus evolution as well as provide guidelines for the development of antiviral therapeutics. This study provides a comprehensive examination of the capsid phylogenies, with a novel comparative analysis of amino acid motifs and interactions conserved across the viral family, viral genera and picornaviruses of the same host species. The functions of conserved motifs were deduced by the *in silico* prediction of interacting residues within the crystal structures with subsequent structural analysis, of representative protomers of enteroviruses, Foot-and-Mouth-Disease-Virus and Theiler's Virus. Findings in this study suggest that the capsid proteins might be evolving independently from the replication proteins through possible inter-typic recombination of functional protein regions. Additionally the study predicts that protomer assembly is facilitated through a network of multiple subunit-subunit interactions. Specifically, 30 interacting motifs were predicted to contain residues involved in interprotein interactions. The study identified 50 interacting residues conserved across the enterovirus capsids, with 26 universally conserved residue-residue interactions and 43 interactions sustained through conservative site mutations. The presented results may serve as fundamental guidelines for the development of economically feasible antivirals specifically targeting virus assembly.

Use of Supercomputational Simulation of Dynamic Protein Interaction to Generate Knowledge-Driven Targeted Fusion Proteins for Treatment of Infectious Diseases

Paolo Carloni[1], Johan Van Weyenbergh[2], Rolf Fendel[3], Theo Thepen[3], **Stefan Barth**[4].
[1]Forschungszentrum Juelich, Juelich, NRW, Germany, [2]Oswaldo Cruz Foundation (FIOCRUZ), Salvador, Bahia, Brazil, [3]Fraunhofer IME, Aachen, NRW, Germany, [4]University of Cape Town, Cape Town, Western Cape, South Africa.

Human granzyme B (hGB) is a serine protease involved in immune-mediated apoptosis. Its cytotoxicity makes it potentially applicable in for novel targeted therapies. However, the effectiveness of hGB can be hampered by the cytosolic expression of a natural protein inhibitor, human Serpin B9 (hSB9). Thus, we used computational approaches to identify hGB mutations that can affect its binding to hSB9 without significantly decreasing its catalytic efficiency. Alanine-scanning calculations allowed us to identify residues of hGB important for the interaction with hSB9. Some variants were selected, and molecular dynamic simulations on the mutated hGB in complex with hSB9 in aqueous solution were carried out to investigate the effect of these variants on the stability of the complex. The point mutation R201K demonstrated strongly reduced sensitivity towards hSB9. By fusing this rationally designed human enzyme to disease-specific antibodies, we generated recombinant therapeutics e.g. targeting CD64 for treatment of Leishmaniasis and MSP4 for Malaria treatment. Further details on the activity of corresponding fusion proteins will be presented.

Structural Characterisation of an HIV-1 Broadly Neutralising Antibody Epitope in the gp120-gp41 Interface

Constantinos Kurt Wibmer[1,2], Jason Gorman[3], Gabriel Ozorowski[4], Jinal N. Bhiman[1,2], Daniel J. Sheward[5], Gordon M. Joyce[3], Debra H. Elliot[6], Julie Rouelle[6], Ashley Smira[6], Nonkululeko Ndabambi[5], Aliaksandr Druz[3], Salim S. Abdool Karim[7], James E. Robinson[6], Andrew B. Ward[4], Carolyn Williamson[5,7], Peter D. Kwong[3], Lynn Morris[1,2,7], Penny L. Moore[1,2,7].
[1]National Institute for Communicable Diseases(NICD), of the National Health Laboratory Service (NHLS), Johannesburg, Gauteng, South Africa, [2]University of the Witwatersrand, Johannesburg, Gauteng, South Africa, [3]National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA, [4]CHAVI-ID, IAVI Neutralizing Antibody Center, and CAVD, The Scripps Research Institute, La Jolla, CA, USA, [5]University of Cape Town and NHLS, Cape Town, Western Cape, South Africa, [6]Tulane University Medical Center, New Orleans, LA, USA, [7]University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa.

Current estimates suggest that 35 million people are infected with HIV-1 worldwide. A preventative vaccine is therefore greatly sought after, and will likely need to induce broadly neutralising antibodies (bNAbs). These antibodies target conserved regions of the HIV-1 envelope trimer, but are rare in natural infection and often exhibit unusual features, suggesting they may be difficult to elicit by vaccination. Here we isolated a bNAb from an HIV-infected donor with broadly neutralising plasma, CAP248, and identified its epitope through the analysis of viral escape pathways, and structural biology.

Monoclonal antibody CAP248-2B was isolated by B-cell culture and screened for activity in an Env-pseudotyped neutralisation assay. HIV-1 single-genome gp160 sequences from longitudinal CAP248 plasma samples were used to identify viral escape mutations, which were validated by mutagenesis. The CAP248-2B Fab structure was determined by protein crystallography, and docked into a Fab-trimer negative-stain EM complex to predict specific interactions.

Although the neutralising activity of CAP248-2B recapitulated the plasma breadth, it was significantly less potent due to incomplete neutralisation maxima. Unlike other bNAbs, these low neutralisation plateaus were not affected by glycan heterogeneity. The Fab crystal structure revealed a highly flexible CDR-H3 and long CDR-L3 (19 amino acids) that jutted away from the other CDRs. Viral escape mutations accumulated in the gp120 C-terminus, which together with the gp41 C-terminus, comprised the CAP248-2B epitope as determined by EM. CAP248-2B only partially competed with other bNAbs targeting the gp120-gp41 interface, suggesting an overlapping but distinct epitope. Mutations that abrogated CAP248-2B neutralisation conferred enhanced sensitivity to other bNAbs targeting the gp41 membrane proximal external region.

Further structure guided understanding of CAP248 virus-antibody co-evolution may thus provide a blueprint for the simultaneous induction of multiple gp41 targeting bNAbs, which could potentially increase vaccine coverage.

How to Kill a Mocking Bug—Structural Insights into Tc Toxin Complex Action

Stefan Raunser.
MPI Dortmund, Dortmund, Germany.

Tripartite Tc toxin complexes of bacterial pathogens perforate the host membrane by forming channels that translocate toxic enzymes into the host, including humans. The underlying mechanism is complex but poorly understood. In my talk I will present the first high-resolution structure of a complete 1.7 MDa Tc toxin complex composed of TcA, TcB and TcC. TcB and TcC form a large cocoon, in which the toxic domain resides and is autoproteolytically cleaved. Binding of TcB/TcC to the pore-forming and receptorbinding TcA opens the cocoon and results in a continuous protein translocation channel, in which the toxic domain is secreted. Our results allows us for the first time to understand key steps of infections involving Tc toxins at molecular level and shed new light on the interaction of bacterial pathogens, such as the plague pathogen Yersinia pestis, with their hosts.

References:
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High Resolution, High Throughput Structural Modeling of T Cell Receptor Specificity and Cross-Reactivity: Implications for Immunotherapy

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T cell receptors (TCRs) recognize antigenic peptides bound and presented by class I or class II major histocompatibility complex proteins (peptide/MHC complexes). TCR recognition of a peptide/MHC complex defines specificity and reactivity in cellular immune responses. While structurally similar to antibody Fab fragments, there are key differences between TCRs and antibodies. Notably, TCRs do not undergo affinity maturation, and unlike mature antibodies, TCRs display a balance of specificity and cross-reactivity. Cross-reactivity is necessary given the limited size of the TCR repertoire relative to the universe of potential antigens, yet specificity is a fundamental feature of immunity. Many pathogens, particularly genetically unstable viruses, take advantage of TCR specificity for immune escape. In this context, there is increasing desire to engineer TCRs for therapeutic purposes. Design goals for engineered TCRs include efficient recognition of key antigens as well as known escape variants. Simultaneously, engineered TCRs must be biased against related self-antigens to avoid autoimmunity. The objective of this work is to develop the means to perform high resolution, high throughput modeling of TCR specificity and cross-reactivity in order to facilitate TCR targeting, identify cross-reactive antigens, and understand and combat immune escape. Our methodology combines large-scale experimental assessments of TCR cross-reactivity with computational modeling, structural biology, and biophysical analyses. Our results demonstrate the potential of this approach and highlight possible uses for the immunotherapy of genetically unstable viruses such as HCV and HIV, as well as other conditions with genomic instability.

Biophysical Methods and Fragment-based Drug Discovery for Infectious Disease: Targeting Mycobacterium Tuberculosis and Mycobacterium Abscessus

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Structure-guided fragment-based screening techniques have proved effective in lead discovery not only for classical enzyme targets but also for less "druggable" targets such as protein-protein interfaces. They also have the advantage of allowing optimisation of a range of physical chemical properties that allow optimisation for a range of absorption, distribution, metabolism, excretion and toxicology (admet) properties; these are proving particular challenges in tuberculosis drug discovery.

As the initial screening involves small fragments with very low, often millimolar affinities, biophysical methods such as isothermal calorimetry (ITC), analytical ultracentrifugation (AUC), thermal shift, surface plasmon resonance (SPR), nuclear magnetic resonance (NMR) and X-ray crystallography are used to explore chemical space of potential ligands. The approach involves a fast initial screening of a library of around 1000 compounds, followed by a validation step involving more rigorous use of related methods to define three-dimensional structure, kinetics and thermodynamics of fragment binding. The use of high throughput approaches does not end there, as it becomes a rapid technique to guide the elaboration of the fragments into larger molecular weight lead compounds.

I will discuss progress in using these approaches for targets in Mycobacterium Tuberculosis and Mycobacterium Abscessus. I will review our work in using fragment-based methods for protein-protein interfaces and discuss the challenges of the interface surfaces in terms of potential binding sites where fragment-based methods can efficiently explore protein landscapes; these tend to have clusters of small but deep pockets rather than the well-defined clefts of traditionally druggable targets.

Novel Approaches to the Aerobiology of Tuberculosis Transmission

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South Africa has the highest tuberculosis notification and death rate of any country worldwide. The city of Cape Town alone has more TB notifications annually than USA, Canada, UK and France combined. TB transmission rates have remained at levels recorded in industrial cities of Europe and North America a century ago. Transmission is determined by the combination of the prevalence of infectious TB in the community, the social and environmental factors enabling air exchange from infective individuals, and the concentration of TB bacilli in the exhaled air of infectious.
Our knowledge of the airborne nature of respiratory disease transmission owes much to the pioneering experiments of Wells and Riley over half a century ago. However,
these in vivo animal studies may have considerably underestimated the potential infectivity of TB cases.
In order to better characterize the factors driving TB transmission in a Cape Township
social environments conducive for potential TB transmission in Cape Town were identified using a combination of social mixing studies and the use of carbon dioxide as a natural tracer gas as a proxy for TB exposure. The volumes of air exchanged between individuals were calculated during different activities and seasons.
The potential infectivity of TB patients was explored by sampling devices installed in a Respiratory Aerosol Sampling Chamber (RASC) that enabled representative sampling and isolation of airborne particles and organic matter from TB patients. Preliminary results from the first 10 TB patients showed the presence of airborne bacilli on scanning electron microscopy, the presence of culturable TB organisms and high levels of TB DNA in the expired air of these patients.

Comprehensive Mutational Analysis of PncA SNPs Conferring in Vitro and in Vivo Pyrazinamide Resistance in M. Tuberculosis

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Pyrazinamide (PZA) is an integral component of chemotherapy for both drug-susceptible and drug-resistant tuberculosis. Unfortunately, the requirement of acidic media significantly complicates the reproducibility of phenotypic drug-susceptibility testing (DST), thus hindering its widespread use. A faster, molecular diagnostic for identifying PZA susceptibility is urgently required. The primary resistance mechanism to PZA is variants in PncA. This enzyme encodes the bacterial pyrazinamidase that is required for conversion of PZA to its active form, pyrazinoic acid (POA-). Unfortunately, single-nucleotide polymorphisms (SNPs) occur across the entire length of pncA in clinically resistant isolates. The phenotypic consequences of these mutations are unclear. To address this, we have developed an *in vitro* and *in vivo* screen to unbiasedly assay for phenotypic drug-susceptibility of all pncA SNPs. We constructed a library of pncA variants using random PCR mutagenesis to complement a *ΔpncA* strain of *M. tuberculosis*. The *in vitro* selection was performed using a range of PZA concentrations (4-500 μg ml^{-1}) in a BD BACTEC MGIT 960 PZA Kit. A complementary *in vivo* screen was also performed by infecting mice by tail vein injection or aerosolization. Treatment with 150 mg ml^{-1}PZA or a saline control was then administered for up to 42 days. Resistant clones from both the lungs and spleens were evaluated. Illumina sequencing was performed to identify enriched SNPs following *in vitro* and *in vivo* selection. Our results have enabled us to identify SNPs conferring phenotypic resistance to PZA and has allowed us to classify these clones as high- or low-level resistance mutations. Importantly, structurally modeling these SNPs onto PncA has furthered our mechanistic understanding of PZA resistance. These results will enable the development of a comprehensive genetic based diagnostic for PZA susceptibility.

Identifying Vulnerable Steps in the CoA Biosynthesis Pathway of M. Tuberculosis

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Enzymes in the pantothenate and coenzyme A (CoA) biosynthesis pathways have attracted considerable interest as potential targets for the development of drugs against a number of human pathogens, including *M. tuberculosis*. However, although potent inhibitors have been developed against pantothenate synthase (PanC), pantothenate kinase (PanK), these have failed to translate into compounds with significant whole-cell activity. In addition to issues of permeability, metabolism and efflux, such target-led approaches to TB drug discovery are confounded by a lack of understanding of target vulnerability.

In this talk, I will describe the combined genetic, physiologic and metabolomic approach we have taken to identify vulnerable steps in the pantothenate and CoA biosynthesis pathway in *M. tuberculosis*. The impact of target depletion on the viability of *M. tuberculosis* has been assessed using a set of conditional mutants in various steps in the pathway. While transcriptional silencing of *panB*, *panC* or *coaE* was bacteriostatic, coaBC silencing was apparently bactericidal in *M. tuberculosis* in vitro, as deduced by CFU enumeration. CoaBC was similarly shown to be required for growth and persistence of *M. tuberculosis* in mice, based on quantification of organ bacillary loads. However, taking advantage of the fact that *M. tuberculosis*is capable of CoaBC bypass through CoA salvage, we showed that *coaBC* silencing results in a 'non-growing but metabolically active' (NGMA) state from which non-culturable bacilli can be partially and transiently rescued by CoA salvage. The response of *M. tuberculosis* to CoA depletion is being further explored by metabolomic analyses which have elucidated similarities and differences in the way in which the organism adapts metabolically to depletion of different targets in the biosynthetic pathway. These findings have significant implications for TB drug discovery, which will be discussed.

Targeting the Membrane Proteome of mTB for Structure based Approaches to Function

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There are currently 803 transmembrane proteins in the mTB genome. There is no atomic structure for even one of these proteins, yet they govern the signaling, entry and exit from the cell. These proteins govern functions that are variously important, sometimes essential for the viability and virulence of mTB. They govern the import of nutrients and secretion systems for the export of virulence factors, and proteins that are adapted to export drugs used to treat tuberculosis. We describe a system to select among the integral membrane proteome of mTB with the end goal of determining their structure at atomic level, sufficient to determine interaction with fragments of molecules and compounds from libraries intended to block critical and essential functions in mTB alone, avoiding interference with the human host by developing selectivity. A high throughput cloning and expression for a selected and focused set of mTB membrane proteins are presented. Examples illustrate how atomic structures of integral membrane protein can be pursued and determined by X-ray crystallography, and the prospects of understanding mechanisms of them by other means. These include the generation of antibody Fab fragments from phage displayed libraries both as tools to modulate activity, and as structural aids to crystallization of electron cryo-microscopy. Illustration of recent applications of these methods and what can be learned from these approaches are presented.
http://www.msg.ucsf.edu/stroud/index.htm

Kinetic Regulation of Open Promoter Complexes by Mycobacterial Transcription Factors

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CarD is an essential and global transcriptional regulator in mycobacteria. While its biological role is unclear, CarD functions by interacting directly with RNA polymerase (RNAP) holoenzyme promoter complexes. Here, using a fluorescent reporter of open complex, we quantitate RP_o formation in real time and show that *Mycobacterium tuberculosis* CarD has a dramatic effect on the energetics of RNAP bound complexes on the *M. tuberculosis rrnA*P3 ribosomal RNA promoter. The data reveal that *Mycobacterium bovis* RNAP exhibits an unstable RP_o that is stabilized by CarD and suggest that CarD uses a two-tiered, concentration-dependent mechanism by associating with open and closed complexes with different affinities. Specifically, the kinetics of open-complex formation can be explained by a model where, at saturating concentrations of CarD, the rate of bubble collapse is slowed and the rate of opening is accelerated. The kinetics and open-complex stabilities of CarD mutants further clarify the roles played by the key residues W85, K90 and R25 previously shown to affect CarD-dependent gene regulation *in vivo*. Lastly, in contrast to *M. bovis* RNAP, *Escherichia coli* RNAP efficiently forms RP_o on *rrnA*P3, suggesting an important difference between the polymerases themselves and highlighting how transcriptional machinery can vary across bacterial genera. In future work, we aim to expand our biophysical studies of CarD to other essential mycobacterial transcription factors to gain a more complete understanding of transcriptional regulation in this important human pathogen.

Raman Biosensing for TB Diagnosis

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The global burden of tuberculosis (TB) today stands at ~9 million cases per annum worldwide. TB is currently the leading cause of mortality in South Africa and the causative agent, Mycobacterium tuberculosis (M.tb), is the most common opportunistic infection in HIV-infected persons; in South Africa today ~70% of new active TB case are HIV positive. Inaccurate diagnosis of TB disease and inability to monitor treatment response continues to be a confounder in disease transmission, patient mortality & morbidity, time to treatment initiation, and assessment of treatment response. Hence, new TB diagnostics designed for use at point-of-care in resource-poor settings represent a major unmet global health priority.
In the absence of any reliable biomarker-based tests for TB, the gold standards for accurate diagnosis continue to rely on detection of whole pathogen, either via smear microscopy, culture or nucleic acid amplification. However, these each have problems associated with sensitivity, speed, cost and suitability for use a point of care in low resource settings. In this paper, we will therefore describe research aimed at developing a novel, hand-held DNA aptamer-based surface enhanced Raman scattering (SERS) biosensor able to give a quantitative, label-free spectroscopic readout on M.tb bacillary load, whilst simultaneously confirming pathogen identity in real time at point of care.
To create the components of our platform, we have used both conventional and bead-based SELEX approaches to generate nM affinity DNA aptamers to M.tb cell surface markers. We have then developed several different, nanostructured, wide area noble metal surfaces and have combined these with electrochemical SERS techniques, the result being highly reproducible, quantitative SERS data. We have also developed a simple, field-deployable sample prep device for capture of M.tb bacilli from sputum and delivery on to our SERS biosensor. Results generated in each of these areas will be discussed.

Combining Structural Genomics and Drug Discovery to Develop New TB Drugs

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Although there are multiple effective drugs available for treating tuberculosis (TB), current strategies are greatly complicated by the long chemotherapy treatment that lasts several months. Widespread patient non-compliance has contributed to the emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) TB strains. There is a clear need for novel fast acting drugs that are capable of eliminating an infection in just a few weeks.

Our lab, in conjunction with the TB Drug Accelerator and the Structural Guided Drug Discovery Consortium, has focused on the identification of new drug targets. Our long-term goal is to identify fast acting lead compounds that would simplify chemotherapy regimens for treating resistant infections. A significant step forward has been the use of whole cell actives for high-throughput screens and subsequent target identification methods for drug development. We now have two compounds in lead-optimization for TB.

Light Forge: A Microfluidic High Throughput Platform for Rapid and Affordable Detection of Drug Resistant Strains of Tuberculosis

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Light Forge is a new scalable microfluidic platform developed at K-RITH for the genomic interrogation of *Mycobacterium tuberculosis* strains using Real-Time PCR and High Resolution Melt Analysis (HRMA) on a chip. We have used this system to identify clinical Tuberculosis strains that are resistant to Rifampicin (a frontline drug in treatment of tuberculosis) relative to a susceptible strain H37Rv based on mutations on the RpoB gene. Lightforge has the potential to contribute towards a low-cost solution to diagnosis of multidrug resistant tuberculosis- a critical global healthcare challenge. Lightforge has detected mutations linked to rifampicin resistance including single nucleotide polymorphisms (SNPs) in a manner consistent with commercial systems. In preparation for diagnosis of clinical isolates, the Lightforge approach is now being expanded to include detection of resistance to other TB drugs including fluoroquinolones and isoniazid based on mutations in the GyrA, KatG and Mab-inhA.

Energy Metabolism in Mycobacterium Tuberculosis and the Infected Host Cell

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A major obstacle to the development of successful therapeutic intervention strategies for tuberculosis (TB) is the lack of a mechanistic understanding of how M. tuberculosis (Mtb) maintains a persistent, non-replicating state in humans for years, insensitive to antimycobacterial drugs, to then unexpectedly resume growth and cause disease. Since numerous host factors can affect Mtb physiology and energy metabolism, it is likely that the mechanisms used by the bacilli to maintain energy balance during active disease are critical towards the outcome of disease. What are the redox and bioenergetics states of infected host cells, and Mtb? How do we define and measure it? Studies have shown that changes in bioenergetic metabolism accompany a wide range of human diseases, and targeting shifts in bioenergetic metabolism may hold strong therapeutic potential. My laboratory have used a combination of quantitative metabolomic, transcriptomic, bioenergetic, EPR and mass spectrometric methods to examine mechanisms whereby Mtb balances the cytoplasmic redox state and maintains bioenergetic homeostasis. I will discuss approaches for characterizing the intracellular redox and bioenergetic status of Mtb and the implications this knowledge have for studying the mode of action of antimycobacterial drugs, and pathogenicity. Lastly, I will discuss how metabolic flux analysis can be exploited for the non-invasive study of real-time Mtb bioenergetics.

Visualizing the Mycobacterial Mutasome

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Previous work in our laboratory identified a DNA damage-inducible mutagenic DNA repair system that is required for adaptive mutagenesis, including the development of drug resistance, in *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB). Moreover, genetic studies established that this so-called "mycobacterial mutasome" comprises a minimum of three essential components: a *dnaE2*-encoded PolIIIα subunit, and *imuB*- and *imuA*'- encoded accessory factors. Current work aims to investigate the recruitment dynamics and sub-cellular localization of the mutasome components following exposure of bacilli to genotoxic stress. To this end, we constructed a panel of *M. smegmatis* (*Msm*) reporter mutants encoding fluorescently tagged mutasome proteins. The DNA damage survival and induced mutagenesis functions of the recombinant proteins were assessed, and fluorescence visualized and quantified in a series of DNA damage assays. In addition, population-wide expression characteristics were assessed by flow cytometry. Results indicate that expression of enhanced green fluorescent protein (EGFP) from the DNA damage-responsive imuA' promoter initiates 60 minutes post exposure to mitomycin C (MMC), a known genotoxin, with maximal EGFP expression achieved 360 minutes post treatment. These results indicate that *Msm* responds rapidly to DNA damage, with dynamics and level of expression correlating with the amount of damage incurred. Moreover, cellular localization of mutasome components indicates differential recruitment and localization of ImuA' and ImuB in MMC-exposed cells. In combination, our results establish the utility of combining fluorescence imaging with functional genetics to elucidate the mechanisms regulating expression and activity of a major error-prone damage tolerance pathway in *Mtb*.

Actions of Plasmodium Falciparum on Its Human Erythrocyte Host Studied by Electron Tomography

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In the blood stage of malaria infection, parasites invade erythrocytes and multiply inside the parasitophorous vacuole. When mature, they escape from the host cell for further rounds of invasion. This escape requires the sequential rupture of vacuolar and erythrocyte plasma membranes, in a process called egress, which is triggered by a cascade of protease activation. Selective inhibition of different steps in this cascade blocks parasite egress. The inhibitor Compound 1 blocks the first stage of egress, leading to accumulation of mature parasites inside the vacuole, whereas the cysteine protease inhibitor E64 blocks the second step of exit, resulting in clusters of parasites contained only by the erythrocyte membrane. We used electron and X-ray tomography, electron energy loss spectroscopy and fluorescence microscopy of late stage infected cells to study membrane disruption in egress. We find evidence for leakage of cell contents across the vacuole membrane when egress is blocked by compound 1, indicating localised disruption to the vacuole membrane prior to its rupture. With E64 treatment the vacuole membrane is ruptured to form extensive, multi-layered vesicles. The results reveal substages in egress, starting with permeabilisation of the vacuole membrane immediately preceding its breakdown into swirl-like fragments, followed by breakdown of the erythrocyte membrane.
We have also used electron tomography to examine the cell surface structures known as knobs, a major virulence factor mediating cytoadherence in P. falciparum infection. In infected erythrocyte ghosts, these knob structures are supported by a spiral framework with multiple connections to the red cell cytoskeleton, suggesting how shear forces could be transmitted from adhesion sites to the cytoskeleton.

Structure-inspired Disruption of Proper Folding of an Essential Malaria Parasite Protein

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Successful drug design involves potent and selective binding of small molecules to active-sites of drugable targets such as *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (*Pf*DHFR-TS). In addition to identification of new drug targets, such as *P. falciparum* dihydroorotate dehydrogenase (*Pf*DHODH), it would be helpful to identify new ways to selectively attack existing high-value parasite protein targets.

Many protozoans, including malaria parasites, code for more than one enzyme on the same multifunctional protein, while host cells have separate corresponding enzymes. Here, we examine whether proper folding and catalytic activity of the trailing thymidylate synthase (*Pf*TS) domain relies on the leading enzyme, dihydrofolate reductase (*Pf*DHFR). Guided by the protein structure of the bifunctional enzyme and cell-free protein expression of individual domains, truncated *Pf*TS mRNA was translated either by itself, with*Pf*DHFR mRNA, or with purified *Pf*DHFR protein. Activity assays showed that *Pf*DHFR protein was required as a template for functional folding of the *Pf*TS domain. Structural interactions between the folded *Pf*DHFR and nucleation sites for proper initiation of*Pf*TS folding were identified by a combination of structure biology, “non-active site” mutations on *Pf*DHFR, and identification of inhibitory synthetic peptides based on predicted critical inter-doman contact residues. These insights open exciting new ways to target species-specific protein-protein interactions in malaria parasites and possibly other pathogens.

Modelling Blood Glucose Concentration in Malaria Patients

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We use a hierarchical modelling approach to construct mathematical models for disease states at the whole-body levels. The objective of the study is to use these models to simulate effects of drug-induced inhibition of reaction steps on the whole-body physiology. We illustrate the approach for glucose metabolism in malaria patients, by merging two detailed kinetic models for glucose metabolism in the parasite *Plasmodium falciparum* and the human red blood cell with a coarse-grained model for whole-body glucose metabolism.

The detailed kinetic model for glucose metabolism in *P. falciparum* was recently constructed in our group (Penkler et al., 2015) on the basis of enzyme kinetic experimental data and has been validated in isolated parasites and with infected red blood cells. The model gives accurate predictions of glucose metabolism in infected red blood cells, and has been successfully tested in its ability to pinpoint drug targets in the metabolic pathway. The model has been linked to a genome scale metabolic model for the parasite to identify biomarkers.

In conclusions, the hierarchical modelling framework is capable of bridging the detailed enzyme kinetic level (where drugs operate) with the physiological, whole body level, reflecting the disease state.

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Structural Biology of Some Virus Pathogens

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My group study a number of viruses, some of which present significant threats to human and animal health. Most of our basic research is aimed at understanding aspects of the virus lifecycle and host interactions. However the biological mechanisms uncovered sometimes present therapeutic opportunities, which range from small molecule antivirals, through therapeutic proteins (eg antibodies) to improved vaccines. At present picornaviruses are major targets of our work: small icosahedral viruses that include an increasing number of genera and increasing structural and mechanistic diversity. I will present some snapshots of different picornaviruses to highlight how evolution can mix and match biological mechanisms in the context of a broadly conserved structural framework for the mature capsid. Whilst there are some effective vaccines against picornaviruses, which often elicit strong antibody responses against intact viruses, these are based on 50 year old technology and are usually produced in high level containment. In contrast there are no currently licensed anti-virals for any picornavirus. I will present case studies of how structural understanding seems to be helping towards both structure-guided novel antivirals, against HFMDVs (EV71 and CVA16)¬ – major public health threats in East Asia and the structure-guided design of improved vaccines, in particular (i) FMDV, where despite a vaccine the virus remains a massive problem for farming globally and in particular in South Africa and (ii) poliovirus, where whilst the disease may be almost eliminated due to a massive sustained operation by WHO the virus lives on across the globe. I hope also to consider the question of how neutralizing antibodies work, and again pose the question – can this understanding be useful? – perhaps by allowing us to aim for vaccines that give greater cross protection.

Inhibition of Parasitic Farnesyl Diphosphate Synthases (FPPS)

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Farnesyl diphosphate synthase (FPPS), an essential enzyme involved in the biosynthesis of sterols (cholesterol in humans and ergosterol in yeasts, fungi and trypanosomatid parasites) as well as in protein prenylation, has been identified as a possible target for antiparasitic drug development. FPPS is inhibited by bisphosphonates, a class of drugs used in humans to treat diverse bone-related diseases. The development of bisphosphonates as antiparasitic compounds targeting FPPS is being considered an important route for therapeutic intervention. X-ray crystallographic and calorimetric studies of complexes of FPPS from Trypanosoma cruzi (the etiologic agent of American trypanosomiasis; Chagas disease) and Leishmania major (the causative agent of cutaneous leishmaniasis) are used to characterize binding of bisphosphonates as potential therapeutic inhibitors of these enzymes. Calorimetric studies showed that binding of bisphosphonates to these enzymes is entropically driven suggesting that one route for design may involve increasing specific interaction of the compounds for the parasitic FPPSs to compensate for the unfavorable enthalpy. Comparison of the structures of TcFPPS and LmFPPS to the human FPPS provides new information for the design of bisphosphonates more specific for inhibition of the parasitic FPPS.

Using Molecular Biophysics to Understand Plasmodium Actin Dynamics and Cell Motility

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Plasmodium is the causative agent of malaria, a devastating tropical disease. Sporozoites, the infectious forms transmitted by mosquitoes, are deposited in the skin of a mammalian host during probing for a blood meal and rely on high motility speeds (approximately 1-3 μm/s) to exit from the dermis into blood capillaries. Sporozoites display an uncommon form of locomotion known as gliding motility, whereby the force of an actin-myosin motor is transmitted through associated transmembrane adhesins to the substrate surface. Unlike its mammalian counterparts, *Plasmodium* actin filaments appear to be inherently unstable and undergo rapid turnover; with this dynamism being crucial for parasite motility. We use advanced microscopy, biophysical methods and molecular genetics to probe sporozoite motility and to understand the nature of actin dynamics in this process. Actin chimeras (whereby different regions were swapped between mammalian and *Plasmodium* forms) were introduced into the parasite genome, replacing the *actin 1* gene. We identified subdomain 4 of actin as an important contributor towards parasite motility, as the respective chimera failed to efficiently invade the insect's salivary glands and showed aberrant 'stop-go' motility pattern. Further, these observations emphasize the importance of this region in (un)stable filament dynamics.

Structural and Molecular Biology of Type IV Secretion Systems

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Type IV secretion (T4S) systems are molecular machines used for the transport of macromolecules across the bacterial cell envelope. T4S systems are highly versatile. Conjugative T4S systems translocate DNA from a donor to a recipient bacterium and contribute to bacterial genome plasticity, spread of antibiotic resistance or other virulence trait among bacterial pathogens. In some bacteria such as Helicobacter pylori (Cag PI), Brucella suis (VirB/D), or Legionella pneumophila (Dot, Icm), T4S systems are directly involved in pathogenicity as they mediate the secretion of virulence factors (DNA or toxins) into host cells. The archetypal T4S system, the VirB/D system, was defined in Agrobacterium tumefaciens where it is naturally responsible for the delivery of the T-DNA to the plant host-cell. The A. tumefaciens VirB/D system comprises 12 proteins (VirB1 to 11 and VirD4). Recently, structures of large complexes formed by several of these proteins have become available shedding unprecedented light on T4S system secretion mechanism.

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Bacterial Infections at Atomic Resolution

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Infecting a multicellular organism is not easy for a bacterium, faced by many physical barriers and a highly developed, multi-tiered immune system. Bacteria nevertheless succeed by producing and secreting dedicated proteins, or so-called virulence factors, that help them to manipulate the host processes to their advantage. We are interested in how virulence factors are designed to bind to host receptors and proteins to re-programme host responses and outcomes in their favour. For this purpose we generally analyse disease-related protein complexes in which one component is of bacterial origin and the other is the corresponding host target. We predominantly use macromolecular crystallography to investigate how bacteria infect humans and other mammals at the molecular and atomic level. In addition, we quantify the binding affinity to explain how the bacterial virulence factor is able to outcompete any physiological binding partners of the host factor.
Organisms that we are currently working on include Mycobacterium tuberculosis, the causative agent of tuberculosis, enterotoxigenic Escherichia coli (ETEC) causing dysentery mostly in small children, Listeria monocytogenes, a food-borne pathogen leading to blood poisoning, meningitis and miscarriages of unborn babies, as well as Legionella pneumophila, the cause of Legionnaires' disease. Our studies include structural analysis, biophysical investigation of protein-protein interactions of bacterial virulence factors with host proteins, as well as cell-based infection studies.

Calcium Phosphate Nanoparticle (CPNP)-entrapped Tetracycline: A Potential Drug against Diarrheal Diseases

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Diarrheal diseases, caused by bacterial genii Escherichia, Vibrio, Salmonella and Shigella, represent a major health problem in developing countries. The broad-spectrum antibiotic, tetracycline, was once the most effective drug against diarrhea. Emergence of bacterial resistance to tetracycline nowadays limits its use. In most cases, resistance generation is mediated by a family of cell membrane proteins, which block entry of tetracycline into cell cytosol. Therefore, to bring back obsolete tetracycline in further use, we venture to synthesize a nano-form of the antibiotic by loading it within calcium phosphate nanoparticle (CPNP), because CPNP has high membrane-penetrating and biodegradable properties and nowadays is used as a potential carrier of DNA, RNA, proteins and therapeutics into different cells. Nano-formulation was done through a co-precipitation method of preparation of CPNP, in presence of tetracycline, using calcium nitrate and di-ammonium hydrogen phosphate as precursors and Na-citrate as stabilizer. Synthesized Tet-CPNP was characterized by DLS, TEM, AFM, FTIR, EDS, spectrofluorimetry and dialysis techniques. Size of the spherical shaped NPs was 10-15nm. About 20% of the maximally added tetracycline (500µg/ml) was entrapped within CPNP. There was sustained release of entrapped tetracycline over seven days, implying higher antibacterial efficacies of Tet-CPNP. Bctericidal activity of nano-particulate tetracycline was investigated by agar plating, spectrophotometry, phage contrast-fluorescence microscopy and AFM technique. Minimum bactericidal concentrations of Tet-CPNP on multiple antibiotic (including tetracycline) resistant bacteria like Escherichia coli, Salmonella kentuckey and Shigella flexneri were 20-40µg/ml. NP-mediated cell filamentation and cell membrane disintegration caused cell killing. Death of Shigella-infected Zebra fish larvae was stalled by Tet-CPNP treatment. All these results implied that our nano-formulation might reclaim a nearly obsolete, cheap antibiotic to further potential function, making it highly useful to developing countries with limited health care budgets.

Microengineering for Microbiology

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Bacterial behavior is highly individualistic in the sense that individual cells living in the same environment can exhibit markedly different phenotypes. Mutation and genetic exchange are important drivers of bacterial individuation but these events are relatively rare. At higher frequencies, genetically identical cells often display metastable variation in their growth rates, response kinetics, stress resistance, and other quantifiable phenotypes. These cell-to-cell differences arise from non-genetic sources: unequal partitioning of components at cell division, stochastic fluctuations in gene expression, phenotypic memory of past events, etc. Time-dependent variation at the single-cell level generates phenotypic diversity at the population level. This diversity contributes to bacterial persistence in fluctuating and stressful environments because it increases the odds that some individuals may survive a potentially lethal change that would otherwise extinguish the population. A medically relevant example of this phenomenon is the refractoriness of bacterial infections to antibiotic therapy, which has been attributed to spontaneous phenotypic variants that survive despite prolonged exposure to bactericidal antibiotics. Bacterial persistence is not due to antibiotic resistance mutations in the classical sense, and it is not clear why some cells tolerate antibiotics that kill their genetically identical siblings. Our studies are focused on the mechanistic basis of bacterial persistence. We use correlated optical and atomic force microscopy in conjunction with purpose-built microfluidic and microelectromechanical systems for single-cell time-lapse imaging and nanomechanical measurements of bacteria subjected to antibiotics and other environmental stresses.

Drug Tolerance in Mycobacteria Replicating in a Microdialyser Mediated by an Efflux Mechanism

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Tuberculosis (TB), caused by infection with Mycobacterium tuberculosis is the world's deadliest curable disease, responsible for an estimated 1.5 million deaths annually. A major challenge in controlling TB is the requirement for prolonged (6 to 9 months of) multidrug therapy to overcome drug-tolerant mycobacteria that persist in human tissues, although the same drugs can sterilize genetically identical mycobacteria growing in axenic culture within days. Recent studies have shown that the intracellular (or intramacrophage) mycobacterial sub-population, which makes up an essential component of human TB infection, is significantly more tolerant to antibiotics compared to the extracellular population. To investigate intramacrophage drug tolerance, we present a microdialyser—a microfabricated physical cell culture system that mimics confinement of replicating mycobacteria, such as in a macrophage during infection. Furthermopre, unlike bactericidal antibiotics, bacteriostatic drugs ultimately depend upon the immune system for sterilization, and are therefore poor treatment options where the immune system is compromised, as in the case of co-infection with HIV. Distinguishing between bacteriostatic and bactericidal action of antimicrobial drugs can be cumbersome using conventional drug susceptibility testing methods but the microdialyser can rapidly resolve this distinction for antimicrobial agents.

Elucidating the Differences between Eukaryotic and Prokaryotic Type II Pantothenate Kinases

Lizbe Koekemoer, Erick Strauss.
University of Stellenbosch, Stellenbosch, South Africa.

Coenzyme A (CoA) is a ubiquitous cofactor essential to all living organisms for its role in numerous metabolic processes including energy metabolism and fatty acid biosynthesis. CoA is biosynthesized de novo from pantothenic acid (Vitamin B5) in 5 enzymatic steps. Although these steps are largely conserved amongst different organisms, the enzymes are not and slight variations are found amongst the enzymes that catalyses the same step in different organsims. These differences are what we are exploiting in the development of selective inhibitors that targets CoA biosynthesis in pathogenic organisms, but do not affect the production thereof in humans.
This project focuses on the first enzyme in the CoA biosynthesis pathway, panthothenate kinase (PanK), that catalysis the ATP dependant phosphorylation of pantothenic acid to 4'-phosphopantothenic acid. PanKs are unique in that there are three distinct PanK types (type I, II and III) which differ in all aspects from molecular level to 3D structure. The type II PanKs are the eukaryotic version of this enzyme, although versions thereof are also found in the pathogen responsible for nosocomial infections, Staphylococcus aureus, and in selected Bacilli. Although grouped together into the same type, the kinetic profiles we obtained for the prokaryotic PanKs differ greatly from the kinetic profiles obtained with the human PanKs and also from each other. These differences between the eukaryotic and prokaryotic enzymes can be explained from available structural data, but this was a surprising result for the prokaryotic PanKs. In this study we set out to explain the structural basis for the observed differences between the prokaryotic PanKs by doing mutagenesis studies, CD analysis and co-crystallization with various ligands. From these results we gained new insight into the method of ligand binding and the required enzyme conformation for activity.

Post-Translational Modification of a Nucleoid Associated Protein Regulates Cell State in Mycobacteria

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Although there are many known mechanisms by which eukaryotic cells achieve epigenetic inheritance, the mechanisms by which prokaryotic cells generate heritable, semi-stable differences in state remain poorly understood. Here we demonstrate that with a population of mycobacterial cells, there are privileged subpopulations of cells that are more tolerant of antibiotic killing than the majority of cells. We show that these differences in cell state are heritable and semi-stable. We further demonstrate that there is extensive post-translational modification of the nucleoid associated protein, HupB, at predicted DNA binding residues. Mutation of the sites of post-translational modification disrupts the formation of subpopulations of antibiotic tolerant cells. We propose that metabolically driven modification of HupB acts as an epigenetic regulator, controlling the formation of a specialized subpopulation of antibiotic tolerant cells.

XChem: From Crystals to Potent Molecules with X-Rays and Poised Synthesis

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Fragment-based lead discovery is now a well-established as a powerful approach to early drug or lead discovery: since small (<250Da) compounds ("fragments") tend to bind relatively promiscuously, hits can be readily identified by screening against comparatively small compound libraries (100s-1000s). What remains challenging is that hits typically bind weakly: not only must the screening technique be sufficiently sensitive, but potency can only be achieved through considerable synthetic elaboration. Historically, the most sensitive primary screening technique of all, direct observation in crystal structures, has been too challenging to be achievable by but a few labs world-wide. Equally, no consensus has yet emerged on systematic strategies for synthetic follow-up.
<!--250Da-->Now, beamline I04-1 at Diamond Light Source has established X-ray screening as a routine medium-throughput experiment with a capacity of up to 500 crystals/day (from soaking to dataset), a facility being offered to Diamond users since April 2015, with dedicated weekly beamtime. The highly streamlined process includes image recognition for crystal targeting, soaking by acoustic dispensing, robot-assisted harvesting, unattended X-ray data collection, automatic data integration, and pan-dataset electron density analysis for detecting hits. The technology was developed as a joint research project with the Protein Crystallography group of the SGC at Oxford University, and has been validated on a series of diverse targets, all of which have yielded hits.
Moreover, a "poised" fragment library has been developed that provides clear and robust routes to first-shell follow-up: combined with new algorithms for prioritizing compounds, the ultimate ambition is to establish how potency can be achieved cheaply from very limited initial experiments. If achievable, this would have a major impact all aspects of biological research.

Structure-based Discovery of Novel DNA Gyrase B Inhibitors

Matjaz Brvar[1], Andrej Perdih[1], Tihomir Tomasic[2], Nace Zidar[2], Lucija PeterlinMasic[2], Janez Ilas[2], Danijel Kikelj[2], Marko Anderluh[2], **Tom Solmajer**[1].
[1]National Institute of Chemistry, Ljubljana, Slovenia, [2]Faculty of Pharmacy University of Ljubljana, Ljubljana, Slovenia.

The emergence of bacterial resistance to most of the clinically used antibiotics and the urgent need for the discovery of potent antibacterials with broad spectrum of efficacy and improved safety profile has revived the research in this field [1]. One of the well established targets is the DNA gyrase B[2], a topoisomerase with an ATP-ase activity. In our ongoing effort to identify low molecular weight inhibitors of DNA gyrase B we used structure-based design approach. Starting from the available structural information[3] we identified a series of novel indolinone-2-ones,[4] 2-2-amino-4-(2,4 dihydroxyphenyl)thiazoles,[5] and more recently, 4'-methyl-N2-phenyl-[4,5'-bithiazole]-2,2'-diamine inhibitors of gyrase B with a low micromolar inhibitory activity[6]. These inhibitors were subsequently extensively characterized by various biophysical techniques (differential scanning fluorimetry, surface plasmon resonance and microscale thermophoresis). Further studies resulted in discovery of 4,5-dibromopyrrolamides, indolamides and 4,5,6,7-tetrahydrobenzo[1,2-d]thiazoles[7] as ATP competitive DNA gyrase B inhibitors. Details of a nanomolar inhibitor 4-(4,5-dibromo-pyrrole-2-carboxamido)benzamide binding mode were revealed by high-resolution crystal structure of the complex with E. coli DNA gyrase B.[8] .References[1] E.D. Brown, G. D. Wright, Chem. Rev. 2005, 105, 759.[2] M. Oblak, M. Kotnik, T. Solmajer, Curr. med. chem., 2007, 14, 2033.[3] R.J. Lewis, D.B. Wigley et al., EMBO J. 1996, 15, 1412.[4] M. Oblak, T. Solmajer et al., Bioorg & Med. Chem. Lett. 2005, 15 5207.[5] M. Brvar, T. Solmajer et al., Bioorg. Med. Chem. Lett. 2010, 20, 958.[6] M. Brvar,T. Solmajer et al., J. Med. Chem. 2012, 55, 6413.[7] T. Tomasic, N., T. Solmajer et al., – J. Med.Chem.2015, Articles ASAP .[8] N. Zidar, T. Solmajer et al., - J. Med. Chem.2015, Articles ASAP

Mycobacterium Tuberculosis Growth in Dead Infected Cells Results in a Positive Feedback Loop Driving Additional Cycles of Host Cell Death

Deeqa Mahamed[1], Steven Skroch[1], Lance Oom[1], Gopelkrishna Sreejit[1], Sameshnee Chinnasamy[1], Oana Catinas[1], Myshnee Naicker[1], Sanisha Rampersad[1], Colisile Mathonsi[1], Jessica Hunter[1], Alex Pym[1], Gil Lustig[1], **Alexander Sigal**[1,2].
[1]KwaZulu Natal Research Institute for TB and HIV, Durban, South Africa, [2]Max Planck Institute for Infection Biology, Berlin, Germany.

Mycobacterium tuberculosis causes heterogeneous infection in the same lung, ranging from infection foci where infection is controlled to foci with active infection and host cell necrosis. To understand what causes this heterogeneity, we used long-term live cell imaging to follow infection of human macrophages. I will discuss our yet unpublished results indicating that initiation of a cascade of cell death involving positive feedback provides a mechanism for the co-existence of active and quiescent infection foci within the same individual.

Complex Long-Distance Effects of Mutations that Confer Linezolid Resistance in the Large Ribosomal Subunit

Simone Fulle, Jagmohan S. Saini, Nadine Homeyer, **Holger Gohlke**.
Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany.

The emergence of multidrug-resistant pathogens will make current antibiotics ineffective. For linezolid, a member of the novel oxazolidinone class of antibiotics, 10 nucleotide mutations in the ribosome have been described conferring resistance. Hypotheses for how these mutations affect antibiotics binding have been derived based on comparative crystallographic studies. However, a detailed description at the atomistic level of how remote mutations exert long-distance effects has remained elusive.
Here, we show that the G2032A-C2499A double mutation, located > 10 Å away from the antibiotic, confers linezolid resistance by a complex set of effects that percolate to the binding site.[1,2] By extensive all-atom molecular dynamics simulations and free energy calculations, we identify U2504 and C2452 as spearheads among binding site nucleotides that exert the most immediate effect on linezolid binding. Structural reorganizations within the ribosomal subunit due to the mutations are likely associated with mutually compensating changes in the effective energy. Furthermore, we suggest two main routes of information transfer from the mutation sites to U2504 and C2452. Between these, we observe cross-talk, which suggests that synergistic effects observed for the two mutations arise in an indirect manner. These results should be highly relevant for the development of oxazolidinone derivatives that are active against linezolid-resistant strains.

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Super Resolution Microscopy Reveals a Preformed NEMO Lattice Structure that is Collapsed in a Genetic Disease

Musa Mhlanga.
CSIR Biosciences, Pretoria, South Africa.

The NF-κB pathway is one of most important signaling cascades in various living organisms, with critical roles in cancer and the immune and inflammatory response. In clinical settings, it has therefore become increasingly necessary to understand the mechanism of disease caused by mutations in genes of this pathway. However, certain aspects of this cascade, notably the rapidity and efficiency with which it is executed remain unexplained. Several lines of evidence have led to the hypothesis that the regulatory/sensor protein NEMO is responsible for this efficiency by acting as a binary switch that depends on polyubiquitin chains. In this study,we use super-resolution microscopy to visualize the existence in non-stimulated cells of higher-order NEMO lattice structures dependent on the presence of polyubiquitin chains, which allow proximity based trans-autophosphorylation leading to cooperative activation of the signaling cascade. We also show that NF-κB activation results in both qualitative and quantitative modification of these structures. These data evoke the formation of higher order structures in signal transduction as key insulators of noise in signal transduction cascades, permitting Hill function responses to external stimuli.

Open Access Chemical Probes of Chromatin Regulators

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University of Toronto, Toronto, Canada.

Regulation of gene expression via chromatin associated factors and alterations of the cellular epigenome are fundamental to most biological processes, to many disease mechanisms and to host-pathogen interactions and immune evasion. We are taking a protein family approach to understand how chromatin regulatory proteins recognize specific histone tail sequences and their posttranslational modifications. Proteins such as histone methyltransferases, demethylases, acetyltransferases and bromodomains and chromodomains mediate nuclear signaling networks that regulate epigenetic cellular states and gene expression programs. Systematic structural and biophysical analyses of these human and parasite protein families and their binding partners are revealing key features of selectivity and regulation among these factors, enabling structure-based development of potent, selective, cell-active small molecule inhibitors of individual epigenetic regulatory proteins. I will describe the various biophysical methods we use for characterizing human and parasite epigenetic regulators and provide examples of their manipulation in human cells using selective epigenetic chemical probes.

Insights into Molecular Switch: Crystal Structure Analysis of Wild Type and Fast Hydrolyzing Mutant of EhRabX3, a Tandem Ras Superfamily GTPase from Entamoeba Histolytica

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[1]Indian Institute of Science Education and Research (IISER) Bhopal, Bhopal, Indiana, India, [2]National Institute of Infectious Diseases, Shinjuku-ku, Tokyo, Shinjuku-ku, Tokyo, Japan.

See abstract: 22-POS Board 22

Moving Forward New Medicines and New Targets in Malaria

Timothy Wells.
Medicines for Malaria Venture, Geneva, Switzerland.

Drug discovery in malaria in the last decade has been built on two pillars and both of these have shown success for some of the projects we have been collaborating on. First, structure-based drug design has been used to optimize new molecules. Using the three dimensional structures of dihydrofolate reductase new inhibitors have been designed to overcome resistance, and the structure of dihydro-orotate dehydrogenase new classes of compound have been optimized which are active against the parasite both in vitro, and in studies in patients and volunteers. On the other hand, technological developments in phenotypic screening mean that almost 6 million compounds have now been screened directly against the parasite. This has yielded several new membrane bound targets, including the sodium channel PfATP4 and the lipid kinase PI4-kinase. These represent high value targets for structural evaluation, and for structure based optimization, especially if such models can be used to suggest new chemical scaffolds. The other output from this process has been the availability of a collection of high value hits, called Malaria Box which has been used as a test set by several groups to understand better the pharmacological impact of these compounds on the parasite.

Bacterial Manipulations of the Host Cell Proteome

Michael Starnbach.
Harvard Medical School, Boston, USA.

Intracellular bacterial pathogens directly alter host cells in order to survive. While microarray analysis has been used to identify changes to host transcription during infection, it has remained difficult to catalog post-transcriptional alterations that also occur. We have applied the global protein stability (GPS) platform and quantitative proteomics to identify changes in host protein stability following infection with the obligate intracellular pathogen Chlamydia trachomatis. Our results suggest that C. trachomatis profoundly remodels the host proteome independently of changes in transcription and some of these regulated proteins are essential for bacterial replication. These direct proteomic approaches can now be applied to examine a broad range of host-pathogen interactions and to begin developing potential host-based therapeutics.

On the Mechanism of the Amidases – Consequences for Sulfhydryl Catalysis and Drug Design

B T. Sewell, S W. Kimani, R Hunter.
University of Cape Town, Cape Town, South Africa.

The amidases, are thiol enzymes that catalyze the hydrolysis and condensation of non-peptidic amide functional groups. Amidases occur in both prokaryotic and eukaryotic organisms, where they play important physiological roles including the synthesis of metabolites, protein post-translational modification, vitamin and co-enzyme metabolism and protein and amino acid deamination. Enzymes in this family find application as biocatalysts in the fine chemical industry, and as tools for drug synthesis. In addition, the glutamine dependent NAD+ synthetase in Mycobacterium tuberculosis, which contains an amidase domain, has been suggested as a potential drug target. We have studied the mechanism of a model amidase from a psychrophile: Nesterenkonia species (NitN). The enzyme has four catalytically essential residues: a cysteine, two glutamates and a lysine. We have investigated the geometry and interactions in the active site using X-ray crystallography and quantum mechanical modeling. Our observations strongly indicate that the generally accepted view that assistance by a glutamate acting as a general base catalyst is needed for thiolate generation in order for the nucleophilic addition step to proceed, is incorrect. Our results clearly demonstrate that realization of stereoelectronic alignment of the sulphur lone pair orbital and the amide LUMO allows the initial nucleophilic addition step of the hydrolysis to proceed via a neutral thiol (sulfhydryl group), so long as there is general acid catalysis on the carbonyl oxygen. In this regard the reaction, rather than being driven primarily by the nucleophilicity of the sulfur as a thiolate, may proceed via a combination of neutral sulfur nucleophilicity with stereoelectronic alignment in tandem with mild carbonyl electrophilicity enhancement as a result of the general acid catalysis.

POSTER ABSTRACTS

POSTER SESSION I

Monday, November 16, 17:30 – 19:30
Old Wine Cellar of The Spier Wine Estate

All posters are available for viewing during all three poster sessions; however, below are the formal presentation times when presenters are required to remain in front of their poster boards to meet with attendees.

Abdalrahman, Tamer	1-POS	Board 1
Barnard, Leanne	3-POS	Board 3
Brown, David	5-POS	Board 5
Cruz, Victor	9-POS	Board 9
Elangovan, Ravikrishnan	13-POS	Board 13
Fanucchi, Stephanie	15-POS	Board 15
Hatherley, Rowan	16-POS	Board 16
Lubbe, Lizelle	21-POS	Board 21
Magagula, Loretta	24-POS	Board 24
Mandyoli, Lungelo	25-POS	Board 25
Morth, Jens Preben	31-POS	Board 31
Quentin, Dennis	37-POS	Board 37
Shobo, Adeola	38-POS	Board 38
Sigauke, Lester	39-POS	Board 39
Singh, Saurabh	40-POS	Board 40
Sundberg, Eric	42-POS	Board 42
Weber, Brandon	45-POS	Board 45

Posters should be set up on the morning of November 16 and removed by 19:30, November 19.

1-POS Board 1

Computational Simulation of Mechanical Interactions between a Cell and its Environment Considering Focal Adhesions and Substrate Stiffness

Tamer Abdalrahman[1], Laura Dubuis[1,2], Neil Davies[2], Jason Green[2], Thomas Franz[1].
[1]Division of Biomedical Engineering, University of Cape Town, Observatory, Western Cape, South Africa, [2]Cardiovascular Research Unit, University of Cape Town, Observatory, Western Cape, South Africa.

Introduction
Cell and viral mechanics including cell-environment, cell-cell and cell-virion interactions may play important roles in aetiology of infectious diseases. Exploring these interactions experimentally is often challenging, and computational modelling offers a complementary approach of inquiry.

Methods
The three-dimensional geometry of a fibroblast, distinguishing cytosol and nucleus, was reconstructed from confocal micrographs (ScanIP, Simpleware Ltd, Exeter, UK) of a cell cultured two-dimensionally and stained with phalloidin (for actin fibres) and Hoechst (for nucleus). The geometry was imported in ABAQUS 6.12 (Dassault Systèmes, Providence, USA) and complemented with a 0.01 μm thick membrane enveloping the cytosol and a flat substrate (thickness: 10 μm). Focal adhesions (FA) were represented with estimated sizes and locations using cohesive elements. Mechanical properties of the cell components from literature were used, and the elastic modulus of the substrate was varied (E_{Sub} = 0.01, 0.14, 1, and 10 MPa). The substrate was stretched to $\lambda = 1.1$ (equivalent to a strain of $9.5e^{-2}$) and the resulting deformation of the cell was assessed for different substrate moduli.

Results
The largest maximum principal strain predicted for E_{Sub} = 0.01, 0.14, 1, and 10 MPa, respectively, was $4.58e^{-4}$, $5.27e^{-4}$, $5.33e^{-4}$ and $5.34e^{-4}$ in the membrane, $1.18e^{-3}$, $1.33e^{-3}$, $1.34e^{-3}$ and $1.34e^{-3}$ in the cytosol, $6.88e^{-6}$, $8.17e^{-6}$, $8.27e^{-6}$ and $8.29e^{-6}$ in the nucleus, and 1.87, 1.96, 1.97 and 1.97 in the FA.

Discussion
The maximum FA strains are likely to be overestimated due to absence of a detachment criterion in the FA formulation. Nevertheless, the results indicate increased cell deformation with increased substrate modulus for a simple case and present a point of departure for the advancement of the models and methods towards the assessment of more complex in cell and virion mechanics.

3-POS Board 3

Developing Degradation Resistant Antimicrobials

Leanne Barnard[1,2], Willem Van Otterlo[2], Erick Strauss[1].
[1]Stellenbosch University, Stellenbosch, South Africa, [2]Stellenbosch University, Stellenbosch, South Africa.

The emergence of multidrug-resistant organisms is one of the main driving forces for the continuous development of new antimicrobial chemotherapies. Previous research established that Coenzyme A (CoA) promotes the growth of various disease-causing bacteria, including *Staphylococcus aureus*, *Plasmodium falciparum* and *Mycobacterium tuberculosis*. Consequently, the CoA biosynthetic pathway is seen as a prospective target for antimicrobial chemotherapies. The first committed step in CoA biosynthesis entails the ATP-dependent phosphorylation of pantothenic acid (Vitamin B5) to 4'-phosphopantothenic acid by pantothenate kinase (PanK). Recent *in vitro* studies have provided evidence that PanK is inhibited by a class of pantothenic acid analogues, namely N-substituted pantothenamides. However, this promising antimicrobial activity is lost when such tests are performed *in vivo* due to enzymatic degradation of the pantothenamides by pantetheinase enzymes.

This study focused on the design and synthesis of new potent inhibitors (based on the pantothenamide scaffold) that are resistant to degradation caused by the pantetheinase enzymes. This will be achieved by making modifications to current potent pantothenamide growth inhibitors to protect the amide bond from hydrolysis. Specifically, the amide bond will be modified to be more sterically hindered though the addition of methyl groups, or by replacing it with a bioisostere moiety that should withstand pantetheinase degradation.

From ten proposed compounds, we successfully synthesized 9 derivatives to date which include bioisosteres such as sulfonamides, thioamides, hydrazides as well as methylations either on the α-carbon, β-carbon or the amide bond. These compounds we tested to determine whether they still act as potent inhibitors of *S. aureus* and *Sa*PanK-II. Furthermore, we used the *Sa*PanK-II crystal structure (PDB 4M7X) to rationalize why some of the analogues acted as poor substrates for *Sa*PanK-II and as poor inhibitors for *S. aureus*.

5-POS Board 5

JMS: Creating and Running Complex Computational Pipelines on High Performance Computer Clusters

David Brown, David Penkler, Thommas Musyoka, Özlem Tastan Bishop.
Rhodes University, Grahamstown, South Africa.

Modern computing has enabled research that was previously considered unfeasible. Parallel algorithms have been developed to run over powerful multicore machines. For even more computing power, these machines can be aggregated together into large high performance computing (HPC) clusters. On these clusters, jobs can be spread out across a large number of nodes instead of being executed on a single machine. This can substantially decrease the time required to execute resource intensive modeling and simulation jobs – a common requirement in the field of biophysics. It is also useful when a large number of much smaller jobs need to be executed. Unfortunately, running jobs on a cluster involves a steep learning curve. Jobs must be submitted via software systems known as resource managers. These systems are usually run via the command line and require expertise that most researchers do not have. To solve this problem, we have developed JMS (Job Management System), a web-based front-end to an HPC cluster. JMS allows users to run, manage and monitor jobs via a user-friendly web interface. It also lets users create new tools that can be pipelined together with existing tools to create complex computational workflows. These workflows can be saved, versioned and reused as needed. All tools, workflows and jobs can be shared with other users to create a highly collaborative work environment. In addition, tools and workflows can be made public via external interfaces. Although applicable to any field, JMS is currently being tailored toward structural bioinformatics with the introduction of tools and workflows for homology modelling, docking studies, and molecular dynamics. JMS has been open-sourced and is freely available at https://github.com/RUBi-ZA/JMS.

9-POS Board 9

Multiscale Simulation to Understand the Bactericidal Action of the AS-48 Antimicrobial Peptide

Victor Cruz, Javier Ramos, Javier Martinez-Salazar.
IEM-CSIC, Madrid, Spain.

The AS-48 bacteriocin is a cyclic peptide with selective antimicrobial activity towards Gramm-positive and Gramm-negative bacteria[1].
Although the structure of the peptide has been elucidated by NMR[2] and X-ray crystallography[3], little is known about the precise mechanism of action. Some electroporation data suggest that the peptide is able to promote pore formation in bacterial membranes[4].
We performed Molecular Dynamics simulations at atomistic and coarse-grained scales to study the structural characteristics of AS-48 and its interaction with different lipid bilayers. The Force Fields used were Gromos 53A6 for the atomistic resolution and Martini[5] for the mesoscale representation.
Previous work on self-assembling simulations had revealed the ability of the amphoteric bacteriocin to promote two kinds of pores with different size[6].
The results show that AS-48 preferentially binds bilayers composed by anionic rather than zwitterionic lipids, as is the case for bacterial membranes.
In summary, the simulation protocols help to understand the mechanistic implications of the AS-48 bioactivity and will be able, for example, to propose mutations that could improve the AS-48 antimicrobial action.

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13-POS Board 13

Spot Immuno-Magnetic Capture for Isolation and Detection of Salmonella Typhi

Ravikrishnan Elangovan[1], Saurabh Singh[3], Mohita Upadhyaya[3], Vikas Pandey[2], Shalini Gupta[2], Vivekanandan Perumal[3].
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Circulating tumor cells, fetal cells or pathogen cells in peripheral blood can be present at concentration less than 10 cells/ml, and are classified as rare cells. Sensitive detection of circulating tumor cells or pathogen cells could empower physician to execute effective treatment. Clinically relevant concentration for detecting circulating tumor cells or S. typhi cells in blood can be as low as 0.2-2 cells/ml. At this low concentration, even the presence of a single cell becomes a Poisson distribution, to diagnose with high confidence one need to analyze a larger sample volume, typically 5-10 ml of patient blood is used for a single test. Current methods for rare cell detection include micro/nano patterned single use chips manufactured in clean room facility and involve automated scanning for detection. These methods are expensive and not affordable in developing countries. We have developed a low cost, milli-fluidic chip and immuno-magnetic enrichment method that allows enrichment of rare cells by 100,00X/ml. We have demonstrated the principle by capturing fluorescently labeled S. typhi cells using 100 nm magnetic particles with anti-typhi antibodies. Sample was processed at 200µl/min with 90% capture efficiency to a 1.6 mm capture spot with final volume of 100 nl. 100000X target enrichment allows us to improve the limit of detection of fluorescence imaging from 100000 CFU/ml to 1 CFU/ml.

15-POS Board 15

The UMLILO Long ncRNA Exploits Pre-formed 3D Chromatin Folding to Coordinate Rapid Chemokine Gene Activation

Stephanie Fanucchi[1,2], Ezio T. Fok[1,2], Emiliano Dalla[3], Youtaro Shibayama[1,2], Stoyan Stoychev[4], Maxim Imakaev[5], Ken W. Sung[6], Musa M. Mhlanga[1,2,7].
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Long non coding RNAs (LncRNAs) are emerging as key intermediates that control gene regulation by coordinating 3D chromatin structure. Here we report a new super-enhancer resident enhancer-like lncRNA, UMLILO, which is brought in close proximity to the chemokine genes by pre-formed chromosomal contacts. Despite lacking a homolog in mice, we show that depletion of UMLILO by siRNA or CRISPR-mediated replacement with an EGFP reporter is sufficient to abrogate chemokine transcription in human cells. By acting in cis, UMLILO uses the local 3D chromatin compaction of the pre-formed chemokine TAD to direct the WDR5-MLL1 complex across the chemokine promoters, facilitating their H3K4me3 activation. In this way, we reveal how pre-formed chromatin loop organization can act as a topological platform to insulate a key transcriptional pathway from gene-intrinsic noise, to achieve rapid and robust chemokine expression. Remarkably, by replacing UMLILO with HOTTIP at its endogenous genomic location, we show the activity of UMLILO can be substituted with a different WDR5-interacting lncRNA. As aberrant expression of these chemokines underlies multiple disease states, such as severe sepsis, adjustment of chemokine levels by altering UMLILO activity may represent a valuable therapeutic strategy.

16-POS Board 16

An Interactive Online Homology Modelling Tool to Assist with Protein Structural Studies

Rowan A. Hatherley, David K. Brown, Ozlem Tastan Bishop.
Rhodes University, Grahamstown, South Africa.

Homology modelling has become a fundamental technique in protein structural studies and in silico drug development involving proteins with no solved structures available in the Protein Data Bank (PDB). Advances in structural prediction have led to the development of a number of automated modelling servers, which focus on modelling protein structures with only a sequence as required input. These can incorporate threading and ab initio techniques which are especially useful for challenging targets, when homology modelling may not be suitable. Unfortunately, the advanced algorithms of these tools leave the user unable to follow the modelling process or adjust parameters to achieve a better model. Presented here is a homology modelling tool which has been designed to allow users to interactively control each stage of the modelling process, using the program MODELLER. The tool aims to be transparent, allowing users to know which programs are being used and what parameters are being passed to these. The scripts for the tool were written in Python and integrate a number of different programs. Collectively these help users to identify and evaluate templates, to perform alignments, to perform modelling and evaluate the models produced. Users are given the ability to revisit any of the steps in the process and make modifications as they see fit. A web interface has been developed that provides academic users with access to this tool for their own research purposes. It has been designed as a single-page application and is easy to use, intuitive and allows modelling results to be reproducible. This tool has been designed for use by researchers with varying degrees of experience in structural bioinformatics and will be a great help to protein structural studies.

21-POS Board 21

The Influence of Angiotensin Converting Enzyme Mutations on the Kinetics and Dynamics of N-Domain Selective Inhibition

Lizelle Lubbe, Trevor B. Sewell, Edward D. Sturrock.
University of Cape Town, Cape Town, South Africa.

Angiotensin-1-converting enzyme (ACE) is a zinc metalloprotease that plays a major role in blood pressure regulation via the renin-angiotensin-aldosterone system. ACE consists of two domains with differences in inhibitor binding affinities despite their 90% active site identity. While the C-domain primarily controls blood pressure, the N-domain is highly selective for cleavage of the antifibrotic *N*-acetyl-Ser–Asp–Lys–Pro. Inhibitors, such as 33RE, that selectively bind to the N-domain thus show potential for treating fibrosis without affecting blood pressure. The aim of this study was to elucidate the molecular mechanism of this selectivity using *in vitro* and *in silico* techniques.

ACE inhibition by 33RE was characterized using a continuous kinetic assay with fluorogenic substrate. The N-domain displayed nanomolar (K_i = 11.21±0.74 nM) and the C-domain micromolar (K_i = 11278±410 nM) inhibition, thus 1000-fold selectivity. Residues predicted to contribute to selectivity based on the N-domain-33RE crystal structure were mutated to their C-domain counterparts. S_2 subsite mutation drastically decreased affinity (K_i = 2794±156 nM) due to loss of hydrogen bonds, yet did not entirely account for selectivity. Additional substitution of all unique S_2' residues completely abolished selectivity (K_i = 10009±157 nM). Interestingly, these residues do not directly interact with 33RE. All six mutants were therefore subjected to molecular dynamics simulations in the presence and absence of 33RE. Trajectory analyses highlighted the importance of S_2' subsite residues in formation of a favourable contact face between the two ACE subdomains and thus a stable, closed, ligand-bound complex.

This study provides a molecular basis for the inter-subsite synergism responsible for 33RE's 1000-fold N-selectivity and aids the future design of novel inhibitors for fibrosis treatment.

24-POS Board 24

LncRNA Discovery in Host-Pathogen Interactions

Loretta Magagula[1,2,3], Janine Scholefield[1,2], Youtaro Shibayama[1,2], Joana Cruz[4], Frank Brombacher[3], Musa Mhlanga[2,3,4].
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In the last year alone, a handful of studies have identified long noncoding RNAs (lncRNAs) linc-Cox2, Lethe, PACER and THRIL as central molecular players in host cell innate immune response against microbial infection. Gene regulation has emerged as a prevailing theme in lncRNA functioning. These discoveries and the vast numbers of uncharacterized lncRNAs identified by high-throughput next-generation transcriptome sequencing technologies, set a precedence for further investigation and characterization of lncRNAs in infection biology. Importantly, lncRNAs may serve as important diagnostic markers of infection as well as therapeutic targets. These aspects, although extensively being explored in cancer research, have been neglected in infection biology, particularly in microbial infection.

In this study, RNA-Sequencing (RNA-*Seq*) was used to identify subtle vairations in transcriptional activity, with particular emphasis on lncRNA differential expression, and uncover their physiological relevance during *Listeria monotocytogenes* infection. To this end, an RNA-Seq dataset of *Listeria*-infected HeLa cells was subjected to several variations of data analysis lncRNA discovery pipelines. Potential lncRNA functioning was hypothesized using a derivation of the Rinn & Chang "guilt by association" approach in which lncRNA functioning was hypothesized based on known functions of tightly co-expressed protein coding mRNAs. "Guilty" lncRNAs were then knocked down in the HeLa cells using transcription activator-like nucleases (TALENs) to validate their candidacy as infection-regulating lncRNAs. Preliminary investigations conducted in this study have revealed potential *Listeria* infection-inhibiting lncRNA candidates in the HeLa cell model. Furthermore, we are currently exploring the use of a physiologically relevant cellular model, induced pluripotent stem cell (iPSC) monocyte derived macrophages (MDMs), to validate identified lncRNA candidates.

25-POS Board 25

Structural Characterization of EtpA, an Adhesin from Enterotoxigenic Escherichia Coli (ETEC)

Lungelo Mandyoli, Wolf-Dieter Schubert,
University of Pretoria, Pretoria, Gauteng, South Africa.

Enterotoxigenic *Escherichia coli* (ETEC) are the most common bacterial pathogens causing diarrhoea in developing countries and in travelers to endemic countries. They cause hundreds of thousands of deaths, mostly in children. As part of its infection strategy, ETEC invades and colonizes small intestinal epithelial cells where it secretes heat-labile and/or heat-stable enterotoxins, inducing diarrhoea. The ability of ETEC to invade human epithelial cells is a hallmark of its pathogenicity and is controlled by a set of plasmid and chromosome encoded virulence factors. They include EtpA, a 170 kDa plasmid encoded autotransporter. During infection, EtpA functions as an adhesin that links flagellin at the tip of ETEC flagella to the host cell surface. ETEC hence interacts with host cells through its flagella appendages to deposit its toxins. Antibodies targeting either EtpA or the conserved regions of flagellin impair delivery of the heat-labile toxin *in vitro*, and prevent intestinal colonization of mice following gastrointestinal challenge with ETEC. EtpA is thus critical to the pathogenicity of ETEC. In our study we are aiming to elucidate the structure of EtpA to explain how it is able to perform its bridging function. We have cloned and are producing a truncated version of EtpA (57 kDa) termed N-terminal EtpA or N-EtpA as a C-terminal His6-tagged fusion protein in *E. coli* TOP10 cells. The protein is purified to homogeneity by metal affinity chromatography (MAC) using Ni-NTA and size exclusion chromatography (SEC) on a Superdex 75 10/30 column. Biophysical characterization of N-EtpA using circular dichroism spectroscopy (CD) revealed the typical spectrum of a β-helical protein. Recording CD spectra at increasing temperatures indicate N-EtpA to be highly thermal stable retaining its conformation up to 95℃. Crystallization experiments of N-EtpA are currently under way.

31-POS Board 31

MgtA from E. Coli Show Strong Cardiolipin Dependent Activity and is Stimulated by Free Mg^{2+} in the Sub Micromolar Range

Saranya Subramani, Harmonie Perdreau-Dahl, **Jens Preben Morth**.
Oslo University, Oslo, Norway.

Clusters of virulence genes, called pathogenicity islands, are incorporated into the genome of pathogenic microorganisms. The clusters are normally absent in the non-pathogenic microorganism of the related species. Pathogenicity islands containing genes associated with bacterial pH and magnesium homeostasis have been shown to play a crucial role in bacterial virulence, thus emphasizing the important role of magnesium and acidification of the local environment during bacterial virulence and survival within the host cell.
The Magnesium transporter A (MgtA) is a specialized P-type ATPase important for with magnesium import. The gene mgtA is upregulated when low magnesium levels or low pH in the periplasmic space activate the two-component system PhoQ/PhoP. PhoQ/PhoP is linked with virulence in most pathogenic bacteria. These studies demonstrate, for the first time, that MgtA is highly sensitive to free magnesium levels in solution and follow a kinetic model that suggest initial activation at sub micromolar levels by free magnesium (1-10 μM) followed by strong substrate inhibition at elevated magnesium levels, already at 1 mM, indicating that MgtA may act as a cytoplasmic magnesium sensor as well as a transporter. The activity of MgtA is highly dependent on the presence of phosphatidylglycerols (PG) lipids and in particular cardiolipin. Overexpressed MgtA in E. coli furthermore confirm that MgtA co-localize with the cardiolipin/PG lipid rafts in the plasma membrane. In the presence of CL the dependencies between ATP, free Magnesium and pH have been explored over a wide range. MgtA belongs to the P3B –type ATPase subfamily that include members from eubacteria, archaea, fungi and plants.

37-POS Board 37

Structural Studies of a Toxin Loaded Bacterial Type VI Secretion (T6S) Effector Complex Using Electron Microscopy

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Participating in interbacterial competition and mediating virulence are two major tasks of almost every bacterium in order to survive in their respective environmental niche. To do so Gram-negative bacteria developed sophisticated protein secretion machineries for translocating a variety of effector proteins across their two membranes into the cytoplasm/periplasm of the host cell.

One of the latest discovered secretion systems is the T6S apparatus, contributing to the virulence of several human pathogens like V. cholera and P. aeruginosa. It uses a unique translocation mechanism, which is functionally and structurally related to the effector delivery of bacteriophages. Upon contraction of an outer sheath, an inner tube, consisting of stacked Hcp hexameric rings, is propelled outwards and finally pierces the target cell. Located at the tip of the inner tube, is a tapering VgrG trimer attached, building the basis for the secreted effector complex.

In this study, we show for the first time the architecture of a Tse6 (Type VI secretion exported 6, a predicted transmembrane protein)-loaded VrgG complex using negative stain EM. Surprisingly, Tse6-mediated toxicity requires the binding to an essential housekeeping protein, translation elongation factor Tu (EF-Tu). Furthermore we show, that a putative chaperoning protein, EagT6, is part of the complex. The assignment of the subunit localization within the complex is supported by nanogold and antibody labeling experiments.

Additionally, we observed the adoption of different conformations in the presence/absence of detergent hinting to a conformational change upon membrane contact.

Our electron microscopic studies combined with biochemical results provide structural and mechanistic insights into these medically relevant protein complexes and will equip us with a better understanding of disease transmission, finally laying the foundation for the development of new therapeutic strategies and treatment options for patients.

38-POS Board 38

MALDI MSI and LCMS/MS as Tools for Gatifloxacin Distribution in Healthy Rat Brain

Adeola Shobo, Tricia Naicker, Linda Bester, Sanil D. Singh, Glenn Maguire, Hendrik G. Kruger, Thavendran Govender.
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High mortality rates from tuberculosis (TB) remains a major concern worldwide. Besides the scourge of pulmonary TB, extra-pulmonary TB (EPTB) is increasing in developed as well as third-world countries. Tuberculosis meningitis (TBM) is the most common form of EPTB in human immunodeficiency syndrome (HIV) co-infected patients, and morbidity and mortality remains high in affected adults and children, despite the initiation of effective anti-TB therapy. This is possibly a consequence of reduced penetration of orally administered TB drugs in the CNS, resulting in treatment failure and drug resistance. The use of matrix-assisted laser desorption ionization-mass spectrometry imaging techniques as a suitable alternative for the localization of drug-tissue distribution for gatifloxacin will be able to answer questions regarding its distribution because other available methods require nuclear labeling and the detection of gamma rays produced by labeled compounds to localize the compound and hence causing a modification to the original structure of the active molecule. The aim of this study was to determine the brain distribution and the pharmacokinetic profile of gatifloxacin in healthy Sprague-dawley rats using matrix assisted laser desorption ionization mass spectrometry imaging (MALDI MSI) and quantitative liquid chromatography tandem mass spectrometry (LCMS/MS). The MALDI MSI results showed the drug gradually diffused into the brain via the blood brain barrier and into the cortical regions of the rat brain reaching Cmax of 228.48 ng/ml at 120 min post-dose. As time elapses the drug slowly leaves the brain following the same path as it followed on its entry into the brain and finally concentrates at the frontal cortex. Thus we have shown that MALDI MSI is a valuable tool in the study of drug localization, which requires less complicated sample processing with better resolution for drug distribution studies.

39-POS Board 39

Lester Sigauke.
University of Cape Town, Cape Town, South Africa.

Withdrawn

40-POS Board 40

Portable Immuno-Capture Device for Early Stage Typhoid Diagnosis in 6 hrs

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Typhoid fever is a global health problem and is caused by Salmonella typhi, a gram-negative bacterium and a very similar Salmonella serotype paratyphi A. Currently diagnosis of typhoid done by (a) blood culture test (b) detection of specific antibody response by widal®, typhidot® and tubex® assay. Antibody response based assay can confirm typhoid only after 4-7 days of infection. The blood culture assay is the gold standard for typhoid diagnosis but has limitation of long (48-78 hrs) processing time for final results. Typhoid patients have the causative organism S. typhi in their blood but its concentration is as low as 1 CFU/ml in early stage of infection, and there is no existing diagnostic method to detect this lower concentration in same day. We have developed a portable device for early stage typhoid diagnosis within 6 hr. This process involves culture growth of patient blood in broth media for 5.5 hrs and then immuno-magnetic enrichment of the target cells using portable device. The enriched target cells was then visually confirmed by lateral flow immunoassay.

Portable device and capture chip: To enrich target cells in cultured sample, we have developed a portable immuno-magnetic enrichment device and a capture chip. The device consists of an automated linear positioner with a magnet cassette mounted on it. Specific arrangement of permanent magnets in cassette allow rapid magnetic capture. Capture chip can be placed on the cassette after loading the sample, pre-incubated with immuno-magnetic particles. Now the linear movement of magnet cassette concentrate target cells into a 50µl recovery chamber of chip.

42-POS Board 42

Enzymatic Manipulation of Antibody Fc-Mediated Effector Functions

Eric Sundberg.
University of Maryland School of Medicine, Baltimore, USA.

In order to evade host immune mechanisms, many bacteria secrete a diversity of immunomodulatory enzymes. Streptococcus pyogenes, one of the most common human pathogens, secretes a large endoglycosidase, EndoS, which removes carbohydrates in a highly specific manner from IgG antibodies. This renders antibodies incapable of eliciting host effector functions through either the complement pathway or via Fc γ receptor signaling, providing the bacteria with a survival advantage. On account of this antibody-specific modifying activity, EndoS is currently being developed as a promising injectable therapeutic for autoimmune diseases that rely on autoantibodies. Additionally, EndoS is a key enzyme used in the chemoenzymatic synthesis of homogenously glycosylated antibodies with tailored Fc γ receptor-mediated effector functions. Despite the tremendous utility of this enzyme, the molecular basis of EndoS specificity for, and processing of, IgG antibodies has remained poorly understood. We have recently determined the high-resolution X-ray crystal structure of EndoS, which provides the first mechanistic insight into its unique enzymatic properties. Based on this structure, we rationally designed chimeric endoglycosidases in which we exchanged the glycosidase domain of EndoS with that of EndoF1 in order to create enzymes that exhibit high specificity for antibody substrates while changing their glycan specificity to that of EndoF1 (high mannose type) from that of EndoS (complex biantennary type). Using mass spectrometry and surface plasmon resonance assays, we found these engineered enzymes to be highly specific and efficient for the glycoprotein substrates for which we designed them. This novel glycoprotein engineering strategy for constructing chimeric endoglycosidases that are able to manipulate the glycan composition on IgG antibodies provides new opportunities to engineer antibodies with unique glycan compositions for previously unachievable therapeutic applications.

45-POS Board 45

Identification and Characterization of Protein Components of Bacteroides Fragilis Fimbriae

Bruna Galvao, **Brandon Weber**, Valerie Abratt.
University of Cape Town, Cape Town, South Africa.

Bacterial surface structures involved in attachment, such as fimbriae, are considered important virulence factors. The aim of this study is to isolate and identify the protein components of the fimbriae of *Bacteroides fragilis* for structural characterisation. Evaluation of fimbriae production was performed by growing *B. fragilis* strains on liquid and solid brain heart infusion (BHI) and Wilkins Chalgren media. Cells were visualised by transmission electron microscopy to assess which conditions gave rise to the production of fimbriae. Fimbriae were isolated by gentle shearing from the surface of the bacteria and assessed by TEM and SDS-PAGE. Protein bands of interest were excised and analysed by LC MS/MS. Antibodies were raised to the recombinant version of one of the candidate fimbrial proteins and used for immunogold localization studies to confirm the role of this protein as a component of the observed fimbrial structures.
Production of fimbriae by *B. fragilis* was shown to be dependent on the bacterial strain and growth conditions used, with the optimum combination observed for strain *B. fragilis* 638R grown on solid BHI medium. These fimbriae were observed by TEM *in situ* on the cells, in the sheared and precipitated protein fractions. The isolated fimbrial proteins, examined using SDS-PAGE, revealed four distinct protein bands. These were analysed by LC MS/MS and revealed a number of candidate fimbrial proteins. One of these, encoded by ORF BF638R_2242, shows homology to both the FimA and Mfa1 fimbrial proteins of the anaerobic oral pathogen *Porphyromonas gingivalis* and was chosen for further study. The role of the putative fimbrial protein as a component of the observed fimbrial structures was investigated by immunogold labelling studies.
One strong fimbrial gene candidate (FimA) has been identified so far and is the subject of ongoing research.

POSTER SESSION II

Tuesday, November 17, 17:30 – 19:30
Old Wine Cellar of The Spier Wine Estate

All posters are available for viewing during all three poster sessions; however, below are the formal presentation times when presenters are required to remain in front of their poster boards to meet with attendees.

Chandra, Mintu	7-POS	Board 7
Eltabakh, Soad	14-POS	Board 14
Jackson, Laurelle	17-POS	Board 17
Marjomäki, Varpu	26-POS	Board 26
Mc Arthur, Chanelle	28-POS	Board 28
Mofrad, Mohammad	29-POS	Board 29
Mokhawa, Mokhawa	30-POS	Board 30
Muthinja, Mendi	33-POS	Board 33
Negishi, Yutaka	34-POS	Board 34
Ozer, Asuman Nevra	35-POS	Board 35
Penkler, David	36-POS	Board 36
Tastan Bishop, Ozlem	43-POS	Board 43
Varma, Sameer	44-POS	Board 44
Wells, Gordon	46-POS	Board 46
Williams, Alison	47-POS	Board 47
Zondagh, Jake	48-POS	Board 48

Posters should be set up on the morning of November 16 and removed by 19:30, November 19.

7-POS Board 7

Insights into Molecular Switch: Crystal Structure Analysis of Wild Type and Fast Hydrolyzing Mutant of EhRabX3, a Tandem Ras Superfamily GTPase from Entamoeba Histolytica

Mintu Chandra[1], Vijay K. Srivastava[1], Yumiko S. Nakano[2], Tomoyoshi Nozaki[2].Sunando Datta[1]
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Background: The enteric protozoan parasite, Entamoeba histolytica, is the causative agent of amoebic dysentery, liver abscess and colitis in human. Vesicular trafficking plays a key role in the survival and virulence of the protozoan and is regulated by various Rab GTPases. EhRabX3, a catalytically inefficient amoebic Rab protein, is unique among the eukaryotic Ras superfamily by virtue of its tandem domain organization. A detail structural analysis revealing sub-atomic description of the protein is essential for understanding how the classical GTP/GDP cycle is influenced by the domain organization in 3-dimensional space and why this atypical GTPase is catalytically dead.
Methodology/Results: Here, using Se-Met SAD phasing, we report the crystal structures of GTP-bound wild type EhRabX3 and its fast hydrolyzing mutant in GDP-bound form. Interestingly, only the N-terminal G-domain showed nucleotide bound to its active site. The overall structure showed that the central beta sheets of NTD and CTD are oriented at right angle to each other. Compared to other Ras superfamily members, both G-domains displayed large deviation in switch II, perhaps due to non-conservative substitution in this region. The bulky side chain of Val71 occluded Lys73 from the nucleotide binding pocket, providing a rationale for the diminished GTPase activity of EhRabX3. Replacement of Val71 and Lys73 by conventional counterparts, Ala and Gln, respectively, resulted in large enhancement in its GTPase activity. Moreover, the existence of an intra-molecular disulfide bond in EhRabX3 is found to be critical for maintaining the structural integrity and function of this unique Rab protein.
Conclusions: Structure-guided functional investigation of cysteine mutants could provide physiological implications of the disulfide bond and allow us to design potential inhibitors for the better treatment of intestinal amoebiasis.

14-POS Board 14

Blood Levels of 8-Isoprostane F2 Alpha in Chronic Hepatitis C

Soad Eltabakh, Fathalla Ismael, Hanan Abdelaziz, Hala Maged, Rasha Ghazala.
Alexandria University, Alexandria, Egypt.

BACKGROUND/PURPOSE: The mechanism by which HCV causes liver damage is mediated through immunological means, direct viral toxicity and induction of oxidation stress (OS) . 8-Isoprostane F2 Alpha (8-Isop) is an important marker to assess the OS in vivo and used as extensively to qualify lipid peroxidation. The purpose of the study was to determine the relation of 8-Isop to the severity of HCV related liver diseases. METHODS: Fifty subjects were evaluated and divided into 5 groups, 10 in each group : G1 : Chronic hepatitis C without cirrhosis. G2 : Chronic hepatitis C with child A cirrhosis. G3 : Chronic hepatitis C with child B cirrhosis. G4 : Chronic hepatitis C with child C cirrhosis. G5 : Volunteers with no evidence or history of liver disease .All subjected to : determination of 8-Isop and lipid profile. RESULTS: Statistical comparison between the mean value of 8-Isop in the studied groups using the F test showed significant increase in groups 2, 3, and 4 than in G5. Also there was significant increase of 8-Isop in G1 than G2, and in G2 than G3, also, in G3 than G4. On the other hand, there was significant association between the severity of HCV liver disease and low cholesterol, TG, LDL, and VLDL. COCLUSION: This results highlight the importance of OS , marked by 8-Isop in the pathogenesis and severity of HCV related liver disease. Also, the findings reported the importance of lipid profile parameters and its relation to disease severity. Further studies are needed entailing the association between lipid levels, liver histo-pathological characters, and virological parameters to predict the response to the therapy. Reference: Spengler U, Nattermann J. Immunopathogenesis in hepatitis C virus cirrhosis.Clin Sic (London) 2007; 112(3):141-155.

17-POS Board 17

Survival of the Weakest: Less Fit Virus Stabilized in the Face of Drug during Robust HIV Infection

Laurelle Jackson[1,2], Andrew Young[3], Mikael Boulle[1], Fabio Zanini[4], Richard Neher[4], Gil Lustig[1], Alex Sigal[1,2,5].
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Current models of drug resistance evolution contend that mutant virus with a higher than wild type fitness will dominate the viral population in the presence of the drug. Counter to this expectation; clinically observed frequencies of highly resistant mutants do not reach 100% of the viral population within the patient. To understand this and examine whether it is dependent on heterogeneity in infection environments, we evolved resistance to efavirenz (EFV), a first line therapy drug, under two different infection conditions: when infection was robust (infected cells consist of 20% of the total cells) and low (infected cells 2% of the total). Counter-intuitively, in robust infection the addition of the drug resulted in delayed evolution of drug resistance and a lower steady-state fraction of mutant virus. This was evident in the mutant virus replication ratio, which decreased as the proportion of mutant in the population increased, until it reached the wild type replication ratio. In contrast, addition of the drug under low infection conditions led to rapid selection of a drug resistant mutant which plateaued at a higher mutant to wild type ratio. To understand the mechanism behind the decrease in mutant fitness, we investigated cell death in robust infection in the face of EFV. We observed that while the proportion of infected cells decreased monotonically in low infection, robust infection showed a peak in the proportion of infected cells at 20nM EFV. EFV increased the number of live infected cells by reducing HIV mediated cell death. The drug resistant mutant lost this protective effect. This implies that in environments where HIV infection is robust (lymph nodes) some drug may be beneficial for the virus and evolution of highly resistant variants will be attenuated.

26-POS Board 26

Site-Specific Probes for Enteroviruses for Detailed Imaging in Light and Electron Microscopy

Varpu Marjomäki[1,2], Mari Martikainen[1,2], Kirsi Salorinne[3,2], Tanja Lahtinen[3,2], Sami Malola[4,3], Jaakko Koivisto[4,3], Mika Pettersson[3,2], Perttu Permi[1,3,2], Hannu Häkkinen[3,4,2].
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We are focused on understanding the mechanisms and cellular factors behind enterovirus infection. Our previous results have suggested that enteroviruses follow a macropinocytic entry to host cells. In order to follow virus uncoating and other details in the infectious pathway, covalently conjugated probes for virus capsid or genome are needed. On the other hand, visualization of virus opening in cellular structures profits from dynamics probes that are released upon virus uncoating. We have developed a protocol for site-specific covalent conjugation of atomically monodisperse gold clusters with 1.5 nm metal core to viral surfaces (PNAS 2014). Water-soluble Au102(para- mercaptobenzoic acid)44 clusters, functionalized by maleimide linkers to target cysteines of viral capsid were conjugated to echovirus 1 and coxsackievirus B3 without compromising the infectivity. Quantitative analysis showed ordering of the bound gold clusters on the viral surface and a clear correlation between the clusters and the cysteine sites close to the viral surface. Another site-specific probe was developed for the hydrophobic pocket of enteroviruses. A derivative of Pleconaril was conjugated to fluorescent labels and Au102. The probe mildly stabilized the virus particle and caused a delay in the virus uncoating, but could not however inhibit the receptor binding, cellular entry or infectivity of the virus. The hydrophobic pocket binding was proven by STD and tr-NOESY NMR methods and TEM. The virus-fluorescent probe accumulated in endosomes but was seen to leak from the virus-positive endosomes from the capsid proteins suggesting that, like the physiological hydrophobic content, the probe may be released upon virus uncoating. Our results collectively thus show that the gold and fluorescently labeled probes may be used to track and visualize the studied enteroviruses during early phases of infection opening new avenues to follow virus uncoating in cells.

28-POS Board 28

Construction of an Automated Cost-Effective Microfluidic Chip that is Able to Capture and Concentrate Human Immunodeficiency Virus-1 Particles

Chanelle Mc Arthur, Frederick Balagadde.
K-rith, Durban, South Africa.

Discovered in 1981, HIV (Human Immunodeficiency Virus) has become a global pandemic, with approximately 35 million people living with the disease by 2013. To date, Sub-Saharan Africa remains the most affected region, with almost 1 in every 20 adults living with HIV. Currently, HIV diagnosis and monitoring is fraught with many challenges. Viral load assays demand resources such as money, a high level of expertise to perform the tests and interpret data, time, equipment, laboratory space and proper infrastructure, all of which are not feasible in many regions of Africa. We propose a new microfluidics based approach to viral load monitoring that has the potential to become a global resource in biological and medical science. Microfluidics allows for the scalability of tests that require microliter volumes of reagents leading to reduced total costs. Using multilayer soft lithography, we were able to create a microfluidic device containing structural sieve valves capable of trapping HIV-1 capturing microspheres. Once the microspheres were captured, we introduced different concentrations of HIV-1 particles. The successful capturing and concentrating of HIV-1 particles was identified and quantified by fluorescent intensity. We were successfully able to carry out viral load experiments via automation on a microfluidic device that has the capability of processing hundreds of patient samples providing qualitative and quantitative data.

29-POS Board 29

The Interaction of RNA Helicase DDX3 with HIV-1 Rev-CRM1-RanGTP Complex during the HIV Replication Cycle

Hanif Mahboobi, Alex Javanpour, **Mohammad Mofrad**.
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Nucleocytoplasmic macromolecullar shuttling is regulated by the nuclear pore complex (NPC), which acts as a highly selective channel perforating the nuclear envelope in eukaryotic cells. The human immunodeficiency virus (HIV) exploits the nucleocytoplasmic pathway to export its RNA transcripts across the NPC to the cytoplasm. Despite extensive study on the HIV life cycle and the many drugs developed to target this cycle, no current drugs have been successful in targeting the critical process of viral nuclear export, even though HIV's reliance on a single host protein, CRM1, to export its unspliced and partially spliced RNA transcripts makes it a tempting target. Due to recent findings implicating a DEAD-box helicase, DDX3, in HIV replication and a member of the export complex, it has become an appealing target for anti-HIV drug inhibition. We present a hybrid computational protocol to analyze protein-protein interactions in the HIV mRNA export cycle. Using this method, we highlight some of the most likely binding modes and interfacial residues between DDX3 and CRM1 both in the absence and presence of RanGTP. This work shows that although DDX3 can bind to free CRM1, addition of RanGTP leads to more concentrated distribution of binding modes and stronger binding between CRM1 and RanGTP.

30-POS Board 30

Homology Modelling of Trypanosoma Cathepsin B like Proteases as a Prelude to Enhibitor Docking Studies

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Trypanosoma brucei (T. brucei) is a protozoan parasite that causes Human African Trypanosomiasis (HAT), a fatal disease if left untreated. Parasite resistance to drugs and drug toxicity necessitates the need for novel methods. This study aims to use bioinformatics approaches to carry out comparative sequence and structural analysis of Trypanosoma brucei cathepsin B-like (TbCatB) protease and its homologs from T. congolense, T, cruzi, T. vivax and H. sapiens as a prelude to docking studies. TbCatB is a papain family C1 cysteine protease which belongs to Clan CA group and has emerged as a potential HAT drug target. Sequences of TbCatB (PDB ID 3HHI) homologs were retrieved by a BLAST search. Human cathepsin B (PDB ID: 3CBJ) was selected from a list of templates for homology modelling found by HHpred. MODELLER version 9.10 program was used to generate a hundred models for T. congolense, T, cruzi and T. vivax cathepsin B like proteases using 3HHI and 3CBJ as templates. The best models were chosen based on their DOPE Z scores before validation using MetaMQAPII, ANOLEA, PROCHECK and QMEAN6. The DOPE Z scores of the models were -1.02, -0.55, and -1.39 while PROCHECK results indicate that 99.9%, 99.4% and 100 % of residues are in allowed regions for T. congolense, T. cruzi and T. vivax cathepsin B like protease models respectively. Evaluation of the models indicate the models are stable and of good quality. Screening of South African natural compounds identified compounds with good binding energies in TbCatB, simultaneously having poor binding in human Cathepsin B (with estimated difference in binding of more than1.0 kcal/mol)

33-POS Board 33

Shape and Force: Key to Transmission of the Malaria Parasite

Mendi Muthinja[1], Katharina Quadt[1], Catherine Moreau[1], Jessica Kehrer[1], Mirko Singer[1], Ulrich S. Schwarz[2], Joachim P. Spatz[3], Freddy Frischknecht[1].
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The malaria-causing parasite (Plasmodium) is transmitted to vertebrate hosts by the infectious bite of a female Anopheles mosquito. The infectious forms, known as sporozoites are deposited in the skin and move at high speed (1-2 μm/s) to find and enter blood vessels [1]. Once in the blood they are transported to the liver, where they enter hepatocytes to differentiate into blood cell invading forms. The pre-erythrocytic stages of the malaria parasite are clinically silent but critical for establishing infection in the mammalian host. Sporozoites migrate using an uncommon mode of locomotion called gliding motility [2], which enables them to penetrate host tissues. Sporozoites are polarized crescent shaped cells that typically move in circles on two-dimensional substrates in vitro. In order to investigate sporozoite morphology and motility, we generated transgenic parasites expressing altered or fluorescently tagged proteins that are implicated in maintaining curvature or gliding. We use micro-patterned pillar arrays [3] as surrogate 3D tissues and blood capillary mimetics to study the role of curvature in sporozoite motility. To investigate forces that can be exerted by the sporozoite we employ laser tweezers, which enabled us to dissociate retrograde flow of adhesins with force production. Our transgenic parasite lines combined with actin-modulating drugs revealed how sporozoites facilitate optimal force transmission for gliding motility.

References

1. Douglas et al., Trends Parasitol, 2015
2. Montagna et al., Front Biosci, 2012
3. Hellmann et al., Plos Path, 2011

34-POS Board 34

DNA Methylation Analysis to Reveal Latent HIV Reactivation Mechanism in Single Cell

Yutaka Negishi[1], Musa Mhlanga[2].
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Despite the suite of effective anti-HIV drugs available, curation of HIV infection is still difficult due to the presence of latent provirus in resting memory T cells. This latent reservoir is established early stage during HIV infection and remains a major barrier to curing HIV infection because latent HIV is unaffected to anti-HIV drugs. One approach to make anti-HIV drugs more effective is reactivating latent HIV artificially. Although some reagents can reactivate latent HIV, it is difficult to reactivate all latent HIV. Therefore, understanding reactivation mechanism of latent HIV in single cell level is important to cure the HIV.
Here, we propose to develop a novel technique able to reveal the methylation status of latent HIV in single cell level based on the adaptation of the padlock FISH approach. DNA methylation in the HIV provirus 5′ or 3’ long terminal repeat (LTR) is considered to be a mechanism of transcriptional suppression that allows retroviruses to evade host immune responses and anti-HIV drugs. In our technique, DNA is digested with a DNA methylation sensitive restriction enzyme such as HpaII, and subsequently generated single strand by lambda 5’-3’exonuclease. The padlock probes, which contain the sequence of HIV 5’ and 3’ LTR near the restriction site and detection sequence, are annealed to the target DNA and the ends of the probes are circularized by ligation. Then, phi29 DNA polymerase amplifies circularized DNA by rolling-circle amplification and amplified DNA product is detected by hybridization of fluorescent-labelled oligonucleotides. In this way, we will be able to specifically visualize integrated provirus that is methylated and reveal methylation status in 5’ and 3’ LTR. Our technique will provide a novel approach to develop novel drugs to reactivate latent HIV.

35-POS Board 35

Prediction of Cleavage Specificity in Proteases by Biased Sequence Search Threading

Gonca Ozdemir Isik, **Asuman Nevra Ozer**.
Marmara University, Istanbul, Turkey.

The assessment of substrate specificity in infectious disease-related proteases is crucial in drug development studies, where interpreting the adaptability of residue positions can be useful in understanding how inhibitors might best fit within the substrate binding sites. In this work, the substrate variability and substrate specificity of the Human Immunodeficiency Virus 1 (HIV-1) protease, the Hepatitis C Virus (HCV) NS3/4A serine protease and the Adenovirus 2 (AdV2) cysteine protease were investigated by the computational biased sequence search threading (BSST) methodology. Available crystal structures and template structures for the substrate-bound proteases, which were created in silico by performing various peptide building and docking procedures followed by energy minimization and molecular dynamics simulations, were utilized. BSST was performed starting with known binding, nonbinding and random peptide sequences that were threaded onto the template complex structures, and low energy sequences were searched using low-resolution knowledge-based potentials. Then, target sequences of yet unidentified potential substrates were predicted by statistical probability approaches applied on the low energy sequences. The results show that the majority of the predicted substrate positions correspond to the natural substrate sequences with conserved amino acid preferences. Overall, supported by the successful outcomes with the case studies of HIV-1 protease, HCV NS3/4A serine protease and AdV2 cysteine protease here, BSST seems to be a powerful methodology for prediction of substrate specificity in protease systems.

36-POS Board 36

Towards Discovering Novel Drug Target Sites and Small Compound Inhibitors of Heat Shock Protein 90 (Hsp90): A Structural Bioinformatics Approach

David L. Penkler, Ozlem Tastan Bishop.
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Hsp90 is a molecular chaperone heavily implicated in maintaining cellular homeostasis, ensuring the correct folding, stabilization and activation of a host of different client proteins, many of which are involved in important biological processes. In diseases such as cancer and malaria, infected cells undergo a vast barrage of environmental insults such as, hypoxia, temperature and pH variation, and oxidative outbursts, which in most cases would arrest the normal function and progression of the cell, an outcome largely avoided through cellular rescue by Hsp90. Given its importance it is thus not surprising that Hsp90 has gathered much attention as a potential drug target. To date the vast majority of known Hsp90 inhibitors include small molecules which actively compete for the ATP binding site located on the N-terminal of the protein. The objective of this study was to investigate natural compounds as potential inhibitors that putatively target functional sites on Hsp90 other than the ATP binding pocket. Whole protein in silico molecular docking experiments were performed using 574 natural compounds from the SANCDB (www.sancdb.rubi.ru.ac.za) against both human and Plasmodium falciparum cytosolic orthologs. Subsequent clustering analysis revealed several strong lead candidate compounds specific to putative Hsp90-Hop interaction sites on human and parasite models. Further in silico sequence and structural analysis of these bound target sites revealed two distinct binding pockets in close proximity to specific Hop interacting residues located in the middle domain of both organisms. In depth molecular dynamics simulations were done to validate the suitability of 20 re-docked lead compound hits for use as putative Hsp90 inhibitors. Here we present the discovery of several South African natural compounds as potential inhibitors, specific to binding pockets involved in Hop-Hsp90 binding.

43-POS Board 43

Ozlem Tastan Bishop.
Rhodes University, Grahamstown, South Africa.

Withdrawn

44-POS Board 44

Entropy-Driven Biological Processes: Signaling Mechanisms in Controlled Entry of Enveloped Viruses into Host Cells

Sameer Varma, Priyanka Dutta, Mohsen Botlani, Nalvi Duro.
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The activities of many proteins, including GPCRs, T-cell receptors and nuclear transcription factors, are controlled by shifts in their conformational densities, and not just through changes in their minimum-energy structures. A primary challenge faced in the study of such proteins and their response functions to biological stimuli concerns the characterization of their thermal motions. Here we will present the development of new generalized methods to evaluate differences between conformational ensembles (JCTC 2013, 9:868; Proteins 2014, 82:3241). In addition, we will present how we are using these methods in conjunction with accelerated conformational sampling techniques and wet-lab experiments to illuminate the molecular details underlying the regulated entry of enveloped paramyxoviruses into host cells. Paramyxoviruses, such as the Measles, and the emerging, highly-lethal Nipah, regulate their entry into host cells via a combination of two separate protein-protein interactions. The signal for their entry originates at the interface formed between one of their membrane proteins and those of the host cell. This protein-protein interface sandwiches a substantially large amount of water, which we find is vital to the inception of the signal (JPCB 2014, 118:14795). The signal then transduces from the receptor binding domain of this viral protein to another domain, traversing a distance > 2 nm, where it activates a second viral membrane protein that facilitates virus-host membrane fusion. A large part of this intricate allosteric signal is entropic in nature, as also evident from crystallographic studies that reveal minor host-induced changes in viral protein structure (RMSD < 0.2 nm). Our investigations are providing the first atomic-level insights into these signaling processes, and we anticipate that our methods will also benefit the study of other entropically-driven biomolecular machines.

46-POS Board 46

Potential Function and Prevalence of Intra/Inter-Protein Carboxylic-Acid Dimers

Gordon Wells[1], Hongjie Yuan[2], Scott Myers[2], Yesim Tahirovic[3], David Menaldino[3], Thota Ganesh[3], James Snyder[3], Dennis Liotta[3], Stephen Traynelis[2].
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The *N*-methyl-D-aspartate receptor (NMDAR) is expressed in most neurons of the mammalian CNS. However, overstimulation of NMDAR by its associated neurotransmitter glutamate often results in cell-death. Ischemic brain injury sites are characterised by increased glutamate concentrations and lowered pH due to poor CO_2 clearance. Thus site-specific NMDAR inhibitors may augment current interventions for treating ischemia.
A recent study[1] describing context dependent inhibitors of the NMDAR implicated an intra-protein carboxylic acid dimer as key to increased inhibition at lower pH. This pH dependent activity is desirable at the site of ischemic brain tissue, as this potentially allows selective reduction of NMDAR induced neurotoxicity at the injury site, while minimizing off-target effects in healthy tissue. Comparison of protein crystal structures solved at different pH values revealed a pair of glutamate residues adopting a geometry consistent with a neutral carboxylic-acid dimer at the lower pH. Subsequent mutagenesis of this pair reduced pH dependent inhibition, suggesting that the carboxylic-acid dimer functions as a pH sensor.
This prompted a search for other dimers within the Protein Data Bank (PDB). A further 40 plus proteins were found with Asp/Glu pairs adopting a geometry consistent with a carboxylic-acid dimer. Approximately half of these interactions are mediated via metal chelation, with no nearby metals apparent for the rest. The carboxylic-acid dimers are found in both solvent exposed and buried locations in a wide variety of proteins.
Carboxylic acid dimers therefore represent a hitherto unrecognised intra/inter-protein interaction that may be functionally significant. Furthermore, pH sensitivity highlights the potential for exploiting drug targets that may only present themselves during the altered physiological conditions induced by communicable diseases (e.g. severe malaria induced acidosis)

[1] http://dx.doi.org/10.1016/j.neuron.2015.02.008

47-POS Board 47

Novel Expression System for HIV-1 Subtype C Protease

Alison Williams, Ikechukwu Achilonu, Yasien Sayed.
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HIV-1 is the most common form of HIV and can be subdivided into groups and subtypes. The subtype of interest for this study, subtype C, can be found in southern Africa. HIV-1 protease is the main drug target and belongs to the class of aspartic proteases. The aim of this study was to develop a novel purification system using a thioredoxin His-tagged fusion protein to improve the protease yield. The study looked at a clinical variant (designated L38↑N↑L) and wild-type protease. The L38↑N↑L variant was found in a drug naïve infant whose mother was exposed to ARV therapy as part of the prevention of mother-to-child transmission (PMTCT) initiative. This double of Asn and Leu results in a protease with each subunit containing 101 amino acids rather than 99. The wild-type and variant proteins were successfully overexpressed in BL21 (DE3) pLysS E.coli cells and purified using nickel charged IMAC. The secondary structure was characterised using far-UV circular dichroism and consisted of β-sheets. A cleavage assay was conducted using a fluorogenic substrate and both the wild-type and the variant were found to be active. The percentage active sites were determined using isothermal titration calorimetry and were found to be 13% for wild-type protease and 8% for the L38↑N↑L variant. Subsequently, the quaternary structure was characterised using size exclusion high performance liquid chromatography wild-type protease was found to be predominately monomeric and the L38↑N↑L variant was found to be dimeric.

48-POS Board 48

Antiretroviral Drug Susceptibility of a Hinge Region Variant of HIV-1 Subtype C Protease

Jake Zondagh, Yasien Sayed.
University of Witwatersrand, Johannesburg, 2050, South Africa.

In South Africa, HIV-1 subtype C (HIV-1 C-SA) is responsible for the majority of HIV infections. The viability of this virus depends on HIV protease, an enzyme which cleaves essential viral polyproteins, thereby activating them. Inhibitors targeting this enzyme provide therapeutic benefits to HIV infected individuals. Unfortunately, inhibitors may become ineffective over time due to the emergence of drug resistant mutations. In this study, a possible drug resistant variant of HIV-1 C-SA protease was analysed. The protease variant N37T↑V is unusual, since it contains an amino acid substitution, as well as an additional amino acid insertion at the 37th codon. A novel method of purification was developed specifically to overexpress and purify HIV-1 variant proteases such as N37T↑V. Structural characterisation of the variant was achieved utilising far-UV circular dichroism, fluorescence spectroscopy and high performance liquid chromatography. Fluorogenic assays were performed to determine the catalytic activity of the enzyme. Results indicate that the mutation is structurally non-disruptive. The N37T↑V protease has a sixfold decrease in fluorogenic substrate processing activity and a threefold decrease in turnover number compared to the wild-type protease. Moreover, the variant has a fivefold increase in catalytic efficiency compared to the wild-type protease.

POSTER SESSION III

Thursday, November 19, 17:30 – 19:30
Old Wine Cellar of The Spier Wine Estate

All posters are available for viewing during all three poster sessions; however, below are the formal presentation times when presenters are required to remain in front of their poster boards to meet with attendees.

Banerjee, Mousumi	2-POS	Board 2
Broadley, Simon	4-POS	Board 4
Burgess, Jeremy	6-POS	Board 6
Cloete, Ruben	8-POS	Board 8
Cumming, Bridgette M	10-POS	Board 10
Dames, Sonja Alexandra	11-POS	Board 11
De Welzen, Lynne	12-POS	Board 12
Kimani, Serah	18-POS	Board 18
Koch, Anastasia	19-POS	Board 19
Lamprecht, Dirk	20-POS	Board 20
Luthuli, Brilliant	22-POS	Board 22
Mackenzie, Jared	23-POS	Board 23
Mazorodze, James Hove	27-POS	Board 27
Mukherjee, Raju	32-POS	Board 32
Singh, Vinayak	41-POS	Board 41

Posters should be set up on the morning of November 16 and removed by 19:30, November 19.

2-POS Board 2

Rapid Identification of Mycobacterium Tuberculosis Bacilli from Clinical Samples Using Maldi-Tof Mass Spectrometry

Mousumi Banerjee, Nelson D. Soares, Jonathan Blackburn.
University of Cape Town, Cape Town, Western Cape, South Africa.

Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI- TOF MS) has become a regular diagnostic tool in clinical microbiology to identify bacterial species (Biotyping) along with conventional phenotyping and gene sequencing. However, the limitation in sensitivity (105-106 bacteria / μl) and time consuming culture based amplification makes this method expensive and sometimes complicated particularly, for the slow growing bacteria like M. tuberculosis. In the past 6 months, I have been working on development of a rapid, highly sensitive and comparatively less expensive method to identify M. tuberculosis bacilli in clinical isolates using commercial MALDI-TOF instrument. In this method we have identified key surface lipids and the membrane protein markers from limited number of bacilli after extracting them with organic solvent; this MALDI data suggests the set of identified lipids is sufficient to discriminate M.bovis BCG from M.smegmatis bacilli although further work is required to explore this differentiation in more quantitative detail. The key lipids were then fragmented by MS-MS and searched against an 'in-house' generated database. The identified protein markers are now being confirmed by 'state-of –art' LC-MS/MS, with resultant peptide mass spectra being matched against a universal database for identification of the parent proteins. MALDI-TOF signal enhancement was achieved by decreasing the noise level by changing laser diameter, laser power and number of laser shots. So far we have been successful in identifying as low as an estimated 10 bacilli, which is encouraging for the future identification of bacteria in aerosolised samples.

4-POS Board 4

Structure Determination of Enzymes Involved in Mutagenesis in Mycobacterium Tuberculosis

Simon Broadley[1], Digby Warner[2,3], Trevor Sewell[1,3].
[1]University of Cape Town, Cape Town, Western Cape, South Africa, [2]University of Cape Town, Cape Town, Western Cape, South Africa, [3]University of Cape Town, Cape Town, Western Cape, South Africa.

Drug resistance in *Mycobacterium tuberculosis* (MTB) arises through the acquisition of spontaneous mutations in antibiotic target or related genes. This places enormous importance on the need to understand the DNA metabolic pathways in MTB, and identifies mutagenic repair mechanisms as compelling targets for novel anti-TB drugs.
The DNA damage-inducible C family DNA polymerase, DnaE2, has been implicated in virulence and the emergence of antibiotic-resistant MTB mutants in vivo. DnaE2 operates as part of a three-component "mutagenic cassette" comprising ImuB - a pseudo Y-family polymerase - and ImuA', a RecA-like protein of unknown function.
The aim of this study is to obtain crystal structures of these proteins as well as the *dnaE1*-encoded replicative polymerase in order to gain insight into the comparative geometries of the DnaE1 and DnaE2 active sites, and to elucidate potential interacting domains in DnaE2, ImuA', and ImuB.
To date, ImuA' and DnaE1 have been solubly expressed in *E. coli* with maltose-binding protein tags. Thermostability studies (which provide an indication of proper protein folding and a measure of stability in crystallization buffers) have been inconclusive and indicate the need for further investigation. MTB proteins are notoriously difficult to express properly folded in large quantities; therefore, optimised codon usage for expression of MTB genes in E.coli has been explored to improve folding stability. Biophysics also suggests that slower expression increases the probability of proper folding; in addition, other expression vectors, strains and conditions have been tested. Provided purified, soluble protein is obtained in sufficient quantities, crystallisation conditions will be screened using a mosquito pipetting robot and hits will be optimised by exploring the crystallisation phase diagram. Crystal cryoprotectant conditions will be optimised using the diffractometer at UCT and the final, high-quality data sets obtained at a synchrotron.

6-POS Board 6

Crystallization and Structural Characterization of Mycothiol S-Conjugate Amidase from Mycobacterium Tuberculosis

Jeremy G. Burgess, Bryan T. Sewell, Brandon W. Weber.
University of Cape Town, Cape Town, South Africa.

Mycothiol (MSH) is a protective thiol found within Mycobaterium tuberculosis and other Actinomycetes. MSH is an intracellular protectant, used to counteract oxidative stress and to detoxify endogenous toxins and xenobiotics. Accordingly, enzymes needed for MSH biosynthesis and metabolism have received interest due to their potential as novel drug targets. Mycothiol S-conjugate amidase (Mca), is a zinc metalloenzyme and member of the LmbE-like superfamily, which catalyzes the hydrolysis of mycothiol S-conjugates (MSR) to glucosaminyl-inositol (GlcN-Ins) and an acetylcysteine S-conjugate (AcCyS-R), which is expelled from the cell. Mca shares minor overlapping substrate specificity with MshB, a deacetylase involved in MSH biosynthesis and a fellow member of the LmbE-like superfamily. Several unconserved residues in the GlcN-Ins binding site of Mca and MshB are thought to be responsible for the observed differences in substrate specificity for Mca and MshB, but their precise contributions are unknown. Site directed mutagenesis and activity assays will be used to analyze the effect of substitution of these residues in Mca with their MshB counterparts. Native Mca was previously produced in E.coli as a fusion protein with maltose binding protein (MalE). The fusion protein was purified by affinity chromatography and cleaved using Tobacco Etch Virus protease. Free Mca activity was demonstrated through a discontinuous assay. Early sparse-matrix crystallization screens with native Mca were unsuccessful. Stabilizing additives identified through thermostability assays were included in later crystallization screens, but the conditions require optimization. Additional attempts to crystallize Mca will include (a) generation of inactive Mca mutants and incubation with a favoured substrate, and (b) removal of disordered regions within Mca, including the final twenty C-terminal residues.

8-POS Board 8

Structural and Functional Effects of Nucleotide Variation on the Tuberculosis Drug Metabolizing Enzyme Human Arylamine N-Acetyltransferase 1 Protein

Ruben Cloete[1], Wisdom Akurugu[1,3], Cedric Werely[2], Alan Christoffels[1].
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The human arylamine N-acetyltransferase 1 (NAT1) determine the duration of action of amine-containing drugs by influencing the balance between detoxification and metabolic activation of these drugs. Single nucleotide polymorphisms (SNPs) in NAT1 have been implicated in inter-ethnic and inter-individual variation of phenotypic profiles in patients being treated for tuberculosis (TB). The effects of six non-synonymous SNPs on the structure and function of NAT1 was tested. Atomistic simulation studies and stability calculations using both GROMACS and Site Directed mutator (SDM) were performed to supplement the routinely used SIFT and POLYPHEN-2 algorithms.

Analysis of the four novel SNPs identified within South African mixed ancestry population revealed two (N245I and V231G) that affect NAT1 protein function while the other two (R242M and E264K) showed contradictory results.

To determine the effect of mutations on stability of the protein structure, we used the crystal structure of NAT1 (PDBID: 2IJA) as the wild type structure and modified the cysteine 68 residue to be acetylated in complex with coenzyme A and para-aminobenzoic acid. The wild type and mutant structures were submitted to the web-server SDM. Three of the novel SNPs showed slightly to highly destabilizing effects (-0.58 to -5.09kcal/mol) while the experimentally validated SNPs associated with fast and slow acetylation (I263V and R64W) showed destabilizing and stabilizing effects -0.98 and 1.19kcal/mol, respectively.

For a conclusive result, we propose the use of simulation studies to quantify the effect of SNPs on the protein structures and function using appropriate measures. This may be validated with in-vitro experiments. Findings from this study might inform a strategy of incorporating genotypic data (i.e, functional SNP alleles) with phenotypic information (slow or fast acetylators) to better prescribe effective tuberculosis treatment.

10-POS Board 10

Energy Metabolism of Mycobacterium Tuberculosis Infected Macrophages: Potential for Use as a Biomarker of Disease Progression and Severity

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Bioenergetics has become central to understanding the pathology of human diseases such as neurodegeneration, diabetes, cancer and cardiovascular diseases. Recent studies have proposed the use of a single value termed the "Bioenergetic Health Index" (BHI) calculated from an oxidative phosphorylation profile to be used as a biomarker to assess patient health with prognostic and diagnostic value. From this perspective, our objective was to investigate if and how *Mycobacterium tuberculosis* (*Mtb*) infection modulates the energy metabolism of human macrophages derived from peripheral blood monocytes and the potential of monitoring bioenergetic metabolism as a biomarker of active infection. In order to differentiate *Mtb* infection from infections with other non-pathogenic mycobacterial species, *M. bovis* BCG infection (BCG), the strain used for vaccination, was investigated in parallel. We utilized extracellular flux analysis to measure oxygen consumption rate (OCR) as a measure of oxidative phosphorylation and extracellular acidification rate (ECAR) as a measure of glycolysis and the TCA cycle of the macrophages in real time and in a non-invasive manner. Mitochondrial modulating compounds revealed respiratory dysfunction in *Mtb* infected macrophages, in line with the greater dependency of *Mtb* infected macrophages on glycolysis as demonstrated by the greater sensitivity of these macrophages to 2-deoxyglucose that inhibits hexokinase II in glycolysis. Analysis of monocytes isolated from peripheral blood of healthy volunteers and a tuberculosis patient prior to treatment disclosed a significantly reduced spare respiratory capacity that reduced the calculated BHI. Future studies include monitoring the BHI of peripheral circulating monocytes isolated from tuberculosis patients prior to and during treatment to assess the value of BHI to monitor disease progression and response to treatment.

11-POS Board 11

NMR Characterization of the Natively Unfolded N-Terminus and the Redox-Sensitive Rubredoxin Motif of Mycobacterial Protein Kinase C–A Tuberculosis Drug Target

Sonja Alexandra Dames[1,2], Matthias Wittwer[1].
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Protein kinase G (PknG) is a eukaryotic-like ser/thr kinase blocking the degradation of pathogenic organisms such as Mycobacterium tuberculosis within host macrophages. Because PknG is secreted into the host cytosol, it is an ideal target for tuberculosis drugs since they do not have to pass the only low permeable mycobacterial cell wall.
The catalytic ser/thr kinase domain (~147-390) is N-terminally flanked by a rubredoxin-like metal-binding motif (RD, ~74 –147) and C-terminally by a tetratricopeptide repeat domain (TPRD). The N-terminal 73 residues are intrinsically disordered (NORS) and harbor the only site (T63) phosphorylated in vivo[1]. Both, deletions or mutations in the NORS or the redox-sensitive RD significantly decrease PknG-mediated mycobacterial survival in the host[1,2]. It has been shown that PknG is more active in the presence of an oxidizing agent[1]. Here, we present the NMR characterization of the NORS region and its chemical shift assignment as well as of the redox-regulated un- and refolding of the RD and its ability to interact with membrane mimetics. The NMR data is complemented by standard kinase assays. Based on our data controlled unfolding of the RD by oxidization may regulate the kinase activity. The detected membrane mimetic interactions may play a role for PknG localization.

References:
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12-POS Board 12

Whole Transcriptome Analysis of Clinical Isolates of Mycobacterium Tuberculosis Using RNA-Seq

Lynne De Welzen[1], Vegard Eldholm[2], Keira A. Cohen[3,1], Kashmeel Maharaj[1], Kaitlin Stouffer[1], Alexander S. Pym[1].
[1]KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH), Durban, KwaZulu-Natal, South Africa, [2]Norwegian Institute of Public Health, Oslo, Norway, [3]Pulmonary and Critical Care Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

Background: Drug resistance of *Mycobacterium tuberculosis* (*M.tb*) has become an epidemic of global proportions. While much drug resistance can be attributed to known mechanisms, some resistance remains unexplained. Investigation of transcriptional adaptations among clinical isolates of drug resistant *M.tb* may identify additional mechanisms of drug resistance.
Aims: To identify transcriptional changes in *M.tb* that lead to drug resistance or compensate for loss of fitness due to a drug resistance conferring mutation.
Methods: Twelve clinical isolates of *M.tb* were selected for RNA-seq. These included Beijing and KZN spoligotypes, with varying drug susceptibilities. RNA sequencing and full genome sequencing was conducted on the Illumina HiSeq 2000 platform to identify mutations that could account for transcriptional changes. Sequenced reads were aligned to the *M.tb* reference genome H37Rv, and fold change differences were calculated using DNASTAR. A dual colour fluorescent protein promoter assay, in conjunction with flow cytometry, was used to assess the promoter activity of intergenic mutations.
Results: Within the Beijing spoligotype the number of genes that were up or down regulated, relative to drug susceptible strains, was greater in MDR and PXDR strains compared to mono resistant strains. A genomic-transcriptomic analysis identified 3 out of 81 and 2 out of 37 intergenic mutations for the Beijing and KZN spoligotypes respectively that were associated with a 4-fold or greater differential regulation in the adjacent gene. The fluorescent reporter assay identified a significant decrease in promoter activity relative to wild-type in a mutation associated with Rv3854c.
Discussion: The evolution of drug resistance can result in a large variation in the global transcriptional profile of XDR strains relative to drug susceptible strains. The minority of these are due to promoter mutations, suggesting the involvement of other global regulatory pathways.

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Substrate Specificity in the Amidases of Biomedical Importance – Insights from the Crystal Structures of a Model Bacterial Amidase

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Nitrilase superfamily amidases predominantly catalyze the conversion of amides to their corresponding acids and ammonia, with only a small subset performing the reverse amidase activity of condensation of non-peptidic amide bonds. These enzymes perform diverse but essential metabolic roles in vivo including detoxification of small molecules, protein post-translational modifications, protein degradation, and vitamin and co-enzyme biosynthesis, among others. Of importance are the medically-relevant amidases like the NAD+ synthetase and apolipoprotein N-acyltransferases from Mycobacterium tuberculosis and other pathogenic organisms, which play critical roles in NAD+ cofactor homeostasis and biosynthesis of mature membrane triacylated lipoproteins respectively, and have therefore been considered as potential targets for inhibitor design. Very little is however known about substrate specificity and catalysis in these enzymes. Using active site mutants of a model amidase from Nesterenkonia species, we have been able to understand how amidases recognize and bind amide substrates, as well as the contribution of the active site pocket size and geometry to substrate specificity and catalysis. Findings from this work will be presented. Dr Kimani's participation has been made possible by WIRSAM SCIENTIFIC, agents for Rigaku.

19-POS Board 19

High Resolution Snapshot of Genetic Diversity within Mycobacterium Tuberculosis in a Region of High HIV Co-Infection

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Infection with HIV greatly increases the risk of becoming infected with tuberculosis (TB) even before a decrease in CD4+ T-cell numbers, and co-infection leads to acceleration of both diseases. The complexity of the biological interactions between HIV, Mycobacterium tuberculosis (Mtb) and the human immune system are incompletely understood. Our study was designed to investigate differences in genetic microdiversity of Mtb samples from HIV-infected and HIV-uninfected patients. Whole genome sequencing (WGS) data for 190 strains (with almost equal numbers isolated from HIV-infected and HIV-uninfected individuals) was generated. No profound differences were observed in clustering patterns, or in overall genetic diversity between Mtb strains isolated from HIV-infected or HIV-uninfected individuals. Lineage 2 Mtb strains isolated from HIV-infected individuals contained a higher number of SNPs in epitope encoding genes than those isolated from HIV-uninfected individuals. However, a similar trend was observed for essential genes, therefore definitive inferences about antigenic variation could not be drawn from SNP counts. Formal analysis of selection is underway using models available as part of the HyPhy package. Preliminary results from a MEDS analysis indicate differential selective pressures on genes important for host-pathogen interactions between Mtb strains isolated from HIV-uninfected or HIV-infected groups. Recombination – which can confound detection of selection – was evaluated the Recombination Detection Package, and found to be minimal. The impact of HIV on the TB rates, particularly in sub-Saharan Africa, has been devastating. These data may elucidate pathways that are important for the biological interactions between these two diseases and the human immune system. Moreover, selection tests as well as recombination analyses have been infrequently reported for Mtb, and are also therefore novel.

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Bedaquiline, Q203, and Clofazimine: Novel Insights into Effects on M. Tuberculosis Respiration

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Understanding how different conditions and antibiotic compounds affect *Mycobacterium tuberculosis* (*Mtb*) energy production through oxidative phosphorylation (OXPHOS) and carbon catabolism has substantial implications for drug discovery and optimization. We have developed a bioenergetic assay by existing adapting extracellular flux (XF) technology, previously used primarily to study eukaryotes, to non-invasively provide real-time data on *Mtb*'s O_2 consumption rate (OCR, a measure of OXPHOS) and extracellular acidification rate (ECAR, a measure of carbon catabolism). This fully integrated multi-well technology can now be used to rapidly investigate, under relevant physiological conditions, compounds that target *Mtb* energy production.
We have made the surprising discovered that the two new anti-mycobacterial drugs bedaquiline (BDQ) and Q203, respectively targeting Complex V and Complex III of the mycobacterial electron transport chain (ETC), cause an increase in *Mtb*'s OCR up to approximately four-fold above that of untreated cells. The increase in OCR is not transient and is maintained for at least 16 hours after drug addition. The increase in OCR is even more pronounced at lower O_2 tensions, similar to those found in a host macrophage or granuloma. This effect is not observed for clofazimine (CFZ), another mycobacterial ETC targeting drug. Also, the increase in OCR is not associated with the production of ATP and is not due to classic uncoupling of the mycobacterial ETC. Furthermore, there is also a sustained increase in ECAR after the addition of BDQ and Q203, an indication that OXPHOS is not meeting energy requirements. Together, the OCR and ECAR results suggest that O_2 consumption, while stimulated, is no longer effectively coupled to energy production.
In sum, we have developed an innovative method to explore the effects of new compounds that target *Mtb* bioenergetics.

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Confinement-Induced Drug-Tolerance in Mycobacteria Growing in Miniaturized Bioreactors

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A considerable challenge in controlling tuberculosis is the prolonged multidrug chemotherapy (6 to 9 months) required to overcome drug-tolerant mycobacteria that persist in human tissues, although the same drugs can sterilize genetically identical mycobacteria growing in axenic culture within days. An essential component of TB infection involves intracellular *Mycobacterium tuberculosis* pathogens that multiply within macrophages and are significantly more tolerant to antibiotics compared to extracellular mycobacteria. We have developed the microdialyser, a new system of culturing mycobacteria in bioreactors with volume comparable to membrane-bound compartment of a macrophages. The microdialyser can support 120 independent cultures with mycobacterial populations ranging from one to over 1000 cells at the same time. Using this system we have uncovered an epigenetic drug-tolerating phenotype that appears when mycobacteria are cultured in the space-confined bioreactors but disappears in larger volume bioreactors. Therefore, macrophage-induced drug tolerance by mycobacteria may be an effect of growth in space confined environment among other macrophage-specific mechanisms.

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Antibiotic Resistance and its Cost in E. Coli Cells Expressing Tap—A Multidrug Efflux Pump of Mycobacterium Tuberculosis

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Drug resistance is a serious global health problem, with 2 million people each year being infected with drug resistant bacteria in the US alone. It is believed that efflux pumps are systems that Tuberculosis microbes as well as other microbes use to survive antibiotics by extruding the drug molecules that enter the bacteria. To date, the study of the *Mycobacterium tuberculosis (M.tb)* efflux system is plagued with problems such as functional redundancy that have frustrated efforts to determine the functions of any single pump. We propose a new approach to discovering the role of each *M.tb* efflux pump. By conditionally expressing *M.tb* pumps in *E.coli* strains that are deficient in transporter proteins and highly sensitive to drugs, we can characterize each mycobacterial efflux pump in terms of substrate specificity, cognate inhibitors and efflux capacity. This is done through the use of Microfluidics and novel microchemostat technology. This technology involves the use of a microfluidic chip that can grow bacterial cells in perpetuity, allowing us to systematically monitor the long-term dynamics of each tuberculosis efflux pump, with the ability to resolve gene expression differences between individual cells. Using these *E.coli* constructs, we have characterized the Tap-like efflux pump Rv1258—an *M.tb*multidrug efflux pump. Cells expressing Tap had increased resistance to streptomycin and gentamicin relative to wild type cells, with a greater level of resistance observed with the latter antibiotic. Our results also indicate that whilst conferring increased resistance to antibiotics, efflux pump expression comes at a fitness cost to the bacteria. This fitness cost induces a non-growth state that may also independently confer tolerance to antibiotics that require active microbial growth to be potent.

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Directing Warburg: Mycobacterium Tuberculosis Redirects Host Energy Metabolism in the TB Lung

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The physiological consequences of hypoxia and acidosis in Mycobacterium tuberculosis (Mtb) infection are poorly understood. We hypothesized that Mtb redirects host metabolism from oxidative phosphorylation towards increased aerobic glycolysis (the "Warburg Effect") resulting in elevated lactate levels and extracellular acidity. To test this hypothesis, we used extracellular flux analysis with an XF96 machine that measures oxygen consumption rate, as a measure of oxidative phosphorylation and extracellular acidification rate as a measure of glycolysis. Mtb infection induced a shift from oxidative phosphorylation to aerobic glycolysis. This shift induced extracellular acidity as demonstrated using a fluorescently labelled pH (low) insertion peptide (pHLIP). Mtb-infected mice were injected with fluorescently labelled pHLIP peptides and the lungs examined ex vivo using near-infrared fluorescence (NIRF) imaging. Immunohistochemistry was performed using antibodies against GLUT1 (a glucose transporter), HIF-1α (hypoxia inducible factor), MCT4 (monocarboxylate transporter 4), LDHA (lactate dehydrogenase A) and NHE1 (sodium hydrogen exporter 1). We used a metabolomics approach to quantify intermediates of the glycolytic and tricarboxylic acid (TCA) cycle in Mtb-infected and uninfected mice. Lastly, we measured differential expression of select genes involved in glycolysis, TCA cycle, acidosis and hypoxia. We observed significantly increased fluorescence intensity in lesions of Mtb-infected lungs compared to uninfected mice via NIRF imaging. Immunostaining revealed increased levels of GLUT1, LDHA, MCT4, NHE1 and HIF-1α in Mtb-infected vs uninfected mice. Further, select genes involved in glycolysis, TCA cycle, acidosis and hypoxia were upregulated in Mtb-infected mice, consistent with immunohistochemistry and pHLIP NIRF imaging. We conclude that Mtb results in a shift from host oxidative phosphorylation to glycolysis, resulting in acidic extracellular pH changes due to accumulation of lactate.

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Small Hydrophilic Molecule Permeation in Mycobacterium Tuberculosis

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Despite originating from different chemical classes, "new chemical entities" identified by screening for compounds with whole-cell activity against Mycobacterium tuberculosis share a number of features: they are relatively hydrophobic, and frequently appear to target a small set of "promiscuous" targets – specifically, DprE1, MmpL3, Pks13 and QcrB – which are either located in the periplasm, or are associated with the mycobacterial plasma (inner) membrane. The relative paucity of hydrophilic compounds identified by phenotypic screening is thought to be attributable, at least in part, to the formidable permeability barrier presented by the complex, lipid-rich mycobacterial cell wall, which comprises the mycobacterial outer membrane (mycomembrane), periplasmic space, and plasma membrane. Discovery-driven mass spectrometry (MS), also known as "shotgun MS", has been widely used for qualitative and quantitative measurements of proteomes. In order to identify outer membrane proteins that may be involved in the uptake of small hydrophilic molecules, including a number of standard anti-TB drugs, we have employed a shotgun MS approach to the mycomembrane sub-proteome of M. tuberculosis. Proteins from the mycomembrane of logarithmic and stationary-phase M. tuberculosis cells were isolated by detergent extraction using the non-ionic detergent, n-octyl-β-D-glucoside, and Triton X-100. Successive tryptic digestion and MS-based searches resulted in the identification of a number of hypothetical and conserved hypothetical proteins. In parallel, we have also employed high density transposon mutagenesis coupled with deep sequencing (Tn-Seq) to identify the conditionally essential genes under the specific growth condition and hence can be implicated for uptake of small molecules including inorganic nitrate. The identification and preliminary functional characterization of a subset of these proteins is described here.

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Chlorpromazine Potentiates the Activity of Spectinomycin against Mycobacterium Tuberculosis

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The aim of this study is to explore the mechanism of action of spectinomycin based on a renewed interest to repurpose or reposition the drug for tuberculosis (TB) treatment. Spontaneous spectinomycin resistant *Mycobacterium tuberculosis* (*Mtb*) mutant strains were generated followed by sequencing of 16S ribosomal RNA <*g class="gr_ gr_40 gr-alert gr_spell ContextualSpelling ins-del multiReplace" id="40" data-gr-id="40">rrs gene* and *rpsE* gene. Transition (A1183G), transversion (G1379T), and novel cytosine insertion (926C) mutations occurred in the <g class="gr_ gr_11 gr-alert gr_spell ContextualSpelling ins-del multiReplace" id="11" data-gr-id="11">*rrs* gene. Mutant strain susceptibility tests with anti-TB drugs revealed <g class="gr_ gr_12 gr-alert gr_gramm Grammar only-ins doubleReplace replaceWithoutSep" id="12" data-gr-id="12">absence of cross-resistance. Synergistic interactions initially observed in a combination of spectinomycin and chlorpromazine (CPZ) against *Mtb* (wild-type) were absent against the mutant strains confirming that spectinomycin was acting “on target.” Moreover, utilizing combination assays, we determined that a synergistic interaction between spectinomycin and CPZ results from CPZ-mediated inhibition of Rv1258c, reinforcing the value of inhibiting efflux as a viable strategy in new TB drug development.