UNIVERSITY OF CAMBRIDGE

CAMBRIDGE INFECTIOUS DISEASES

Cambridge Infectious Diseases
4th Annual
Meeting of Minds

Thursday 13th November 2014
Divinity School, St John's College Cambridge

Programme

8.30	*Registration and Coffee*	
9.00	Introduction	
Session 1:	**Mycobacteria Chair: Professor Ken Smith, Department of Medicine**	
9.15	Insights into human tuberculosis from the zebrafish	Prof Lalita Ramakrishnan, Department of Medicine
10.00	Autophagic killing of mycobacteria: genetics, pitfalls and therapies	Prof Andres Floto, Cambridge Institute for Medical Research
10.30	Development of new drugs to combat TB using a target focused approach.	Dr Vitor Mendes, Department of Biochemistry
11.00-11.30	*Tea & Coffee*	
Session 2:	**Enabling Technologies Chair: Professor Lisa Hall, Department of Chemical Engineering and Biotechnology**	
11.30	Automated imaging and micromanipulation: towards finding malaria's weak spot	Dr Pietro Cicuta, Department of Physics
12.00	Developing bioinformatic web/mobile applications to address the global genetic epidemiology, population and evolutionary biology of micro-organisms	Dr David Aanensen, Imperial College London
12.30	Superresolution imaging methods to study molecular mechanisms of disease	Prof Clemens Kaminski, Department of Chemical Engineering and Biotechnology
1-2pm	*LUNCH*	
Session 3:	**Social and historical perspectives of infectious diseases Chair: Dr Rob Doubleday, Centre for Science and Policy**	
2.00	Migration and smallpox	Dr Romola Davenport, Department of Geography
2.30	Intestinal Parasites throughout Human Evolution	Dr Piers Mitchell, Department of Archaeology and Anthropology
3.00	Teens Against Polio: Mobilizing Youth for Vaccination in Post-War America	Dr Stephen Mawdsley, Department of History
3.30-4pm	*Tea & Coffee*	
Session 4:	**Biological Structures Professor Clare Bryant, Department of Veterinary Medicine**	
4.00	Herpesviruses and super resolution microscopy	Dr Colin Crump, Department of Pathology
4.30	Structural biology of pathogenic bacterial spores	Dr Graham Christie, Department of Chemical Engineering and Biotechnology
5.00	Gene expression in the remnant chloroplast of Plasmodium	Prof Christopher Howe, Department of Biochemistry
5.30-6.30	*Drinks Reception*	

Abstracts

Session 1: Mycobacteria
Chair: Professor Ken Smith, Department of Medicine

9.15 **Insights into human tuberculosis from the zebrafish**
Prof Lalita Ramakrishnan, Department of Medicine

10.00 **Autophagic killing of mycobacteria: genetics, pitfalls and therapies**
Prof Andres Floto, Cambridge Institute for Medical Research

Autophagy is an evolutionarily conserved process used by cells to degrade cytoplasmic protein aggregates and organelles, but can also target intracellular bacteria, including *M. tuberculosis* (MTB), for lysosomal degradation. I will discuss how genetic variants may alter the capacity for autophagic killing and thus determine an individual's risk of acquiring MTB, how inadvertent inhibition of autophagy may render individuals more susceptible to mycobacterial infection, and whether chemical induction of autophagy might provide a novel therapeutic strategy for drug resistant infections.

10.30 **Development of new drugs to combat TB using a target focused approach.**
Dr Vitor Mendes, Department of Biochemistry & Center for Neuroscience and Cell Biology, University of Coimbra, Portugal.

Tuberculosis (TB) is a deadly infectious disease mainly caused by the tubercle bacillus *Mycobacterium tuberculosis*, which has afflicted humanity since ancient times. Although the number of TB-related deaths is declining each year, its incidence in many countries is still a major cause of concern. The intrinsic drug resistance mechanisms of *M. tuberculosis*, together with acquired drug resistance due poor chemotherapy choices and lack of treatment compliance have created a severe drug resistance problem that rendered some of the *M. tuberculosis* strains virtually untreatable. The identification of new essential gene functions together with structural knowledge of these targets is an important strategy for rational drug design in an organism where the exploration of many of the traditional chemical libraries has often failed to deliver hits. Using a target focused fragment based approach, which iteratively combines computational, biophysical, biochemical, and crystallographic methods we have been targeting essential pathways in this organism in an attempt to develop new drugs to combat TB.

11.00-11.30 *Tea & Coffee*

Session 2: Enabling Technologies
Chair: Professor Lisa Hall, Department of Chemical Engineering and Biotechnology

11.30 **Automated imaging and micromanipulation: towards finding malaria's weak spot**
Dr Pietro Cicuta, Department of Physics

Erythrocyte invasion by *Plasmodium falciparum* merozoites has been studied intensively, but our cellular understanding of invasion has been limited by the fact that invasion occurs very rapidly: it is generally complete within one minute, and shortly thereafter the merozoites, at least in *in vitro* culture, lose their invasive capacity. The rapid nature of the process, and hence the narrow time window in which measurements can be taken, have limited the tools available to observe invasion. We employ automated imaging, and for the first time optical tweezers, to study individual invasion events, showing that newly released *P. falciparum* merozoites, delivered via optical tweezers to a target erythrocyte, retain their ability to invade. Even spent merozoites that had lost the ability to invade still retain the ability to adhere to erythrocytes, and also can still induce transient local membrane deformations in the erythrocyte membrane. We use this technology to measure the strength of the adhesive force between merozoites and erythrocytes, and to probe the cellular mode of action of known invasion inhibitory treatments. These data have interesting implications for our current understanding of the cellular process of invasion, and demonstrate the power of optical tweezers technologies in unravelling blood stage biology of malaria.

12.00 **Developing bioinformatic web/mobile applications to address the global genetic epidemiology, population and evolutionary biology of micro-organisms**
Dr David Aanensen, Imperial College London

Recent advances in DNA sequencing technology provide the ability to obtain the DNA sequences of large numbers of isolates of bacterial pathogens and open the way to detailed analysis of the spread of strains of pathogens (e.g. the spread of MRSA within and between hospitals or within and between countries and globally). Making sense of these data by communities of researchers requires the development of web applications that logically store and query the genome sequences of very large numbers of isolates of individual pathogen strains and allow a range of sophisticated analyses to be carried out that provide insights into the relationships within and between sets of closely related isolates. Using *Staphylococcus aureus* as an exemplar, in this talk I will demonstrate several web applications aimed at storing and querying genetic and epidemiological data aimed at ease of use and identification of high-risk clones for use within public health.

12.30 **Superresolution imaging methods to study molecular mechanisms of disease**
Prof Clemens Kaminski, Department of Chemical Engineering and Biotechnology

Misfolding and aggregation of peptides and proteins is a characteristic of many neurodegenerative disorders, including Parkinson's Disease (PD) and Alzheimer's (AD). Their common feature is that normally unstructured and soluble proteins, misfold and aggregate into insoluble amyloid fibrils, which make up the deposits in the brains of patients suffering from these devastating illnesses. A key requirement to gain insight

into molecular mechanisms of disease and to progress in the search for therapeutic intervention is a capability to image the aggregation process and structure of ensuing aggregates *in situ*. In this talk I will give an overview of research to gain insight on the aggregation state of alpha synuclein (relevant to PD) beta-amyloid and Tau (relevant to AD) *in vitro*[1], in cells[2,3], and in live model organisms [4]. In particular we wish to understand how these and similar proteins nucleate to form toxic structures and to correlate such information with phenotypes of disease[3]. I will show how direct stochastic optical reconstruction microscopy, *d*STORM, and multiparametric imaging techniques, such as spectral and lifetime imaging, are capable of tracking amyloidogenesis *in vitro*, and *in vivo*, and how we can correlate the appearance of certain aggregate species with toxic phenotypes[5]. Using multiparametric imaging methods we follow the trafficking of aggregates between cells and see how the misfolded state propagates from cell to cell. I will show how such information at the molecular level guides our understanding of disease pathology in humans.

1. Pinotsi D, Büll AK, Galvagnion C, Dobson CM, Kaminski-Schierle GS, Kaminski CF, "Direct Observation of Heterogeneous Amyloid Fibril Growth Kinetics via Two-Color Super-Resolution Microscopy," Nano Letters (2013), 14 (1), 339–345
2. Kaminski Schierle GS, van de Linde S, Erdelyi M, Esbjörner EK, Klein T, Rees E, Bertoncini CW, Dobson CM, Sauer M, and Kaminski CF, "In Situ Measurements of the Formation and Morphology of Intracellular ß-Amyloid Fibrils by Super-Resolution Fluorescence Imaging", J. Am. Chem. Soc., 133 (33), pp 12902–12905 (2011)
3. Esbjörner, E.K., Chan, F., Rees, E., Erdelyi, M., Luheshi, L.M., Bertoncini, C.W., Kaminski, C.F., Dobson, C.M., and Kaminski Schierle, G.S., "Direct Observations of Amyloid β Self-Assembly in Live Cells Provide Insights into Differences in the Kinetics of Aβ(1–40) and Aβ(1–42) Aggregation," Chemistry & Biology (2014).
4. Kaminski Schierle GS, Bertoncini CW, Chan FTS, van der Goot AT, Schwedler S, Skepper J, Schlachter S, van Ham T, Esposito A, Kumita JR, Nollen EAA, Dobson CM, Kaminski CF, "A FRET sensor for non-invasive imaging of amyloid formation in vivo", ChemPhysChem, 12(3), 673–680, (2011)
5. Michel CH, Kumar S, Pinotsi D, Tunnacliffe A, St George-Hyslop P, Mandelkow E, Mandelkow E-M, Kaminski CF, Kaminski Schierle GS, "Extracellular Monomeric Tau is Sufficient to Initiate the Spread of Tau Pathology", J. Biol. Chem. (2014), 289: 956-967.

1-2pm *LUNCH*

Session 3: Social and historical perspectives of infectious diseases
Chair: Dr Rob Doubleday, Centre for Science and Policy

2.00 **Migration and smallpox**

Dr Romola Davenport, Cambridge Group for the History of Population and Social Structure

The more lethal form of smallpox, variola major, was a major cause of mortality in European cities before vaccination (adopted widely from 1798). Smallpox infection conferred lifelong immunity on survivors and was a disease of childhood amongst the urban-born, accounting for 20-30 per cent of deaths under ten in English cities in the eighteenth century. However many urban victims were adult migrants from rural areas where smallpox was not endemic. Evidence from age at burial of smallpox victims has revealed a puzzling geography of smallpox infection before 1800. In southern England, where population density was higher and the population was relatively urbanised, smallpox remained a rare epidemic disease in small towns and villages and killed adults as well as children. However in the north and south-west adult victims were extremely

rare, despite a pattern of more dispersed and lower density populations in these areas. Smallpox appears to have circulated more regularly in these more remote communities and we evaluate the reasons for these geographical patterns with respect to the relatively low infectiousness of smallpox and the ability of the virus to persist outside a human host, and the implications of these characteristics for the control of smallpox in both the pre-vaccine and vaccination eras.

2.30 **Intestinal Parasites Throughout Human Evolution**
Dr Piers Mitchell, Department of Archaeology and Anthropology

The remains of some parasites can survive thousands of years in archaeological contexts. We may find them in ancient latrines, the pelvic soil of skeletons, mummies, or coprolites. From an archaeological and historical perspective, detecting them can enable us to better understand health in past populations, and how ancient peoples spread parasites around the planet as they undertook migrations. From the perspective of clinical science and medicine, we have the potential to demonstrate how parasites may be evolving, by comparing the morphology and genetics of ancient and modern parasites. It has been argued that allergies may sometimes be triggered by the absence of parasites in many modern societies, so knowledge of which parasites have been infecting humans throughout our evolution can potentially help us with research into treatments for allergies. In order to explore these concepts we will present some of the fascinating discoveries made in the ancient parasites lab here in Cambridge, from those of King Richard III to the earliest cities of the prehistoric Middle East.

3pm **Teens Against Polio: Mobilizing Youth for Vaccination in Post-War America**
Dr Stephen Mawdsley, Department of History

In the late 1950s, a health charity, known as the National Foundation for Infantile Paralysis (March of Dimes), organized American teens into volunteer divisions to fight polio, as well as tame adult anxieties surrounding juvenile delinquency. The alliance that developed permitted the NFIP to increase its influence and revenue, while granting teens an opportunity to assert their cultural power and challenge negative stereotypes. Although the NFIP nurtured and at times dominated the relationship, young volunteers joined for their own reasons and shaped the program to suit their own aspirations and interests.

3.30-4.00 *Tea & Coffee*

Session 4: Biological Structures
Chair: Professor Clare Bryant, Department of Veterinary Medicine

4.00 **Herpesviruses and super resolution microscopy**
Dr Colin Crump, Department of Pathology

Herpes simplex virus type-1 (HSV-1) is one of the most widespread pathogens among humans. Although the structure of HSV-1 has been extensively investigated, the precise organization of tegument and envelope proteins remains elusive. We have developed super-resolution imaging by direct stochastic optical reconstruction microscopy

(*d*STORM) in combination with a model-based analysis of single-molecule localization data to determine the position of specific proteins within virus particles. Using multi-colour *d*STORM imaging we can resolve different protein layers within individual HSV-1 particles using and discriminate envelope glycoproteins from tegument proteins both in purified virions and in virions present in infected cells. Particle averaging of purified viruses and model-based analysis of the radial distribution of tegument proteins VP16, VP1/2 and pUL37 and envelope protein gD has enabled us to uncover new structural details of HSV-1. The techniques we have developed now provide the ability to investigate many virus-host protein interactions at nanometre resolution.

4.30 **Structural biology of pathogenic bacterial spores**
Dr Graham Christie, Chemical Engineering and Biotechnology

Spores of the bacterial orders *Bacillales* and *Clostridiales* represent nature's toughest cells. While most endospore forming bacteria are relatively benign saprophytic organisms, a handful of species are pathogenic, with the spore being responsible for transmission of diseases such as anthrax, tetanus, pseudomembranous colitis, and various food-poisoning conditions, including botulism. Despite occupying an extremely wide range of habitats, spores of all species share a common multi-layered cellular ultrastructure. The most notable structures include a thick layer of modified peptidoglycan that surrounds the spore protoplast, and which is responsible for maintenance of metabolic dormancy. The cortex itself is surrounded by a proteinaceous shell, or spore coat, which has roles in spore adhesion and the exclusion of harmful enzymes and chemicals. The current talk will look at the assembly and architecture of the outer layers of the spore during sporulation, which has been examined using electron and fluorescent microscopy techniques. An update on current understanding of the enzymatic activities responsible for degrading spore structures during spore germination, and the potential for development of novel sporicidal or therapeutic approaches that target this area, will also be presented.

5.00 **Gene expression in the remnant chloroplast of Plasmodium**
Prof Christopher Howe, Department of Biochemistry

Apicomplexan parasites such as *Plasmodium* contain a remnant chloroplast derived by endosymbiotic acquisition of a red alga. Although *Plasmodium* is no longer photosynthetic, its remnant chloroplast retains a genome whose expression is essential for parasite survival and represents a target for antimalarial drugs. Closely related alveolate protists, such as the photosynthetic Apicomplexa *Chromera* and *Vitrella*, and peridinin-containing dinoflagellate algae also have genome-containing chloroplasts, but across the group as a whole there is a remarkable diversity in chloroplast genome organization. Some members of the group show unusual post-transcriptional modification of chloroplast transcripts, including editing and addition of a 3' polyU tail. I shall look at the distribution of these different processing pathways across the group, and try to interpret it in an evolutionary context.

5.30-6.30 *Drinks Reception*

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