Mini-Symposium: Transformative Measurement Methods and Experimental Approaches for Bacterial Biofilm

Day 1: 29 August 2017

Title: Mechanism of biofilm formation

Fitnat Yildiz, University of California - Santa Cruz, USA

Abstract: Biofilms, surface attached microbial communities composed of microorganisms and the extra-polymeric substances they produce, enhance growth and survival of microorganisms in natural settings. Biofilms contribute to dissemination, environmental survival, and transmission of human pathogens. One such pathogen is Vibrio cholerae, the causative agent of the disease cholera. Production of biofilms by V. cholerae requires extracellular matrix components Vibrio exopolysaccharide (VPS) and matrix proteins RbmA, RbmC, and Bap1, RbmA is critical for development of biofilm architecture; however, we do not yet understand the molecular mechanisms by which RbmA contributes to biofilm formation. Here, we show that RbmA binds VPS directly to control the VPS-dependent formation of higher-order structures that modulate biofilm architecture and that V. cholerae biofilms can be fine-tuned by RbmA structural dynamics.

Title: Identification of biofilm-specific functions: Grail or hoax?

Jean-Marc Ghigo, Institut Pasteur, France

Abstract: Surface-attached bacterial biofilm communities are widespread in natural environments and also contribute to biofouling and infections in medical and industrial settings. Considering the profound phenotypic modifications during the switch from planktonic to community lifestyle, bacterial biofilms are hypothesized to constitute reservoirs of new biological functions that could be used to diagnose, combat or even use bacterial biofilms. However, besides the characteristic high tolerance to antibiotics and stress, biofilm physiology is still poorly characterized, due to the intrinsic difficulty of analyzing or perform measurements in this dense and highly heterogeneous bacterial environment. We will present our approach to explore biofilm functions and identify molecules indicative of physiological adaptations to biofilm conditions.

Title: LSPR-based biosensor for bacterial biofilm analysis

Riccardo Funari, Micro/Bio/Nanofluidics Unit, OIST, Japan

Bacterial biofilms are groups of closely-packed microorganisms, often attached to

surfaces, characterized by a slimy extracellular matrix providing high resistance to conventional antibiotic therapies. For this reason, these structures have a strong impact in medicine, industry and food processing, thus there is a strong need of novel detection methodologies providing cheap, fast and reliable analysis of the system of interest. To face this issue, we propose a LSPR-based device suitable for monitoring the biofilm formation as well as measuring the response of this biological structure to a set of disturbing agents like antibiotics and detergents. The high sensitivity and intrinsic flexibility of this technique make our device a promising tool for further applications in sensing and medicine.

Title: Structure, Function, and Inhibition of E. coli Amyloid-integrated Biofilms

Lynette Cegelski, Stanford University, USA

Abstract: The biofilm extracellular matrix (ECM) is typically constructed as an insoluble, noncrystalline and heterogeneous assembly surrounding microbial cells, and poses a challenge to quantitative compositional analysis by conventional methods. Solution-based methods, e.g. solution-state NMR, HPLC, mass spec, analyze only soluble components that are typically liberated through harsh and often perturbative processing of biofilms and usually do not permit a total accounting of matrix parts. We addressed this challenge and introduced new biochemical protocols and NMR approaches to define and quantify ECM composition. Beginning with uropathogenic E. coli, we observed supramolecular basket-like structures that encapsulated single cells and established that the isolated matrix was composed of the two major components, curli and a modified form of cellulose, in a 6:1 ratio by mass. Through the NMR analysis, we made the unanticipated discovery that the polysaccharide portion consists of a modified form of cellulose – phosphoethanolamine (pEtN) cellulose in which one-half of the glucose units contain a pEtN modification. We have identified the gene required for installation of the modification and established that the modification is required for biofilm formation. Thus, solids tate NMR of the intact polysaccharide was able to identify this biologically important modification that evaded detection by conventional approaches. We complement our chemical and structural analyses with microscopy, biochemical analyses, and interfacial rheology in order to reveal how the physical properties and biological functions of biofilms depend on composition and architecture. We are using this approach to probe the modes of action of biofilm inhibitors, including a number of promising compounds identified through our own highthroughput screening. I will report on the biosynthetic production of pEtN cellulose, cooperation of curli and pEtN cellulose in bacterial adhesion and biofilm production,

and small molecules that inhibit biofilm formation and can serve as chemical tools to probe biofilm assembly and function

Title: Quantifying the strength of adhesion of E. Coli onto bladder cells

Gerry Fuller, Stanford University, USA

Abstract: Uropathogenic Escherichia coli (UPEC) are the major causative agents of urinary tract infections, employing numerous molecular strategies to contribute to adhesion, colonization, and persistence in the bladder niche. Identifying new strategies to prevent adhesion and colonization is a promising strategy to inhibit bacterial pathogenesis and to help preserve the efficacy of available antibiotics. This approach requires an improved understanding of the molecular determinants of adhesion to the bladder urothelium. We designed experiments using a custom-built Live Cell Monolayer Rheometer (LCMR) to quantitatively measure individual and combined contributions of bacterial cell surface structures (type 1 pili, curli, and modified cellulose) to bladder cell adhesion. Using the UPEC strain UTI89, isogenic mutants, and controlled conditions for the differential production of cell surface structures, we discovered that curli can promote stronger adhesive interactions with bladder cells than type 1 pili. Moreover, the co-production of curli and cellulose enhanced adhesion. Complementary biochemical experiments support a model wherein cellulose serves a Velcro-like function to promote curli association with the bacterial cell surface, resulting in increased adhesion strength at the bladder cell surface.

Title: Evolution of bacterial communities associated with termites

Tom Bourguignon, Evolutionary Genomics Unit, OIST, Japan

Abstract: Like any other organisms, insects live in a microbial world, and have established numerous relationships with eukaryotic and bacterial cells. One such charismatic relationship involves termites and the microbial communities colonizing their gut, that allow them to digest the most abundant biomolecule on Earth: ligno-cellulose. The gut microbiome of termites is a complex assemblage, containing upwards of 1000 species of bacteria and archaea. It has fascinated scientists for over a century, yet, how gut bacterial communities evolve is not yet fully understood. In this talk, I will summarize our present understanding of the world smallest bioreactor, the termite gut. I will also present new results revealing how termites acquire their gut bacteria, and how termites and bacteria have evolved together during the last 140 Million years.

Day 2: 30 August 2017

Title: Spatial organization of transcriptional control and c-di-GMP signaling in E. coli macrocolony biofilms

Regine Hengge, Humboldt University of Berlin, Germany

Abstract: Bacterial macrocolony biofilms fold into highly stable three-dimensional macroscopic structures. This morphogenesis depends on an extracellular matrix, which in the case of E.coli consists of amyloid curli fibers and the exopolysaccharides cellulose. This matrix assembles into a complex 3D architecture controlled by a regulatory network of different sigma subunits of RNA polymerase, a transcription factor cascade (RpoS-MlrA-CsgD) and cdi-GMP signaling. In order to directly visualize the activities of different sigma factors – and thereby also different physiological states of growth - within the 3D space of macrocolony biofilms, we fused gfp to (i) a standard ribosomal promoter, (ii) to generic artificial RpoD- and (iii) RpoS-dependent promoters, (iv) to the promoter of pdeH (yhjH), which not only serves as a reporter for RpoF (FliA) activity, but also encodes the most strongly expressed c-di-GMPdegrading phosphodiesterase in E. coli which maintains a low cellular c-di-GMP level, and (v) to the promoter of csgD, encoding a key biofilm transcription factor required for curli and cellulose production. In cryosectioned biofilms these reporters revealed long-range physiological heterogeneity in the form of a complex physiological stratification that essentially follows gradients of nutrients and oxygen, as well as a pronounced short-range heterogeneity of matrix production in transition zones between regions of growth and stationary phase. Genetically redesigning this biofilm architecture - e.g. by expressing matrix also in vegetatively growing zones or by eliminating short-range heterogeneity of matrix production - showed this intricate matrix architecture.

Title: Investigating bacterial social interactions through spatio-temporal imaging and analysis

Andrew Utada, University of Tsukuba, Japan

Abstract: It has become increasingly clear that bacteria exist primarily as members of surface-adhered social communities, called biofilms, rather than as free-living individuals. These communities are encased in an extracellular matrix that provides protection from harsh environmental conditions, predation, enables resource sharing, and facilitates intercellular communication. However, depending on the local conditions bacteria will transition between these two states; this interplay is fundamental to the ecology of bacteria. We use brightfield, laser scanning confocal microscopy, and

label-free reflection confocal microscopy to visualize biofilm development from the earliest stages of surface contact to more complex 3D structures. We combine this with the use of knock-out mutants, single-cell level tracking algorithms, and icrofluidics to analyze these highly complex systems over space and time.

Title: Using advanced microscopy to decipher the bacterial cell division machinery

Bill Soederstroem, Structural Cellular Biology Unit, OIST, Japan

Abstract: It has been 25 years since the discovery of FtsZ and its role in bacterial cell division. Since then have more then 30 proteins been associated with the cell division machinery, ‘the divisome’. This machinery is assembled in a ring-like structure at midcell each round of division. Though the general functions of many of these proteins are now known, is their interplay and relative organization within the divisome is still largely unknown. I will present new super resolution results on the divisome acquired in a novel way. By immobilizing cells in a vertical position can we capture whole divisome rings in one image, without the need of advanced post processing (as needed in 3D reconstruction methods). This approach greatly reduces the risk for imaging artifacts, and importantly allows for new ways of addressing decade-old questions about the internal organization of the divisome. By using a combination of immunofluorescence and fluorescent proteins fusions have we quantitatively analysed various divisome components with Stimulated Emission Depletion (STED) nanoscopy and time-lapse Structured Illumination Microscopy (SIM). Lastly, I will briefly discuss how we use correlative cryo-fluorescence and cryo-electron microscopy (cryo-CLEM) as a new tool in our studies of the bacterial cell division process.

Title: Unraveling the contribution of different cell-surface factors to uropathogenic Escherichia coli biofilm formation

Mark Schembri, The University of Queensland, Australia

Abstract: Uropathogenic Escherichia coli (UPEC) are the major cause of urinary tract infections, a disease of major significance to human health and increasingly associated with antibiotic resistance. UPEC pathogenesis and persistence in the urinary tract involves a complicated process involving adherence, aggregation, extracellular and intracellular lifestyles, resistance to host innate immune factors and biofilm formation. The formation of biofilms by UPEC is linked to the coordinated expression of multiple and diverse surface factors such as fimbriae (e.g. type 1 fimbriae and curli), autotransporter proteins (e.g. antigen 43), polysaccharides (e.g. capsule) and flagella. These surface factors are subjected to various levels of regulatory control at different

stages during biofilm development. We have studied the role of fimbrial and autotransporter adhesins in UPEC biofilm formation using a combination of molecular and structural approaches. Type 1 fimbriae are well-characterised structural organelles that belong to the chaperone-usher fimbrial class and mediate biofilm formation predominantly via the action of a tip-located FimH adhesin. Autotransporter proteins, a highly abundant group of outer membrane and secreted proteins, also contribute significantly to this phenotype. Our recent work has described the structure-function relationships of antigen 43, a self-associating autotransporter protein. The crystal structure of the functional passenger domain of antigen 43 revealed it adopts a twisted L-shaped beta-helical structure firmly stabilized by a three-dimensional hydrogen-bonded scaffold, and we have shown that this confirmation drives the formation of cell aggregates via a molecular Velcrolike handshake mechanism. Our more recent work has identified and mapped the interaction of a novel monoclonal that inhibits antigen 43 - antigen 43 interactions. The role of fimbriae and autotransporter adhesins in UPEC biofilm formation will be discussed in context with new insights into the regulatory control of other surface factors such as the polysaccharide capsule. Overall, this knowledge will enable us to understand the complex regulatory mechanisms that control the development of heterogeneous UPEC biofilms.

Title: Chemical biology approaches for biofilm eradication

Liang Yang, Nanyang Technological University, Singapore

Abstract: A bacterial biofilm is a surface attached community of microorganisms embedded in and protected by an extracellular matrix of self-made biomolecules. The US National Institute of Health (NIH) has estimated that 65---80% of all microbial infections involve bacterial biofilms. Biofilm-based bacteria can evade the otherwise detrimental actions of immune responses and develop into chronic infections. Because the present day’s armory of conventional antimicrobials cannot efficiently eradicate biofilms, there is an urgent need to understand the fundamental mechanism of antibiotic resistance by biofilms. One major obstacle to study biofilm physiology is the heterogeneity in biofilms, which often confounds our efforts to target specific aspects of biofilm biology. Bis---(3’---5’)---cyclic dimeric GMP (c---di---GMP) is a global, intracellular secondary messenger that controls biofilm differentiation. High intracellular levels of c---di---GMP stimulate bacteria to form biofilms by enhancing synthesis of adhesive structures and biofilm matrix components while low intracellular levelsfacilitate motility and chemotaxis.The c---di---GMP signaling system has been investigated for its roles in resistance to oxidative stress and host immune clearance.

Induction of the c---di---GMP signaling pathways was monitored by means of RNA---sequencing based transcriptomics. Enhanced intracellular c---di---GMP content was observed duringP. aeruginosa infections, and the data suggest that this may play a role in impairing immune mediated clearance. High intracellular c---di---GMP content was shown to enhance resistance to oxidative stress as well as to increase siderophore production via the rsmY and rsmZ regulatory, non---coding RNAs (ncRNA). Reduced c---di---GMP content of bacterial cells was found to significantly improve the killing by hydrogen peroxide of different pathogens. The existence of physiologically---differentiated subpopulations in bacterial biofilms provides an “insurance effect” against antibiotic treatment. Enrichment of antibiotic resistant subpopulationsin bacterial biofilms often leads to persistent infections. An advanced proteomics approach was developed by our group, which uses pulsed stable isotope labeling with amino acids (pulsed---SILAC) to separate and systematically examine the antibiotic sensitive and resistant subpopulations from the same biofilm. Quorum sensing and type IV pili---mediated migration were found to contribute significantly to colistin--- resistance in Pseudomonas aeruginosa biofilms. Erythromycin, which inhibits the motility and QS ofP. aeruginosa, was shown to promote biofilm eradication by colistin. This pulsed---SILAC technology is particular suitable for studying the killing efficacies of combinatorial, antibiotic treatments of biofilms and their dispersed cells.

Day 3: 31 August 2017

Title: Early diagnosis of mycobacterial infection via a new biomarker: the activity of essential mycobacterial enzymes

Megan Yi Pong Ho, The Chinese University of Hong Kong, Hong Kong

Abstract: Rapid and precise detection of pathogenic bacteria is critical for effective antibiotic treatment at an early infection stage, thus preventing the spreading of bacterial disease. While an array of diagnostic approaches has been developed for this purpose, challenges linger particularly when accessibility and time-to-results are considered collectively. Take diagnosis of tuberculosis (TB) for example, culturing the mycobacteria from the specimen/samples followed by microscopic examinations of colonies is the most commonly adapted clinical gold standard, but the long culture time of mycobacteria (up to 8 weeks) often delays the reporting. A handful of technological innovations have been recently introduced to accelerate the process of identifying the presence of bacteria, including quartz crystal microbalance, surface plasmon resonance, surface enhanced Raman scattering, and fluorescence spectroscopy. However, the

performance of these techniques rely heavily on complex purification procedures to isolate bacteria from the biological matrices, hampering their applications in the field settings. To address these challenges, we present a newly developed quantum dots (QDs) based DNA nanosensor for rapid and specific identification of a bacterial enzyme, mycobacterial topoisomerase, which is essential for bacterial cell survival. The cleavage activity of mycobacterial topoisomerase has been investigated as a novel class of biomarker in this report. We have demonstrated the possibility to quantify the cleavage activity of the mycobacterial enzyme in less than an hour, without pre-processing sample purification or post-processing signal amplification. With this being said, the cleavage induced signal response has proven reliable in crude biological matrices, such as whole cell extracts prepared from Escherichia coli and human Caco-2 cells. Significant clinical value is anticipated by extending this assay for the detection, as well as the monitoring of treatment outcome, for mycobacteria-associated diseases, such as TB, caused by infections of Mycobacterium tuberculosis. Further, the proposed nanosensor is highly transformable for the detection of contaminating mycobacteria in water or food, which may have a broad appeal to the food industries and agricultural sectors.

Title: Inexpensive and fast pathogenic bacteria screening using electronic biosensors

Nikhil Bhalla, Micro/Bio/Nanofluidics Unit OIST, Japan

Abstract: While pathogenic bacteria contribute to a large number of globally important diseases and infections, current clinical diagnosis is based on processes that often involve culturing which can be time-consuming. Therefore, innovative, simple, rapid and low-cost solutions to effectively reduce the burden of bacterial infections are urgently needed. Here we demonstrate a label-free sensor for fast bacterial detection based on metal–oxide–semiconductor field-effect transistors (MOSFETs). The electric charge of bacteria binding to the glycosylated gates of a MOSFET enables quantification in a straightforward manner. We show that the limit of quantitation is 1.9×105 CFU/mL with this simple device, which is more than 10,000-times lower than what is achieved with electrochemical impedance spectroscopy (EIS) and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-ToF) on the same modified surfaces. Moreover, the measurements are extremely fast and the sensor can be mass produced at low costs as a tool for initial screening of pathogens.

Title: How Bacillus subtilis structures its local environmen

Cait Macphee, University of Edinburgh, UK

Abstract: The Gram-positive soil bacterium Bacillus subtilis forms an architecturally complex, highly hydrophobic biofilm that resists wetting by water, solvents, and biocides. This property is conferred by a small secreted protein called BslA, which self-assembles into an organised lattice at an interface and forms a coat across the surface of the biofilm. This is mediated by the exposure of a hydrophobic “cap” when the protein reaches the air-biofilm interface. We have recently demonstrated that BslA is a multifunctional protein across multiple axes: 1) dimerization is required for hydrophobicity, whereas monomeric protein is sufficient for biofilm complex architecture; 2) the “cap out” form of the protein renders a surface layer hydrophobic whereas we can infer that a “cap in” form is present in the wetting protein layer at the base of the biofilm where the protein is exposed to water; and 3) stable lateral interactions between BslA molecules, which can be measured in vitro, appear to be required for architectural complexity, whereas unstable lateral interactions (reflected in unstable film formation in vitro) nonetheless are sufficient to give rise to hydrophobicity if dimeric protein is present. After genetically separating biofilm structure from hydrophobicity, we were able to demonstrate that it is the hydrophobicity of the biofilm and not the overall structure that protects the residents in the community from biocides. It is remarkable that B. subtilis achieves such complex architectures and functions with only a limited number of components; we are currently investigating the properties of the other major protein component, TasA.

Title: How the microscale physical environment shapes the secret lives of bacteria

William M. Durham, University of Sheffield, UK

Abstract: Modern microbiology relies on growing cells in homogeneous liquid cultures and agar plates. While these have taught us a tremendous amount about microbial life,in nature bacteria experience complex chemical and hydrodynamic landscapes that are not found in homogeneous laboratory conditions. This talk will present two projects that use microfluidic experiments and mathematical models that show how bacteria exploit heterogeneity in their natural environments. The first project investigates how hydrodynamics mediates bacterial competition in porous environments like soil. In porous environments bacterial biofilms face a fundamental challenge: dividing too rapidly can redirect flow, and the dissolved nutrients that it carries, to competing biofilms. We combine experiments, physical models, and game theory to show how this interaction favours genotypes with a specific “evolutionary stable” growth rate. Our

results suggest that growing slower can actually give cells a competitive advantage in porous environments, which is where >97% of bacteria on Earth live. The second half of the talk will present how bacteria within biofilms use grappling hook like appendages called pili to navigate chemical gradients. Using massively parallel cell tracking, we resolve both the molecular and behavioural basis of pili-based chemotaxis. Our experiments show that cells can navigate chemical gradients with submicron precision, suggesting that they directly sense changes in concentration over the length of their bodies. Taken together, these two studies offer new insights into the microscale processes that shape the composition of bacterial communities and offer new tools to manipulate them to our advantage.

Title: Scrutinizing electrical properties of bacterial biofilms

Lukasz Szydlowski, Biological Systems Unit, OIST, Japan

Abstract: Bacterial biofilms show astounding ability to transfer rapidly signals over large distances, up to centimetres' range. This ability is often performed by ion signalling, causing depolarization of cell membranes. In other cases, highly conductive pili are formed, which allow extracellular electron transfer (EET) between either the same or different bacterial species. Microbial Electrogenic Technology (MET) utilizes EET in either electricity generation, as in the case of Microbial Fuel Cell (MFC) or "electrotrophy", as in the case of Microbial Electrolysis Cells (MEC). Bacterial biofilms covering electrodes are crucial in both cases and are flexible enough to switch from electron generation to electron reception. MFCs are emerging technology of energy-efficient wastewater treatment as compared to the commonly applied aerobic methods. The electricity generation can also be utilized by means of biosensor development, where subsequent power production would instantaneously indicate the presence of organic substrates. MEC, on the other hand, offer an alternative of nutrient recovery, e.g. ammonia and phosphorus from the waste or allow degradation of recalcitrant substrates e.g. lignocellulose. We have identified major electrogenic bacteria comprising various electrode biofilms, mainly belonging to the class of deltaproteobacteria, e.g. Geobacter spp. We are interested in discovering mechanisms facilitating EET within biofilms, as well as improve this process by means of metabolic pathway engineering.