8th National Infection Biology Meeting / Microbiology Meeting – NIB 2019

14th -15th of October 2019

Aronsborgs Konferenshotell, Bålsta, Sweden

Conference book

NDPIA
National Doctoral Programme in Infections and Antibiotics

Svenska Föreningen för Mikrobiologi (SFM)

Picture on the frontpage: Aronsborg konferenshotell

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Welcome to the 8th National Infection Biology Meeting /

Swedish Microbiology Meeting - NIB 2019!

The National Infection Biology network has been supported and coordinated by The Laboratory for Molecular Infection Medicine Sweden (MIMS; www.mims.ume.se) since 2011 and NIB meetings have been organised every third year. The 5th NIB meeting was held 2011 in Umeå, the 6th meeting was held 2014 in Göteborg/Marstrand and the 7th meeting co-organised with the National Doctoral Programme in Infections and Antibiotics (NDPIA) was held at Djurönäset in the Stockholm archipelago at Djurönäset Conference Hotel.

Other national meetings relevant to infection or microbiology include meetings organised by the Swedish Society for Microbiology (SFM) and the annual NDPIA network meetings.

For this year's meeting, NIB/SFM/NDPIA join forces to co-organise the National Infection Biology meeting with a long-term ambition to organise the NIB-meetings biannually.

We wish you a successful meeting and welcome your input on the format of this years meeting.

Welcome!

Organizing Committee:
Oliver Billker, Umeå University, MIMS
Eva-Maria Diehl, Umeå University, MIMS/NDPIA
Åke Forsberg Umeå University, NDPIA
Fredrik Kahn, Lund University, NDPIA
Keira Melican, Karolinska Institutet, SFM
Barbara Sixt, Umeå University, MIMS
Staffan Svärd, Uppsala University, SFM

Monday 14 October 2019

10.20 **Welcome address**

Keira Melican

Chair Swedish Society for Microbiology (SFM), Karolinska Institutet

Åke Forsberg

Director NDPIA, Umeå University

10.30-12.15 Session I

Chairpersons Keira Melican and Åke Forsberg

10.30 ***Staphylococcus aureus* and Atopic Dermatitis – understanding the molecular basis of skin colonisation**

Joan Geoghegan

Department of Microbiology, Moyne Institute of Preventive Medicine, School of Genetics and Microbiology, Trinity College Dublin, Ireland

11.00 **Bacteria-derived hydrogen peroxide suppresses inflammasome-dependent innate immunity**

Saskia Erttmann

Department of Molecular Biology, Umeå University

See abstract and poster no 38

11.15 ***Helicobacter* species infections cause glycosylation changes which affect the *Helicobacter* binding ability mucins from pig and human stomachs**

Sara Lindén

Department of Medical Biochemistry and Cell Biology, University of Gothenburg

11.30 **Immune resolution dilemma: Host antimicrobial factor calprotectin induces tissue damage in a peritonitis infection model**

Nathalie Uwamahoro

Department of Clinical Microbiology, Umeå University

See abstract and poster no 14

11.45 **Streptococcal sagA activates a pro-inflammatory response in mast cells by a sublytic mechanism**

Christopher Von Beek

Department of Medical Biochemistry and Microbiology, Uppsala University

12.00 **Modulation of competence for natural transformation by pneumococcal bacteriophages**

Geneviève Garriss

Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet

See abstract and poster no 27

12.15 – 13.30 Lunch (free poster viewing)

Meet the experts (keynote speakers) opportunity for PhD students and Postdocs

13.30- 15.00 Session II

Chairpersons: Mikael Sellin and Åsa Sjöling

13.30 **Subversion of infection associated macropinosomes by *Shigella* and *Salmonella* determines their distinct intracellular niches.**

Jost Enninga
Institute Pasteur, Paris, France

14.00 **Barcoded consortium infections resolve cell type-dependent *Salmonella* host cell invasion mechanisms**

Maria Letizia Di Martino
Department of Medical Biochemistry and Microbiology, Uppsala University
See abstract and poster no 74

14.15 **Exploring the host reaction to a new host-targeting, small molecular inhibitor against Zika virus**

Aleksandra Pettke
Department of Oncology and Pathology, Karolinska Institutet
See abstract and poster no 51

14.30 **Biofilm expression patterns to microenvironmental cues**

Ferdinand Xiankeng Choong
Department of Neuroscience, Karolinska Institutet
See abstract and poster no 7

14.45 **DNA methylation during encystation of *Giardia intestinalis***

Laura Rojas
Department of Cell and Molecular Biology, BMC, Uppsala University
See abstract and poster no 49

15.00 **Coffee – poster session**

Poster walks in 10 groups (see separate programme)

From ca 16.00 Free poster viewing (even numbers at posters first 30 min, odd numbers at posters second 30 min)

17.00-18.45 Session III

Chairpersons: Staffan Svärd and Helen Wang

17.00 **High-throughput phenotyping of *Leishmani*a knockout mutants *in vitro* and *in vivo***

Eva Gluenz

Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

17.30 **Biology of *Cryptosporidium*, a leading global cause of diarrhea in children**

Boris Striepen

University of Pennsylvania School of Veterinary Medicine, Pathobiology, University of Pennsylvania, U.S.A.

18.00 **An interplay between inflammation, oxidative stress and cellular aging in a controlled human malaria challenge study**

Aurelie Miglar

Department of Medicine, Karolinska Institutet

See abstract and poster no 43

18.15 **Designing a systematic screen for fertility genes in *Plasmodium berghei***

Claire Sayers

Department of Molecular Biology, Umeå Universitet

See abstract and poster no 20

18.30 **To understand pregnancy malaria is to understand epigenetic regulation in parasites**

Madle Sirel

Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet

See abstract and poster no 65

19.00 ***Pre-dinner mingle***

19.30 ***Dinner - followed by networking /poster viewing/open bar***

Tuesday, 15 October 2019

08.45 - 10.30 Session IV

Chairpersons: Barbara Sixth and Fredrik Kahn

08.45 **_Chlamydia_ hijacks regulators of the actin cytoskeleton to remodel epithelial cell-cell junctures**

Raphael Valdivia

Duke University School of Medicine, Molecular Genetics and Microbiology, Durham, NC, U.S.A.

09.15 **The _Chlamydia_ inclusion membrane protein CpoS recruits Rab GTPases to subvert the host cellular surveillance system**

Karsten Meier

Department of Molecular Biology, Umeå University

See abstract and poster no 18

09.30 **A novel antibacterial compound with antibiotic effect in _Chlamydia_ infected mice**

Åsa Gylfe

Department of Clinical Microbiology, Umeå University

See abstract and poster no 6

09.45 **Dissemination of antibiotic resistance genes from antibiotic producers to pathogens**

Xinglin Jiang

Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark

See abstract and poster no 12

10.00 **Panacea - a universal antitoxin domain**

Gemma Atkinson

Department of Molecular Biology, Umeå University

10.15 **Quantification of low-abundance proteins by targeted mass spectrometry reveals dynamics of bacterial toxin-antitoxin systems**

Niilo Kaldalu

Institute of Technology, University of Tartu, Estonia

See abstract and poster no 35

10.30-11.30 Coffee, networking and poster viewing

11.30-13.00 Session V

Chairpersons: Sören Molin and Debra Milton

11.30 ***Pseudomonas aeruginosa* adaptation in cystic fibrosis airways**

Helle Krogh Johansen

Dept of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

12.00 **Transcriptional profiling of *Pseudomonas aeruginosa* infections**

Susanne Häußler

Dept of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

12.30 **Within-host adaptation mediated by intergenic evolution in *Pseudomonas aeruginosa***

S. M. Hossein Khademi

Department of Clinical Sciences, Lund University

See abstract and poster no 3

12.45 **Blood, fat and smears: apolipoprotein E as an endogenous alternative to antibiotics against Gram negative infections?**

Malin Elvén

Department of Clinical Sciences, Lund University

See abstract and poster no 39

13:00 – 14:15 Lunch (free poster viewing)

Meet the experts (keynote speakers) opportunity for PhD students and Postdocs

14.15-15.30 Session VI

Chairpersons: Oliver Billker and Susanne Häussler

14.15 **Discreet *Salmonella typhimurium* invasion of the murine gut absorptive epithelium**

Mikael Sellin

Medical Biochemistry and Microbiology, Uppsala University

14.45 **The transcriptional regulator CsvR controls the modulation of Enterotoxigenic *Escherichia coli* virulence by bile salts**

Enrique Joffré

Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet

See abstract and poster no 10

15.00 **Infection with genotoxin-producing *Salmonella enterica* synergises with loss of the tumor suppressor APC in promoting genomic instability in colonic epithelial cells**

Anna Bergonzini

Department of Molecular Biology, Umeå University

See abstract and poster no 48

15.15 **Practical and effective detection of bio-aerosols carrying mycobacteria using a novel, ionization-based air sampler**

Nuno Sousa

Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet

See abstract and poster no 21

15.30 **Closing of the meeting**

Keira Melican and Åke Forsberg

16.00 ***Bus to Arlanda***

Jost Enninga

Director of Research
Research Unit "Dynamics of Host-Pathogen Interactions"
Institute Pasteur, Paris, France
jost.enninga@pasteur.fr

Dr. Jost Enninga studied biochemistry at the University of Hannover and the Hannover Medical School in Hannover, Germany, and performed his PhD studies in the Laboratory of Cell Biology at the Rockefeller University (USA) and under the supervision of Guenter Blobel where he focused on the transport of biological macromolecules between cellular compartments using cell biological approaches. Afterwards, Jost wanted to apply his cell biological knowledge to the investigation of host-pathogen interactions. So in spring 2004 he came to the Institute Pasteur, Paris, to work with Guy Tran Van Nhieu and Philippe Sansonetti on the secretion of type III effectors from Gram negative bacteria. At the beginning of 2008, Jost went on searching for new adventures, so he started his own independent research team in the Department of Cell Biology and Infection at the Institut Pasteur in Paris.

Besides his research, Jost is dedicated to scientific teaching having co-organized a sequence of hands-on imaging courses at the Institute Pasteur and abroad.

Jost Enninga's long standing research interest has been to decipher the principles how intracellular pathogens subvert host pathways to establish their niches at cellular and molecular level. To achieve this, he has developed novel imaging technologies that can capture the host-pathogen cross talk at the single cell level at high spatiotemporal resolution.

His research team has identified how the molecular cascades in the host cell is exploited by a variety of bacteria, such as *Shigella flexneri*, *Salmonella enterica* and *Mycobacterium tuberculosis* to gain access to the host cytosol.

For more information, please visit:

https://research.pasteur.fr/en/member/jost-enninga/

Joan Geoghegan

Assistant Professor
Department of Microbiology, School of Genetics and Microbiology
Trinity College Dublin, Ireland
geoghegj@tcd.ie

Dr. Joan Geoghegan's research focuses on *Staphylococcus aureus*, an important pathogen of humans. During her PhD and postdoctoral training at Trinity College Dublin, Joan developed expertise in using biochemical and biophysical approaches to dissect the molecular basis of interactions between staphylococcal proteins and host molecules. Her current research involves characterizing the factors that contribute to the success of *S. aureus* by studying proteins involved in promoting attachment to host cells and surfaces, biofilm formation and bacterial survival in neutrophils and macrophages.

Her group is investigating the molecular basis of protein-mediated biofilm formation in *MRSA*. They have found that it is possible to prevent staphylococci from establishing biofilms by targeting the protein linkages that hold the bacteria together and the interactions that facilitate adherence to surfaces. These findings offer new opportunities for the development of compounds to prevent biofilm formation by staphylococci

A major research interest in the Geoghegan lab is in understanding how copper tolerance genes contribute to the ability of *MRSA* to colonize and persist during human infection. Joan Geohegan and her team are investigating how copper tolerance genes are carried on mobile genetic elements and allow *MRSA* to resist the antibacterial effects of copper to help the bacteria to withstand killing by human immune cells. This is related to the group's interest in antibiotic resistance in pathogenic bacteria and the link between metal and antibiotic use in food-production animals and antibiotic resistance in human pathogens. The main aim of this work is to identify new targets for the treatment and prevention of *S. aureus* infection.

For more information, please see:
https://www.tcd.ie/Microbiology/research/joan-geoghegan/

Eva Gluenz

Royal Society University Research Fellow and Group Leader
Research Lecturer
Sir William Dunn School of Pathology, University of Oxford
Fellow by special election
St. Edmund Hall, University of Oxford
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Eva Gluenz earned a MSc in Biology from the University of Bern, Switzerland, in 2000. She obtained her PhD at London School of Hygiene and Tropical Medicine in 2005. From 2004-2011, she was a post-doctoral research associate at the Sir William Dunn School of Pathology, University of Oxford and in 2011, started her own research group as a Royal Society University Research Fellow and Research Lecturer at the Dunn School.

The Gluenz lab studies the single-celled parasites *Leishmania*, which cause disease in humans and animals in over 88 countries around the world. Leishmaniasis is a neglected disease, associated with poverty and conflict. There is currently no vaccine and an urgent need for better drug treatments.

Leishmania are transmitted by blood feeding sand flies and in the mammalian host they enter macrophages of the host and replicate intracellularly. Eva Gluenz and her colleagues study the molecular cell biology of this parasite to understand how it can cycle between insect vector and mammalian host and how the parasites modulate host-cell functions to survive in macrophages. The research focuses on three main areas:

1. Structure and function of the *Leishmania* flagellum. Cilia and flagella are cellular projections built around a microtubule axoneme whose molecular architecture is highly conserved across eukaryotes. When the *Leishmania* parasite is engulfed by a macrophage, it changes shape and the flagellum turns from a device built for into a structure resembling a sensory cilium. The scientists aim to dissect the mechanisms that govern this change in flagellar motility structure and test their hypothesis that the amastigote flagellum serves as a sensory organelle in host-parasite interactions.
2. Identification of genes and pathways important for survival in a macrophage. The Gluenz lab used RNA-sequencing to map gene expression patterns in the insect- and mammalian-infective forms of *Leishmania mexicana*. Comparative analyses allowed us to define differences in gene expression patterns between the different parasite forms and we now seek to investigate their functions.
3. Development of genetic tools. Eva Gluenz and her lab have developed a CRISPR-Cas9 high throughput genome editing toolkit for *Leishmania* and related protists and is using these tools to harness the information from genome, transcriptome and proteome data and dissect the cell biology of *Leishmania*.

For more information, please visit:
http://users.ox.ac.uk/~path0389/

Susanne Häussler

Department of Clinical Medicine
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Susanne Häußler studied medicine at the Medical Faculty of the University of Lübeck and at the Medical School Hannover (MHH), where she obtained her doctorate (Dr. med.) in 1995. Afterwards, she worked in the field of internal medicine in a hospital in Vechta for one year and started specializing in the field of medical microbiology and infection epidemiology at the department of medical microbiology at the MHH. In 2002, Susanne Häußler finished her specialization and she completed her habilitation thesis in 2004.

Her research interests are in the area of the pathogenicity of acute and chronic *Pseudomonas aeruginosa* infections. From 2003 to 2004, she worked on that topic as a project leader in the department of Cell and Immune Biology at the German Research Centre for Biotechnology (GBF). From 2005 to 2012, she was the head of the junior group "Chronic Pseudomonas Infections" at the GBF (since 2006 Helmholtz Centre for Infection Research, HZI) with a close connection to the MHH.

In January 2009, Susanne Häußler became Professor in "Pathophysiology of Bacterial Biofilms" of the MHH at TWINCORE, Centre for Experimental and Clinical Infection Research in Hannover. Since 2012, she is the head of the department "Molecular Bacteriology" at the HZI and full professor at the MHH.

Since August 2019, she is establishing a Novo Nordisk Foundation funded lab at the Rigshospitalet in Copenhagen, which aims at the development of novel molecular diagnostics to fight antimicrobial resistance.

For more information, please visit:
https://www.twincore.de/forschungsgruppen/molekulare-bakteriologie/

Helle Krogh Johansen

Clinical Professor
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Rigshospitalet- Diagnostisk Centre
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Dr. Johansen graduated as a medical doctor from the University of Copenhagen in 1987 and received her DMSc from the University of Copenhagen in 1996. She became a specialist in clinical microbiology in 2000 and has worked as a consultant at Rigshospitalet, a tertiary referral hospital, taking care of more than 320 children and adult cystic fibrosis (CR) patients since 2004.

Helle Krogh Johansen's research has for the last three decades focused on clinical and translational biomedical aspects of CF. Recently she introduced genome sequencing and bioinformatics as a new clinical diagnostic tool of *Pseudomonas aeruginosa* in CF patients in the Copenhagen CF clinic. She is the author/co-author of more than 160 scientific articles in peer-reviewed international journal and 25 Cochrane reviews.

She was chairperson for The Danish Society for Clinical Microbiology from 2006 to 2012 and she has presented more than 220 posters and been an invited speaker at more than 85 international conferences, and been the leader of several round table discussions, and moderator at several sessions at international conferences.

For more information, please visit:
https://ikm.ku.dk/english/contact/ansatte/?pure=en/persons/33067

Søren Molin

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Søren Molin earned a Master Degree (1972) and a PhD in Microbiology from the University of Copenhagen (1979), he was Assistant and Associate Professor until 1983 when he became Full Professor of Microbiology at the Department of Systems Biology at the Technical University of Denmark (DTU). From 2011-2015, he was Professor and Scientific Section Director, Novo Nordisk Foundation Center for Biosustainability, DTU, and since 2016, he is Professor and Group Leader at the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark. In March 2018, he stepped down as full professor and entered a part-time contract with DTU Biosustain.

Dr. Molin was a member in the Forskningsrådet for Natur og University (Danish Natural Science Research Council). He is Member of Danish Academy of Natural Sciences, The Academy of Technical Sciences, The Royal Danish Academy of Sciences. Dr. Molin received several awards and among these are: The Novo Nordisk Prize in 2014, Researcher of the Year 2006/2007 and has also been awarded the Most Cited Danish Microbiologist (included in ISIHighlyCited.com). Professor Molin has been engaged in teaching at all levels at Danish universities for 40 years and has supervised approximately 40 PhD students.

Søren Molin's research activities cover broad aspects of microbiology and has done so for 40 years of academic work at Danish universities. The initial research activities focused on growth physiology and control of macromolecular synthesis in *E. coli* (DNA, RNA and protein), including early applications of genetic engineering in the 1970s. Later, his research turned to extracellular proteins, secretion, and stress responses in *Pseudomonas*. In the 1990s, Søren Molin's laboratory conducted pioneering research on biofilms where they developed and introduced molecular and imaging tools for studies of bacterial biofilm development. These activities are still the foundation for Dr. Molin's on-going research, which also comprises cell-cell interactions and evolutionary processes. He has further developed the field of microbial adaptation and evolution during chronic infection. His current research activities are based on the assumption that fundamental studies of bacterial physiology and ecology are essential to understand and eventually interfere with such microbial infections.

During the last 10 years, Søren Molin has been actively engaged in translational studies of long-term bacterial infections in human airways focused on 1) the adaptive processes driving bacteria towards chronic infections states, and 2) the transfer of biological knowledge and methods to clinical microbiology applications.

For more information, please visit:
https://www.dtu.dk/english/service/phonebook/person?id=1652&tab=1

Mikael Sellin

Associate Senior Lecturer
Department of Medical Biochemistry and Microbiology (IMBIM)
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Mikael Sellin studied towards a MSc degree at Umeå University and Karolinska Institute and later earned his PhD at the Department of Molecular Biology, Umeå University in 2011. From 2011 until 2015, he was a post-doc at the Institute of Microbiology, ETH Zürich, Switzerland. 2015-2016, he worked as a Senior Scientific Associate at the same department. Since 2016, Mikael Sellin heads a research group at Uppsala University, Department of Medical Biochemistry and Microbiology.

The Sellin lab studies the microbe – host interactions that drive progression of intestinal infectious disease. Instead of exclusively studying the pathogen – host cell interplay under traditional cell culture settings, the Sellin lab aspires to understand the relevant molecular underpinnings under conditions that more closely resemble the intact gut. In this work, the Sellin lab employs organotypic tissue culture, analysis of intact infected tissues, bacterial genetics approaches, and state-of-the-art live microscopy. The work focuses on clinically relevant enteropathogens – i.e. Salmonella and Shigella species – and how these bacteria invade, propagate and spread within the gut mucosa.

For more information, visit:
https://www.imbim.uu.se/research-groups/infection-and-defence/sellin-mikael/

Boris Striepen

Professor
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Boris grew up in Ruhrort where the German rivers Rhine and Ruhr meet, an industrial area then dominated by coal and steel. He studied biology at the universities of Bonn and Marburg and conducted undergrad research on liver flukes in Bonn and Nagana in Bobo Dioulasso, Burkina Faso. Boris earned a PhD summa cum laude for work on parasite biochemistry with Ralph Schwarz, was a postdoc with David Roos studying parasite cell biology, and joined the faculty of the Center for Tropical & Emerging Global Diseases at the University of Georgia in 2000 until 2017.

He rose to Distinguished Research Professor prior to joining the faculty of the University of Pennsylvania.

Since 2017, Boris Striepen is Professor at the Department of Pathobiology, Department of Pathobiology, the Department of Microbiology and the Center for Global Health at the University of Pennsylvania in Philadelphia.

Boris studies the cell and molecular biology of apicomplexan parasites and directs an internationally respected research laboratory. His current research focus is the parasite *Cryptosporidium*, a leading global cause of diarrhea and mortality in young children. His lab pioneered molecular genetics for this important infection and leads a range of interdisciplinary efforts to understand fundamental parasite biology and to advance urgently needed translation towards drugs and vaccines. Boris is also engaged in education and training. He taught undergraduate, graduate and veterinary classes, directed an NIH training grant program in parasitology, and served as faculty and director of the Biology of Parasitism summer research course at the Marine Biology Laboratories in Woods Hole, MA. Boris is married to a social worker with remarkable patience for scientists and has three children, two are scientists – all are awesome.

For more information, please, visit:
https://www.striepenlab.org/

Raphael H. Valdivia

Professor of Molecular Genetics and Microbiology
Amgen Faculty Mentor
CMB – Molecular Genetics and Microbiology
Third Year Mentor – Molecular Medicine Study Program (MolMed)
Duke University School of Medicine, Durham, U.S.A.

Raphael Valdivia, PhD, received his B.S. in Microbiology from Cornell University in 1991. He moved to California to begin graduate studies with Stanley Falkow in the Department of Microbiology and Immunology at Stanford University. In Dr. Falkow's laboratory, Dr. Valdivia devised a fluorescence-based screen to identify virulence factors that are induced when pathogens enter a host cell and which are involved in remodeling the host cell's endosomal compartments. In the process, he developed new generations of green fluorescent proteins that are widely used today. In 1998, Dr. Valdivia moved to the University of California, Berkeley, to study endosome dynamics and biogenesis in the laboratory of Randy Schekman. Dr. Valdivia used the formidable tools of genetics and biochemistry of the yeast *Saccharomyces cerevisiae* to uncover novel transport pathways between endosomal compartments.

Dr. Valdivia also serves as an editor in multiple journals (PLoS Pathogens, Pathogens and Disease, the Journal of Bacteriology, and Infection and Immunity), is a standing member of NIH review panels, and was elected Fellow of the American Association for the Advancement of Science (AAAS) in 2012. Dr. Valdivia is the founding Director of the Duke Center for the Genomics of Microbial System (GeMS) and Vice Dean for Basic Sciences at the School of Medicine.

Dr. Valdivia's laboratory focuses on identifying and characterizing the bacterial factors that are secreted into the host cell cytoplasm to manipulate cellular functions. They use a combination of cell biology techniques, biochemistry, genetics, genomics, proteomics and molecular biology to determine the function of virulence factors that reveal novel facets of the cell biology of host-pathogen interactions. The ultimate goal is to understand how these obligate intracellular bacterial pathogens manipulate host cellular functions to replicate, disseminate and cause disease.

A second area of focus in Dr. Valdivia's research group is the development of new methods to perform genetic analysis in many of the microbes that reside in our gut. Understanding how the collection of genetic information of microbes associated with our bodies (microbiomes) impact our health is one of the new frontiers in microbiology. The group is currently studying how bacteria that proliferate in the mucus layers of our gut contribute to nutrient homeostasis and immunological health.

For more information, please visit:
https://medschool.duke.edu/about-us/our-faculty/raphael-h-valdivia

Short talks

From the 83 submitted abstracts, The Organizing Committee selected 22 projects for short oral presentations.

Some of these speakers will not show posters. Their abstracts are presented in this section.

For all other short talk presentations, please, see the poster abstract.

Helicobacter species infections cause glycosylation changes which affect the *Helicobacter* binding ability to mucins from pig and human stomachs

Gurdeep Chahal[1], Medea Padra[1], Chunsheng Jin[1], Mattias Erhardsson[1], Janos Padra[1], Anders Thorell[2], Niclas G Karlsson[1], Sara K Lindén[1]

[1]University of Gothenburg, Department of Medical Biochemistry and Cell Biology, Gothenburg, 41390 Sweden, [2]Karolinska Institutet, Department of Clinical Science at Danderyds Hospital and Department of Surgery, Ersta Hospital, Stockholm, Sweden

H. pylori infects the stomach of half the world's human population and *H. suis* is the most prevalent non-*Helicobacter pylori Helicobacter* species in the human stomach. Infection with both species is associated with chronic gastritis, peptic ulcer disease, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Here, we show that *H. pylori* and experimental *H. suis* infection change the gastric mucin glycosylation. The overall ability of mucins from *H. pylori* infected humans to bind *H. pylori* is increased compared to mucins from non-infected individuals. Among mucins positive for the glycan structure Lewis b, which bind to *H. pylori* via the Blood group binding adhesin (BabA), mucins from infected individuals had increased binding capacity compared to non-infected Lewis b positive individuals. This was in line with that the level of Lewis b was higher among mucins from infected individuals. In contrast, the binding ability of Lewis b negative mucins from infected individuals had decreased *H. pylori* binding ability compared to mucins from non-infected individuals. Additionally, Lewis b was associated with increased *H. pylori* J99 wt adhesion, but the relation was not linear, and binding occurred also to Lewis b negative samples. In line with the results from the Lewis b negative mucins, the *H. suis*-binding ability of mucins from *H. pylori*-infected humans and *H. suis* infected pigs was lower than that of non-infected individuals. Thus, *Helicobacter* species infections impair the mucus barrier by decreasing the binding ability of the mucins to *H. suis* and to BabA negative *H. pylori*. In contrast, the enhanced Leb dependent ability of human gastric mucins from infected individuals, suggests an increased defence against BabA-positive *H. pylori*. Previous studies have demonstrated downregulation/loss of BabA during infection however, suggesting that BabA positive bacteria can avoid this defence.

Streptococcal sagA activates a pro-inflammatory response in mast cells by a sublytic mechanism

Christopher von Beek[1], Waern I[2], Eriksson J[3], Melo FR[1], Robinson C[4], Waller AS[4], Sellin ME[3], Guss B[5], Pejler G[1,2].

[1]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, [2]Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden, [3]Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden, [4]Department of Bacteriology, Animal Health Trust, Kentford, Newmarket, UK, [5]Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Mast cells are implicated in the innate pro-inflammatory immune defense against bacterial insult, but the mechanisms through which mast cells respond to bacterial encounter are poorly defined. Here we addressed this issue and show that mast cells respond vividly to wild-type *Streptococcus equi* by up-regulating a panel of pro-inflammatory genes and by secreting pro-inflammatory cytokines. However, this response was completely abrogated when the bacteria lacked expression of *sagA*, whereas the lack of a range of other potential virulence genes (*seeH*, *seeI*, *seeL*, *seeM*, *hasA*, *seM*, *aroB*, *pyrC*, *recA*) had no effect on the amplitude of the mast cell responses. The *sagA* gene encodes streptolysin S, a lytic toxin, and we next showed that the wild-type strain but not a *sagA*-deficient mutant induced lysis of mast cells. To investigate whether host cell membrane perturbation per se could play a role in the activation of the pro-inflammatory response we evaluated the effects of detergent- and pneumolysin-dependent lysis on mast cells. Indeed, exposure of mast cells to sublytic concentrations of all these agents resulted in cytokine responses of similar amplitudes as those caused by wild-type streptococci. This suggests that sublytic membrane perturbation is sufficient to trigger full-blown pro-inflammatory signaling in mast cells. Subsequent analysis showed that the p38 and Erk1/2 signaling pathways had central roles in the pro-inflammatory response of mast cells challenged by either *sagA*-expressing streptococci or detergent. Altogether, these findings suggest that *sagA*/streptolysin S-dependent mast cell membrane perturbation is a mechanism capable of activating the innate immune response upon bacterial challenge.

Panacea - a universal antitoxin domain

Chayan Kumar Saha[1], Steffi Jimmy[1], Constantine Stavropoulos[1], Tatsuaki Kurata[1], Kathryn Turnbull[1,] Vasili Hauryliuk[1], Gemma C. Atkinson[1]

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Toxin-antitoxin systems (TAs) are typically comprised of two closely linked genes encoding a toxic protein or RNA and its antidote, the antitoxin - also protein or RNA. TAs provide a regulatory mechanism of severe but reversible growth rate reduction, and while specific functions are often unknown, roles have been documented for certain TA pairs in plasmid maintenance and resistance to bacteriophages. TAs are most common in bacteria, but are also found in genomes of archaea and bacteriophages. Using our computational tool FlaGs, standing for Flanking Genes, which detects conservation of gene neighbourhood over large evolutionary distances, we are uncovering a vast network of combinations of different toxin and antitoxin domains. Most strikingly, we have discovered an antitoxin protein domain that can be paired with over 300 different toxin domains, and is present in many lineages across the bacterial tree of life with additional representatives also in phages and archaea. The list of toxin domains includes some well-known toxins such as mRNA endonucleases MazF and MqsR, but most are currently unknown. Using toxin neutralisation microbiological assays, we have confirmed 12 predicted novel TA pair types as bona fide TA systems involving a common antitoxin domain and novel toxins. Given its apparent function as a universal antidote, we have named this domain Panacea. We are now working to understand the mechanism of action of Panacea, and the many novel toxins it neutralises, while also analysing the evolutionary processes of TA partner swapping.

Poster No. 1

Studies on citrullinated LL-37: detection in human bronchoalveolar lavage fluid, antibacterial effects and biophysical properties

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Background: Arginine residues of the antimicrobial peptide LL-37 can be citrullinated by peptidyl arginine deiminases (PADs). Notably, citrullinated LL-37 has not been detected in human biological material. In addition, functional and biophysical properties of citrullinated LL-37 are not fully explored.

Aim: To detect citrullinated LL-37 in human bronchoalveolar lavage (BAL) fluid and to determine antibacterial and biophysical properties of citrullinated LL-37.

Material and methods: BAL fluid was obtained from human healthy volunteers exposed to lipopolysaccharides (LPS). Synthetic peptides were used for bacterial killing assays, transmission electron microscopy, isothermal titration calorimetry, mass-spectrometry and circular dichroism.

Results: Citrullinated LL-37 was detected in BAL fluid by targeted proteomics. It lacked bactericidal effects against *Escherichia coli* and did not cause membrane leakage in human red blood cells. Isothermal calibration showed that both native and citrullinated LL-37 interacted with LPS in a biphasic mode with different kinetic profiles. Both peptides had similar α-helical secondary structures, however, citrullinated LL-37 appeared to be more stable at higher temperatures, as shown by circular dichroism. ***Conclusions:*** Citrullinated LL-37 is present in the human lung and the most striking effect of citrullination was impaired bacterial killing, demonstrating the importance of a net positive charge for antibacterial and membrane lysing effects.

Poster No. 2

The global RNA-binding protein ProQ promotes SPI-2 gene expression through the transcriptional regulator SlyA

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Salmonella enterica is a food-borne bacterial pathogen and one of the major causes of gastroenteritis. To establish an infection in the human gut, Salmonella needs to precisely orchestrate the expression of a large number of virulence genes, the majority of which are encoded in Salmonella pathogenicity island 1 (SPI-1) and 2 (SPI-2). This is achieved by transcription factors acting at the level of DNA, and post-transcriptional regulators such as RNA-binding proteins (RBPs) and small RNAs. Global RBPs are key post-transcriptional regulators that control many important cellular processes, including virulence, in a variety of bacterial species. In *Salmonella*, Hfq, CsrA, and ProQ are key players during the physiological transition from extracellular to intracellular replication (Ansong *et al*., 2009; Lawhon *et al*., 2003; Sittka *et al*., 2008). Still, the mechanisms by which these RBPs act, in particular the recently discovered protein ProQ, are not fully understood. A recent study showed that ProQ promotes SPI-2 gene expression (Westermann *et al*. 2019), but the underlying molecular mechanism has not been described. Using qRT-PCR, we could confirm that ProQ is a positive determinant for SPI-2 gene expression. To test if ProQ acts upstream of SPI-2, we analysed the expression of several known transcriptional activators of SPI-2 genes. Deletion of *proQ* had negligible effects on the expression of *phoP*, *phoQ*, *ompR*, and *envZ*. In contrast, the absence of ProQ strongly impaired expression of the *slyA* mRNA, while complementing a *proQ* deletion strain with plasmid-expressed ProQ restored the *slyA* mRNA to wild-type levels. Using a transcriptional SPI-2 reporter (P*ssaG-gfp*), we could confirm that SlyA is strictly required for ProQ-dependent activation of SPI-2 genes. Importantly, this also applies to intracellular *Salmonella* during infection of human U937 cells. Recently, *in vivo* RNA-protein cross-linking identified a ProQ binding site at the 3' end of *slyA* mRNA (Holmqvist *et al*. 2018), suggesting a direct effect on *slyA* expression. In conclusion, we propose that ProQ promotes expression of genes involved in intracellular survival by interacting with the *slyA* mRNA.

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Poster No. 3

Within-host adaptation mediated by intergenic evolution in Pseudomonas aeruginosa

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Bacterial pathogens evolve during the course of infection as they adapt to the selective pressures that confront them inside the host. Identification of adaptive mutations and their contributions to pathogen fitness remains a central challenge. Although mutations can either target intergenic or coding regions in the pathogen genome, studies of host adaptation have focused predominantly on molecularevolutionwithincodingregions,whereastheroleofintergenicmutationsremainsunclear. Here,weaddressthisissueandinvestigatetheextenttowhichintergenicmutationscontribute tothe evolutionary response of a clinically important bacterial pathogen, *Pseudomonas aeruginosa*, to the host environment, and whether intergenic mutations have distinct roles in host adaptation. We characterize intergenic evolution in 44 clonal lineages of *P. aeruginosa* and identify 77 intergenic regionsinwhichparallelevolutionoccurs.Atthegeneticlevel,wefindthatmutationsinregionsunder selection are located primarily within regulatory elements upstream of transcriptional start sites. At the functional level, we show that some of these mutations both increase or decrease transcription of genes and are directly responsible for evolution of important pathogenic phenotypes including antibiotic sensitivity. Importantly, we find that intergenic mutations facilitate essential genes to become targets of evolution. In summary, our results highlight the evolutionary significance of intergenic mutations in creating host-adapted strains, and that intergenic and coding regions have different qualitative contributions to thisprocess.

Poster No. 4

The role of Guanylate-binding proteins (GBPs) during intracellular infection with *Francisella tularensis*

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Francisella tularensis (*F. tularensis*) is a facultative intracellular Gram-negative bacterium and the causative agent of the zoonotic disease tularemia. IFN-γ is crucial to control infection with this bacterium. However, it is unclear how the critical role of IFN-γ for control of cytosolically localized bacteria is executed, but recent studies on *Francisella novicida (F. novicida),* a bacterium closely related to *F. tularensis*, showed that GBP2 and GBP5, two major proteins from the GBP family, targeted cytosolic *F. novicida* and promoted their lysis. The bacterial genomic DNA released into host cytosol was proposed to activate the AIM2 inflammasome. The role of GBPs for control of *F. tularensis* infection is much less studied. In view of the lack of understanding regarding how the antibacterial mechanisms are mediated by GPBs and the lack of knowledge regarding their role for control of other *Francisella* strains, both of these questions are critical for a comprehensive understanding of the role of GBPs.

In this study we specifically assessed how GBP2 affects intracellular growth of *F. tularensis* LVS or SCHU S4 and *F. novicida* in mouse cells, and if the AIM2 inflammasome is required for the GBP2 antibacterial activities in *F. tularensis* strains. Preliminary data on mouse macrophage cells indicates that GBP2 are important to restrict intracellular growth of the *F. tularensis* LVS strain and *F. novicida*, however, the SCHU S4 strain is not affected by the GBP2-mediated control.

Poster No. 5

The autoregulation of *Escherichia coli* RelA through intramolecular interactions

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When subjected to amino acid starvation, *Escherichia coli* cells rapidly produce the alarmone nucleotide (p)ppGpp. Accumulation of (p)ppGpp potentiates transcriptional rewiring and a dormant cellular phenotype that promotes survival during stress conditions. RelA, the enzyme responsible for this stress response, has to be tightly regulated in order to avoid aberrant (p)ppGpp synthesis in non-stressed conditions. Conversely, it is well documented that in the absence of stress, the N-terminal domain (NTD) of RelA constitutively produces (p)ppGpp. The C-terminal domain (CTD) has thus been proposed to regulate the N-terminal synthase domain off the ribosome, but there are currently conflicting reports regarding the mechanistic interplay. Here, we present data that shows high ectopic expression of RelA-CTD inhibits translation in a (p)ppGpp-independent manner, suggesting that CTD directly interacts with the ribosome. Furthermore, we reveal that growth permitting low level expression of RelA-CTD does not sequester the activation of native RelA, indicating that the activation of RelA is not regulated by dimerization through the CTD as previously proposed. Our findings show that the (p)ppGpp synthetic activity of RelA is negatively regulated by the presence of the RRM subdomain of the CTD, suggesting existence of possible intramolecular interaction between RRM and NTD. Considering the detrimental effects of unchecked RelA-NTD (p)ppGpp synthesis, or untimely association of the CTD with the translation machinery, the autoinhibition of RelA is vital for maintaining high cellular fitness.

A novel antibacterial compound with antibiotic effect in *Chlamydia* infected mice

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Novel antibiotic targets are important for combating infections. ***Chlamydia trachomatis*** is a significant human pathogen with limited antibiotic treatment options. We have previously shown that acylated sulfonamides inhibit ***Chlamydia*** fatty acid synthesis (FAS II), an interesting target for novel antibiotics. The current study investigated the efficacy of this compound class ***in vivo***. Drug profiling of the acylated sulfonamides including chemical and metabolic stability in liver microsomes predicted good drug-like properties. Pharmacokinetics in mice showed high exposure after parenteral administration of compound ME0619. In mice, ME0619 was metabolized to sulfamethoxazole and thus functioned as a prodrug of the folate synthesis inhibitor in addition to the inhibition of FAS II. Sulfamethoxazole inhibits ***Chlamydia*** growth ***in vitro*** but the effect ***in vivo*** is not known. We determined pharmacokinetics of sulfamethoxazole in mice and confirmed that a dose of 3 mg/kg resulted in a slightly higher exposure compared to the amount of sulfamethoxazole formed after administration of 10 mg/kg ME0619. Mice vaginally infected with ***C. trachomatis*** were thereafter treated with either 10 mg/kg ME0619, 3 mg/kg sulfamethoxazole or vehicle by intraperitoneal injections for 7 days. ME0619-treatment was effective with significantly more mice clearing the infection during treatment (16/19 mice), while there was no significant difference between sulfamethoxazole treatment (5/19) and vehicle (1/19). Our data show that ME0619 was effective for treatment of vaginal ***C. trachomatis*** infection ***in vivo*** and validates fatty acid synthesis as an interesting antibiotic target in ***Chlamydia***.

Biofilm expression patterns to microenvironmental cues

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Biofilms are a fundamental form of microbial life. Approximately 80% of all bacterial and archaeal cells on Earth exist as biofilms. Biofilm formation is closely associated with the surrounding micro-environment, wherein the external cues trigger the expression of concert of genes, evoking a multitude of physical and biochemical responses. *Salmonella*, the prominent cause of food poisoning, assembles curli and cellulose into an extracellularmatrix (ECM) that promotes cell community behavior. Curli in particular, is involved in many pathogenic processes of Salmonella, participating in persistence, cell aggregation, host cell adhesion and invasion, and they are potent inducers of the host inflammatory and immune responses. Using optotracing, a novel tool for tracking ECM formation, this study shows the development of biofilms on the air-solid interface in real-time. By tracking the curli specific fluorescence released by optotracers, we examine the development of wrinkles, ridges and channels, and how they associate with micro-environmental parameters such as salinity, viscosity, carbon source, pH, volume and surface area. By simultaneously tracking bacteria division, this study also elucidates thegrowth patterns of bacteria and biofilms on solid medium, previously only recorded in liquid medium. Understanding the association the micro-environment with biofilm ECM formation and the resultant morphotypes will contribute to biofilm control and the prevention of salmonelldosis.

Poster No. 8

Characterization of the secreted cysteine protease CP17516 as a potential virulence factor

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Giardia intestinalis is an intestinal protozoan parasite causing diarrhoea infections in humans. Worldwide there are an estimated 280 million symptomatic *Giardia* infections (giardiasis) annually, affecting mainly young children in developing countries. Despite this, *Giardia* virulence factors and disease mechanisms and are poorly characterized. However, secreted proteins are known to contribute to the parasites pathogenicity. Of these proteins many cysteine proteases (CPs) were identified to be highly upregulated and secreted during co-infections with intestinal epithelial cells (IECs) *in vitro*. CPs have a clear link to *Giardia* virulence and have been shown to be responsible for the degradation of several immune factors as well as contribute to the disruption of the intestinal epithelial barrier. The uncharacterized CP, CP17516, was shown to be secreted during *Giardia's* interaction with IECs by two independent secretome studies. This secreted CP is glycosylated and contains a secretion signal peptide (SSP). Interestingly, CP17516 has an unusual active site lacking the common cysteine and histidine residues (C101S and H245L) found in most other CPs. Therefore, we started to characterize the potential function of CP17516 during infection. First, we localized the protein in trophozoites to the endoplasmic reticulum and cytoplasmic vesicle-like structures (a localization pattern similar to other characterized CPs) by epitope tagging. After this we went on to characterize its activity. Recombinant CP17516 expressed by *Pichia pastoris* showed no activity towards any tested substrate. Further, we attempted to perform substrate phage display but no substrate specificity could be identified. Therefore, it appears that this CP might have a different function than previously known and characterized CPs. Currently we are investigating if and what host or parasite proteins CP17516 binds to during an infection. Our goal is to co-immunoprecipitate CP17516 with its potential host receptor. Further we are using live-imaging to track the protein during a co-infection with IECs *in vitro*.

Poster No. 9

Cross-resistance between colistin and the antimicrobial peptide LL-37 in carbapenemase-producing *Klebsiella pneumoniae*

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Background: Carbapenem-resistance is rapidly spreading among multidrug-resistant Gram-negative pathogens. Colistin is an important treatment option, but resistance against colistin is increasing. Colistin is an antimicrobial peptide (AMP) thus cross-resistance between the endogenous AMP LL-37 and colistin might occur. However, literature data is not conclusive in this issue. This study aimed to examine it, also to test how colistin resistant strains interact with other innate antimicrobial effector systems, including hydrogen peroxide (H_2O_2), serum and whole blood.

Materials and methods: Two carbapenemase-producing *Klebsiella pneumoniae* (same sequence type, virulome, and plasmid content); one colistin-susceptible (Col-S) and one colistin-resistant (Col-R) were used. *K. pneumoniae* ATCC 25955 was used as a Col-S control. Antimicrobial susceptibility assays were performed for all strains using standard CFU-killing assays with LL-37 at different concentrations (3 mg/L - 100 mg/L), hydrogen peroxide (H_2O_2), serum and whole blood. Growth-curves were generated using BioScreen.

Results: The Col-R strain exhibited a higher CFU count for LL-37 compared to the Col-S and ATCC strains at concentrations above 50 mg/L and the difference gradually decreased at lower concentrations. There was no difference between the Col-S and Col-R strains with regard to susceptibility to H_2O_2, serum or whole blood. However, the ATCC control strain was more resistant to serum and whole blood than the other strains. Reciprocally, the ATCC-strain was more susceptible to H_2O_2 than the carbapenemase-producing strains. When the complement system was inhibited by sodium polyanethole sulphate the killing of all three strains in whole blood was inhibited.

Conclusion: We observed cross-resistance between colistin and LL-37 at concentrations > 50 mg/L of LL-37. No difference between the examined strains in relation to survival in serum, whole blood or H_2O_2-exposure were observed. Thus, the potential risk of cross-resistance between colistin and LL-37 might have lower impact than expected, since these strains are readily killed by other innate effector systems.

The transcriptional regulator CsvR controls the modulation of Enterotoxigenic Escherichia coli Virulence by Bile Salts

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Enterotoxigenic *Escherichia coli* (ETEC) is one of the four most important agents that cause moderate-to-severe diarrheal among children < 5 years in endemic countries. It is commonly associated with traveler's diarrhea. ETEC cause disease primarily by attaching to the human small intestine through surface appendages called colonization factors (CFs), after which bacterial toxins stimulate intestinal secretion resulting in diarrhea. Virulence in ETEC is influenced by a highly regulated transcriptional cascade which translates its interaction with host factors into the modulation of virulence. We discovered that the host factor, bile and the bile component glyco-conjugated cholate (NaGCH) altered the expression of 61 genes in CS5+CS6 ETEC isolates. The most striking finding was the >100-fold increase of gene expression of the CS5 operon (csfA-F), a putative transcription factor csvR (a member of the AraC/XylS family), and the putative ETEC virulence factor cexE gene. iTRAQ-coupled LC-MS/MS proteomic analysis verified in the induction of the plasmid borne virulence proteins CS5 and CexE and also showed that NaGCH affected the expression of bacterial membrane proteins. NaGCH also induced bacteria to form a biofilm-like aggregation, increased their adherence to epithelial cells through the induction of CS5 and reduced their motility. These effects are not observed in our csvR mutant indicating that this transcription factor is essential for ETEC virulence in a bile dependent manner. RNA sequencing of the csvR mutant also showed > 300 genes differentially expressed (+/- 2-fold, $p < 0.05$), of which the majority are encoded in the bacterial chromosome and involved in chemotaxis and motility. Among the plasmid encoded genes, we found transcription factors and virulence genes associated. Our results indicate that CS5+CS6 ETEC uses bile present in the small intestine as a signal through the activation of CsvR to initiate colonization of the epithelium and unravel a novel bile-dependent regulatory network governed by CsvR.

Poster No. 11

Sublethal antibiotic concentrations as drivers of the evolution of antibiotic resistance in *Escherichia coli*

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With the widespread global use of antibiotics, it is possible to find substantial antibiotic concentrations in a number of different environments, including humans, animals, food, water and soils. Additionally, several studies regarding the effect of antibiotics in bacteria have shown that low levels of antibiotic exposure can select for resistant bacteria. However, how they acquire and develop such resistance still remains poorly defined. In this study, we examined how long-term exposure to sublethal concentrations of some commonly used antibiotics select for mutants with reduced antibiotic susceptibility. Thus, for 8 different clinically important antibiotics (mecillinam, tetracycline, imipenem, cefotaxime, nitrofurantoin, trimethoprim, trimethoprim/sulfamethoxadole and fosfomycin), and with drug concentrations up to several hundred-fold below the minimal inhibitory concentration of susceptible bacteria, we show rapid enrichment for highly resistant bacteria in the *Escherichia* coli strain MG1655 exposed to these antibiotics.

These results provide us with fundamental knowledge regarding resistance evolution at different drug concentrations and the potential impact low drug levels have in maintaining and generating clinically relevant resistances. Thus, sub-MIC levels of antibiotics that are far below the MIC of susceptible bacteria can both contribute to the maintenance of existing resistances as well as select *de novo* for resistant mutants from a susceptible population.

Poster No. 12 - short talk

Dissemination of antibiotic resistance genes from antibiotic producers to pathogens

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Elucidating the origins of antibiotic resistance genes (ARGs) and the mechanisms mediating their spread to pathogens has become a public health priority. It has been hypothesized that some ARGs found in pathogenic bacteria derive from antibiotic-producing actinobacteria. Here we provide bioinformatic and experimental evidence supporting this hypothesis. We identify genes in proteobacteria, including human and farm animal pathogens, that appear to be closely related to actinobacterial ARGs known to confer resistance against clinically important antibiotics. Furthermore, we identify two examples of recent horizontal transfer of actinobacterial ARGs to proteobacterial pathogens. Based on this bioinformatic evidence, we propose and experimentally test a 'carry-back' mechanism for the transfer, involving conjugative transfer of a carrier sequence from proteobacteria to actinobacteria, recombination of the carrier sequence with the actinobacterial ARG, followed by natural transformation of proteobacteria with the carrier-sandwiched ARG. Our results support the existence of ancient and, possibly, recent transfers of ARGs from antibiotic-producing actinobacteria to proteobacteria, and provide evidence for a defined mechanism.

Poster No. 13

Novel c-di-GMP-dependent Metabolic Capabilities in Extraintestinal Pathogenic *Escherichia coli* Due to Loss of Global Stress Regulation

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The species *Escherichia coli* is defined by its immense variety of commensal, pathogenic and environmental strains. The conventional signalling via bis-(3',5')-cyclic dimeric guanosine monophosphate (cyclic di-GMP or c-di-GMP) in *E. coli* controls sessility-motility changes linked to commensalism and/or pathogenicity. Extraintestinal Pathogenic *E. coli* (ExPEC) are "commensals" that can cause an array of infections outside the gastrointestinal tract. To accommodate their pathogenic lifestyle with the commensal one, ExPEC biology is shaped not only by the presence of specific virulence genes and pathoadaptive mutations but also by regulatory adaptations. A subgroup of ExPEC is represented by Neonatal Meningitis-causing *E. coli* (NMEC). We found that NMEC maintain remarkably low levels of c-di-GMP as a result of deficiency in the global stress regulator RpoS. The altered regulation of c-di-GMP signalling in the NMEC strain IHE3034 involved the *ycgG2* gene, coding for an YcgG allozyme with c-di-GMP phosphodiesterase activity. The virulence-associated S-fimbriae were robustly produced in artificial urine whereas deletion of *ycgG2,* or the restoration of RpoS activity, led to a decrease in S-fimbrial expression. The findings hint that the low c-di-GMP level promotes the adaptation of NMEC to reside in the urinary tract. The YcgG2 level was lower in artificial urine compared to LB medium. We also considered that regulation of YcgG2 expression in NMEC might contribute to a potential alteration in metabolic capability. NMEC were capable of aerobic citrate utilization in presence of a co-substrate - a property that normally does not exist in *E. coli*. The main reason for this metabolic capability are two pathoadaptive events – the RpoS inactivation which resulted in the production of the citrate transporter CitT, and the downregulation of YcgG2 which further stimulated the citrate catabolism. Our results suggest that bacteria can exploit CitT for YcgG2-dependent ferric citrate uptake. The absence of RpoS and the presence of YcgG2 suggested for a new c-di-GMP regulatory node that linked metabolic adaptation to the expression of virulence factors – a novel feature of NMEC that could be part of a switch that controls the NMEC transition from commensalism to pathogenicity and vice versa. Here we report the existence of an unconventional c-di-GMP signalling network in ExPEC, its prerequisites and the rationale behind its evolution in ExPEC.

Immune resolution dilemma: Host antimicrobial factor calprotectin induces tissue damage during peritonitis

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Peritonitis is the leading cause of severe sepsis in the surgical intensive care unit, as more than 70% of patients diagnosed with peritonitis develop severe septic shock. A critical role of the host immune system is to return to homeostasis against severe inflammatory responses during infection while effectively deploying antimicrobial agents to contain or clear the infection. S100A8/A9 (calprotectin), is an antimicrobial, heterodimeric protein highly expressed in neutrophils with a wide range of physiological roles in infection-related systemic inflammation. However, the effect of calprotectin on inflammatory collateral tissue damage (ICTD) is less understood. Here we characterize the systemic effect of calprotectin *in vivo*.

Using a murine calprotectin knock-out mouse and the tissue invasive fungal pathogen *Candida albicans* in a disseminated peritonitis model, recombinant S100A8 and S100A9 protein therapy were administered to decipher the role of the proteins in ICTD. We found that the presence of either S100A8 leads to higher host-fungal clearance, and the occurrence of ICTD associated with *C. albicans* infection is dependent on S100A8. ICTD was not observed in the presence of only S100A9 or the absence of both S100A8, though significantly higher host-fungal burden was observed. Treatment with paquinimod, a therapy initially designed to target systemic sclerosis, abolished S100A8 dependent ICTD induced by *C. albicans*. The findings suggested that host modulation of S100A8 during disseminated candidiasis is essential for host resolution of inflammation and survival against ICTD.

Furthermore, S100A9 specific paquinimod also targets S100A8 activity to alleviate ICTD. Future studies on the use of paquinimod in conjunction with current antifungal drugs have the potential to provide a valuable adjuvant therapy for critically-ill candidiasis patients and immunocompromised patients suffering from severe ICTD during systemic infection

Poster No. 15

Acid tolerance of oral biofilms

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OBJECTIVE Dental caries is a major public health problem worldwide. The disease is mediated by microbial biofilms on teeth, which become acidified due to bacterial metabolism of carbohydrates. To survive at low pH, bacteria can activate specific acid-tolerance responses but the mechanisms underlying this are poorly understood. This project explores the ability of a range of oral bacteria to develop an acid tolerance response in biofilms and the role of surface-associated salivary proteins in this process.

METHOD Acid-tolerance of different species of Actinomyces and Streptococci was compared with that of the reference bacteria, *Streptococcus mutans*. Initially, planktonic cultures were exposed to a very low pH that distinguishes non-acid-tolerant from acid-tolerant cells. Biofilms were then established in an Ibidi mini-flow cell system on surfaces coated with salivary proteins and the acid tolerance of biofilm cells compared with that of planktonic cells. It was also assessed if biofilm cells were able to induce an acid-tolerance response (ATR) when exposed to a sub-lethal pH (pH 5.5), allowing them to survive at an otherwise lethal pH. Bacterial survival was visualized with LIVE/DEAD BacLight staining and confocal scanning laser microscopy.

RESULTS Preliminary results show that the different strains tested were more acid-tolerant in biofilms than during planktonic growth. In all strains tested, an ATR was developed after exposure to a sub-lethal pH, enhancing the survival at a lethal pH. Adhesion to salivary proteins did not affect the acid tolerance or ATR, and no enhanced survival was observed.

CONCLUSION This study suggests that biofilm bacteria are more acid-tolerant than their planktonic counterparts, indicating that adherence to a surface is involved in the mechanisms behind acid tolerance. Other oral species may well exhibit acid tolerance levels similar to that of *S. mutans,* indicating possible involvement in caries development. Biofilm growth on salivary proteins does not seem to affect the acid tolerance or ATR developed.

Poster No. 16

Exploring the genetic determinants of synergistic interactions of two or more antibiotics against multi- and extensively drug-resistant Gram-negative bacteria

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In Gram-negative bacteria, major resistance determinants to beta-lactam antibiotics include enzymatic inactivation by beta-lactamases and reduced drug uptake due to deficiencies in outer-membrane porins. Multi- and extensively drug-resistant *E. coli* and *K. pneumoniae* frequently harbour multiple beta-lactamases (ESBLs and/or carbapenemases) in combination with porin loss as well as resistance genes to other classes of antibiotics. Treatment options are thereby limited and combinations of antibiotics are often used clinically. Combinations of antibiotics can be effective as a result of synergistic activities even though each drug alone has insufficient activity.

To date, there is limited knowledge on which antibiotics to combine for best effect. We also lack understanding of the mechanisms behind synergy. Previous studies have almost exclusively been performed using clinical strains. The results from such studies are highly variable, even against strains of the same species harbouring the same enzymatic resistance. Thus, there is a need not only to screen for effective combinations but to explore the genotype-phenotype associations. We aim to explore the genetic determinants of synergistic interactions of two or more antibiotics against multi- and extensively drug-resistant *E. coli* and *K. pneumoniae* with acquired resistance to beta-lactam antibiotics. We focus on combination regimens that include beta-lactam antibiotics, either as double combinations or in triple combination with a beta-lactamase inhibitor and/or an antibiotic of another class.

In addition to testing combinations towards clinical strains, we use constructed strains of well-characterized reference strains in which we systematically vary the genetic set up of beta-lactamases and porins in an otherwise isogenic background. *In vitro* activity of antibiotics will be studied with static automated time-lapse microscopy (oCelloScope) and time-kill experiment as well as in the dynamic hollow fiber infection model. *In vivo* activity of antibiotics will be studied in wax moth larvae (*Galleria mellonella*) infection model. By studying the activity of antibiotic combinations against both clinical and constructed isogenic strains we can assess how genetic determinants for resistance affect synergy and subsequently gain knowledge on which resistance profiles that may be circumvented with different combinations of antibiotics.

Poster No. 17

Vaginal microbiota and HPV infection of adolescent young girls

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Changes in vaginal microbiota with the absence of *Lactobacilli* and increased microbial diversity facilitate sexually transmitted infections. Human papillomavirus (HPV) is the most common sexually transmitted virus. To define the HPV infection associated microbial community in Sweden, we analyzed the microbial community composition of Swedish young women respecting to HPV infection status and 27 HPV subtypes. 16S rRNA gene sequencing was used to characterize the vaginal samples from a youth clinic which covers age 14 to 22 (n=139), and health care centers from age 23-29 (n=179). Microbiota alpha diversity analysis revealed a significantly increased diversity in the HPV+ group compared to HPV- group, especially from young girls infected by oncogenic HPV sub-types. The vaginal microbiome in HPV+ women was characterized by higher levels of signature bacteria, such as *Gardnerella* compared to HPV- women. Our results suggest HPV infection is associated with increased vaginal microbiota diversity and potential microbial markers can be used for HPV infection.

Keywords: Vaginal microbiota; HPV; Adolescent; Sweden; Healthy

Poster No. 18 - short talk

The *Chlamydia* inclusion membrane protein CpoS recruits Rab GTPases to subvert the host cellular surveillance system

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The obligate intracellular bacterial pathogen *Chlamydia trachomatis* is a leading cause of preventable blindness and sexually transmitted diseases. The recently established techniques for molecular genetic analysis of *Chlamydia* spp. allow for the first time an in-depth dissection of the mode of action of the pathogen's virulence factors. It has previously been shown that the inclusion membrane protein CpoS, an effector protein which the bacteria insert into the membrane of their parasitophorous vacuole (inclusion), dampens the host cell's STING-mediated type I interferon (IFN) response and is critical for inhibition of premature host cell death, optimal intracellular bacterial replication, and survival of *C. trachomatis* in the murine genital tract.

Here we describe the identification of CpoS' interaction partners and provide evidence that CpoS' ability to block the type I IFN response in infected host cells is correlated with its ability to mediate recruitment of Rab GTPases to the *Chlamydia* inclusion. More specifically, we demonstrate a role for Rab35 as negative regulator of STING activation and show that Rab35 depletion from infected cells compromises *C. trachomatis*' ability to block type I IFN production. Current research aims to further explore the link between Rab35 recruitment and the dampening of cell autonomous defense responses by using a genetic approach that will enable us to artificially restore Rab35 localization to the inclusion membrane in cells infected with CpoS-deficient bacteria. We furthermore explore the hypothesis that CpoS-deficiency, by altering the pathogen's ability to interact with host membrane trafficking processes, results in an altered lipid composition and reduced integrity of the *Chlamydia* inclusion. This may lead to increased cytosolic access of the bacteria, explaining the enhanced induction of the IFN response.

By exploring the mode of action of the virulence factor CpoS, we hope to identify mechanisms of host-pathogen interaction that in the future could be exploited for the development of novel means to fight *Chlamydia* infections.

Poster No. 19

Anatomy of a standby site: an essential role for ribosomal protein S1 and a secondary structure element for ribosome binding

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Translation initiation involves binding of 30S, fMet-tRNAfMet, and initiator factors to an accessible mRNA ribosome binding site (RBS). Stable structures at RBS's inhibit initiation, yet in some cases, "ribosome standby" can overcome this (1): transient, sequence-non-specific binding to a single-stranded region enables 30S subunits to compete with rapidly folding secondary structure masking an RBS. Standby can work over considerable distances. The best-characterized example is the *tisB/istR-1* locus in *Escherichia coli*. Here, a standby site ≈100 nts upstream of the sequestered RBS in tisB mRNA is required for translation of TisB (2). Under normal growth conditions, the sRNA IstR-1 blocks this site, inhibiting translation. Though standby is strongly supported, direct evidence of ribosomes on standby has been elusive. Here, we report on the anatomy of the *tisB* mRNA standby site, its requirements and functional elements.

Fluorescence anisotropy experiments with a fluorescein-labeled *tisB* mRNA were conducted, and showed that 30S subunits bind the standby site independently of tRNAfMet with high affinity, and addition of IstR-1, or competition with unlabeled mRNA with an active standby site, impairs 30S binding. The ribosomal protein S1 is required for standby. We found that S1-alone binding is reminiscent of that of 30S, being affected by IstR-1 inhibition and mRNA competition. Furthermore, 30S subunits depleted of S1 neither bind the *tisB* mRNA, form 30S initiation complexes (30S-IC), nor support TisB translation *in vitro*. To directly map standby binding, *in vitro* CLIP experiments were conducted with 30S subunits, or S1-alone, on the *tisB* mRNA. As expected, an abundant cluster of reads covered the single-stranded standby region, but unexpectedly also a structural element at the mRNA 5'end. 5'-truncations inactivated functional standby and TisB translation. Anisotropy changes revealed that this was due to decreased affinity of 30S and S1. In conclusion, our results define the anatomy of the natural standby site in *tisB* mRNA and its functional requirements: an RNA structure element, a single-stranded region, and ribosomal protein S1. The long-distance effect of this standby binding event is tentatively explained by S1-dependent directional unfolding towards the downstream RBS.

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Designing a systematic screen for fertility genes in *Plasmodium berghei*

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Sexual reproduction of malaria parasites in mosquitoes is an essential lifecycle stage and vital for disease transmission. Blocking transmission will play a key role in malaria elimination, so a better understanding of fertility is required and reverse genetics is fundamental in identifying the molecular mechanisms involved. Experimental genetics has recently improved in the rodent model malaria parasite, *Plasmodium berghei*, thanks to the *Plasmo*GEM library of barcoded gene-targeting vectors. This has facilitated the phenotyping of hundreds of blood-stage *P. berghei* mutants. Over 900 genes without essential functions during the asexual blood-stage are expected to function at subsequent lifecycle stages. Identifying genes that function specifically in sexual development and transmission is challenging because most fertility genes are predicted to have female- or male-specific functions. Since parasites are diploid from the zygote stage, which form in the blood meal within minutes of a mosquito feeding on an infectious host, a pooled screen design would allow barcodes corresponding to genes that are sterile in one sex to be transmitted to the oocyst stage by the opposite sex. Here we demonstrate a successful design to screen systematically for genes with sex-specific functions. Selection marker-free mutants were generated that were either male- or female-deficient. These were used to mutagenise one sex selectively, that were then crossed with the mutant producing the opposite sex for transmission to oocysts. Barcode outputs were sampled from oocysts of dissected midguts on day seven and 14 post mosquito infection. We have completed a pilot screen that recapitulates existing knowledge of fertility genes, and demonstrates that population bottlenecks during sexual development do not present unsurmountable challenges to the systematic identification of fertility genes through barcode sequencing screens.

Practical and effective detection of bio-aerosols carrying mycobacteria using a novel, ionization-based air sampler

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Aim To develop an air-sampling device that can be used to detect *Mycobacterium tuberculosis*

Introduction Tuberculosis (TB) claims more human life than any other infectious disease. The success of TB is driven by the aerogenic nature of infection and in this context, technologies designed for detection of aerosols containing airborne pathogens could be employed in TB control efforts. Practical, robust methods for detecting *Mycobacterium tuberculosis (Mtb)* in air which are scalable in the complex and resource-limited settings of TB hotspots are currently lacking. We designed an ionization-based air-sampling device for collecting aerosols termed the TB Hotspot DetectOR (THOR).

Methods In a chamber tailored for aerosol experiments, we investigated the performance of the device in collecting aerosolized microspheres, spores of the Anthrax simulant Bacillus atrophaeus and airborne mycobacteria using *M. bovis* BCG as a simulant for *Mtb*.

Results THOR was able to efficiently remove microspheres and bacterial spores from air, resulting in the concentration of these airborne particles onto the unit's collector piece. Furthermore, THOR was capable of collecting bio-aerosols carrying BCG as determined by both electron microscopy and real-time PCR. Importantly, detection of BCG aerosols by PCR was successful across a wide range of BCG concentrations aerosolized. Finally, we field tested THOR in a prison TB hotspot where it was able to detect mycobacterial DNA from patient cough.

Conclusion Collectively, our data report the construction and validation of a novel, easily-scalable device that can be used to monitor bio-aerosols in the environment to enable the identification of the drivers of TB transmission.

Importance Air sampling tools could play an important role in Tuberculosis control by enabling the detection of tubercle bacilli in air, thereby identifying contagious persons and transmission hotspots. We report on the development and validation of a novel air sampler that will serve to improve case finding, identify transmission hotspots and to assess the effectiveness of transmission-blocking strategies.

Poster No. 22

Alarmone synthetases turn toxic

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Bacteria respond to nutritional stress by producing the alarmone (p)ppGpp. This triggers the so-called stringent response that down-regulates protein synthesis and growth and upregulates amino acid biosynthesis. (p)ppGpp is produced and degraded by proteins of the RelA/SpoT homologue (RSH) superfamily, named after the two *Escherichia coli* representatives – multi-domain 'long' RSH factors RelA and SpoT. In addition to long RSHs, bacteria can encode single-domain RSHs: Small Alarmone Synthetases (SAS) and Small Alarmone Hydrolases (SAH). The functional associations of SASs and SAHs are not well-defined. Therefore, to predict potential functional interactors, we developed a Python tool, FlaGs (for Flanking Genes) that analyses the conservation of the genomic neighbourhood surrounding the gene encoding a protein of interest. Using this tool, we made the surprising discovery that SASs can be encoded in toxin-antitoxin (TA) -like bicistronic loci in bacteria and bacteriophages. TA loci are comprised of two genes that are encoded next to each other in the genome, and may be overlapping. When expressed, the toxin protein abolishes bacterial growth – and its toxicity can be efficiently countered by the antitoxin, which is either RNA or protein-based. We have experimentally validated five FlaGs-predicted toxic SAS (toxSAS)-antitoxin pairs, each with a different protein antitoxin. We have uncovered the global evolutionary diversity of toxSAS TA systems using sensitive *in silico* sequence searching and gene neighbourhood analysis. We are now expressing toxSAS TAs for biochemistry and structural analyses, and developing a new version of FlaGs named NetFlaX (Network FlaGs for toxins) to uncover the universal network of all protein-based (Type II) TAs in bacteria and bacteriophages.

Poster No. 23

Persistent transmission of *Plasmodium malariae* and *Plasmodium ovale* species in an area of declining *Plasmodium falciparum* transmission in eastern Tanzania

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A reduction in the global burden of malaria over the past two decades has encouraged efforts for regional malaria elimination. Despite the need to target all *Plasmodium* species, current focus is mainly directed towards *Plasmodium falciparum*, and to a lesser extent *P. vivax*. There is a substantial lack of data on both global and local transmission patterns of the neglected malaria parasites *P. malariae* and *P. ovale* spp. We used a species-specific real-time PCR assay targeting the *Plasmodium* 18s rRNA gene to evaluate temporal trends in the prevalence of all human malaria parasites over a 22-year period in a rural village in Tanzania. We tested 2897 blood samples collected in five cross-sectional surveys conducted between 1994 and 2016. Infections with *P. falciparum*, *P. malariae*, and *P. ovale* spp. were detected throughout the study period, while *P. vivax* was not detected. Between 1994 and 2010, we found a more than 90% reduction in the odds of infection with all detected species. The odds of *P. falciparum* infection were further reduced in 2016, while the odds of *P. malariae* and *P. ovale* spp. infection increased 2- and 6-fold, respectively, compared to 2010. In 2016, non-falciparum species occurred more often as mono-infections. The results demonstrate the persistent transmission of *P. ovale* spp., and to a lesser extent *P. malariae* despite a continued decline in *P. falciparum* transmission. This illustrates that the transmission patterns of the non-falciparum species do not necessarily follow those of *P. falciparum*, stressing the need for attention towards non-falciparum malaria in Africa. Malaria elimination will require a better understanding of the epidemiology of *P. malariae* and *P. ovale* spp. and improved tools for monitoring the transmission of all *Plasmodium* species, with a focus towards identifying asymptomatic carriers of infection and designing appropriate interventions to enhance malaria control.

Poster No. 24

Experimental assessment of infectivity of aerosolized murine noroviruses

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Introduction Noroviruses are the cause for the majority of viral gastroenteritis cases in the world. Due to their low infectious dose and long stability in the environment, they often gives rise to outbreaks, even in hospital wards where legitimate infection control measures are practiced by the staff. Resent research has shown that noroviruses are present in air during outbreaks (Bonifait et al. 2014); however more research is needed to investigate the ability of noroviruses to transmit disease via the airborne route.

Method In this study, a method for evaluating viral infectivity after aerosolization was developed. As there is no well-established cell-cultivation technique for human noroviruses, a murine norovirus (MNV) strain was used as model virus in this study. MNV was aerosolized from a liquid suspension into a flow tube (Figure 1) and collected into phosphate buffer saline (PBS) after 10 seconds as airborne particles. MNV infectivity after aerosolization was assessed by detection of intracellular negative sense RNA in RAW 264.7 cells after 24 h incubation. The complementary negative sense RNA strand of the MNV genome is only present during active replication and can therefore be used as an indication of infection. The collected aerosol samples were added in 10-fold end point dilution series to 24-well cell culture plates. After 24 hours, the supernatant was removed, cells lysed, RNA was extracted, and negative sense RNA detected by a tagged primer according to the method in Vashist et al. (2012).

Result MNV preserved its infectivity after aerosolization, shown by the detection of negative sense specific RT-qPCR in the cell infectivity assay. With a dilution factor in the aerosolisation setup of about 500 times, the viral titers in the collected samples were relatively low, and the infectivity incubation time was therefore optimized for those, resulting in 24 h. Our aerosolization methodology can be further applied to other virus types, or to evaluate the effect of specific parameters in the air environment, such as temperature or humidity.

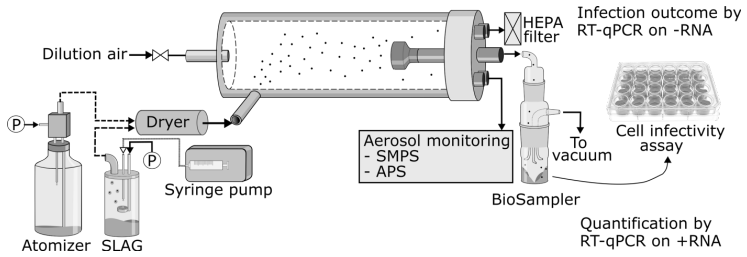

Figure 1. Experimental setup for aerosolization of murine noroviruses (by atomizer or sparging liquid aerosol generator) into a flow tube, and collection into PBS using a BioSampler for further molecular analysis.

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Poster No. 25

Innate lymphoid cells type 3-derived IL-22 boosts NF-kB-induced lipocalin-2 production through STAT3 in human intestinal epithelial cells

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Escherichia coli and *Klebsiella pneumoniae* are opportunistic pathogens that are commonly associated with infections at mucosal surfaces, such as the lung or the gut. The host response against these types of infections includes the release of epithelial-derived antimicrobial factors such as lipocalin-2 (LCN-2), a protein which specifically inhibits the iron-acquisition of *Enterobacteriaceae* by binding and neutralizing the bacterial iron-scavenging molecule enterobactin. Regulation of epithelial antimicrobial responses, including the release of LCN-2, has previously been shown to depend on IL-22, a cytokine produced by innate lymphoid cells type 3 (ILC3) during *Enterobacteriaceae* infections. However, much remains unknown about the extent to which antimicrobial responses are regulated by IL-22 and how IL-22 regulates the expression and production of LCN-2 in intestinal epithelial cells (IECs). Our study demonstrates how IL-22-induced activation of STAT3 synergizes with NF-κB-activating cytokines to enhance LCN-2 expression in human IECs and elucidates how ILC3 are involved in LCN-2-mediated host defense against *Enterobacteriaceae*.

Together, these results provide new insight into the role of ILC3 in regulating LCN-2 expression in human IECs and could prove useful in future studies aimed at understanding the host response against *Enterobacteriaceae* as well as for the development of antimicrobial therapies against *Enterobacteriaceae*-related infections.

Poster No. 26 - short talk

Modulation of competence for natural transformation by pneumococcal bacteriophages

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Bacteriophages are the most abundant biological entity on our planet. Phages have a significant impact on the biology of their host cells by providing virulence factors, modulating gene expression and mediating horizontal gene transfer. During their life cycle, temperate/lysogenic phages integrate into the bacterial chromosome where they normally reside dormant until their excision and replication is triggered by conditions such as exposure to DNA-damaging agents. However, in the context of naturally transformable bacteria, integrated phages are vulnerable to deletion from the chromosome by uptake of DNA from non-lysogenic strains. *Streptococcus pneumoniae* (*Spn*) is the paradigm of naturally transformable organisms and an important human pathogen that causes severe diseases such as pneumoniae, septicaemia and meningitis. Acquisition of extracellular DNA by *Spn* through natural transformation plays a central role in the spread of antimicrobial resistances and vaccine evasion. The competent state is controlled by the auto-inducer CSP and requires the production of a type IV pilus encoded by the *comG* operon. Some *Spn* phages integrate into *comGC*, the gene encoding the major pilin of the competence pilus, preventing the translation of the full-length ComGC protein which is required for transformation.

While determining the genome sequence of the *Spn* clinical isolate BHN1198, which carries ϕ135B integrated into *comGC*, we found evidence of excised phage molecules in the sequenced cell population. Using BHN1198 as a model, we investigated the genetic and transcriptional connections between phage excision and natural transformation.

We show that ϕ135B is a functional bacteriophage, capable of causing cell lysis and releasing phage particles upon induction with the DNA-damaging agent mitomycin C (MMC), and that spontaneous excision of ϕ135B reconstitutes the normal sequence of *comGC* allowing a subpopulation of cells to uptake DNA. Using differential RNA sequencing and RT-qPCR, we describe for the first time a pneumococcal phage gene which responds to the CSP auto-inducer, linking phage and competence gene expression. Finally, we show that other clinical isolates of *Spn* carry prophages integrated into *comGC* and that the majority of these strains retain the ability to uptake DNA. Taken together our results indicate that pneumococcal bacteriophages adopt two strategies to inhibit DNA uptake but that a high level of spontaneous site-specific excision of these phages from *comGC* allows a subpopulation of cells to remain transformable. Finally, our results highlight connections between host quorum-sensing pathways and phage gene expression.

Poster No. 27

Host-parasite interactions during *Plasmodium falciparum* zygote development inside the mosquito host

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Plasmodium falciparum is the most dangerous malaria parasite species and is responsible for 99,7% of malaria cases on the African continent (WHO 2017). The parasite has a complex life cycle that includes the symptom-causing asexual multiplication stage in the blood of the human host, followed by stages of differentiation and Initiation of the sexual stages, which initiate with the sexual precursor cells, the gametocytes. These gametocytes differentiate to male and female gametes once they are taken up by the mosquito vector. The gametes quickly fuse and form a sexually recombining zygote, which is the first developmental stage in the mosquito host. This project focuses on studying zygote development of *Plasmodium falciparum* inside the mosquito host using transcriptional profiling of individual gametes and zygotes during the entire zygote developmental stage. A total of >300 single cells were isolated using a micro-capillary manipulation system and fluorescence-based cell isolation, followed by a modified version of SMART-seq2 preparation and sequencing. Computational analyses focus on identifying genes that are endogenously transcribed in early zygotes as novel biomarkers for targeted drug development. Second, we aim to map the mechanisms and timing involved in *P falciparum* meiosis, and third we aim to elucidate parasite response to stress exerted by the host, during early infection.

Poster No. 28

The transcriptomic landscape of *Salmonella Typhimurium* persistent infection of mice

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Human pathogenic species of *Salmonella* are constituted by two pathotypes causing distinct disease: Non-typhoidal *Salmonellae* (e.g. s*erovar Typhimurium*) responsibly for a self-limiting gastroenteritis and typhoidal Salmonellae (e.g. serovar typhi) causing the systemic disease, typhoid fever, with a potentially fatal outcome. In 4% of *Salmonella* typhi cases, the infected individual will become asymptomatic carrier with bacteria persisting in gallbladder and possibly liver for years. Currently, knowledge on the adaptational requirements of *Salmonellae* to these host niches is largely unknown.

The study *Salmonella* Typhi pathogenesis is complicated by the fact that Typhi is a human obligatory pathogen normally unable to infect mice. Salmonella Typhimurium, however, causes a typhoid-like infection in immunocompetent mice with establishment of persistence in systemic organs including liver and spleen. Employing such models, only the contribution of single genetic determinants to survival of persistent bacteria is known. Instead, using two mouse models of persistent infection, we seek by to describe the transcriptional reprogramming of *Salmonella* from early to late persistent infection of liver and gallbladder. Preliminary data indicate a shift in transcriptomic landscape from one resembling that of growth in macrophages, such as upregulation of virulence genes, to a landscape more directed towards heat-shock stress response and host nutrient metabolism.

Poster No. 29

RyfA is part of a possible type I toxin-antitoxin system in *Salmonella*

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Historically, small open reading frames have been largely unintentionally ignored, because of difficulties in their recognition from the DNA sequence and experimental detection of small protein products. However, there are studies that describe phenotypic effects due to mutations in "intergenic regions" and expression of peptides from what was originally characterized as non-coding RNA genes. These discoveries have revealed the functions of these small open reading frames in many organisms and underlined their biological importance. Recent technological advances allow small protein genes to be detected systematically, through improved computational analyses and advanced experimental approaches such as genome-wide ribosome profiling.

Here we demonstrate that a 38 amino acid peptide is expressed from a gene previously annotated to encode a RyfA sRNA in *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*Salmonella* Typhimurium). Overexpression of RyfA peptide strongly inhibits *Salmonella* Typhimurium growth. RyfA encodes a classical Sec system signal peptide in its N-terminus and overexpression of RyfA evokes Cpx membrane stress response, indicating its translocation across, or insertion into, the cytoplasmic membrane. Our studies are focused on understanding the function of RyfA in *Salmonella* by studying its cellular localization and phenotypic effects of *ryfA* deletion. Although we currently do not know the function of RyfA, we present evidence that its expression is silenced by a STnc2070 sRNA which is transcribed from a gene located directly upstream of *ryfA*. Thus, the RyfA-STnc2070 system resembles a classical type I toxin-antitoxin system, where the expression of a toxic peptide is inhibited by the RNA:RNA interaction between toxin mRNA and small regulatory RNA.

Poster No. 30

Hepatitis C virus core protein down regulates expression of src-homology 2 domain containing protein tyrosine phosphatase

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Hepatitis C virus (HCV) is the major causative pathogen associated with liver cirrhosis and hepatocellular carcinoma and is remarkably efficient at establishing persistent infection, suggesting it has evolved one or more strategies aimed at evading the host immune response. The main virion component core (C) has been implicated in several aspects of HCV pathology including oncogenesis and immune subversion. We have previously reported that expression of C induces an anergic state characterized by specific down-modulation of interleukin 2. Here we demonstrate that expression of C protein in stably transfected T cells conferred a general increase in phosphorylation of TCR-related signaling including ZAP-70, LAT and PLC-gamma. Expression of HCV C correlated with a specific reduction of Src homology domain 2-containing protein tyrosine phosphatase 1 (SHP-1, also known as PTPN6), which has a role as a major feedback-regulator of T cell activation and acts as tumor suppressor in lymphoma and hepatocarcinogenesis. Whereas reduction was observed on both SHP-1 mRNA and protein levels, we were unable to detect any significant effect on differential splicing of SHP-1 isoforms. The reduction of SHP-1 expression could be reversed by treatment with 5-azacytidine, suggesting that the SHP-1 promoter is specifically inactivated by methylation. Our results suggest that HCV suppresses immune responses and facilitate its own persistence by inactivation of the T cell signalosome via suppression of SHP-1.

Poster No. 31

Nervous sensing and inter-organ communication during *E. coli* kidney infection is modulated by the toxin α-haemolysin

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Background: Tissue microbiological studies, whereupon infection is studied within a living host, have revealed that host responses to local bacterial infections includes inter-organ communication between the kidney and the spleen within 8 h of infection. We hypothesize that this rapid inter-organ signaling is, at least in part, mediated by the nervous system.

Methods: GFP$^+$-expressing uropathogenic *Escherichia coli* (UPEC) were microinfused into single proximal tubules in exposed kidneys of anesthetized rats. After 4 h the infection site was examined by *ex vivo* immunofluorescence (IF) analysis. Nervous projections in the renal cortex were identified through IF analysis of fixed, uninfected rat renal tissue. Splenic *Ifng* mRNA expression was determined by qPCR. ATP release from renal epithelial cells (A498) infected with UPEC was investigated in a biomimetic *in vitro* model. Primary sensory nerve cells from mice were used to determine nervous responses to UPEC infection.

Results: Here we demonstrate that a localized kidney infection by UPEC initiates communication between kidney and spleen within 4 h. We show that sensory neurons surround renal tubules and can be activated directly and indirectly by UPEC infection. The UPEC toxin α-haemolysin triggers both pathways, directly stimulating sensory neurons to release the neuropeptide CGRP, and indirectly mediating eATP release from infected kidney epithelial cells. During *in vivo* infection, expression of α-haemolysin is shown to be essential for inter-organ communication between kidney and spleen.

Conclusion: Our work describes an emerging concept in innate immunity, a nervous-driven response to infection, which alerts the host and initiates a complex and coordinated systemic response to a local infection.

Poster No. 32

A small RNA involved in pyrimidine synthesis regulation is controlled by an antisense RNA in *Neisseria meningitidis*

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Neisseria meningitidis is an obligate human pathogen and one of the major causative agents for bacterial meningitis. In asymptomatic carriers, it can be part of the common microflora of the upper respiratory tract. It is not fully understood which factors could influence transition to systemic infection but gene regulation through non-coding RNAs emerges as important factor in bacterial virulence. One such RNA is the *trans*-acting small RNA (sRNA) #59 which base pairs to an mRNA coding for *pyrC* and positively regulates its translation to Dihydroorotase. This might strengthen the fitness of *N. meningitidis* in its natural niche as the protein is involved in the *de novo* biosynthesis of the scarce resource of pyrimidines. While sRNA #59 regulates expression of *pyrC*, its own stability is dependent on a *cis*-encoded antisense RNA (asRNA). Further, two detectable copies of sRNA #59 suggest an RNA processing event which is mediated by Hfq, a pleiotropic RNA chaperone protein. Tight regulation by an asRNA and two copies suggest that sRNA #59 has more targets than *pyrC*. These targets, as well as what triggers expression of sRNA #59 and its asRNA are currently being investigated.

Poster No. 33

Identification of regulators of a defensive host cell death program that is actively suppressed by *Chlamydia trachomatis*

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Cell autonomous immunity is the ability of individual cells to detect invading pathogens and to respond to the threat by inducing cellular defense responses that can block pathogen replication. However, successful intracellular pathogens, for instance *Chlamydia trachomatis*, a leading cause of preventable blindness and sexually transmitted diseases, have often evolved effective counter strategies.

Previous research established that the chlamydial secreted effector protein CpoS is critical in blocking innate immune signalling and induction of premature host cell death, which could otherwise disrupt chlamydial development. The nature of the defensive host cell death program activated during infection with CpoS-deficient bacteria is unknown and is the subject of our current investigations. We strive to identify host proteins that execute this cell death, both by targeted and non-targeted approaches. In the targeted approach, we apply specific cell death assays, pharmacological inhibitors, and knock-out cell models to probe for the involvement of established cell death pathways. Our data suggest that only a proportion of the cells succumbs to apoptosis, while others perish by a so far undefined necrotic mode of cell death that appears to be neither necroptosis nor pyroptosis and could therefore represent a novel death pathway. In the non-targeted approach, we establish a pooled CRISPR/Cas9 positive selection screen that will enable us to identify gene deficiencies that confer host cell resistance to the cell death otherwise induced by CpoS-deficient chlamydiae.

Taken together, these approaches will enable us to identify regulators of defensive host cell death and will thereby shed light onto the mechanisms by which human cells respond to infection by inducing cell death. Most importantly, the study will identify proteins and pathways that, in the future, could be exploited to combat infections by intracellular pathogens.

Poster No. 34

A regulatory RNA contribution to invasive meningococcal disease in Europe, 2010-2018: a molecular epidemiological study

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The strictly human pathogen *Neisseria meningitidis* is a commensal bacterium but can occasionally turn lethal causing septicaemia and meningitis. The mechanisms of how the meningococcus shifts to invasive infection remain poorly understood.

During May 2017 in Stockholm, an adolescent girl succumbed to invasive meningococcal disease and deceased. Concurrently, an adolescent boy was identified as an asymptomatic meningococcal carrier. Both attended the same group trip to the French Alps two months earlier where another adolescent girl contracted meningococcemia. Molecular methods investigating protein expression and capsule production were performed to elucidate virulence mechanisms involvement. Comparative sequence analysis of 4434 European clinical isolates classified as either invasive or carrier from the European meningococcal database was also performed.

Both invasive- and carrier isolates were identified as serogroup C, cc32, with 98·1% loci conservation. Our results showed that the invasive isolate had an eight base-pair tandem repeat deletion in the 5´-untranslated region of the polysaccharide capsular biosynthesis operon and resulted in a hypercapsulation phenotype. The increased capsule production significantly improves the bacterium survival in human serum while impairing its ability to adhere to human pharyngeal cells. Among 4434 reported meningococcal cases in Europe from 2010-2018, the loss of an eight base-pair tandem repeat is three times more prevalent in invasive isolates (16·3%) compared to carrier isolates (5·1%).

Our result suggests meningococci can rapidly switch from asymptomatic colonisation to invasive infection through hypercapsulation, mediated by the loss of an eight base-pair repeat in its polysaccharide capsular biosynthesis regulon. To our knowledge, this is the first reported regulatory RNA to be directly involved in clinical manifestation of meningococcal disease.

Quantification of low-abundance proteins by targeted mass spectrometry reveals dynamics of bacterial toxin-antitoxin systems

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Bacterial toxin-antitoxin (TA) systems are implicated in bacterial virulence and formation of antibiotic tolerant persister cells of pathogenic bacteria. TA systems are composed of toxic proteins (toxins) and their neutralizing counterparts (antitoxins). Toxins stop bacterial growth by targeting a bacterium's own vital functions while antitoxins prevent their activity under normal growth conditions. Antitoxins are much more unstable than toxins and are constantly produced to maintain the antitoxin surplus and enable growth. Inhibition of the RNA- or protein production in bacteria supposedly releases the TA toxins from inhibition.

TA proteins exist in the bacterial cell at a low abundance and cannot be quantified with Western blot or routine shotgun proteomics. Thus, their quantitative dynamics in response to various antibiotics and other stresses has so far gone unstudied. Here we report an absolute quantification of the toxin and antitoxin proteins based on targeted mass spectrometry (MS) method using ^{15}N-labelled spike-in TA proteins and ribosomal proteins. The targeted MS methods were composed based on nano-LC/MS/MS data from purified proteins.

We derived exact intracellular concentrations of three toxins (CcdB, MazF, RelE) and their associated antitoxins (CcdA, MazE, RelB) of *Escherichia coli* K514(F'). We monitored the changes in antitoxin/toxin stoichiometry of these TA systems in response to three different antibiotics (mupirocin, rifampin and spectinomycin) and during growth in a batch culture using different growth media. As expected, the antitoxin concentrations started to decrease in response to antibiotics that inhibit protein- or RNA synthesis. The antitoxin/toxin ratios were high in exponentially growing cultures and dropped when the cultures reached the stationary phase. However, even after several days of starvation, the antitoxin/toxin ratios did not drop below the level that would indicate a fraction of free toxin in the cells.

We expect that the targeted MS quantification is suitable for measuring TA levels in the cell culture infection models where the effects of TA systems have been observed.

Poster No. 36

Metronidazole resistance in *Giardia duodenalis:* Identifying patterns by transcriptomics combined with biochemical analysis of two oxygen-insensitive nitroreductases

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Giardia duodenalis is an intestinal protozoan parasite causing diarrhoea and abdominal cramping in approximately 300 million patients every year. The standard treatment is oral medication with metronidazole, a nitroimidazole drug selectively targeting anaerobic cells. Metronidazole is taken up as prodrug and has to be activated by redox enzymes to its reactive form. Under aerobic conditions this activation reaction is quickly and non-enzymatically reversed explaining the selectivity.

Metronidazole has been used to treat various infections since the early 1960s and only recently resistance became a problem. This slow resistance development is believed to be caused by the rather unspecific mode of action of metronidazole targeting both proteins and nucleic acids inside the cell. In accordance to that, while it is easy to generate resistant *G. duodenalis* mutants in vitro, they survive only up to 10x more drug than the wildtype. Those in-vitro mutants often have severe growth and attachment defects and are therefore seldom able to establish infections in immunocompetent hosts. Vice versa resistant lines isolated from patients are hard to grow and analyse in vitro. Hence, changes in genome and transcriptome of resistant lines created in vitro were explored and searched for common features as possible common resistance mechanisms.

Here we report the transcriptomic analysis of two additional resistant lines as well as one revertant connected to one of these lines. They were grown without metronidazole to establish whether there is a baseline of changes found in those lines enabling them to grow in presence of the drug. In other pathogens oxidoreductases and more specifically nitroreductases have been shown to play an important role in activating the drug. We have therefore started to analyse the proteins showing up in resistant lines with these functions in *G. intestinalis* to hopefully help identify key players of metronidazole metabolism. Expression and purification have been set up and redox activity of GL50803_15307 can be shown. We will continue by testing both enzymes on their ability to reduce different imidazole drugs and look for additional targets implied by the transcriptomic dataset. We have so far specifically looked in differential expression of nitroreductases implying GL50803_22677 and GL50803_8377 as best targets for further investigation. Another oxidoreductase differential expressed in all lines is GL50803_17151, a quinone reductase.

Poster No. 37

Tuning of virulence in *Shigella flexneri* by distinct isoforms of the master regulatory protein VirF

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Enteropathogenic bacteria of the genus *Shigella* cause bacillary dysentery in humans. When S*higella* reaches the site of infection – the lower intestine – it invades colonic epithelial cells, causes inflammation and later disruption of the epithelial cell barrier. Being an opportunistic pathogen, *Shigella flexneri* has highly sophisticated ways of regulating the switch from a non-virulent to a virulent state, in response to a number of environmental stimuli. VirF is an AraC-like regulator, which acts at the top of the *Shigella* virulence gene regulatory cascade. VirF expression is activated upon entry into the host and results in the activation of several operons encoding invasion functions, including a type III secretion system. The VirF protein has been identified in different forms, but only the longest one, $VirF_{30}$, has an established positive regulatory effect on the virulence cascade. It was recently shown that a shorter isoform, $VirF_{21}$, may exhibit auto-regulation of VirF protein expression. In this work, we address the impact of the $VirF_{21}$ protein isoform on *Shigella* virulence. We describe environmental conditions which impact the expression levels of $VirF_{21}$ and show that its overexpression suppresses *Shigella* invasion of intestinal epithelial cells. Therefore, we suggest that the $VirF_{21}$ protein has a tuning role in regulation of *Shigella* virulence.

Bacteria-derived hydrogen peroxide suppresses inflammasome-dependent innate immunity

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Hydrogen peroxide (H_2O_2) plays a major role in host-microbial interactions. Thus far, studies have focused on the endogenous H_2O_2 produced by immune cells to kill microbes. However, microbes also produce H_2O_2. How microbial H_2O_2 influences the dynamics of host-microbial interactions is unknown. Here we show that H_2O_2 released by *Streptococcus pneumoniae*, the causative agent of pneumonia, inhibits inflammasomes, key components of the innate immune system and that this contributes to its ability to colonize the host. Further, we demonstrate that other H_2O_2-producing bacteria such as *Streptococcus oralis*, an oral commensal also blocks inflammasomes. This study uncovers an unexpected role of H_2O_2 in immune suppression and demonstrates how, through this mechanism, bacteria may restrain the immune system to co-exist with the host.

Blood, fat and smears: Apolipoprotein E: role during Gram negative infections unraveled?- Structural properties upon bacterial membrane interaction

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Human apolipoprotein E (apoE) is an amphiphatic glycoprotein. Humans express three common isoforms of this 34 kDa protein (without glycosylations or sialylations); E2, 3 and 4, that differ by two amino acids. Different affinities for the (low density lipid) LDL receptor is one result of these mutations. apoE3 is the most abundant form in healthy individuals. In the peripheral tissues, hepatocytes, macrophages and adipocytes express the most apoE. In the vascular regions of the CNS, astrocytes, microglia and mural cells produces the most of this multi-faceted protein. In plasma, apoE is preferentially accompanying VLDL particles,circulating to clear the blood from cholesterol by binding cell surface receptors. In the brain however, it´s more often found in HDL particles. The last decade, several studies have presented evidence that apoE holds regions with both antibacterial as well as antiviral activities. As for the antibacterial studies, most efforts, from *in silico* to *in vitro*, have emphasized not only but especially the receptor binding region of apoE (residues 130-162), a peptide named COG-133. Interestingly, this peptide has proven to sustain the biological activities of the full-length protein in terms of it´s anti-inflammatory and neuroprotective accomplishments. Based on this background, this study aimed for understanding if the full-length form of plasma apoE have similar bactericidal effects as it´s previously reported truncated variants and if so- does the interaction effect the structure of apoE? The study included the Gram negative strains *E.coli* (ATCC 25922) and *P. aeurginosa* (wt PA01) and Gram positive *S.aureus* (ATCC 29213) where investigation of killing was performed by the Viable Count Assay (VCA). This was further followed up by structural investigations of apoE during the interaction with bacterial membrane components by Circular Dichroism, Blue Native- PAGE and Micro Scale Thermophoresis. The results showed bactericidal effect of full-length apoE (from plasma) against the two Gram negative bacterial strains, but no effect on the Gram positive strain. The interaction with LPS but not LipidA was indicated with BN-PAGE (gels and Western Blots). Increased amounts of alpha helical content upon addition of LPS was observed, which further suggest binding of the two. Preliminary MST measurements has indicated binding, although further optimizations are required. If the bactericidal effect of plasma apoE can be further understood in more detail, a deeper understanding of not only apoE but hopefully other lipid binding proteins could provide a deeper understanding of their rather mysterious role in the immunological response to bacterial infections.

Poster No. 40

DncV synthesizes cGAMP and regulates biofilm formation and motility in *Escherichia coli* ECOR31

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Cyclic dinucleotides (cDNs) act as intracellular second messengers, modulating bacterial physiology to regulate the fundamental life style transition between motility and sessility commonly known as biofilm formation. Cyclic GMP-AMP (cGAMP), synthesized by the dinucleotide cyclase DncV, is a newly discovered cDN second messenger involved in virulence and chemotaxis in *Vibrio cholerae* O1 biovar El Tor. Here we report a novel role for horizontally transferred DncV in cGAMP production and regulation of biofilm formation and motility in the animal commensal strain *Escherichia coli* ECOR31. The rdar (red, dry, and rough) morphotype visualized on LB without salt agar plates was used as a model to assess biofilm formation. Motility was measured on soft agar medium. DncV was purified for enzymatic assay, followed by TLC assay to detect the catalytic activity. The enzymatic products were identified by MS and ESI-MS/MS. *In vivo* produced cDNs were extracted and detected by LC-MS/MS. ECOR31 expresses a semi-constitutive temperature-independent rdar morphotype on agar plates characterized by the extracellular matrix components cellulose and curli fimbriae, which requires activation by the major biofilm regulator CsgD and c-di-GMP signaling. In contrast, C-terminal His-tagged DncV negatively regulates the rdar morphotype and cell aggregation via down-regulation of *csgD* mRNA steady-state level. Furtheron, DncV sequentially promotes and inhibits adhesion to the abiotic surface after 24 h and 48 h of growth, respectively. DncV also suppresses swimming and swarming motility post-transcriptional of class 1 flagella regulon gene *flhD*. Purified DncV produced different cDNs, c-di-GMP, c-di-AMP, unknown product(s), and the dominant species 3'3'-cGAMP. *In vivo* only 3'3'-cGAMP concentrations were elevated upon short-term overexpression of *dncV*. Regulation of rdar biofilm formation and motility upon overexpression of untagged DncV in combination with three adjacent cotransferred gene products suggests a novel temperature-dependent cGAMP signaling module in *E. coli* ECOR31.

Poster No. 41

The differential survival of ESBL carrying *E. coli* in blood suggest a cost of fitness compared to antibiotic susceptible strains

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Background: The concept of ESBL carrying strains being less fit when it comes to evading the immune system has been suggested in the literature, however detailed investigation into the relationship between innate immune defenses and antibiotic resistance is lacking. It is important to know the details of the mechanism by which the antibiotic resistant pathogens are being killed, from survival, growth and complement killing, to leukocyte activation and phagocytosis. By gathering more information about the way the innate immune system recognizes and eliminates the antibiotic resistant strains, the more likely we are to find ways to circumvent this arising worldwide problem.

Materials/methods: 21 strains of clinically isolated *E.coli* strains, either antibiotic susceptible or ESBL producing, were studied for survival in 20% whole blood and serum with CFU counting after 2 hour. The strains were further sequenced with Next Generation Sequencing and characterized.

Results: When the strains were cultured in 20% whole blood, the ESBL producing strains showed significantly lower survival compared to the antibiotic susceptible strains. The survival in serum confirmed earlier results, and heat inactivation of the serum regained the survival of the bacteria. For further analysis, the strains were sequenced and the genomes compared in connection to virulence factors and LPS structure.

Conclusions: ESBL producing *E. coli* strains survive less in blood compared to susceptible bacteria. The same effect was observed when the bacteria was added to human serum, where heat inactivation of the serum resulted in regained growth, indicating difference in complement killing between the two groups and that carrying ESBL enzyme results in a cost of fitness for the bacteria.

Poster No. 42

Roles of small RNAs and associated Argonaute proteins in the battle between potato and *P. infestans*

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Small non-coding RNAs (ncRNAs), e.g. microRNA and siRNAs, associate with their effector protein, Argonaute, to cleave target mRNAs or inhibit their translation initiation. This RNA-protein complex often cross talk in the host-pathogen battles, which is also seen by our group in the potato *Solanum Tuberosum* late blight disease caused by the infection of the oomycete *Phytophthora infestans*. Our recent studies show that *P. infestans* miRNAs can also be bound by the potato Argonaute 1 (StAGO1) during infection, and vice versa. Many *P. infestans* derived small ncRNAs are predicted to target the host resistance (R) genes that are important for host defense. Besides R genes, *P. infestans* small ncRNAs seem to promote the pathogen infection, e.g., one of *P. infestans* miRNA silences its potato mRNA target which made the host more vulnerable to infection. Our transcriptome analyses revealed the differential expression of the 14 predicted StAgo genes upon *P. infestans* infection, suggesting their functions are not redundant to some extend. My interest is to study the function of StAGO10b, e.g. its localization and its associated small RNAs. StAGO10b and StAGO1 are from the same subclade sharing sequence homologies to *Arabidopsis thaliana* AGO1; yet their differential expression upon *P. infestans* infection vary largely. It would be interesting to characterize their functional difference to provide new insights into how these Ago proteins have evolved in potatoes.

An interplay between inflammation, oxidative stress and cellular aging in a controlled human malaria challenge study

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Infectious diseases can potentially affect cellular aging by adding miles to the biological clock. However, the underlying molecular mechanism that mediates the effect of infection on cellular aging is currently unknown. Here, we studied aging markers, cytokines and oxidative stress to mechanistically delineate the association between acute malaria infection and cellular aging in a controlled human infection study. Malaria naïve volunteers were infected with *Plasmodium falciparum* by mosquito bites, administered antimalarial treatment and monitored up to day 64 post-infection. Telomere length, telomerase, cyclin dependent kinase inhibitor 2A (CDKN2A) and oxidative stress were measured by qPCR, and cytokines by flow cytometry (Luminex technology). Our results show that a single low-density malaria infection already leads to cellular aging, but is fully reversible upon successful treatment. Significant increase in inflammatory cytokines, CDKN2A levels and liver injury while reduction in telomere length and antioxidants level were observed after malaria infection. The observed effect was reversed after successful treatment, with telomere length, CDKN2A, cytokine levels, oxidative stress levels, and liver functionality returning to base-line values (before challenge). Parasites density, inflammatory cytokines, CDKN2A levels and liver injury were positively associated with each other while negatively associated with antioxidants levels and telomere length. Our findings indicate the potential interplay between different cellular processes that lead to cellular senescence in malaria infection.

Poster No. 44

Towards target identification for 2-pyridone amides affecting *Chlamydia trachomatis* infectivity

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Substituted 2-pyridone amides alter development of *Chlamydia trachomatis*, resulting in progeny bacteria that are not infectious to new cells. Herein, a custom synthesized probe was used to identify their target proteins. The probe was photo-crosslinked to the target and thereafter covalently attached to a biotin-rhodamine tag via click chemistry. Finally, the probe-protein complex was separated out by streptavidin magnetic coated beads. The pulled down proteins were identified by mass spectrometry. Since *Chlamydia* is an intracellular pathogen, host cell proteins can bind non-specifically to the probe. We applied the pulldown technique in isolated *Chlamydia* inclusions to reduce the background proteins. The resulting candidate proteins are being characterized by *Chlamydia* strains presenting mutations in genes encoding for those proteins.

These compounds present high specificity for *Chlamydia trachomatis*, as they don't have an effect on other highly related *Chlamydia* species such as *Chlamydia muridarum* or *Chlamydia caviae*. Since the genomes of *C. trachomatis* and *C. muridarum* are over 99% similar, the resulting candidate targets can be narrowed down.

Pulldowns in isolated inclusions will result in the characterization of the mode of action of 2-pyridones in *Chlamydia trachomatis*. This approach can lead to the discovery of novel targets of anti-*Chlamydia* compounds, which then can be applied to other intracellular pathogens.

Poster No. 45

Experimental challenge of Atlantic salmon (*Salmo salar*) with the diplomonad parasite *Spironucleus salmonicida* to characterize the infection cycle

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Atlantic salmon (*Salmo salar*), originating from the Baltic Sea, were challenged with the diplomonad fish parasite *Spironucleus salmonicida* in a series of experiments in order to define the infection cycle, with focus on the time-line and putative routes of transmission. An oral intubation infection protocol using axenic parasites was developed together with new diagnostic tools using PCR and specific antibodies. Furthermore, we generated firefly luciferase expressing *S. salmonicida* parasites that could be identified in infected fish using *in vivo* and *ex vivo* imaging. The new tools made it possible to follow *S. salmonicida* infection cycle in details. Three different stages of the infection were identified: initial intestinal stage, followed by a blood stage and a final tissue stage. Parasites intubated into the intestines first attached to the intestinal surface and later detected in the blood 1-3 weeks after infection. Skin lesions and infections of the muscles, internal organs and eyes were detected 4-10 weeks post infection. Replicating trophozoites and several morphologically different forms of *S. salmonicida* cells were detected in the mucus from skin and in *ex vivo* cell-cultures of skin lesion biopsies. This study indicates that *S. salmonicida* uses several alternative routes of transmission. One alternative is the fecal-oral route, similar to other diplomonad parasites but the parasites can potentially also be excreted directly into the surrounding environment from the mucus layer of the skin or from an ulcerated skin lesion. This information, combined with the new diagnostic tools developed will be useful to prevent transmission and control of the parasite in fish farms.

Poster No. 46

The role of CopB protein in *Yersinia* virulence plasmid copy number regulation

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Many bacteria have evolved virulence mechanisms allowing them to invade the host and cause diseases. These virulence factors are plentiful and can take many forms. *Yersinia* genus includes 3 pathogenic species *(Y. pestis, Y. enterocolitica and Y. pseudotuberculosis)*, sharing a common IncFII 70-kb virulence plasmid that encodes several toxins called Yersinia outer proteins (Yops). The Yop proteins are secreted and translocated by a secretion system called Type III Secretion System(T3SS), also encoded on the virulence plasmid. Activation of this secretion system is dependent on contact with eukaryotic cells upon which it deploys and translocates the Yops into the target cell cytoplasm. After contact with the target cell,the Yops expression and secretion will increase in a highly regulated process.

A higher dose of these plasmid encoded *yop* genes is essential for virulence. However, conditions promoting Yops secretion are inhibiting bacterial growth which is incompatible with the course of infection. Wang et all.(2016) have found that *Yersinia* is able to rapidly change its gene expression by changing virulence plasmid copy number according to T3SS induction/repression status. This allows the pathogen to reduce the impact of costly virulence factors ongrowth.

IncFII is a well-studied class of plasmid using the model of plasmid R1 in *E. coli*. The plasmid replication initiation protein RepA initiates replication. Anantisens eRNA,*copA*,controls the level of RepA at the translational level. IncFII plasmids also have an additional control system, CopB that regulates *repA*-mRNA at the transcription level. Wang et all. (2016) have shown that an increase in *copA* antisense RNA levels results in a higher virulence plasmid copy number during infection in a mouse model, while the level of *repA*-mRNA level per plasmid remainsunchanged.

The aim of our project is to investigate further the molecular mechanisms by which the plasmid copy number is regulated. We are able to mimic T3SS inducible condition in *Yersinia in vitro* by shifting the temperature from 26°C to 37°C and simultaneously depleting the calcium in the growth medium. Using this system, we want to investigate the role of CopB in the regulation of plasmid copy number. Preliminary results seem to indicate that a *copB* deletional mutant has an increased plasmid copy number at 26°C, before turning on T3SS. Therefore, we will focus on understanding this mechanism further.:

Poster No. 47

Regulation of biofilm formation in *Shewanella algae*

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Shewanella algae is a marine bacterium and an emerging human pathogen. Biofilm is an important lifestyle for *S. algae* eco-physiology, as it contributes to the colonization of multiple aerobic and anaerobic environments, which is facilitated by an outstanding respiratory versatility. Biofilm formation is also believed to contribute to *S. algae* infectivity.

Little is known about *S. algae* biofilm physiology. The cyclic dinucleotide c-di-GMP is an almost universal second messenger regulating biofilm formation and dispersal in Bacteria. To begin to dissect the complex regulatory networks orchestrating the switch from a planktonic to a biofilm lifestyle in *S. algae*, we have over-expressed c-di-GMP turnover proteins and their corresponding catalytic mutants to show the implication of this signaling pathway on biofilm formation, motility, and cell morphology. Upon whole-genome sequencing and annotation, we have found 63 putative c-di-GMP turnover proteins encoded in the genome of the *S. algae* type strain CECT 5071. Some of these proteins show unprecedented features, including novel N-terminal domains that we are currently characterizing.

Transposon mutagenesis has implied the deep integration of multiple pathways to regulate biofilm formation in *S. algae*. One of such pathways is respiration and dissimilatory reduction of alternative electron acceptors. The inter-connection of these two a priori unrelated processes, biofilm formation and respiration, is not obvious. Here we present that terminal anaerobic electron acceptors trigger biofilm formation in *S. algae* strain-dependently, irrespective of the ability of a strain to use it, affecting the global transcriptome proportionally to biofilm formation levels. Through genetic analyses, we show that terminal reductase activity is required to elicit the biofilm formation response. In addition, using nitrate as a representative case, we show that the catalytic activity of the nitrate reductase is essential for nitrate-induced biofilm formation by preparing a catalytic mutant protein in which three cysteines in the 4Fe-4S cluster were replaced by serine.

Altogether, this work represents the first steps in the characterization of the biofilm physiology of a poorly understood organism with a recognized environmental role and an emerging clinical importance. Our research demonstrates respiration and redox balancing pathways to be a key player in this process in *S. algae*, with important potential implications in processes such as redox specialization and environmental niche colonization.

Infection with genotoxin-producing *Salmonella enterica* synergises with loss of the tumor suppressor *APC* in promoting genomic instability in colonic epithelial cells

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Several commensal and pathogenic Gram-negative bacteria produce DNA damaging toxins that are considered *bona fide* carcinogenic agents. The microbiota of colorectal cancer (CRC) patients is enriched in genotoxin-producing bacteria, but their role in the pathogenesis of CRC is poorly understood. The *adenomatous polyposis coli* (*APC*) gene is mutated in familial adenomatous polyposis and in the majority of sporadic CRCs. We investigated whether the loss of *APC* alters the response of colonic epithelial cells to infection by *Salmonella enterica*, the only genotoxin-producing bacterium associated with cancer in humans. Using 2D and organotypic 3D cultures, we found that APC deficiency was associated with sustained activation of the DNA damage response, reduced capacity to repair different types of damage, including DNA breaks and oxidative damage, and failure to induce cell cycle arrest. The reduced DNA repair capacity and inability to activate adequate checkpoint responses was associated with increased genomic instability in APC deficient cells exposed to the genotoxic bacterium. Inhibition of the checkpoint response and acquisition of genomic instability was dependent on activation of the phosphatidylinositol 3-kinase (PI3K) pathway. These findings highlight the synergistic effect of the loss of APC and infection with genotoxin-producing bacteria in promoting a microenvironment conducive to malignant transformation.

DNA methylation during encystation of *Giardia intestinalis*

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Giardia intestinalis presents a simple life cycle: the environment-resistant cysts and the disease-causing vegetative trophozoites. Between these developmental stages *Giardia* needs to rapidly adapt to establish an infection in a new host. The cyst wall of *Giardia* protects the parasite from the harsh environmental conditions it has to endure outside the host's intestine. On the other hand, when a putative host ingests water or food contaminated with *Giardia* cysts, a signal transduction cascade triggered by the low pH of the stomach allows the release of trophozoites in the upper small intestine. Once in the small intestine, trophozoites produce a symptomatic infection or remain unnoticed in asymptomatic individuals. Differentiation of *Giardia* parasites from trophozoites to cysts is essential for transmission of the parasite.

We have recently characterized the gene expression changes related to this process using RNA seq. We used our newly developed in vitro encystation protocol to generate differentiating cells from each 1.5 h of the 32 h encystation process. Transcriptional changes during the entire differentiation from trophozoites to cysts were studied using RNA sequencing (RNA-seq). A high level of periodicity was observed for up- and down-regulated genes, both at the level of the entire transcriptome and putative regulators. The transcriptomic analyses were complemented by Bisulfite sequencing to characterize the global methylation changes and, analyses of the methylation of selected encystation-regulated genes. These analyses suggest an important role of epigenetic regulation of gene expression during G. *intestinalis* encystation. We also characterize the effect of the DNA methylase inhibitor 5'-aza-2' deoxycytidine (5-aza-dC) on *Giardia* trophozoites and differentiation, observing that de-methylation effects encystation.

Poster No. 50

Improved resolution of complex *in vivo* transcriptomic profiling of persistent *Yersinia pseudotuberculosis* in mice caecum

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We have recently established a mouse model of persistent bacterial infections of *Yersinia pseudotuberculosis*. To achieve this, low dose infection (approx. $1.5x10^6$ CFU/ml) *with Y. pseudotuberculosis* YPIII (pIBX) is administered to 8-week old female FVBn mice, were 20-40% of the infected mice succumb to acute infection with sickness and weight loss during the inital weeks of infection. However, of the total mice population, a subpopulation (10-30%) maintain *Y. pseudotuberculosis* populations, where observation on an *in vivo* imaging system (IVIS) platform recurrently show localization of bacteria in caecal lymphoid follicles. Previous complex *in vivo* gene expression analysis of such small caecal tissue biopsies by our group show an interesting reprogramming of *Y. pseudotuberculosis* from virulent to persistent mode, where persistent *Y. pseudotuberculosis* show a gene expression pattern never have been seen at elevated temperatures in *in vitro* laboratory settings. The gene expression of persistent *bacteria* resembles expression seen *in vitro* at 26°C, accompanied by significant downregulation of T3SS virulence genes, but also upregulation of genes encoding proteins of importance for adaption to new environments. In this study, we replicated this mouse model and considerably improved the complex *in vivo* techniques to improve resolution and most importantly to separate bacteria localized within ceacal tissue from luminal bacteria. Refined caecal tissue separation and "fishing" techniques of persistent *Y. pseudotuberculosis* greatly enriched acquired *Yersinia* transcript reads from these complex samples for further analyses. Here, we present the results of an improved complex RNA-seq analysis method that corroborate earlier findings and pave way to understanding the *in vivo* bacterial physiology and life cycle of this enteropathogen. The results herein will provide fundamental basis for future possible approaches of "taming" bacterial long term infections and identifying potential bacterial targets for new antimicrobials.

Exploring the host reaction to a new host-targeting, small molecular inhibitor against Zika virus

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When Zika virus (ZIKV) re-emerged in the Americas in 2015, it caused a major public health crisis all over the world. Although infection by ZIKV usually causes only mild and short disease with symptoms like cutaneous rush and fever, it has also been linked to serious neurological disorders such as Guillain-Barré syndrome in adults and severe microcephaly in fetuses, if women are infected during pregnancy. As for many other emerging viruses, efficient and specific therapeutic options are missing. This unmet need resulted in a collaboration between Helleday laboratory and Public Health Institute of Sweden to develop new antivirals against emerging RNA viruses. Our newly identified hit compound is a host-targeting, small molecular inhibitor with antiviral properties against ZIKV infected cells.

In addition to its antiviral properties, we have observed increased fitness and survival of both cells and brain organoids after ZIKV infection and treatment with our inhibitor. Since our compound is targeting host proteins, we are hypothesizing that some changes of the host reaction to ZIKV are responsible for this change in fitness. To answer this question, we will explore the close link between infection, and cell death, by studying apoptosis and cell cycle in ZIKV infected and treated cells.

Furthermore, virus infections trigger specific, conserved antiviral host pathways like induction of type 1 interferons. To characterize the changes to the Interferon (IFN) signaling pathway in infected and treated epithelial cells, gene expression levels of IFN alpha and beta will be determined. To further see if potential changes in gene expression are reflected in changes in protein levels, concentrations of the respective cytokines in the supernatant will be measured. Our findings will be validated in brain organoids and in suitable animal models to see if findings on cellular level can be translated to organism level.

Altogether, our approach will shed further light into host responses to ZIKV infection and will drive the development of new therapeutic options for ZIKV infection.

Poster No. 52

Pharmacodynamic studies of intracellular activity of antibiotics against Uropathogenic *Escherichia coli* phagocyted by mice macrophages J774

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One of the major causative agents of urinary tract infections is uropathogenic *Escherichia coli* (UPEC). Internalization of bacteria leads to increased formation of persisters, metabolically inactive cells, which are one of the main reasons of antibiotic treatment failure and recurrent infections. Pharmacodynamic parameters have not been previously determined for intracellular antibiotic activity against intracellular UPEC. The aims of this study were to establish an *in vitro* model of infection of mice macrophages by strain CFT073 and to compare the intracellular and extracellular activity of commonly-used antibiotics. Fluorescent marker was used to gain better insight into bacterial heterogeneity and nature of bacterial subpopulations surviving the antibiotic treatment.

Macrophage cell-line J774 was infected with opsonized and fluorescently labelled CFT073 strain. Non-phagocyted bacteria were eliminated by incubation with 100x MIC of Gentamicin for 1h and washing in 1xPBS. Infected cells were exposed to a broad range of antibiotic concentrations (0.003-100x MIC) for additional 24h and visualized by fluorescent microscopy. For measuring extracellular activity, the bacteria were exposed to the same concentrations of antibiotics in broth, for the same period. The Hill equation of concentration-response curves was used to calculate pharmacodynamic parameters (bacteriostatic concentrations [Cs] and maximal relative efficacies [E_{max}]).

Our results showed that all antibiotics had decreased activity when tested in cell culture model than in broth. The best eradication of intracellular bacteria was observed for Fluoroquinolones (E_{max}= approximately - 3 log CFU/mg of protein). Fluorescent labelling and microscopy analysis revealed that only subpopulation of bacterial cells start to divide in the intracellular niche.

All antibiotics failed to eradicate intracellular UPEC, even when bactericidal in broth. The different obstacles for investigated antibiotics could be insufficient bioavailability or activity of antibiotics in the infected compartment and/or dormant state of majority of intracellular bacteria. Yet, among the drugs tested, Fluoroquinolones were the most effective, killing almost 99.9% of intracellular bacteria.

Poster No. 53

Spontaneous and clinically relevant tet(A)-dependent tigecycline resistance development

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Resistance to the last line antibiotic tigecycline, a third-generation tetracycline, has been observed in the clinic without being fully understood. In the light of the recent approval of new tetracyclines, such as omadacycline, understanding mechanisms of resistance is of increased importance. In order to elucidate new resistance mechanisms, a collection of 35 diverse *Escherichia coli* isolates was screened for the ability to develop resistance to tigecycline. Through this screen, we discovered that amplifications of the common tetracycline resistant determinant *tet(*A), an efflux pump, leads to tigecycline resistance above the clinical breakpoint. We can also show that amplifications of *tet*(A) lead to an increase in MIC to omadacycline.

In this work we also identified a previously undescribed 24-base pair deletion in *tetR*(A) which prevents the development of *tet*(A)-mediated tigecycline resistance. The deletion decreases the expression of Tet(A) with exposure to tigecycline, which is most likely caused by a decrease in affinity towards tigecycline of the mutated TetR(A). The high prevalence of the deletion allele of *tetR*(A), which is observed in up to 40% of *tet*(A) in *E. coli*, can be explained by the lack of a fitness cost or slight fitness advantage compared to wild-type *tet*(A) in presence of low antibiotic concentrations, as determined by competition experiments. The prevalence of the 24-base pair deletion allele of *tetR*(A) varies depending on source. We present a simple multiplex PCR to identify the *tet*(A) allele and potentially aid in clinical decisions.

Poster No. 54

Using different types of antisense oligonucleotides and delivery systems to probe the function of non-coding RNA in the siRNA deficient *P. falciparum*

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The lethal malaria parasite *Plasmodium falciparum* adapts incredibly fast to changes within the human host, but the underlying molecular mechanisms remain elusive. It is well-known that the coding transcriptome of the parasite is hard-wired and virtually non-responsive to stimuli, pointing towards post-transcriptional regulation being key for the rapid adaptation. Non-coding RNAs (ncRNAs) are known to be important post-transcriptional regulation mediators in other cellular systems but the lack of a functional siRNA machinery has prevented efficient targeting of these molecules for functional determination in *P. falciparum*. In addition, although antisense RNA has been used successfully to target parasite transcripts, the effect has been marginal at best. Thus, any successful development of methodologies that allow for fast and large scale scrutinization of the ncRNA biology within the parasite would be highly valuable.

Here we have used different types of next generation antisense oligonucleotides (ASOs) and developed alternative systems to deliver these to intraerythrocytic parasites. ASOs were designed to either degrade, abrogate biogenesis or inhibit the function of different types of ncRNA that we hypothesized to partake in regulation of various cellular processes. Delivered ASOs were evaluated for their transcript silencing potential by monitoring the transcriptome, proteome and phenotype of the treated parasites. Our data suggests that next generation ASOs, if being designed and delivered in optimal ways, have the potential to efficiently target different types of ncRNA of variable abundance within intraerythrocytic *P. falciparum*. In addition, we show that successful ASOs were highly specific with minimal/none off-target effects. By using these ASOs, we were also able to identify adaptive parasite phenotypes linked to the targeted ncRNAs.

Taken together, our work presents an important methodological development for the scrutinization of the elusive ncRNA mediated post-transcriptional layer of gene regulation in *P. falciparum*. We demonstrate that antisense oligonucleotides can be applied to specifically target ncRNAs in order to perturb the epigenome of a protozoan parasite lacking the siRNA machinery. Importantly, this approach has a great potential to be scaled up for high-throughput exploration of the non-coding transcriptome of *P. falciparum*.

Poster No. 55

Functional profiling of a *Plasmodium* genome

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Plasmodium parasites are the causative agents of malaria which continues to kill almost one child every minute. The search for new drug targets urgently requires a deeper understanding of malaria parasite biology, but more than a decade after the completion of the first *Plasmodium* genome sequence, around a third of genes still have no predicted function, and only ~600 genes have been studied by experimental perturbation, leaving still nearly >85% of the genome unexplored. We have recently overcome challenges associated with modifying the AT and repeat-rich genome of the rodent model parasite *P. berghei* at genome scale. Screening large libraries of barcoded mutants we have identified systematically parasite genes required for asexual growth of the blood stage, for the differentiation to infectious transmission stages that reproduce sexually in the mosquito vector and for the obligatory liver stage that initiates infections in the vertebrate host. Phenotyping 2571 barcoded mutants for their competitive fitness during asexual replication in red blood cells revealed that two thirds of parasite genes are required for normal growth of just a single life cycle stage *in vivo*, an unexpectedly large proportion when compared to the much smaller essentialomes of cultured yeast, human cells or bacterial pathogens. We propose that sequential reductions in genome size during the evolution of intra-erythrocytic parasitism have resulted in an increased importance of individual genes, which gives rise to optimism that many new drug targets remain to be discovered to attack malaria parasites and their transmission at multiple life cycle stages. Essential metabolic pathways can now be exploited for drug development by taking into consideration how parasite metabolism gets rewired as the parasite moves between different host species. In a second screen, we looked specifically for genes needed for the developmental switch to sexual forms, which is key for parasites to become infectious to mosquitoes. Many genes with sex-specific functions contain likely nucleic acid binding domains and respond rapidly to induction of the sexual master transcription factor, AP2-G, making them candidate early regulators of the distinct male and female gene expression programmes of the transmission stages. Using single cell transcriptomics of developing gametocytes from selected mutants, we can now distinguish candidate genes for sex determination and differentiation and demonstrate how developmental decisions in a parasite life cycle can be delineated.

Poster No. 56

Alterations in the gut microbiome of HIV infected patients under antiretroviral therapy

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Objective: Millions of individuals globally are infected with HIV and are currently on antiretroviral therapy (ART) for suppressing viral replication and improving host immune responses. The most common ARTs are Nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors. These ARTs traverse the gut and encounter the rich diversity of gut commensals termed as the "human gut microbiome" which is even referred as the "second genome". The involvement of gut microbiome in HIV infection is gradually being recognized. However understanding the direct effect of ARTs on HIV infected individuals has been mostly overlooked in microbiome studies concerned with HIV individuals.

Design: Microbiota composition was determined by 16S rRNA sequencing (Illumina MiSeq) of stool samples from 16 viremic patients after follow-up during ART.

Results: The results indicated that ART directly influenced gut microbiome composition. Moreover, the impact of nucleoside analogues on microbial growth has been hypothesized earlier. The thymidine analog azidothymidine (AZT) or zidovudine (ZDV) was known to possess antibacterial activity against many microbes belonging to family Enterobacteriaceae. ZDV was tested against gut microbes in vitro and was further assessed for its antibacterial effect. Zidovudine showed in vitro antibacterial effect on *E. coli* with an MIC of 0.5-8µg/mL. This showed that ART further decreased gut microbial diversity implicating possible influence of ART on the microbiome composition.

Conclusion: Particularly with the increasing number of individuals on ARTs, this proof of principle can be replicated with other ARTs to investigate their individual or combinatorial effect on gut microbes. This will help us enrich the microbiome population within viremic individuals for improved health. ZDV is one of the prescribed drug in developed countries, therefore their potential effect remains of significance among HIV infected individuals. Our work will fill this gap by studying the antiretroviral drugs interactions with bacteria.

Poster No. 57

Deciphering *Salmonella* invasion mechanisms and host responses in organoid models of the gut epithelium

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Salmonella enterica infections constitute a globally prevalent cause of foodborne intestinal disease. To prevent the infection, it is essential to better understand the initial steps of enterobacterial invasion and the intestinal epithelium response. In the past, most of the cellular and molecular host-pathogen interactions have been studied in simplistic culture settings, using tumor-derived cell lines. These models may not represent the arrangement and physiology of the intestinal epithelium and their response to infection may hence have limited physiological relevance. On the other hand, *in viv*o infection experiments lack precision for dynamic studies of the initial steps of invasion, while also standing the risk of being questionably cruel. To fill this gap, our research relies on recent progress in *ex vivo* culture of primary intestinal epithelium in the form of so called organoids, from mouse as well as human, which we adapted for the study of *Salmonella* invasion events. The combination of this breakthrough culture model with state-of-the-art live microscopy enables the study of cellular/subcellular events during the first minutes of epithelium invasion in real time, what would be unfeasible in animal models. Upon *Salmonella* invasion, we observed both single cell and tissue-scale intestinal epithelium responses. Our present work aims to uncover the cell biological mechanisms underlying *Salmonella* invasion of the physiologically arranged epithelium and the host tissue responses triggered in this process.

Poster No. 58

Characterisation of two novel co-transcribed sRNAs in *Neisseria meningitidis*

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Increasing evidences highlighted the pivotal role of regulatory RNA elements in the fine tuning of gene expression during the infection process in pathogenic bacteria. Recently, RNA elements were discovered controlling important virulence traits of the human pathogen *Neisseria meningitidis*, the causative agent of severe septicaemia and meningitis.

In our RNAseq-based genome wide screening, we identified 129 sRNAs in *N. meningitidis*. One of these candidates termed sRNA40 consists of two distinct consecutive sRNAs that were later named sRNA40A and sRNA40B. Promoters were identified upstream each sRNA, however there is no obvious transcriptional terminator between them. The expression patterns and profiles of these sRNAs were therefore investigated in *N. meningitidis* strains harboring deletions of several genes involved in sRNA synthesis, maturation and turnover. We observed that the exonuclease PNPase and the endonuclease RNase III play a major role in the processing of sRNA40A from a transcript comprising sRNA40A and B. Furthermore, RNase R, polyA polymerase, the stress induced RNase toxin MazF and the RNA chaperone Hfq affect the stability of both sRNAs, revealing a complex concerted action of multiple enzymes to produce and control regulatory RNAs.

We observed that stress conditions, such as salt and oxidative shocks, induce a differential expression of the sRNAs, indicating a potential role in the adaptation to environmental challenges. A computational prediction of sRNAs targets suggests that sRNA40A might regulate the synthesis of outer membrane proteins involved in the interaction with host cells and immune evasion. Whereas, the candidate targets of sRNA40B participate in iron scavenging, an essential function in colonizing human tissues. We are currently validating the targets and investigating the role of the sRNAs in colonization and pathogenesis.

Poster No. 59

Bacterial suspended aggregation in high viscosity environment supports multi-species co-existence by spatial segregation of micro-communities

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Introduction: Cystic fibrosis patients produce copious amounts of sputum in their respiratory tract that obstruct their airways. Bacterial pathogens colonise these thick sputa and cause chronic respiratory infections. Upon colonisation, the bacteria form suspended aggregates. Long considered to be a type of biofilm growth, while these aggregates have shared features with surface-associated biofilms such as elevated tolerance against antibiotics, attachment factors such as extracellular biofilm polysaccharides are not required to develop aggregates and therefore may represent a distinct and uncharacterised growth format of bacteria.

Hypothesis and aims: Bacterial suspended aggregates reduce interspecies competition and promote multispecies microbiota in viscous environments.

Methodology: Inoculation of bacteria in low density into high viscosity media (0.8% w/v agar) leads to formation of suspended bacterial aggregates. Co-inoculation of more than one species in 1:1 ratio will result in mixed aggregates. In this study, we evaluate the resulting ratio of pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus* which are frequent colonisers of cystic fibrosis airways and compare against low viscosity conditions where the bacteria do not aggregate.

Results: Based on microscopy results, individual aggregates appear to be outgrowths derived by clonal expansion from a single bacterium.

Conclusion: The bacteria in the aggregates formulate spatially segregated micro-communities. This prevents competition between one bacterial aggregate to another and may contribute to multi-species co-existence in viscous environments such as on mucosal surfaces that otherwise may involve outcompeting species in spatially mixed environments.

Poster No. 60

Structural basis for ribosomal protection by the *Listeria monocytogenes* ABCF ATPase Lmo0919

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The ribosome is targeted by numerous antibiotic classes. Streptogramin, lincosamide, and pleuromutilin antibiotics all block transpeptidation by binding the peptidyl transferase center (PTC) of the ribosome. A class antibiotic resistance proteins (ARE) belonging to the ATP-binding cassette F (ABCF) family protect the ribosome from PTC blockers by directly displacing the antibiotic. *Listeria monocytogenes* Lmo0919 is ABCF ATPase resistance factor closely related to well-studied Staphylococcal VgaA ARE. The ribosomal interaction of Lmo0919 has not yet been demonstrated experimentally and the full spectrum of antibiotic protection conferred by Lmo0919 in its native *Listeria* host has not been demonstrated.

To understand the molecular mechanism of ribosomal protection by Lmo0919, we used cryo-EM to solve the structure of the ATPase deficient mutant Lmo0919^{EQ2} bound to the *L. monocytogenes* 70S ribosome, and functionally characterize *L. monocytogenes* ribosomal protection by Lmo0919. We characterise the spectrum of antibiotic protection by Lmo0919 in *L. monocytogenes* and demonstrate that expression of Lmo0919 is induced by the translational stress caused by ribosome assembly defects.

Poster No. 61

Biofilm-evolved *Klebsiella pneumoniae* exhibit changes in capsule, fimbriae and c-di-GMP turnover

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Background: *Klebsiella pneumoniae* frequently causes medical device-related infections due to biofilm formation, but the factors affecting it remain understudied in this species. We employed experimental evolution to study the mutations leading to increased biofilm formation and compare to the changes observed during a *K. pneumoniae* hospital outbreak.

Methodology: Surface-attached biofilms were grown on plastic pegs (in-house developed modular version of the Calgary device with insertable pegs). Ten lineages of three clinical strains (IA565, CAS55 and an ESBL-outbreak isolate) were subjected to five cycles of biofilm growth, one cycle lasting for 48 h and involving attachment, maturation, and dispersal. Evolved mutants were whole-genome sequenced and selected mutants were characterised with respect to biofilm formation (CFU/peg and crystal violet staining), fitness in planktonic cultures, resistance to human serum and virulence in *G. mellonella* larvae model.

Results: Most mutants carried single mutations associated with type 3 fimbriae (*mrkD*), c-di-GMP turnover (EAL-domain proteins, e.g. *yhjH*) and capsular polysaccharides (*wzc* tyrosine kinase). IA565 strain also repeatedly had a point mutation in a putative bacteriocin/T6SS effector gene. Evolved mutants had up to 30x higher CFU/peg, but planktonic growth rate did not change. *Wzc* mutants had the most extreme biofilm phenotype (long strings of biomass stretching from the pegs) and colonies completely stuck on agar. Unexpectedly, *wzc* mutants were highly sensitive to human serum in planktonic state. However, we observed resistance to human serum in biofilms formed by these mutants and they were highly virulent in *G. mellonella* larvae. Mutations in *mrkD*, *wzc* and *yhjH* at different positions were also present in isolates from a clonal *K. pneumoniae* outbreak at Uppsala University Hospital.

Conclusion: We identified a range of novel mutations leading to increased biofilm formation in *K. pneumoniae* using an experimental evolution approach. Mutations in the most frequent targets (type 3 fimbriae, c-di-GMP turnover and capsular polysaccharide production) were also observed during the clinical outbreak. This suggests possible clinical relevance with regards to the virulence or persistence of infection and we are currently investigating it in a *G. mellonella* larvae model.

Poster No. 62

Transient colonization of MRSA on the stratum corneum of human skin is controlled by a local IL-8 mediated neutrophil recruitment

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Background: Epi-cutaneous *Staphylococcal* colonization is increasingly recognized as important for priming the host dermal immune response towards this potential pathogen.

Objectives: The significant morphological and immunological differences between human and mouse skin encouraged us to develop a xenograft humanized model in which we study *S. aureus* skin colonization on living human skin.

Methods: In our *in vivo* human skin xenograft mouse model, *S. aureus* was topically applied to human skin. After 2, 5 and 10 days, samples were prepared for viability counts, cytokine analysis (Luminex), immunohistochemistry and flow cytometry (day 5). To study the influence of neutrophils in colonisation, we performed transmigration assays using explant tissues homogenates and induced neutropenia *in vivo* before inoculation of human skin with bacteria.

Results: We have identified an IL-8 mediated pro-inflammatory signalling that is upregulated in response to epi-cutaneous colonization of human skin by *S. aureus* USA300 *in vivo* and *in vitro*. The IL-8 response appears to be produced by the surface layers of keratinocytes and does not require invasive bacteria. This pro-inflammatory signal induces directed transmigration of neutrophils into the thick human epidermis. Blockage of this IL-8 signal *in vitro* is shown to reduce neutrophil transmigration. Neutrophil depletion *in vivo* leads to higher bacterial loads on the skin indicating that this epidermal neutrophil recruitment may function to control bacterial numbers on the skin surface. This work has demonstrated a unique human response to epi-cutaneous *Staphylococcus* and we hypothesise that this sub-clinical response is a way for the tissue to control bacterial numbers.

Poster No. 63

SLIcer of Microbial Ecosystems (SLIME) – a new microfluidic model for studying antibiotic resistance dynamics in biofilms

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It is known that antibiotics at low and non-lethal concentrations (sub-MIC) are widespread and ultimately end up in the environment. Furthermore, recent experimental data has shown that sub-MIC concentrations of antibiotics selectively enrich for highly resistant pathogens, motivating actions to improve the design of therapeutic dosing regimens and treatment. While many studies have examined the effects of sub-MIC with planktonic bacteria, little is known about the effects of sub-MIC concentrations of various antibiotics when bacteria are in a biofilm state. We have developed SLIME, that is a new microfluidic device for studying antibiotic resistance dynamics in bacterial biofilms. Utilizing stereolithography, rapid prototyping of custom 3D-printed molds with various designs was explored to develop SLIME. To confirm the state of bacterial growth, cultured *E. coli* biofilm was stained for cellulose, a major EPS component. Furthermore, various layers of the biofilm were harvested and gene expression analyzed. Thereafter, the SLIME model was used to explore the biofilm response to sub-MIC Ciprofloxacin exposure by competing pairs of susceptible and resistant bacteria in biofilm growth. Our preliminary data suggest that cultivated *E. coli* cells express cellulose within the SLIME chip. Furthermore, harvested biofilm layers all showed higher expression of *rpoS* and *csgD* genes, suggesting that the cells are in a biofilm state of growth. When Ciprofloxacin susceptible and resistant strains were co-cultured and competed against each other in the SLIME model, a higher minimal selective concentration (MSC) of the susceptible strain was observed suggesting different selective effects of Ciprofloxacin in *E. coli* biofilms as compared to planktonic cultures of *E. coli*. Thus, we present a new microfluidic biofilm model called SLIME that is well suited for the study of antibiotic resistance dynamics in biofilms.

Poster No. 64

Characterization of flavohemoprotein in *Giardia* isolates

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Introduction: Metronidazole (MTZ) is used for the treatment of the eukaryotic parasite *Giardia lamblia* and is thought to cause oxidative- and nitrosative stress. The nitrosative stress detoxification protein, flavohemoprotein (FHMP) has previously been found to have high genetic diversity in *Giardia*. The aim of the study was to analyze FHMP haplotype and genetic diversity and crystal structure.

Methods and Materials: Twenty clinical isolates of *Giardia* were cultured at the Robert-Koch Institute, Berlin and then whole genome sequenced by illumina. The FHMP gene was then PCR amplified from eighteen isolates of *Giardia* and finally cloned into competent cells of *E.coli*. The different clones of the gene were then investigated.

The FHMP of *Giardia* assemblage A (E2RTZ4) and B (6U182) was also cloned into vectors for protein expression in *E. coli*, and two constructs, one assemblage A and one assemblage B, were expressed and purified to homogeneity. Structural analysis was done using multi angle light scattering (SEC-MALS), circular dichroism (CD), small-angle X-ray scattering (SAXS) and crystallization trials were started.

Results: FHMP gene coverage varied between isolates and was 1-5 times higher than average of other genes. 116 sequences from eleven assemblage A2 isolates harbored 56 unique haplotypes, while for 45 B sequences in four isolates, 31 unique haplotypes were found.

Adding heme and FAD to the proteins during the purification was a key factor for obtaining a pure and homogeneous sample. CD predicted a folded secondary structure, and T_m between 40-45 °C. SAXS analysis predicted a monomeric and globular shape. Crystallization attempts are ongoing, and small crystals have already been detected.

Discussion: The high genetic variation in FHMP can partly be explained by it being a multicopy gene with up to five copies in some isolates. The high number of unique haplotypes found in the cloning experiments, also indicate a high degree of genetic variation. The multicopy theory will be further investigated using digital PCR. Expressed FHMPs were prone to precipitation while storage and freezing.

High genetic variability in FHMP may allow adaption to nitrosative stress and could play a role in the MTZ susceptibility or MTZ resistance.

To understand pregnancy malaria is to understand epigenetic regulation in parasites

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Semi-immune women in endemic countries are in general protected from severe *Plasmodium falciparum* malaria. This changes drastically during pregnancy when the parasites switch expression of surface antigens to allow for cytoadherence in the new placental niche. The resulting infection is associated with high risks of both maternal and perinatal morbidity and mortality. It is unclear how the malaria parasites establish placental-binding phenotype. We recently revealed an important lead by the discovery of the *Plasmodium* translation enhancement factor (PTEF), which is essential for efficient translation of the parasite antigen (VAR2CSA) that mediates placental binding. To further understand the development of placental malaria, we are investigating the epigenetic regulation of this key factor. Here, we present surprising findings how PTEF's expression is controlled.

Using a unique selection strategy, we were able to generate parasites with gradual placental binding phenotypes differing in PTEF expression levels, ideal for monitoring epigenetic drivers in parasite's with variable phenotypic strength. Chromatin immunoprecipitations revealed that *var2csa* and *ptef* share similar histone modification occupancy upon activation. However, the two loci differed in DNA methylation pattern. Furthermore, the presence of three additional DNA modifications, that have not been identified in *Plasmodium* before (1mA, 3mC and 5hmC) were confirmed. The findings were further supported by a chemical biology approach using compounds targeting chromatin modifying enzymes.

Collectively, these findings suggest that *var2csa* and *ptef* are differentially regulated and point, for the first time, towards DNA methylation playing a role in antigenic variation within the parasite. Our discoveries add an additional piece to the jigsaw puzzle of attempts to understand how malaria during pregnancy is brought about. Further scrutiny of the identified epigenetic modes of PTEF activation may result in new targets of therapeutic intervention, which is crucial in order to reduce the suffering in the most vulnerable demographics, namely the unborn babies and their moms.

Poster No. 66

The EmrKY contribution for *Shigella* intracellular life: a pivotal study for understanding efflux pumps role in *Shigella* invasive process

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Efflux pumps (EPs) are present in all living organisms and represent an important and consistent group of transporter proteins. The importance of efflux pumps in bacteria has always been associated to their ability to extrude a wide range of antibiotic, resulting in a multidrug resistance phenomenon. However, besides antibiotic resistance, efflux pumps (EPs) of several bacterial pathogens likely take part in relevant processes of the microbial physiology, including bacteria-host interactions.

Since in *Shigella*, the causative agent of bacillary dysentery, the role of EPs in the intracellular life have been very poorly investigated, the aim of this work is to analyze their induction during *Shigella* infection of macrophages and epithelial cells and to identify those that are potentially involved in the pathogenesis of *Shigella*.

In the present study, we report that many of the conserved *S. flexneri* EP genes have their expression differentially modulated during the intracellular life. Among them, our findings highlight very promising genes encoding the MFS EmrKY EP as highly and specifically activated in *Shigella*-infecting U937 macrophage-like cells. We demonstrate that the *emrKY* induction occurs in response to a combination of high K+ and mild acidic pH. Both of these physico-chemical features are sensed and transduced by the EvgS/EvgA two-component system, both in laboratory conditions and in infected macrophages. Notably, we show that following *S. flexneri* infection, macrophage cytosol undergoes a mild reduction of intracellular pH, permitting EvgA to trigger the *emrKY* activation. Finally, we obtain evidence suggesting that EmrKY is functional to *Shigella* to better overcome the macrophage obstacles, pointing out the pivotal role of an EP in *Shigella* invasive process.

Poster No. 67

CRISPR/Cas9 screening at the High Throughput Genome Engineering facility

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High Throughput Genome Engineering (HTGE) is a SciLifeLab core facility offering pooled CRISPR/Cas9 screening. The CRISPR- Cas9 system allows targeted gene knockout, editing, or transcriptional modulation. One powerful application of this technology is in pooled screening, where thousands of genes can be targeted in parallel in a population of cells. This population is then enriched for a phenotype of interest, typically by growth advantage, drug selection, or cell sorting. Finally, enriched and depleted guide RNAs are determined by Next Generation Sequencing (NGS). HTGE supports pooled CRISPR/Cas9 screening from planning to data analysis.

In addition to CRISPR knockout screening, which allows loss of function queries of coding genes, HTGE now offers CRISPRi(nhibition) and a(ctivation), in which nuclease-dead Cas9 (dCas9) is fused to transcriptional effectors and targeted to elements of interest. dCas9-based approaches, which do not generate DNA double strand breaks, are preferable in cells with an intact DNA damage response, as p53-induced cell cycle arrest can introduce noise and reduce the power of a CRISPR screen. CRISPRi is particularly useful for targeting ncRNAs and for work in primary cells, while CRISPRa allows for gain-of- function screens. We are also implementing CRISPR/Cas9 screening coupled to single cell transcriptomics. At HTGE we include Unique Molecular Identifiers (UMIs) in all of our guide libraries. These UMIs allow us to track individual transduction events, providing internal replicates and enhanced statistical power and quality control.

Poster No. 68

Inferring mechanism from high-throughput data - The case of CD4 T cells

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CD4 T helper cells are an important part of the adaptive immune response and is involved in many diseases; from cancer and arthrosis to asthma. As such they are among the most studied cell types. The challenge now is to make best use of the large body of produced data and known mechanisms. While some are well-known (e.g. JAK-STAT pathway), and some are emerging (e.g. circadian rhythm), most are only known by a few experts (e.g. compensation of transcriptional regulation during low temperature conditions). These can be important confounders in our models, but hard to capture due to lack of human understanding. To this end we are developing a new computer model with the aim of explaining CD4 T cell phenotypes in different in vivo contexts. This model is able to bridge the limits of human understanding by the computers vast ability to store quantitative information.

To generate our model we are performing in vitro experiments under well-defined conditions. Our goal is to regenerate the known regulatory cascades from high-throughput data, combined with manually annotated data. Tools include for example CRISPR, microscopy, RNA-seq, ChIP-seq and proteomics. A Bayesian first order differential equation framework is then used to capture the essentials, and the output is a model where each fitted parameter corresponds to one or few mechanisms that can be tested in the lab. Positive and negative data can then be added to further restrain the model and thus generated hypotheses. This way we hope our model can be used as a map for systematic exploration of T cell behavior, accelerating our progress.

Poster No. 69

Two domains of nsP3 determine vector specificity of Chikungunya and O'nyong nyong viruses

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O'nyong nyong virus (ONNV) and Chikungunya virus (CHIKV) are closely related alphaviruses. They are transmitted by different mosquito vectors. CHIKV is transmitted by culicine mosquitoes, primarily by *Aedes albopictus* and *Aedes aegypty* while ONNV is the only alphavirus transmitted by anopheline mosquitoes such as *Anopheles gambiae* and *Anopheles funestus*. The difference in vectors is reflected in different spread of these viruses: CHIKV has spread over all tropical region while ONNV has remained restricted to Africa. Nonstructural protein 3 (nsP3) has been identified as the determinant of ONNV vector specificity (1). However, nsP3 consists from three different domains and it is not clear what part(s) of the protein is responsible for the effect. Furthermore, it is not known is the effect reciprocal and what are the molecular mechanisms behind of it.

In order to deeply elucidate this phenomenon, chimeric viruses that contained swapped domain(s) of nsP3 from ONNV and CHIKV, were constructed. All viruses were viable in mammalian cells and replicated to high levels with minimal differences between them. This contrasts to results of the previous research where ONNV harboring CHIKV nsP3 was found to be not viable (1). To detect effects caused by swapping, a novel trans-replication system was developed and used to evaluate the replication and transcription of CHIKV and ONNV RNA templates in mammalian and *Aedes* mosquito cells. It was found that for maximal level of replication the N-terminal macro-domain of nsP3 should be of the same origin as the rest of ns-proteins. The alphavirus unique domain (AUD) and C-terminal hypervariable domain (HVD) also play critical roles in replication and transcription however the effects were host specific. All chimeric replicases were functional in mammalian cells. In *Aedes* cells CHIKV replicase was highly active; in contrast ONNV replicase had very low activity. This activity was considerably increased by replacement either AUD or HVD of ONNV with CHIKV counterparts. Replacement of both AUD and HVD resulted in cumulative effect and replication levels similar to these observed at the presence of CHIKV replicase. These results contribute to understanding the interactions between alphaviruses and their vectors and may help the development of antiviral strategies against alphaviruses.

Keywords: O'nyong nyong virus, Chikungunya virus, nonstructural protein 3, trans-replication system

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Poster No. 70

The regulation of tRNA genes during the intra-erythrocytic developmental cycle in *Plasmodium falciparum*

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The genome of *P. falciparum* is highly AT-biased and concomitantly results in a highly biased codon usage and amino acid composition (AAT and AAA codons alone account for more than 20% of the total codon counts).However, the parasite genome only encodes a non-redundant set of tRNA genes.The lack of correlation between tRNA gene copy number and codon usage, as often observed in single-cell organisms, would suggest that tRNA genes expression to be regulated on the transcriptional level to allow the parasites to cope with the extreme codon usage bias. In this study, we aim to establish a highly quantitative tRNA expression profile of the parasite during the asexual blood stage by comparing three different sequencing protocols that are specially adopted for tRNA sequencing.

Based on the result from the hydro-tRNAseq method, we found that the retrieved sequence reads of different tRNAs is highly disproportional to the usage frequency of their respective codons. This may suggest the potential of regulating the expression of different tRNA genes as a mean to re-program the translatome.

As a parallell in eof investigation, we also aim to assess the effect of queuosine (Q) substitution on translation, a queuine-derived post-transcriptional modification found on the wobble position of Asn, Asp, His and Tyr tRNAs. We found that Q can be rapidly substituted into these tRNAs at nanomolar concentrations, but is entirely dependent on exogenous queuine. The phenotypic consequences of Q substitution were assessed and reportedhere.

Poster No. 71

The intracellular phosphoproteome of *Streptococcus gordonii* DL1

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Objective: *Streptococcus gordonii* is an early colonizer and adheres well to oral surfaces. In *S. mutans*, attachment to a surface leads to multiple changes in protein expression, including downregulation of glycolytic enzymes, which probably facilitates adaptation to the biofilm environment. However, generally the cellular responses associated with substrate sensing in oral streptococci are poorly understood. As a first step in unravelling these mechanisms, we have investigated the intracellular phosphoproteome of *S. gordonii*.

Methods: Intracellular proteins were extracted from *S. gordonii* DL1 in the exponential growth phase by freeze-thaw cycling followed by sonication in a lysis buffer containing urea, CHAPS, IPG buffer pH 4-7 and DTT. After centrifugation, the extract was subjected to isoelectric focusing in the same buffer and then SDS-PAGE in 14% gels. Protein spots were visualized using T-Rex staining and phosphorylated proteins with Pro-Q™ Diamond Phosphoprotein Gel Stain. Spots displaying phosphorylation were identified by LC-MS/MS and protein sequence analysis.

Results: The two-dimensional gel electrophoresis revealed more than 350 intracellular protein spots. Of these, around 40 appeared to correspond to phosphorylated proteins. Mass spectrometry analysis of the 11 most prominent spots identified five different phosphorylated proteins; enolase (4 spots), elongation factor Tu (3 spots), phosphoglucosamine mutase (1 spot), homoserine dehydrogenase (1 spot) and DNA-directed RNA polymerase subunit alpha (1 spot). Of these, the most abundant was enolase.

Conclusion: Thus, we have identified a number of phosphorylated proteins involved in various cellular functions such as glycolysis, amino acid and nucleotide metabolism, cell wall and protein synthesis in *S. gordonii*. Our ongoing studies clearly show that surface adhesin presentation in *S. gordonii* is regulated in response to binding to the salivary glycoprotein MUC5B. Therefore, our future research will focus on changes in the intracellular phosphoproteome during adhesion to this protein.

Poster No. 72

Droplet digital PCR based determination of plasmid copy number during *Y. pseudotuberculosis* infection

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Within the genus Yersinia only *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis* are human pathogens. These three species share a common IncFII virulence plasmid which encodes a type III secretion system (T3SS) and effector proteins called Yops (*Yersinia* outer proteins). During infection, *Y. pseudotuberculosis* upregulates its plasmid copy number (PCN) in a T3SS dependent manner, as recently shown by Wang and colleagues[1]. Although, upregulation of PCN is essential for virulence, the resulting gene dosage effect leads to a metabolic burden which prevents growth. Thus, Yersinia must have evolved a mechanism to circumvent this effect, thereby allowing establishment of infection. However, these previous results were obtained via whole genome sequencing (TruSeq), a very precise but expensive platform, and qPCR, which lacks of preciseness in highly complex samples with low abundant targets.

Here, we optimized droplet digital PCR (ddPCR) which allows us to determine PCN changes during *Y. pseudotuberculosis* infection. Therefore, total DNA extractions of organs were screened, taken from different infected mice over a period of 10 days. The results were confirmed by TruSeq and qPCR or FACS-analysis and CFU-assays, respectively. Our results show, that ddPCR is sensitive enough to reliably detect even small amounts of bacteria in highly complex samples. Independent of background DNA we were able to determine the relative PCN in infected mice organs. Furthermore, the precision of ddPCR enabled us to determine the bacterial load in each of the tested organ at different time points of infection. This method provides a useful tool to screen a greater amount of complex samples in a cost efficient but also highly precise way, compared to TruSeq or qPCR.

Reference:

[1] Wang, H., Avican, K., Fahlgren, A., Erttmann, S. F., Nuss, A. M., Dersch, P., Fallman, M., Edgren, T., & Wolf-Watz, H. Increased plasmid copy number is essential for Yersinia T3SS function and virulence. *Science*. **353**, 492 - 495. (2016).

Poster No. 73

Optotracing *S.aureus*

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Imaging bacteria in clinical or environmental samples is possible using nonspecific organic dyes (i.e. Gram stain). A fluorescent signal specific for the species can be obtained using immunofluorescent staining, which is however, more complicated. Here, we describe a simple method for specific fluorescence labelling of *Staphylococci* based on the optotracing technology. We show that the molecule emits a bright red fluorescence when bound to *S. aureus*. In an assay containing mixed cultures of *S. aureus* and *S*. Enteritidis or *E. faecalis*, *S. aureus* is selectively labeled. As the molecule is fluorescent only when bound to *S.aureus* at physiological pH, the fluorescence intensity is directly related to the amount of *S.aureus* allowing direct quantification. Careful investigation of the structural and chemical properties and the binding behavior in different buffer systems, enabled us to establish a method for live monitoring of *S.aureus* growth using fluorescence emission as a readout. This is the first reported application of optotracing for direct imaging of bacteria and we foresee that this technology could become a valuable tool for microbiologists in academia and clinical laboratories alike.

Barcoded Consortium Infections Resolve Cell Type-Dependent *Salmonella* Host Cell Invasion Mechanisms

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Bacterial host cell invasion depends both on the bacterium's virulence factors and the properties of the target cell. The enteropathogen *Salmonella enterica* Typhimurium (*S*.Tm) invades epithelial cell types in the gut mucosa and a variety of immune cell types at later infection stages. The molecular mechanism(s) of host cell entry have, however, predominantly been studied in epithelial cell lines. *S*.Tm uses a Type-Three-Secretion-System (TTSS-1) to translocate effectors into the host cell cytosol, thereby sparking actin ruffle-dependent entry. The ruffles also fuel cooperative invasion by bystander bacteria. In addition, several TTSS-1-independent entry mechanisms exist, either involving alternative *S*.Tm virulence factors, or the passive uptake of bacteria by phagocytosis. However, it remains ill-defined how *S*.Tm invasion mechanisms vary with host cell context. Here, we developed a single-well, internally controlled and scalable method to map *S*.Tm invasion mechanisms across host cell types at high temporal resolution. The method relies on host cell infections with consortia of chromosomally tagged *S*.Tm wild-type and mutant strains, where the abundance of each strain can be quantified by qPCR or amplicon sequencing. Using this methodology, we quantified co-occurring TTSS-1-dependent, cooperative, and TTSS-1-independent invasion events in epithelial, monocyte, and macrophage cells. We found *S*.Tm invasion of epithelial cells and monocytes to proceed by a similar MOI-dependent mix of TTSS-1-dependent and cooperative mechanisms. Moreover, during the first minutes of interaction, entry into macrophages was also virtually exclusively TTSS-1-dependent. Finally, we identified a generic SopB/SopE/SopE2 effector module responsible for TTSS-1-dependent invasion across both epithelial and phagocytic cell types. The application of this methodology across bacterial species and infection models will provide a scalable means to address host-pathogen interactions in diverse contexts.

Poster No. 75

Regulation of Type 4 pilitation by a *trans*-acting sRNA in *Neisseria meningitidis*

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Neisseria meningitidis is a common bacterial colonizer of the human nasopharynx; however, it can also cause very severe systemic infections with a rapid onset. Different niches within the human host require differential expression of colonization and virulence factors for effective colonisation and infection. The Type 4 pilus is considered the major colonization and virulence factor of *N. meningitidis*. Recent studies have shown that the Type 4 piliation is crucial for the colonization of the blood capillary network. Adaptation-mediated gene expression upon sensing of environmental signals is often regulated by RNA-RNA or RNA-protein interactions.

Through RNA-seq analysis, we identified an approximately 150 nucleotides long RNA transcript consisting only of the 5'-UTR of *fadL* mRNA (encoding a putative long-chain fatty acid transporter). This transcript contains a 132 nucleotide complementary sequence to the 5'-UTR of *pilF* mRNA (encoding the ATPase responsible of Type 4 pili elongation) and a 90 nucleotide complementary sequence to the 5'-UTR of the minor pilin operon. We could show that this RNA transcript functions as a *bona fide trans*-acting sRNA promoting translation of PilF and the minor pilin FimT. Further, we could also show that this sRNA is essential for normal piliation meanwhile overexpression of it leads to decreased adhesion to epithelial cells. We are currently investigating environmental triggers affecting expression of this sRNA.

Our study here sheds light into regulation of neisserial Type 4 piliation and related phenotypes. A better understanding of adaptation-mediated gene expression of major neisserial virulence factors could allow us to design smart interference strategies to disarm pathogenic *Neisseria*.

Poster No. 76

The potential of asymptomatic subjects and non-human reservoirs for Kala-Azar in Bihar, India

Puja Tiwary, Om Prakash Singh, Anurag Kumar Kushwaha, Shakti Kumar Singh, Dhiraj Singh, Rahul Chaubey, Edgar Rowton, Phillip Lawyer, David Sacks, Christine Peterson, Shyam Sundar

Background: Visceral Leishmaniasis in the Indian subcontinent is thought to have an anthroponotic transmission cycle as there is no direct evidence that a asymptomatic or non- humans mammals can be infected with *L. donovani* and transmit infection to the sand fly vector. Recent studies of the potential impact of sand fly feeding on other domestic species in Indian subcontinent found evidence sand fly feeding on dog, cattle, water buffalo and goats, which points towards the possibility of non-human reservoirs.

Methods: In order to answer this critical question, the Bill and Melinda Gates Foundation (BMGF) has invested in the personnel and infrastructure needed to conduct xenodiagnostic surveys using colonized *Phlebotomus argentipes* allowed to feed on animals. Xenodiagnosis on animals within the study area were performed and fed flies were used to detect presence of Leishmania parasites. We have also collected blood from asymptomatic subjects and animals for qPCR and serology.

Results: To date no transmission to sand fly detected using PCR and microscopy. We performed qPCR on blood collected from the dogs and found positive.

Conclusion: These studies will be the first to address the possibility of non-human reservoirs of VL in India that are not inferentially based on evidence of infection, but on direct demonstration of transmission using xenodiagnosis.

Poster Sessions

Group A. Chairpersons Åsa Sjöling and Staffan Svärd

1	**Salma Al Adwani, KI,**	Studies on citrullinated LL-37: detection in human bronchoalveolar lavage fluid, antibacterial effects and biophysical properties
2	**Sofia Berggren, UU**	The global RNA-binding protein ProQ promotes SPI-2 gene expression through the transcriptional regulator SlyA
3	**S. M. Hossein Khademi, LU**	Within-host adaptation mediated by intergenic evolution in *Pseudomonas aeruginosa*
4	**Nasibeh Mohammadi, UmU**	The role of Guanylate-binding proteins (GBPs) during intracellular infection with *Francisella tularensis*
5	**Mohammad Roghanian, UU**	The autoregulation of *Escherichia coli* RelA through intramolecular interactions
6	**Åsa Gylfe, UmU**	A novel antibacterial compound with antibiotic effect in *Chlamydia* infected mice
7	**Ferdinand Xiankeng Choong and Agneta Richter-Dahlfors, KI**	Biofilm expression patterns to microenvironmental cues
8	**Janna Grüttner, UU**	Characterization of the secreted cysteine protease CP17516 as a potential virulence factor

Group B. Chairpersons Barbara Sixt and Raphael Valdivia

9	**Hissa Al Farsi, KI**	Cross-resistance between colistin and the antimicrobial peptide LL-37 in carbapenemase-producing *Klebsiella pneumoniae*
10	**Enrique Joffré, KI**	The transcriptional regulator CsvR controls the modulation of enterotoxigenic *Escherichia coli* virulence by bile salts
11	**Cátia Pereira, UU**	Sublethal antibiotic concentrations as drivers of the evolution of antibiotic resistance in *Escherichia coli*
12	**Xinglin Jiang**	Technical University of Denmark, Dissemination of antibiotic resistance genes from antibiotic producers to pathogens
13	**Nikola Zlatkov, UmU**	Novel c-di-GMP-dependent metabolic capabilities in extraintestinal pathogenic Escherichia coli due to loss of global stress regulation
14	**Nathalie Uwamahoro, UmU**	Immune resolution dilemma: Host antimicrobial factor calprotectin induces tissue damage during peritonitis infection model
15	**Gabriella Boisen, Malmö University**	Acid tolerance of oral biofilms

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16	**Lisa Allander, UU**	Exploring the genetic determinants of synergistic interactions of two or more antibiotics against multi- and extensively drug-resistant Gram-negative bacteria
17	**Li Qin Cheng, KI**	Vaginal microbiota and HPV infection of adolescent young girls
18	**Karsten Meier, UmU**	The Chlamydia inclusion membrane protein CpoS recruits Rab GTPases to subvert the host cellular surveillance system
19	**Cedric Romilly, UU**	Anatomy of a standby site: an essential role for ribosomal protein S1 and a secondary structure element for ribosome binding
20	**Claire Sayers, UmU**	Designing a systematic screen for fertility genes in *Plasmodium berghei*
21	**Nuno Sousa, KI**	Practical and effective detection of bio-aerosols carrying mycobacteria using a novel, ionization-based air sampler
22	**Chayan Kumar Saha, UmU**	Alarmone synthetases turn toxic
23	**Doreen Mutemi, KI**	Persistent transmission of *Plasmodium malariae* and *Plasmodium ovale* species in an area of declining *Plasmodium falciparum* transmission in eastern Tanzania

Group D. Chairpersons Debra Milton and Søren Molin

24	**Malin Alsved, LU**	Experimental assessment of infectivity of aerosolized murine noroviruses
25	**Maarten Coorens, KI**	Innate lymphoid cells type 3-derived IL-22 boosts NF-kB-induced lipocalin-2 production through STAT3 in human intestinal epithelial cells
26	**Geneviève Garriss, KI,**	Modulation of competence for natural transformation by pneumococcal bacteriophages
27	**Mohammed Mubasher, SU**	Host-parasite interactions during plasmodium falciparum zygote development
28	**Rikki Frederiksen, UmU**	The transcriptomic landscape of *Salmonella Typhimurium* persistent infection of mice
29	**Liis Andresen, UU**	RyfA is part of a possible type I toxin-antitoxin system in *Salmonella*
30	**Priya Devi, UU**	Hepatitis C virus core protein down regulates expression of src-homology 2 domain containing protein tyrosine phosphatase
31	**Svava Steiner, KI**	Nervous sensing and inter-organ communication during *E. coli* kidney infection is modulated by the toxin α-haemolysin

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32	**Hannes Eichner, KI**	A small RNA involved in pyrimidine synthesis regulation is controlled by an antisense RNA in *Neisseria meningitidis*
33	**Samada Muraleedharan, UmU**	Identification of regulators of a defensive host cell death program that is actively suppressed by *Chlamydia trachomatis*
34	**Jens Karlsson, KI**	A regulatory RNA contribution to invasive meningococcal disease in Europe, 2010-2018
35	**Niilo Kaldalu, University of Tartu**	Quantification of low-abundance proteins by targeted mass spectrometry reveals dynamics of bacterial toxin-antitoxin systems
36	**Sascha Krakovka, UU**	Metronidazole resistance in *Giardia duodenalis*: Identifying patterns by transcriptomics combined with biochemical analysis of two oxygen-insensitive nitroreductases
37	**Eva Skovajsová, UU**	Tuning of virulence in *Shigella flexneri* by distinct isoforms of the master regulatory protein VirF
38	**Saskia Erttmann, UmU**	Bacteria-derived hydrogen peroxide suppresses inflammasome-dependent innate immunity

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39	**Malin Elvén, LU**	Blood, fat and smears: apolipoprotein E as an endogenous alternative to antibiotics against Gram negative infections?
40	**Fengyang Li, KI**	DncV synthesizes cGAMP and regulates biofilm formation and motility in *Escherichia coli* ECOR31
41	**Harpa Karadottir, KI**	The differential survival of ESBL carrying *E.coli* in blood suggest a cost of fitness compared to antibiotic susceptible strains
42	**Zhen Liao, SLU**	Roles of small RNAs and associated Argonaute proteins in the battle between potato and *P. Infestans*
43	**Aurelie Miglar, KI**	An interplay between inflammation, oxidative stress and cellular aging in a controlled human malaria challenge study
44	**Carlos Nunez Otero, UmU**	Towards target identification for 2-pyridone amides affecting *Chlamydia trachomatis* infectivity
45	**Asgeir Astvaldsson, UU**	Experimental challenge of Atlantic salmon (*Salmo salar*) with the diplomonad parasite *Spironucleus salmonicida* to characterize the infection cycle

Group G. Chairpersons Fredrik Kahn and Helle Krogh Johansen

46	**Tifaine Hechard, UU**	The role of CopB protein in Yersinia virulence plasmid copy number regulation
47	**Alberto Jonatan Martín-Rodríguez, KI**	Regulation of biofilm formation in Shewanella algae
48	**Anna Bergonzini, UmU**	Infection with genotoxin-producing Salmonella enterica synergises with loss of the tumor suppressor APC in promoting genomic instability in colonic epithelial cells
49	**Laura Rojas, UU**	DNA methylation during encystation of Giardia intestinalis
50	**Valerie Diane Valeriano, UmU**	Improved resolution of complex in vivo transcriptomic profiling of persistent Yersinia pseudotuberculosis in mice caecum
51	**Aleksandra Pettke, KI**	Exploring the host reaction to a new host-targeting, small molecular inhibitor against Zika virus
52	**Ivana Kerkez, Tartu University**	Pharmacodynamic studies of intracellular activity of antibiotics against uropathogenic Escherichia coli phagocyted by mice macrophages J774

Group H. Chairpersons Helen Wang and Jost Enninga

53	**Jennifer Jagdmann, UU**	Spontaneous and clinically relevant tet(A)-dependent tigecycline resistance development
54	**Leonie Vetter, KI**	Using different types of antisense oligonucleotides and delivery systems to probe the function of non-coding RNA in the siRNA deficient P. falciparum
55	**Oliver Billker, UmU**	Functional profiling of a Plasmodium genome
56	**Shilpa Ray, KI**	Alterations in the gut microbiome of HIV infected patients under antiretroviral therapy
57	**Pilar Samperio Ventayol, UU**	Deciphering Salmonella invasion mechanisms and host responses in organoid models of the gut epithelium
58	**Francesco Righetti, KI**	Characterisation of two novel co-transcribed sRNAs in Neisseria meningitidis
59	**Yasuhiko Irie, Tartu University,**	Bacterial suspended aggregation in high viscosity environment supports multi-species co-existence by spatial segregation of micro-communities
60	**Kathryn Jane Turnbull, UmU**	Structural basis for ribosomal protection by the Listeria monocytogenes ABCF ATPase Lmo0919
61	**Greta Zaborskyte, UU**	Biofilm-evolved Klebsiella pneumoniae exhibit changes in capsule, fimbriae and c-di-GMP turnover

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63	**Po-Cheng Tang, UU**	SLIcer of Microbial Ecosystems (SLIME) – a new microfluidic model for studying antibiotic resistance dynamics in biofilms
64	**Christina Skår Saghaug, Univ. of Bergen**	Characterization of flavohemoprotein in *Giardia isolates*
65	**Madle Sirel, KI**	To understand pregnancy malaria is to understand epigenetic regulation in parasites
66	**Martina Pasqua, UU**	The EmrKY contribution for *Shigella intracellular* life: a pivotal study for understanding efflux pumps role in *Shigella* invasive process
67	**Soniya Dhanjal, & Jenna Persson KI/ScilifeLab**	CRISPR/Cas9 screening at the high throughput genome engineering facility
68	**Johan Henriksson, UmU**	Inferring mechanism from high-throughput data - The case of CD4 T cells
69	**Sainan Wang, University of Tartu**	Two domains of nsP3 determine vector specificity of Chikungunya and O'nyong nyong viruses

Group J. Chairpersons Agneta Richter-Dahlfors and Mikael Sellin

70	**Sherwin Chan, KI**	The regulation of tRNA genes during the intra-erythrocytic developmental cycle in Plasmodium falciparum
71	**Carolina Robertsson, Malmö University**	The Intracellular phosphoproteome of *Streptococcus gordonii* DL1
72	**Stephan Schneiders, UU**	Droplet digital PCR based determination of plasmid copy number during *Y. pseudotuberculosis* infection
73	**Karen Butina, KI**	Optotracing *S. aureus*
74	**Maria Letizia Di Martino, UU**	Barcoded consortium infections resolve cell type-dependent *Salmonella* host cell invasion mechanisms
75	**Christian Spoerry, KI**	Regulation of Type 4 pilitation by a trans-acting sRNA in *Neisseria meningitidis*
76	**Puja Tiwary , UmU**	The potential of asymptomatic subjects and non-human reservoirs for Kala-Azar in Bihar, India

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The Svenska Föreningen för Mikrobiologi
(The Swedish Society for Microbiology)

Swedish Society for Microbiology - Svenska föreningen för mikrobiologi (SFM)

SFM is a life science membership organization for individuals interested in microbiological sciences. The mission of SFM is to support the progress and growth of microbiology in Sweden and to be a vehicle for development of high quality fundamental and applied research among Swedish microbiologists.

Svenska Föreningen för Mikrobiologi (SFM)

SFM has currently about 500 members, located at universities, university colleges, research institutes and industry active in general microbiology, molecular biology, microbial genetics, microbial ecology, microbial engineering, medical and veterinary microbiology, virology and immunology. SFM subsidises the registration fee for its members at this years NIB/SFM/NDPIA meeting.

For additional information please visit www.mikrobiologi.net/SFM.

Contact person and chair of the SFM board
Keira Melican, Karolinska Institutet

National Doctoral Programme in Infections and Antibiotics (NDPIA)

NDPIA is a National network including more than 400 PhD students and postdocs active in infection biology research in Sweden and at international partner institutions. The programme is a partnership between Umeå University, Uppsala University, Karolinska Institutet, Linköping University, Gothenburg University and Lund University. Through these partners we also engage other universities and institutes in Sweden. In addition the programme also has international partners in Norway and Germany and would like to extend the collaborations to additional Nordic and European partner.

NDPIA
National Doctoral Programme in Infections and Antibiotics

The programme provides meetings/courses/workshops organized in Sweden and together with international collaborators. NDPIA covers the registration fee for its member at the NIB/SFM/NDPIA meetings and also provides travel support. NDPIA is supported by a grant form the Swedish Research Council (VR).

For additional information please visit www.ndpia.se.

Coordinator: Åke Forsberg, Umeå University