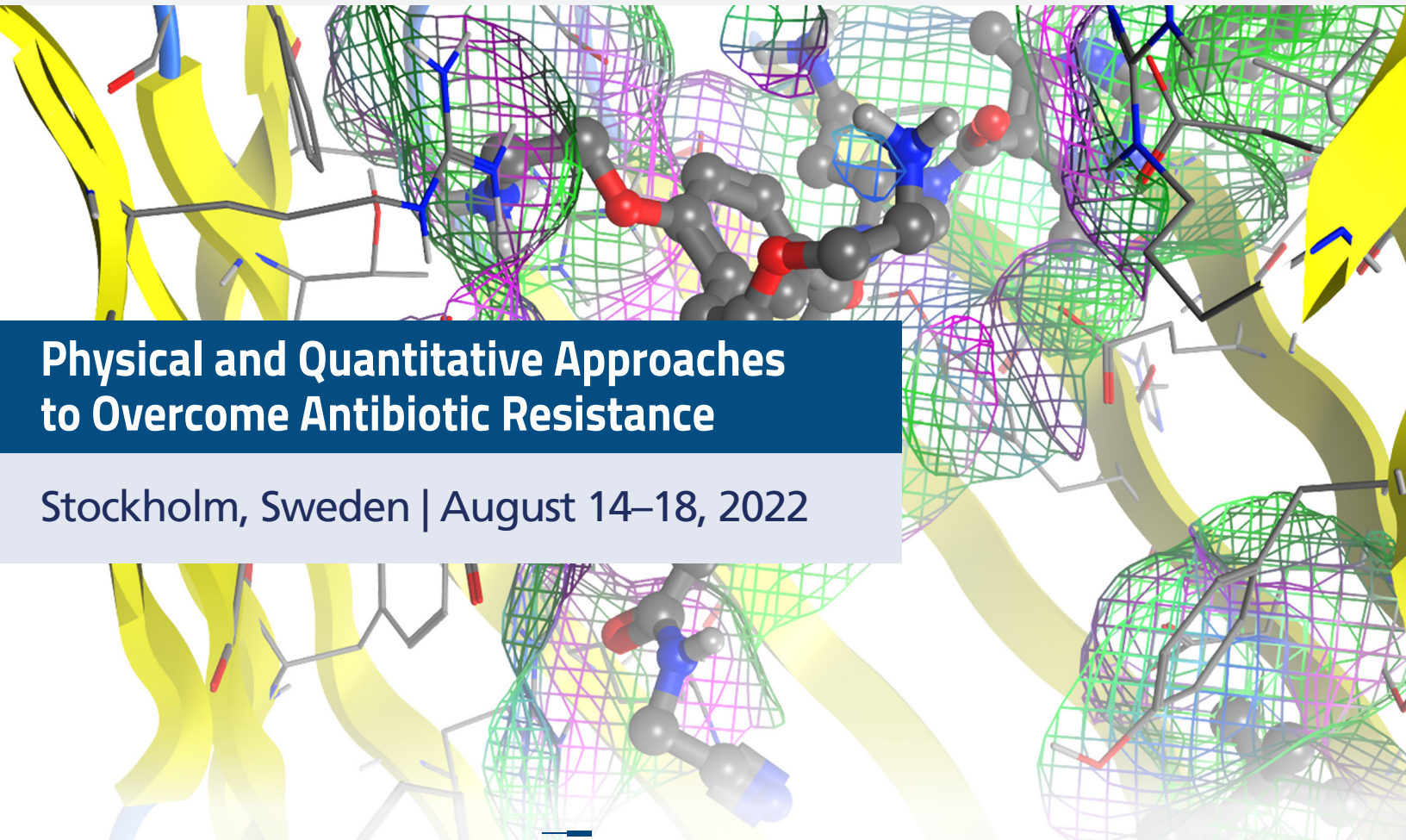

Physical and Quantitative Approaches to Overcome Antibiotic Resistance

Stockholm, Sweden | August 14–18, 2022

Biophysical Society

Biophysical Journal

Swedish Research Council

Wenner-Gren Stiftelserna

Organizing Committee

Peter Kasson, University of Virginia, USA

Joanna Slusky, University of Kansas, USA

Georgios Sotiriou, Karolinska Institute, Sweden

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August 2022

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting on Physical and Quantitative Approaches to Overcome Antibiotic Resistance, co-sponsored by the Swedish Research Council and the Wenner-Gren Foundations. The thematic meetings series provides an opportunity for scientists to meet and exchange ideas in a more intimate setting than large annual meetings, and we greatly hope that this will provide a stimulating venue for discussion.

Antimicrobial resistance is an increasing threat to global health and one that requires creative new solutions. Physical and quantitative sciences have much to offer in this regard, and the goal of this meeting is to bring together physical scientists working on antibiotic resistance from different backgrounds to share their work and inspire each other. The many approaches that have been fruitful include biomedical imaging, engineering approaches, network and community approaches to antimicrobial resistance, as well as structure, measurements, and modeling of antibiotic uptake, efflux, and the bacterial membranes that control many of these interactions.

We hope that these and more will all be stimulating for you, and please take part in the discussions, poster sessions, and informal exchanges offered by the meeting. We value the contributions of all participants, and we hope you enjoy the meeting and Stockholm!

The Organizing Committee

Peter Kasson, University of Virginia, USA
Joanna Slusky, University of Kansas, USA
Georgios Sotiriou, Karolinska Institute, Sweden

Biophysical Society Code of Conduct, Anti-Harassment Policy

The Biophysical Society (BPS) is committed to providing an environment that encourages the free expression and exchange of scientific ideas. As a global, professional Society, the BPS is committed to the philosophy of equal opportunity and respectful treatment for all, regardless of national or ethnic origin, religion or religious belief, gender, gender identity or expression, race, color, age, marital status, sexual orientation, disabilities, veteran status, or any other reason not related to scientific merit.

All BPS meetings and BPS-sponsored activities promote an environment that is free of inappropriate behavior and harassment by or toward all attendees and participants of Society events, including speakers, organizers, students, guests, media, exhibitors, staff, vendors, and other suppliers. BPS expects anyone associated with an official BPS-sponsored event to respect the rules and policies of the Society, the venue, the hotels, and the city.

Definition of Harassment
The term "harassment" includes but is not limited to epithets, unwelcome slurs, jokes, or verbal, graphic or physical conduct relating to an individual's race, color, religious creed, sex, national origin, ancestry, citizenship status, age, gender or sexual orientation that denigrate or show hostility or aversion toward an individual or group.

Sexual harassment refers to unwelcome sexual advances, requests for sexual favors, and other verbal or physical conduct of a sexual nature. Behavior and language that are welcome/acceptable to one person may be unwelcome/offensive to another. Consequently, individuals must use discretion to ensure that their words and actions communicate respect for others. This is especially important for those in positions of authority since individuals with lower rank or status may be reluctant to express their objections or discomfort regarding unwelcome behavior. It does not refer to occasional compliments of a socially acceptable nature. It refers to behavior that is not welcome, is personally offensive, debilitates morale, and therefore, interferes with work effectiveness. The following are examples of behavior that, when unwelcome, may constitute sexual harassment: sexual flirtations, advances, or propositions; verbal comments or physical actions of a sexual nature; sexually degrading words used to describe an individual; a display of sexually suggestive objects or pictures; sexually explicit jokes; unnecessary touching.

Attendees or participants who are asked to stop engaging in harassing behavior are expected to comply immediately. Anyone who feels harassed is encouraged to immediately inform the alleged harasser that the behavior is unwelcome. In many instances, the person is unaware that their conduct is offensive and when so advised can easily and willingly correct the conduct so that it does not reoccur. Anyone who feels harassed is NOT REQUIRED to address the person believed guilty of inappropriate treatment. If the informal discussion with the alleged harasser is unsuccessful in remedying the problem or if the complainant does not feel comfortable with such an approach, they can report the behavior as detailed below.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Reporting a Violation
Violations of this Conduct Policy should be reported immediately. If you feel physically unsafe or believe a crime has been committed, you should report it to the police immediately.

To report a violation to BPS:

- You may do so in person at the Annual Meeting at the BPS Business Office in the convention center.

- You may do so in person to BPS senior staff at Thematic Meetings, BPS Conferences, or other BPS events.

- At any time (during or after an event), you can make a report through http://biophysics.ethicspoint.com or via a dedicated hotline (phone numbers listed on the website) which will collect and relay information in a secure and sensitive manner.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated per the procedure detailed below. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Investigative Procedure

All reports of harassment or sexual harassment will be treated seriously. However, absolute confidentiality cannot be promised nor can it be assured. BPS will conduct an investigation of any complaint of harassment or sexual harassment, which may require limited disclosure of pertinent information to certain parties, including the alleged harasser.

Once a complaint of harassment or sexual harassment is received, BPS will begin a prompt and thorough investigation. Please note, if a complaint is filed anonymously, BPS may be severely limited in our ability to follow-up on the allegation.

- An impartial investigative committee, consisting of the current President, President-Elect, and Executive Officer will be established. If any of these individuals were to be named in an allegation, they would be excluded from the committee.

- The committee will interview the complainant and review the written complaint. If no written complaint exists, one will be requested.

- The committee will speak to the alleged offender and present the complaint.

- The alleged offender will be given the opportunity to address the complaint, with sufficient time to respond to the evidence and bring his/her own evidence.

- If the facts are in dispute, the investigative team may need to interview anyone named as witnesses.

- The investigative committee may seek BPS Counsel's advice.

- Once the investigation is complete, the committee will report their findings and make recommendations to the Society Officers.

- If the severity of the allegation is high, is a possible repeat offense, or is determined to be beyond BPS's capacity to assess claims and views on either side, BPS may refer the case to the alleged offender's home institution (Office of Research Integrity of similar), employer, licensing board, or law enforcement for their investigation and decision.

Disciplinary Actions

Individuals engaging in behavior prohibited by this policy as well as those making allegations of harassment in bad faith will be subject to disciplinary action. Such actions range from a written warning to ejection from the meeting or activity in question without refund of registration fees, being banned from participating in future Society meetings or Society-sponsored activities, being expelled from membership in the Society, and reporting the behavior to their employer or calling the authorities. In the event that the individual is dissatisfied with the results of the investigation, they may appeal to the President of the Society. Any questions regarding this policy should be directed to the BPS Executive Officer or other Society Officer.

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GENERAL INFORMATION

Registration/Information Location and Hours

On Sunday, Monday, Tuesday, Wednesday, and Thursday, registration will be in the Exhibition Area on Level 3 of the Karolinska Institute, Biomedicum. Registration hours are as follows:

Sunday, August 14	17:00 – 19:30
Monday, August 15	08:00 – 17:00
Tuesday, August 16	12:30 – 17:00
Wednesday, August 17	08:00 – 17:00
Thursday, August 18	08:00 – 12:15

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in the Eva & Georg Klein Lecture Hall. Speakers are required to bring their own laptops and adaptors. It is recommended to have a backup of the presentation on a USB drive in case of any unforeseen circumstances. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) All poster sessions will be held in the Exhibition Area on Level 3 of the Karolinska Institute, Biomedicum.

2) A display board measuring 120 cm wide x 150 cm high - Portrait Style (approximately 3.9 feet wide x 4.9 feet high) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as listed in the e-book.

3) Posters should be set up on the morning of Monday, August 15 and removed by noon Thursday, August 18. All posters are available for viewing during all poster sessions; however, there will be formal poster presentations at the following times:

Monday, August 15	14:30 – 15:15	Odd-numbered poster boards
Monday, August 15	15:15 – 16:00	Even-numbered poster boards
Tuesday, August 16	14:30 – 15:15	Odd-numbered poster boards
Tuesday, August 16	15:15 – 16:00	Even-numbered poster boards
Wednesday, August 17	14:30 – 15:15	Odd-numbered poster boards
Wednesday, August 17	15:15 – 16:00	Even-numbered poster boards

4) During the assigned poster presentation sessions, presenters are requested to remain in front of their poster boards to meet with attendees.

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

Meals and Coffee Breaks

There will be a Welcome Reception on Sunday evening from 18:30 – 19:30 in the Exhibition Area on Level 3.

Coffee Breaks (Monday, Tuesday, Wednesday, and Thursday) will be served in the Exhibition Area on Level 3.

Lunches (Monday and Wednesday) will be served in the Exhibition Area on Level 3.

Smoking

Please be advised that smoking is not permitted at the Karolinska Institute, Biomedicum.

Proof of Vaccination and Masks

All participants are to have had their vaccinations verified through CrowdPass. No exemptions will be permitted. Please be prepared to show your approved vaccination QR code from CrowdPass at registration.

To promote the safety of all attendees, the Biophysical Society has a practice of requiring masks at most meetings. However, Swedish government facilities are not permitted to mandate masks. We therefore strongly encourage the use of medical face masks by all attendees to keep fellow meeting-goers safe, but are not implementing a requirement.

Name Badges

Name badges are required to enter all scientific sessions, poster sessions, and social functions. Please wear your badge throughout the meeting.

Internet

Wi-Fi will be provided at the venue. Attendees will receive information at registration.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from August 14-18 during registration hours.

In case of emergency, you may contact the following:

Dorothy Chaconas, BPS Staff
dchaconas@biophysics.org

Umi Zhou, BPS Staff
uzhou@biophysics.org

Georgios Sotiriou, Karolinska Institute
georgios.sotiriou@ki.se

Physical and Quantitative Approaches to Overcome Antibiotic Resistance

Stockholm, Sweden
August 14-18, 2022

All scientific sessions will be held at the Karolinska Institute, Biomedicum
in the Eva & Georg Klein Lecture Hall unless otherwise noted.

PROGRAM

Sunday, August 14, 2022

17:00 – 19:30	**Registration/Information**	**Level 3, Exhibition Area**
18:30 – 19:30	**Welcome Reception**	**Level 3, Exhibition Area**

Monday, August 15, 2022

8:00 – 17:00	**Registration/Information**	**Level 3, Exhibition Area**
9:00 – 9:15	Joanna Slusky, University of Kansas, USA ***Welcome and Opening Remarks***	
Session I	**Bacterial Membranes, Cell Walls, and Transport** Joanna Slusky, University of Kansas, USA, Chair	
9:15 – 9:45	Steven Boxer, Stanford University, USA * ***Protein Electric Fields Regulate Covalent Inhibition of Beta-Lactamases***	
9:45 – 10:15	Mathias Winterhalter, Jacobs University, Germany ***Quantifying the Uptake of Antibiotics into Gram-Negative Bacteria***	
10:15 – 10:45	**Coffee Break**	**Level 3, Exhibition Area**
Session II	**Bacterial Membranes, Cell Walls, and Transport** ***(Continued)*** Joanna Slusky, University of Kansas, USA, Chair	
10:45 – 11:15	Megan O'Mara, The University of Queensland, Australia ***Are Polyunsaturated Lipids an Archilles' Heel in Acinetobacter Baumannii Antimicrobial Resistance?***	
11:15 – 11:45	Kaspar Locher, ETH Zürich, Switzerland ***Structural Basis of Drug Binding to Multidrug Transporters Revealed by Cryo-Electron Microscopy***	
11:45 – 12:00	Sergei Sukharev, University of Maryland, USA * ***Teasing Half-Bilayers: Physicochemical Properties of LPS Monolayers, Electrostatic Asymmetry of LPS-Phospholipid Bilayers and Implications for Drug/Peptide Permeability***	
12:00 – 13:00	**Lunch**	**Level 3, Exhibition Area**
Session III	**Efflux and Drug Resistance** Colin Kleanthous, University of Oxford, United Kingdom, Chair	

13:00 – 13:30	Klaas Martin Pos, Goethe University Frankfurt, Germany ***Structural Insights on Drug Transport and Inhibition of Tripartite Efflux Pumps***	
13:30 – 14:00	Helen Zgurskaya, University of Oklahoma, USA ***The Two-Faced Janus of Multidrug Efflux Substrates and Inhibitors***	
14:00 – 14:15	Andrea Catte, University of Cagliari, Italy * ***Molecular Dynamics Simulations of MexB, MexF and MexY Multidrug Transporters of Pseudomonas Aeruginosa***	
14:30 – 16:00	**Poster Session I and Coffee Break**	**Level 3, Exhibition Area**
Session IV	**Efflux and Drug Resistance *(Continued)*** Colin Kleanthous, University of Oxford, United Kingdom, Chair	
16:15 – 16:30	Vasileios Petrou, Rutgers New Jersey Medical School, USA * ***Structural Basis of Lipid A Modification by the Aminoarabinose Transferase ArnT Linked to Polymyxin Resistance***	
16:30 – 17:00	Georgios Sotiriou, Karolinska Institute, Sweden ***Engineering Responsive Nanomaterials Against Infections***	

Tuesday, August 16, 2022

8:00 – 13:00	**Free Time**	
12:30 – 17:00	**Registration/Information**	**Level 3, Exhibition Area**
Session V	**Engineering Drug Resistance** Peter Kasson, University of Virginia, USA, Chair	
13:00 – 13:30	Anushree Chatterjee, University of Colorado Boulder, USA ***Fast, Smart Therapeutic Solutions for Pandemic Response***	
13:30 – 14:00	Yi Yan Yang, A*STAR, Singapore ***Macromolecule Engineering Approach to Overcoming Antimicrobial Resistance***	
14:00 – 14:15	Fredrik Westerlund, Chalmers University of Technology, Sweden * ***High-Resolution Bacterial Typing Using Optical DNA Mapping for Diagnosing Bacterial Infections***	
14:30 – 16:00	**Poster Session II and Coffee Break**	**Level 3, Exhibition Area**
Session VI	**Engineering Drug Resistance *(Continued)*** Peter Kasson, University of Virginia, USA, Chair	
16:15 – 16:45	Cindy Gunawan, University of Technology, Sydney, Australia ***The Evolution of Bacterial Adaptation Phenomena to Antimicrobial Nanoparticle***	
16:45 – 17:00	Priscilla Gomes, Auburn University, USA * ***The Role of Protein Mechanostability in Antibiotic Resistance***	

Wednesday, August 17, 2022

8:00 – 17:00	**Registration/Information**	**Level 3, Exhibition Area**

Session VII	**Bacterial Communities and Resistance** Georgios Sotiriou, Karolinska Institute, Sweden, Chair
9:00 – 9:30	Peter Kasson, University of Virginia, USA ***Improving Small-Molecule Uptake Using Simulations and Data***
9:30 – 10:00	Sada Boyd, University of California, Los Angeles ***Evaluating the Interaction Between Copper Resistant and Antibiotic Resistant E. Coli***
10:00 – 10:15	Daniel Charlebois, University of Alberta, Canada * ***Nongenetic Resistance Enhances Population Survival While Hindering the Evolution of Drug Resistance***
10:15 – 10:45	**Coffee Break** **Level 3, Exhibition Area**
Session VIII	**Bacterial Communities and Resistance *(Continued)*** Birgitta Henriques-Normark, Karolinska Institute, Sweden, Chair
10:45 – 11:15	Roy Kishony, Technion - Israel Institute of Technology, Israel ***Predicting and Inverting Antibiotic Resistance***
11:15 – 11:45	Kevin Wood, University of Michigan, USA ***Steering Bacterial Pathogens Through the Phenotype Space of Multidrug Resistance***
11:45 – 13:00	**Lunch** **Level 3, Exhibition Area**
Session IX	**Antimicrobial Targets** Helen Zgurskaya, University of Oklahoma, USA, Chair
13:00 – 13:30	Joanna Slusky, University of Kansas, USA ***Plugging TolC Antibiotic Efflux***
13:30 – 13:45	Gnana Gnanakaran, Los Alamos National Laboratory, USA * ***Predicting Permeation of Compounds Across the Outer Membrane of Pseudomonas Aeruginosa***
13:45 – 14:15	Birgitta Henriques-Normark, Karolinska Institute, Sweden ***Pneumococcal Interactions with the Host as a Target for Therapy***
14:30 – 16:00	**Poster Session III and Coffee Break** **Level 3, Exhibition Area**
Session X	**Antimicrobial Targets *(Continued)*** Helen Zgurskaya, University of Oklahoma, USA, Chair
16:15 – 16:45	Alejandro Vila, CONICET-Instituto de Biología Molecular y Celular de Rosario, Argentina ***The Adaptive Success of New Delhi Metallo-Beta-Lactamase Depends on the In-Cell Kinetic Protein Stability***
16:45 – 17:00	Adéla Melcrová, University of Groningen, The Netherlands* ***Lateral Organization of Bacterial Membranes as Antimicrobial Target***

Thursday, August 18, 2022

8:00 – 12:15	**Information**	**Level 3, Exhibition Area**
Session XI	**Membranes, Permeation, and More!** Georgios Sotiriou, Karolinska Institute, Sweden, Chair	
9:00 – 9:30	Pierre Santucci, The French National Centre for Scientific Research (CNRS), France ***Intracellular Localisation of Mycobacterium Tuberculosis Affects Efficacy of the Antibiotic Pyrazinamide***	
9:30 – 10:00	Michaela Wenzel, Chalmers University of Technology, Sweden ***Fluorescence Live Cell Imaging Approaches to Studying Antibiotic Mechanisms of Action and Resistance***	
10:00 – 10:15	Georgina Plant, University of Bristol, United Kingdom * ***Investigating the Use of Sub-Cellular Fluctuation Imaging with Neisseria Gonorrhoeae***	
10:15 – 10:45	**Coffee Break**	**Level 3, Exhibition Area**
Session XII	**Membranes, Permeation, and More!** ***(Continued)*** Georgios Sotiriou, Karolinska Institute, Sweden, Chair	
10:45 – 11:15	Colin Kleanthous, University of Oxford, United Kingdom ***Principles of Bacterial Outer Membrane Organization***	
11:15 – 11:45	James C. Gumbart, Georgia Institute of Technology, USA * ***Modeling the Assembly of the AcrAB-TolC Multidrug Efflux Pump***	
11:45 – 12:00	Nandan Haloi, University of Illinois Urbana-Champaign, USA * ***Investigating Molecular Mechanisms of Antibiotic Permeation Through Outer Membrane Porins in High Dimensional Conformational Space***	
12:00 – 12:15	Peter Kasson, University of Virginia, USA Georgios Sotiriou, Karolinska Institute, Sweden **Closing Remarks and *Biophysical Journal* Poster Awards**	

**Contributed talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

PROTEIN ELECTRIC FIELDS REGULATE COVALENT INHIBITION OF BETA-LACTAMASES

Steven G. Boxer; Zhe Ji[1];
[1]Stanford University, Chemistry, Stanford, CA, USA

Beta-lactamases can use their protein machinery to hydrolyze some beta-lactam antibiotics rapidly, yet are less proficient towards other substrates, and are even trapped by efficient inhibitors. We sought to understand how covalent inhibitors function by studying the physical basis for their differences in reactivity from substrates, using TEM-1 as a model beta-lactamase. While penicillin G, a b-lactam substrate, is subject to a two-step hydrolysis mechanism, enzyme acylation and hydrolytic deacylation, avibactam as a covalent inhibitor can perform rapid acylation but sluggish deacylation, trapping many b-lactamase targets in the inactive acyl-enzyme state. We examine the different reactivities of penicillin G and avibactam under the framework of electrostatic catalysis. Electric fields projected onto a bond involving charge displacement can stabilize its transition state and therefore enhance the rate. Using the vibrational Stark effect to quantify the magnitude of electric fields, we observed that C=O in avibactam, the key bond undergoing reactions, experiences high electric fields as that in penicillin G does in the Michaelis complex (see Kozuch poster), but contrastingly lower fields in the acyl-enzyme, consistent with the observation of fast acylation and slow deacylation. These electric fields are mainly exerted by hydrogen bonds between the avibactam C=O and protein backbone amides. By replacing a backbone amide with an ester using amber suppression, we quantified the role of the hydrogen bond in exerting electric fields and accelerating reactions. Compared with penicillin G, avibactam's C=O experiences a lower electric field by 67 MV/cm when passaging towards deacylation, leading to 10^6-fold rate diminution. Our studies provide physical insights into the long residence time of covalent inhibitors—electrostatic stabilization can contribute more than intrinsic bond stability. We envision that active site electric fields can act as a general, quantitative descriptor to guide the design of covalent drugs.

QUANTIFYING THE UPTAKE OF ANTIBIOTICS INTO GRAM-NEGATIVE BACTERIA

Mathias Winterhalter
Jacobs University, Germany

No Abstract

ARE POLYUNSATURATED LIPIDS AN ARCHILLES' HEEL IN ACINETOBACTER BAUMANNII ANTIMICROBIAL RESISTANCE?

Megan L O'Mara[1,3]; Hugo I MacDermott-Opeskin[1]; Bart A Eijkelkamp[2]; Katie A Wilson[1];
[1]Australian National University, Research School of Chemistry, Canberra, Australia
[2]Flinders University, Molecular Sciences and Technology, Adelaide, Australia
[3]The University of Queensland, Australian Institute for Bioengineering and Nanotechnology, Brisbane, Australia

Gram-negative bacteria such as Acinetobacter baumannii sequester host lipids from the site of infection for incorporation into lipid synthesis pathways, altering the membrane lipidome. Changes in membrane composition from the incorporation of host-derived polyunsaturated fatty acids (PUFAs) help restore sensitivity to antimicrobials in several species of Gram-negative bacteria. Using coarse-grained simulations based on lipidomic data of A. baumannii inner membrane collected under three different growth conditions, we show PUFA-incorporation alters membrane biophysical properties, increasing the phase separation between ordered and disordered lipid domains resulting in thinner, less ordered membranes. We show that the changes in A. baumannii membrane biophysical properties on the incorporation of PUFA-containing lipids alters the conformational cycling of RND multidrug efflux pumps and restores sensitivity to some antimicrobials. Finally, we examine the interaction of antimicrobial peptides (AMP) with the simulated A. baumannii membranes to identify the effect of lipid saturation and alterations in membrane properties has on AMP-induced membrane disruption.

STRUCTURAL BASIS OF DRUG BINDING TO MULTIDRUG TRANSPORTERS REVEALED BY CRYO-ELECTRON MICROSCOPY

Kaspar Locher[1];
[1]ETH Zurich, Institute of Molecular Biology and Biophysics, Zurich, Switzerland

Multidrug transporters recognise and extrude toxic compounds from cells. The accepted substrates are generally hydrophobic but also include compounds that contain charged groups. To understand the interaction of drugs with these transporters, high resolution structural insight is required. In the past, X-ray crystallography was primarily used to determine structures of multidrug transporters. Unfortunately, the high detergent concentrations during crystallisation experiments often prevented the visualisation of bound drugs, in particular because the affinity of the substrates to the transporters are not high. Using single particle cryo-electron microscopy, we have determined several structures of bacterial and eukaryotic multidrug transporters. Because the studies were performed using nanodisc-reconstituted rather than detergent-solubilised protein, we could visualise bound drugs. For multidrug ABC transporters, we found that a single substrate molecule is generally bound at a central binding pocket. In contrast, several inhibitors bound in pairs. These studies provide key insight to understand the broad specificity of multidrug transporters, which may provide avenues to explore novel antibiotic approaches.

TEASING HALF-BILAYERS: PHYSICOCHEMICAL PROPERTIES OF LPS MONOLAYERS, ELECTROSTATIC ASYMMETRY OF LPS-PHOSPHOLIPID BILAYERS AND IMPLICATIONS FOR DRUG/PEPTIDE PERMEABILITY

Sergei Sukharev[1]; Hannah Cetuk[1]; Jake Rosetto[1]; Joseph Najem[4]; Alison J Scott[2]; Myriam L Cotten[3]; Robert K Ernst[2];
[1]University of Maryland, Biology, College Park, MD, USA
[2]University of Maryland, School of Dentistry, Baltimore, MD, USA
[3]William and Mary, Applied Science, Williamsburg, VA, USA
[4]The Pennsylvania State University, Mechanical Engineering, University Park, PA, USA

The outer bacterial membrane and its leaflet asymmetry were critical evolutionary inventions that allowed Gram-negative bacteria to occupy multiple ecological niches. The stratified and cross-linked structure of the outer lipopolysaccharide (LPS) leaflet functions as the major impediment for penetration of foreign substances. Yet, many drugs are characterized by a direct permeation mechanism through the LPS layer, for which partitioning/intercalation between LPS molecules is the obligatory first step. In this presentation, we focus on two major parameters of LPS layers that may affect drug intercalation: (1) the lateral compressibility and its determinants, and (2) the electrostatic potential of the LPS layer including its dominant dipole component. We analyze monolayer compression isotherms and report the molecular areas and compressibility moduli for Lipid A, Re-, Rd- and Rc-LPS isolated from E. coli, which indicate a strong influence of the carbohydrate chain length on monolayer mechanics. We report differential effects of Ca2+ and Mg2+ on the character of monolayer compression. Under high ionic strength, the surface potential of Rc-LPS was ~100 mV lower than that of E. coli phospholipids, indicating the difference in the interfacial dipole. We developed a technique allowing to reliably form completely asymmetric LPS/phospholipid droplet interface bilayers (DIBs). Consistently, the electrostatic potential difference between the LPS and phospholipid sides of asymmetric DIB was ~-110 mV, suggesting a constant electrostatic bias inside the outer bacterial membrane. Finally, we report a survey of affinities for LPS and molecular intercalation areas for seven classes of antibiotics and one class of antimicrobial peptides (Piscidins). The data reveal the stronger preference of Piscidins toward LPS as opposed to mammalian/vertebrate cell surfaces as the reason for their specificity against gram-negative microorganisms.

STRUCTURAL INSIGHTS ON DRUG TRANSPORT AND INHIBITION OF TRIPARTITE EFFLUX PUMPS

Klaas Martin Pos[1];
[1]Goethe University Frankfurt, Institute of Biochemistry, Frankfurt am Main, Germany

Tripartite efflux pumps in Gram-negative bacteria play a prominent role in the resistance against multiple antibiotics. These efflux systems comprise an inner membrane transporter as a substrate binding/transport and energy-coupling determinant, a periplasmic adaptor protein, and an outer membrane channel. Transport of drugs across the outer membrane and its coupling to the electrochemical gradient across the inner membrane is dependent on the presence of all three protein components. In Escherichia coli, the inner membrane transporter AcrB of the AcrAB-TolC tripartite efflux pump recognizes and transports a wide selection of toxic compounds including bile salts, organic solvents, detergents, dyes, and multiple antibiotics. Using structural analysis and other biophysical/biochemical methods, we obtained insight into the molecular basis of this substrate promiscuity and the distinct transport pathways of drugs through AcrB. Moreover, functional and structural analysis on two classes of effective efflux pump inhibitors revealed their discrete binding sites within AcrB, rendering the AcrAB-TolC efflux machinery inactive.

THE TWO-FACED JANUS OF MULTIDRUG EFFLUX SUBSTRATES AND INHIBITORS

Helen I Zgurskaya[1];
[1]University of Oklahoma, Chemistry and Biochemistry, Norman, OK, USA

Antibiotics are miracle drugs that can cure infectious bacterial diseases. However, their utility is challenged by antibiotic resistant bacteria emerging in clinics. Such bacteria as Gram-negative and Mycobacteriales species are intrinsically resistant to most clinical antibiotics and can further gain multidrug resistance through mutations and plasmid acquisition. These species stand out by the presence of additional external outer membranes (OM). Although formidable, the OM is a passive permeability barrier that can reduce penetration of antibiotics but cannot affect intracellular steady-state concentrations of drugs. The two-membrane envelopes are further reinforced by active efflux transporters that expel antibiotics from cells against their concentration gradients. Active efflux of drugs is the major mechanism of antibiotic resistance in bacterial pathogens that act synergistically with the low permeability barrier of the OM. In this presentation, we summarize the progress in understanding the mechanism of efflux pumps from Resistance-Nodulation-Division (RND) superfamily and their kinetic advantages from synergistic relationships with passive permeation barriers. The ability to transfer various substrates across the OM at the expense of the proton-motive force acting on the inner membrane and engagement of accessory proteins for their functions are the major mechanistic advantages of these pumps. Both the RND transporters and their accessory proteins are targeted in the discovery of efflux pump inhibitors that in combinations with antibiotics potentiate antibacterial activities. We discuss intriguing relationships between substrates and inhibitors of efflux pumps, as both types of ligands face similar barriers and binding sites in the transporters and accessory proteins and both types of activities are often present in the same chemical scaffold. Recent mechanistic insights, both empirical and computational, led to identification of features that distinguish efflux pump inhibitors from the substrates. These findings suggest the path for optimization of efflux inhibitory activities in antibiotics and other chemically diverse compounds.

MOLECULAR DYNAMICS SIMULATIONS OF MEXB, MEXF AND MEXY MULTIDRUG TRANSPORTERS OF PSEUDOMONAS AERUGINOSA

Andrea Catte[1]; Venkata K Ramaswamy[1]; Attilio V Vargiu[1]; Giuliano Malloci[1]; Paolo Ruggerone[1];
[1]University of Cagliari, Department of Physics, Monserrato (Cagliari), Italy

The secondary multidrug transporters of the resistance-nodulation-cell division (RND) superfamily mediate multi-drug resistance in Gram-negative bacteria like Pseudomonas aeruginosa. This pathogen expresses four main polyspecific RND transporters, namely MexB, MexD, MexF and MexY, with partly overlapping specificities. However, only the structure of the former has been resolved experimentally to date. The lack of data about the structure and the dynamics of most transporters has limited a systematic investigation of the molecular determinants defining their activities. In a previous work [Ramaswamy et al, Front. Microbiol. 9:1144 (2018)], we employed computational methods to compare the main putative recognition sites (named access and deep binding pockets, AP and DP respectively) in MexB and MexY. In this work, we expand the comparison by performing extended molecular dynamics simulations of MexB, MexY and MexF embedded in a more realistic model of the inner phospholipid membrane of P. aeruginosa, using updated force-fields and newly developed protocols. Moreover, to elucidate how the structures and the dynamics of these transporters define their substrate specificity, we conduct a comparative dynamic fragment-based mapping on these three proteins. In addition to analyzing the binding of probes on access and distal/deep binding pockets, we investigated for the first time the accumulation of fragments at various entrance and exit channels of each protein. Our results highlight similarity and differences in the distribution of multi-functional sites in the AP and DP of the three transporters binding sites. Moreover, our findings pinpoint a peculiar behavior of MexF vs. MexB/Y regarding the features of the entrance gates of the periplasmic and transmembrane channels. Altogether, our results allow to rationalize the partial redundancy and the specificities of the substrate profiles of the three Mex transporters.

STRUCTURAL BASIS OF LIPID A MODIFICATION BY THE AMINOARABINOSE TRANSFERASE ARNT LINKED TO POLYMYXIN RESISTANCE

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Lipid A, the major lipidic component of the lipopolysaccharide (LPS) decorating the outer membrane of Gram-negative (GN) bacteria, can be modified by addition of diverse chemical moieties. Such modifications lead to altered host recognition, evasion of host defenses, and resistance to antimicrobial agents. Modification of the phosphates of Lipid A with the aminoarabinose moiety 4-amino-4-deoxy-L-arabinose (L-Ara4N) leads to charge modification of the outer membrane and is responsible for bestowing resistance against natural cationic antimicrobial peptides (CAMPs) and polymyxin-class antibiotics to GN bacteria. Polymyxins are cationic peptides that associate with the outer bacterial membrane through electrostatic interactions with the phosphate groups of Lipid A. They are currently used as last resort antibiotics, either as monotherapies or in combination with other antibiotics, against multidrug resistant (MDR) GN bacteria. The enzymatic transfer of L-Ara4N to Lipid A to "cap" its phosphate groups is catalyzed by ArnT, an inner membrane lipid-to-lipid glycosyltransferase, and is the major contributor for development of polymyxin resistance in Escherichia coli and Salmonella enterica. Using single particle cryo-electron microscopy (cryoEM) we have determined the structure of ArnT from S. enterica in two states: i) bound to both the acceptor ligand Lipid A and the donor undecaprenyl phosphate (UndP), and ii) only bound to Lipid A, after mutating one of the coordinating residues for UndP. These structures are the first to capture the Lipid A-bound state of ArnT. They allow us to fully characterize substrate binding in the glycosyltransferase ArnT, and to accurately localize the active site of the enzyme. By comparing these structures to existing structures of ArnT from C. metallidurans, we provide further insights towards understanding the structural basis of catalysis and the substrate binding cycle of the glycosyltransferase ArnT.

ENGINEERING RESPONSIVE NANOMATERIALS AGAINST INFECTIONS

Georgios Sotiriou
Karolinska Institute, Sweden

No Abstract

FAST, SMART THERAPEUTIC SOLUTIONS FOR PANDEMIC RESPONSE

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The rapid rise of multidrug-resistant (MDR) superbugs and novel strains of viruses and the declining antibiotic and antiviral pipeline are serious challenges to global health. Rational design of therapeutics can accelerate development of effective therapies against infectious pathogens and dampen the impact of pandemics. In this talk, I will describe multi-pronged systems, synthetic biology, and nano-biotechnology based approaches being devised in our lab to rationally engineer therapeutics that can overcome antimicrobial resistance in MDR bacteria as well as respond to SARS-CoV2 virus by developing antivirals in real-time. We have engineered antisense therapeutics that inhibit desired genes in a species-specific manner for targeted inhibition. Using this approach, we have built a platform that can accelerate therapeutic development in less than a week. We have shown that we can create novel antibiotics that can kill a range of WHO (World Health Organization) top priority I MDR pathogens, as well as reduce infection from SARS-COV2. I will also present a nano-biotechnology based approach involving development of a unique semiconductor material-based quantum dot-antibiotic (QD ABx) which, when activated by stimuli, release reactive oxygen species to eliminate a broad range of MDR bacterial clinical isolates. We have validated both these platforms in number of pre-clinical studies and continue to advance these therapeutic modalities further. The platforms and inter-disciplinary approaches presented in this talk offer novel methods for rationally engineering new therapeutics to combat disease challenges.

MACROMOLECULE ENGINEERING APPROACH TO OVERCOMING ANTIMICROBIAL RESISTANCE

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With the increased prevalence of antimicrobial resistance, there is an urgent need for development of innovative antimicrobial therapeutics. In this talk, biodegradable antimicrobial polymers, which are based on biodegradable guanidinium-functionalized polycarbonates or polypeptides, will be discussed. These polymers were synthesized via organocatalytic living ring-opening polymerization. This synthetic platform yields polymers/polypeptides with well-defined molecular weight and structure, which allows for study of structure-activity relationship. We used confocal microscopy, SEM, TEM and bacterial RNA-Seq to study antimicrobial mechanism of the polymers. Unlike quaternary ammonium- or primary amine-functionalized polymers that killed bacteria via a membrane-disruption mechanism, the guanidinium-functionalized polymers killed bacteria via membrane translocation followed by precipitation of intracellular proteins and genes. Bacterial RNA-Seq was also performed to study drug resistance development after repeated use of the polymers in comparison with small molecular antibiotics. Unlike antibiotics, multiple treatments using these polymers do not cause resistance. The synthetic macromolecules were engineered to fine tune hydrophobicity, hydrophilicity and structure for optimal antimicrobial activity and toxicity mitigation. The macromolecules with optimal compositions have strong activity against multidrug-resistant (MDR) bacteria without inducing significant toxicity. The optimized macromolecules demonstrated efficacy in an MRSA-infected skin wound infection mouse model. These macromolecular therapeutics hold potential for use in the treatment of MDR infection.

HIGH-RESOLUTION BACTERIAL TYPING USING OPTICAL DNA MAPPING FOR DIAGNOSING BACTERIAL INFECTIONS

My Nyblom[1]; Anna Johnning[2]; Karolin Frykholm[1]; Zahra Abbaspour[1]; Marie Wrande[3]; Albertas Dvirnas[4]; Tobias Ambjörnsson[4]; Christian Giske[5]; Linus Sandegren[3]; Erik Kristiansson[2]; **Fredrik Westerlund**[1];
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[5]Karolinska Institute, Laboratory Medicine, Stockholm, Sweden

High-resolution identification of bacteria is important for diagnosing bacterial infections, but can be challenging with existing methods. We use Optical DNA Mapping (ODM) to identify bacteria in clinical isolates with sub-species resolution. We have previously demonstrated that ODM can be used to accurately identify bacteria in a sample on the species level [1]. We here demonstrate that the method is applicable for high-resolution typing on the sub-species level for E. coli, K. pneumoniae and S. pyogenes. Pathogenic bacteria from clinical samples are enclosed in agarose plugs to keep DNA molecules intact during the cell lysis. Large (>100 kb) DNA molecules are extracted and a single step competitive binding-based labelling is applied to create a sequence specific emission intensity profile along the DNA. To record the intensity profile, the DNA is stretched in nanofluidic channels and imaged using fluorescence microscopy. Experimental intensity profiles are compared to a reference database, and bacteria present in the sample are identified based on discriminatively matching profiles. Bioinformatics tools are used to create a phylogenetic tree, based on which typing with taxonomic resolution higher than species can be performed. For E. coli we have a true positive rate close to 100 % at a taxonomic resolution where the E. coli species is divided into 89 different groups. This means that we can efficiently identify particularly pathogenic E. coli, such as the endemic ST131. Similar performance was observed also for K. pneumoniae and S. pyogenes. The method can also efficiently identify and characterize bacteria. By including a Cas9 restriction step, the plasmid carrying a specific (resistance) gene can be identified. Since the method is applicable directly to clinical samples, such as urine, and is very efficient in identifying bacteria in complex mixtures, we foresee that it can be an important future diagnostic tool.

THE EVOLUTION OF BACTERIAL ADAPTATION PHENOMENA TO ANTIMICROBIAL NANOPARTICLE

Cindy Gunawan[1]; Elizabeth Valentin[1]; Riti Mann[1]; Oliver McNeilly[1]; Georgios Sotiriou[2]; Mehrad Hamidian[1]; Scott A Rice[3];
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[2]Karolinska Institutet, Department of Microbiology, Tumor and Cell Biology, Stockholm, Sweden
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Silver nanoparticle (NAg) with its broad spectrum antimicrobial efficacy has been used as alternative technologies to control pathogenic growth. NAg has been used in medical devices to fight infections, however, the nanoparticle has also been incorporated in arrays of consumer products, often without clear antimicrobial targets. This widespread use of NAg has caused concerns, as whether, just like in the case of antibiotics, bacteria will develop resistance to the important antimicrobial.Methods. Bacterial pathogens, in their free-living and biofilm forms of growth, were subjected to long-term exposures (30-50 days) to increasing NAg concentrations. The development of adaptation phenotypes were determined by assessing the changes in the nanoparticle minimum inhibition concentration (MIC) for resistance trait, as well as in the minimum duration for killing (MDK) 99% and 99.99% of the cell population for tolerance and persistence trait, respectively. Molecular basis studies (gene mutations, RNAseq, metabolomics) were carried out for insights into the adaptation mechanisms. Our study found that bacteria has the natural ability to adapt to the complex toxicity mechanisms of NAg. The nanoparticle targets multiple cellular components through the activity of the leached soluble silver and the solid silver particulates. Gram-positive and Gram-negative bacteria can develop stable resistance traits to NAg as a result of prolonged exposures, and grow in an otherwise toxic concentrations of the nanoparticle. The team found that bacteria modify their physiological growth behaviour and stress responses, which are linked to mutations in their genomes. The mutations are indicated to alter the expression levels of specific genes, affecting the cellular defence pathways. The discoveries present the need to elucidate and target the cellular signalling mechanisms that trigger the defence pathways, ultimately overcoming the adaptation phenomena. With no development of new antibiotics over the last 30 years, we need to preserve the efficacies of existing antimicrobials.

THE ROLE OF PROTEIN MECHANOSTABILITY IN ANTIBIOTIC RESISTANCE

Priscila SFC Gomes[1]; Diego Enry B Gomes[1]; Lukas F Milles[2,3]; Hermann E Gaub[3]; Rafael C Bernardi[1];
[1]Auburn University, Department of Physics, Auburn, AL, USA
[2]University of Washington, Institute for Protein Design, Seattle, WA, USA
[3]Ludwig-Maximilians-University, Lehrstuhl für Angewandte Physik and Center for Nanoscience, Munich, Germany

Gram-positive pathogenic bacteria have an arsenal of virulence factors to target and adhere to their host. Among these virulence factors, adhesins play critical roles during infection participating actively on the formation of biofilm. The extreme mechanostability of the interaction between pathogenic adhesins and proteins of the human extracellular matrix have been shown to pose a major challenge to traditional drug-development routes. Here, we show that adhesins from methicillin resistant Staphylococcus aureus (S. aureus) strains (MRSA) are more resilient to shear forces than those of methicillin susceptible strains (MSSA). Combining a myriad of state-of-the-art computational biology approaches we show that, although methicillin does not act on the adhesins, the MRSA strains have mutations on these proteins that give them extreme mechanostability. In fact, the complex formed between adhesins and proteins of the human extracellular matrix are the strongest protein interactions known, surpassing by an order of magnitude the strength of streptavidin-biotin. To discover that, we employed bioinformatic tools to retrieve and align nearly 200 proteins of the bacterial adhesin superfamily. Using AI-based protein structure prediction, we modelled adhesins of interest from MSSA and MRSA strains, together with their human target. Using NAMD, steered molecular dynamics (SMD) simulations were performed using a wide-sampling paradigm. This protocol allowed us to investigate how adhesins can sense forces and become activated to resist high shear hydrodynamic force loads found during host infection. For representative strains, experimental validation was given by single molecule force spectroscopy experiments. In summary, the extreme mechanostability of all strains with a pattern of higher forces for the MRSA strains was observed. With increasing prevalence of multidrug resistant bacterial infections, this new finding could be exploited for the development of antiadhesion strategies as an innovative alternative to antibiotics.

IMPROVING SMALL-MOLECULE UPTAKE USING SIMULATIONS AND DATA

Peter Kasson
University of Virginia, USA

No Abstract

EVALUATING THE INTERACTION BETWEEN COPPER RESISTANT AND ANTIBIOTIC RESISTANT E. COLI

Sada Boyd
University of California, Los Angeles

No Abstract

NONGENETIC RESISTANCE ENHANCES POPULATION SURVIVAL WHILE HINDERING THE EVOLUTION OF DRUG RESISTANCE

Joshua Guthrie[1]; Harold Flohr[1]; **Daniel Charlebois**[1,2];
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Rising rates of resistance to antimicrobial drugs threatens the effective treatment of infections across the globe. Drug resistance has been established to emerge from nongenetic mechanisms, such as "persistence" in quiescent microbes and fluctuations or “noise” in gene expression in actively replicating cells, as well as from genetic mutations. However, it is still unclear how nongenetic drug resistance affects the evolution of genetic drug resistance. Using deterministic and stochastic population models that incorporate nongenetic and genetic forms of drug resistance, as well as resource competition between these subpopulations, we find that nongenetic resistance can enhance survival while at the same time hinder the evolution of genetic resistance. Nongenetic resistance in the presence of subpopulation competition is found to increase the fixation time of drug resistance mutations, while increasing the probability of mutation before population extinction during drug exposure. Intense intraspecific competition during drug treatment leads to extinction of susceptible and nongenetically resistant subpopulations. We are presently experimentally investigating these findings using genetically engineered budding yeast (Saccharomyces cerevisiae) that carry synthetic gene circuits to control drug resistance genes. These well-characterized synthetic gene circuits enable the precise control of gene expression mean and noise levels during drug resistance evolution experiments. Overall, these findings are advancing our understanding of antimicrobial resistance and leading to new therapeutic strategies to improve the outcome for patients with drug-resistant infections.

PREDICTING AND INVERTING ANTIBIOTIC RESISTANCE

Roy Kishony;
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Antibiotic resistance is growing as a major public health concern. Predicting antibiotic resistance and the evolutionary paths leading to resistance is key for our ability to control the spread of drug resistant pathogens. I will describe a series of experimental-computational methodologies for following and identifying recurrent patterns in the evolution of antibiotic resistance in the lab and in the clinic. Combined with machine-learning approaches applied to electronic patient records, these tools can lead to predictive diagnostics of antibiotic resistance and personalized treatments of microbial infections.

STEERING BACTERIAL PATHOGENS THROUGH THE PHENOTYPE SPACE OF MULTIDRUG RESISTANCE

Kevin Wood
University of Michigan, USA

No Abstract

PLUGGING TOLC ANTIBIOTIC EFFLUX

Joanna Slusky,
University of Kansas, USA

No Abstract

PREDICTING PERMEATION OF COMPOUNDS ACROSS THE OUTER MEMBRANE OF PSEUDOMONAS AERUGINOSA

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One of the major obstacles in the antibiotic discovery pipeline is the lack of understanding on how to breach antibiotic permeability barriers of Gram-negative pathogens. We have combined mechanistic and machine learning approaches to predict outer membrane (OM) permeation in P. aeruginosa. In the first study, we rationally identify a "chemical vocabulary" specifically related to OM permeability without employing known rules. Specifically, we compute a fragment-based representation of compounds and use a combination of sparse regression and a hierarchical cleansing proceed to select a subset of relevant fragments, which are responsible for OM permeation. By synergizing theory, computation, and experiment, we are able to validate our predictions and to explain the molecular mechanism behind identified fragments promoting compound entry and select candidate compounds from an external library that permeate across OM. In the second study, we generate predictive models that identify specific molecular descriptors that can predict the likelihood that a given compound is capable of OM permeation. While part of these descriptors is computed using traditional approaches based on the physicochemical properties intrinsic to the compounds, all-atom molecular dynamics simulations are used to derive additional bacterium-specific biophysical descriptors. Specifically, properties related to enthalpy, entropy and diffusion are calculated in different subregions of the OM model of P. aeruginosa. A statistical analysis based on hierarchical clustering, rank correlations, and a random forest classifier, finds a set of biophysical descriptors with prediction accuracy of 96%. Our results show the potential to predict small molecule permeation across the OM of P. aeruginosa with high precision and accuracy.

PNEUMOCOCCAL INTERACTIONS WITH THE HOST AS A TARGET FOR THERAPY

Birgitta Henriques-Normark
Karolinska Institute, Sweden

No Abstract

THE ADAPTIVE SUCCESS OF NEW DELHI METALLO-BETA-LACTAMASE DEPENDS ON THE IN-CELL KINETIC PROTEIN STABILITY

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Protein stability is essential for biological function. In contrast to the vast knowledge on the thermodynamics of protein stability in vitro, little is known about the factors governing the kinetic stability, that defines the lifetime of the native state of proteins within the cell. Here we show that the kinetic stability of the metallo-β-lactamase NDM-1 in the bacterial periplasm is optimized to face metal restriction at the host-pathogen interface. NDM-1 is one of the main responsible of providing resistance to carbapenems in pathogenic bacteria. Despite its high stability in vitro, the non-metalated (apo) NDM-1 is recognized and proteolyzed by the protease Prc due to the flexibility of its C-terminal domain. Zn(II) binding renders the protein refractory to degradation by quenching this flexibility. Apo-NDM-1 is anchored to the outer membrane, a localization that renders it less accessible to Prc and less prone to aggregate. Membrane anchoring also protects apo-NDM-1 from the quality control protease DegP, which degrades misfolded, non-metalated NDM-1 precursors. More recent clinical variants of NDM accumulate mutations at the C-terminus that quench its flexibility therefore enhancing their stability towards proteolysis. This work provides direct evidence of how the kinetic stability of a protein optimized within the bacterial cell, and links metallo-β-lactamase-mediated resistance with the cellular metabolism in the periplasm. On a broader perspective, this reveals that knowledge of the protein physiology in the cell is essential to understand protein kinetic stability.

LATERAL ORGANIZATION OF BACTERIAL MEMBRANES AS ANTIMICROBIAL TARGET

Adéla Melcrová[1]; Josef Melcr[2]; Sourav Maity[1]; Mariella Gabler[1]; Jonne van der Eyden[1]; Niels de Kok[3]; Arnold Driessen[3]; Siwert-Jan Marrink[2]; Wouter Roos[1];
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Staphylococcus aureus is one of the leading human pathogens that developed strains resistant to commonly used antibiotics. Cell wall active antimicrobial peptides and their mimics—peptidomimetics—are promising tools in the fight against these infections. Here we scrutinize the functional mechanism of the antimicrobial action of a tetrapeptide based peptidomimetic with a potential in clinical use by combining Atomic Force Microscopy (AFM), High-Speed Atomic Force Microscopy (HS-AFM) and Molecular Dynamic (MD) simulations. In particular, we study its activity on membranes extracted from S. aureus cells, and on anionic membrane models matching our lipidomic analysis of the S. aureus extracts. Our joint experimental/theoretical approach reveals a two-step mechanism of its activity. First, the peptidomimetic self-assembles into stable aggregates with high selectivity for anionic membranes such as those of S. aureus. Second, the peptidomimetic incorporates into the membrane and thereby dissolves lateral membrane domains, affecting the membrane lateral organization. These lateral domains fulfil essential functions in living cells such as protein sorting, signaling, and cell-wall synthesis. Moreover, resistance of S. aureus to penicillin, methicillin, and related drugs is caused by low affinity penicillin binding proteins (PBP2a) that oligomerize in the membrane lateral domains. Disintegration of the domains leads to deletion of the vital membrane functions, and possible renewal of susceptibility to methicillin and penicillin. Last but not least, we observe that the active mechanism of the peptidomimetic resembles activity of small disinfectants such as ethanol, towards which no bacterial resistance has ever been developed. In summary, we identify and describe a new mechanism of antimicrobial activity that (i) deletes vital functions of the bacterial membrane, (ii) can possibly be used to renew the susceptibility to already known and tested antibiotics, and (iii) has the potential of no-resistance-generation.

INTRACELLULAR LOCALISATION OF MYCOBACTERIUM TUBERCULOSIS AFFECTS EFFICACY OF THE ANTIBIOTIC PYRAZINAMIDE

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To be effective, chemotherapy against tuberculosis (TB) must kill the intracellular population of the pathogen, Mycobacterium tuberculosis. However, how host cell microenvironments affect antibiotic accumulation and efficacy remains unclear. By combining, high-content fluorescence microscopy with correlative light, electron, and ion microscopy (CLEIM), we investigate how various microenvironments within human macrophages affect the activity of pyrazinamide (PZA), a key antibiotic against TB. We show that PZA accumulates heterogeneously among individual bacteria in multiple host cell environments. We also demonstrate that correlative SEM-NanoSIMS imaging can be used to identify anti-TB drugs distribution and interaction at a subcellular resolution. Finally, by developing a dual-live imaging approach with pharmacological and genetic perturbations, we show that Mtb can maintain its intracellular pH independently of the surrounding pH in primary human macrophages. We show that unlike bedaquiline (BDQ), isoniazid (INH) or rifampicin (RIF), the front-line drug pyrazinamide (PZA) displays antibacterial efficacy by acting as protonophore which disrupts intrabacterial pH homeostasis in cellulo. By using Mtb mutants with different subcellular localisation, we confirmed that intracellular acidification is a prerequisite for PZA efficacy in cellulo. Our results may explain the potent in vivo efficacy of PZA, compared to its modest in vitro activity, and its critical contribution to TB combination chemotherapy.

FLUORESCENCE LIVE CELL IMAGING APPROACHES TO STUDYING ANTIBIOTIC MECHANISMS OF ACTION AND RESISTANCE

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In order to better guide the development of new and better antibacterial treatment options, it is pivotal to understand the fundamental mechanisms underlying both effective antibiotic activity and resistance development. In the past, the mechanisms of antibiotics have often been viewed with a strong single-target focus and, consequently, were typically investigated with specific activity assays, often in artificial in vitro systems. However, the development of new drugs based on highly specific single-target interactions has not yielded the anticipated success. Following the realization that successful antibiotics often target more than just a single target molecule, antibiotic lead development has shifted towards compounds with more complex mechanisms of action. While such candidates promise slower resistance development, they also pose new challenges for drug development as their mechanisms of action are harder to investigate and understand. This is aggravated by mounting evidence that antibiotics exhibit complex and multifaceted mechanisms when studied in living bacterial cells, which often cannot be appropriately captured in in vitro assays. In response to this challenge, we have been working on developing and compiling suitable live cell imaging and spectroscopy techniques that allow insight into such complex and multiple mechanisms of action in living bacterial cells. While we have focused on cell envelope targets, we have by now established an array of assays to assess both cell envelope-bound and intracellular processes as well as general mechanisms such as oxidative stress. Here, we present the power of these techniques for mode of action analysis of complex antibiotic mechanisms using examples of both new antibiotic candidates and established antibiotics that exhibit more complex mechanisms than previously thought.

INVESTIGATING THE USE OF SUB-CELLULAR FLUCTUATION IMAGING WITH NEISSERIA GONORRHOEAE

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The microscopy technique SCFI (Sub-Cellular Fluctuation Imaging) has been developed at the University of Bristol for use as a rapid antibiotic susceptibility test (AST). We aim to investigate how this technique can be applied to the pathogenic organism Neisseria gonorrhoeae, due to its high level of antimicrobial resistance, following a successful proof of concept with Escherichia coli. The future implementation of this technique in clinical settings would eliminate the need for overnight culture of samples, decreasing the time taken to identify effective antimicrobials. The evanescent field of a totally internally reflected laser measures internal nanoscale fluctuations of individual bacterial cells that are immobilised in a microfluidic channel. Analysis of these fluctuations indicates the growth phase of each bacterium, therefore distinguishing it as either dead or alive. The technique can also distinguish between different live growth phases, and therefore, measurements of a bacterial population result in a metabolic status for the entire sample.Results presented here show that different growth phases of N. gonorrhoeae can be identified with statistical significance, such that populations of dead (paraformaldehyde treated), stationary and exponential phase bacteria can be identified. Fluctuations observed are significantly lower than those found with samples of E. coli. We hypothesise that this disparity is due to the differing morphologies of the bacterial species, and as such the amount of biological material the laser travels through. However, studies into the effect of the orientation of the diplococcus N. gonorrhoeae with respect to the orientation of the evanescent field, indicate no statistical difference in fluctuation levels.Results of the application of antimicrobials with E. coli show that the SCFI technique can determine antibiotic killing. SCFI will be used similarly with N. gonorrhoeae with various antibiotics on resistant and susceptible separate strains.

PRINCIPLES OF BACTERIAL OUTER MEMBRANE ORGANISATION

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The asymmetric outer membrane of Gram-negative bacteria is an impermeable barrier that is a major factor in antibiotic resistance. The protection afforded by the outer membrane is necessarily compromised by the insertion of numerous b-barrel outer membrane proteins (OMPs) – ~2% of the Escherichia coli genome for example encodes OMPs - that mediate exchange of small nutrients and metabolites with the environment, import essential molecules such as vitamins and metal ions, maintain and stabilise the membrane, hydrolyse antimicrobial peptides and adhere to surfaces during biofilm formation and pathogenesis. OMPs are known to cluster into supramolecular islands that exhibit spatiotemporal organisation whereby 'old' OMPs are sequestered to the poles whereas 'new' OMPs are inserted preferentially at midcell. How OMP islands form and the basis for spatiotemporal organisation are both unknown. My talk will focus on recent work from my lab where we have uncovered principles that underpin these elements of outer membrane organisation

MODELING THE ASSEMBLY OF THE ACRAB-TOLC MULTIDRUG EFFLUX PUMP

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The multidrug efflux pumps of Gram-negative bacteria are one type of a number of complexes that span the periplasm, coupling both the inner and outer membranes to expel toxic molecules. The most well characterized example of these tripartite pumps is AcrAB-TolC complex of Escherichia coli. However, there are still many uncertainties regarding how the complex assembles. Using molecular dynamics simulations and free-energy calculations, we have elucidated multiple aspects of the assembly process. A three-dimensional potential of mean force (PMF) reveals how the conformation of the adaptor protein, AcrA, adjusts upon binding to the inner-membrane-bound AcrB. We also find that in the fully assembled pump, the peptidoglycan cell wall localizes to the interface between AcrA and the outer-membrane channel, TolC, suggesting that peptidoglycan may play a stabilizing role for AcrA during an intermediate stage of assembly. These results indicate potential features of AcrA to target for inhibition of efflux pump formation.

INVESTIGATING MOLECULAR MECHANISMS OF ANTIBIOTIC PERMEATION THROUGH OUTER MEMBRANE PORINS IN HIGH DIMENSIONAL CONFORMATIONAL SPACE

Nandan Haloi[1,5,9]; Archit K Vasan[1,5,6]; Emily Geddes[4,7,8]; Rebecca J Ulrich[4,7,8]; Arjun Prasanna[3,7]; Mary E Metcalf[3]; Po-Chao Wen[2,5,6]; William W Metcalf[3,7]; Diwakar Shukla[8,10,11]; Paul Hergenrother[4,7,8]; Emad Tajkhorshid[1,2,5];
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[8]University of Illinois Urbana-Champaign, Cancer Center at Illinois, Urbana, IL, USA
[9]KTH Royal Institute of Technology, Applied Physics, Stockholm, Sweden
[10]University of Illinois Urbana–Champaign, Department of Chemical and Biomolecular Engineering, Urbana, IL, USA
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Antibiotic resistance of Gram-negative bacteria is largely attributed to the low permeability of their outer membrane (OM). Effective antibiotics typically permeate OM porins; thus, understanding porin permeation mechanisms would aid antibiotic development. Although molecular dynamics (MD) simulations can provide key structural information on molecular processes, simulating OM porin permeation is challenging due to its high dimensionality. Since OM porins spontaneously transition between open/closed states, an understanding of (1) porin functional states regulating permeation (Vasan & Haloi et al. PNAS, 2022) and (2) their antibiotic permeation pathways is necessary (Haloi & Vasan, et al. Chemical Science, 2021). To investigate the first aspect, we sampled the conformational landscape of the apo porin using MD simulations and Markov state models. We found large-scale motion of the internal loop to opposing sides of the porin regulates transition between energetically stable open and closed states. Furthermore, mutations of key residues involved in the transition alter the dynamic equilibrium of the porin, as found in our MD simulations, and regulate antibiotic permeation inside the cell, as observed in our whole-cell accumulation assays. The identified open state was then used to investigate the high OM porin permeability of primary amine-appended antibiotics. We compared the permeation of aminated and amine-free antibiotic derivatives by incorporating MD simulation with our Monte Carlo and graph theory-based algorithm designed to improve sampling of conformationally flexible drugs. We found that the primary amine facilitates permeation by enabling the antibiotic to align its dipole to the luminal electric field of the porin and form favorable electrostatic interactions with highly-conserved charged residues of the internal loop. The importance of these interactions was validated with experimental mutagenesis

and whole-cell accumulation assays. Overall, our study demonstrating the regulation of antibiotic permeation by porin dynamics and direct antibiotic-protein interactions could help design effective antibiotics against resistant bacteria.

POSTER ABSTRACTS

Monday, August 15
POSTER SESSION I
14:30 – 16:00
Level 3, Exhibition Area

All posters are available for viewing during all poster sessions, however, below are the formal presentations for Monday. Presenting authors with odd-numbered poster boards should present from 14:30 – 15:15 and those with even-numbered poster boards should present from 15:15 – 16:00. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 14:30 – 15:15 | Even-Numbered Boards 15:15 – 16:00

Admane, Nikita	**1-POS**	**Board 1**
Cea, Pablo	**4-POS**	**Board 4**
Cory, Michael	**7-POS**	**Board 7**
Gentile, Rocco	**10-POS**	**Board 10**
Kuo, Katie	**13-POS**	**Board 13**
Manioglu, Selen	**16-POS**	**Board 16**
Pavlova, Evgeniya	**19-POS**	**Board 19**
Saral Sariyer, Aysegül	**22-POS**	**Board 22**
Shlyonsky, Vadim	**25-POS**	**Board 25**
Vikraman, Devika	**28-POS**	**Board 28**

Posters should be set up on the morning of Monday, August 15 and removed by noon on Thursday, August 18. All uncollected posters will be discarded.

1-POS Board 1

MODULATORY EFFECTS OF A QUINOLONE ALKALOID ON THE AMYLOIDOGENIC STRUCTURAL TRANSITIONS OF SMALL BASIC PROTEIN STRENGTHENING THE STAPHYLOCOCCAL BIOFILM MATRIX

Nikita Admane[1]; Sumit Biswas[1]; Ram Kothandan[2];
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[2]Kumaraguru College of Technology, Coimbatore, India

The spread of detrimental nosocomial and implant-associated infections is mainly attributed to the antibiotic-resistant bacterial biofilm formation by communities of opportunistic bacterial pathogens like Staphylococcus epidermidis. An 18-kDa small basic protein (Sbp) and its amyloid fibrils account for strengthening the biofilm architecture and scaffolding the S. epidermidis biofilm matrix, making it resistant to antibiotic treatment. Our study puts forward novel insights on the amyloidogenic structural transitions of Sbp and also reports amyloid core of the protein which may induce misfolding and aggregation. Herein, we describe the novel amyloid modulatory potential of Camptothecin (CPT), a quinoline alkaloid which binds stably to Sbp monomers. This heteromolecular association of Sbp with CPT further destabilizes the protein redirecting it towards unstructured aggregate formation. Molecular dynamics simulations reveal that Camptothecin interrupts with electrostatic interactions, averts β-sheet transitions and interrupts with the intermolecular hydrophobic associations between the exposed amyloidogenic hydrophobic cores of Sbp. Overall, this study presents the first report detailing the amyloid modulatory effects of Camptothecin which may serve as a structural scaffold for the tailored designing of novel drugs targeting the antibiotic resistant biofilm matrix of S. epidermidis. The findings of this study further warrant investigating the amyloid modulatory effects of other non-toxic CPT analogues on Sbp aggregation. This will open new avenues for the rational discovery of non- toxic therapeutic agents targeting amyloid fibrils consolidating antibiotic resistant biofilm assembly on foreign body implants.

4-POS Board 4

TOWARDS THE IDENTIFICATION OF BINDING SITES IN THE NISIN IMMUNITY COMPLEX NISFEG

Pablo A Cea[1]; Julia Gottstein[2]; Sander H.J. Smits[2]; Holger Gohlke[1,3];
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[2]Heinrich Heine University Düsseldorf, Institute of Biochemistry, Düsseldorf, Germany
[3]Forschungszentrum Jülich, John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC), Institute of Biological Information Processing (IBI-7: Structural Biochemistry) & Institute of Bio- and Geosciences (IBG-4: Bioinformatics), Jülich , Germany

Nisin is a potent peptidic antibiotic produced by Lactococcus lactis. The nisin biosynthetic operon also encodes for a set of immunity proteins that protect the producing strain against the toxic effects of its own antibiotic. One of these immunity proteins is the NisFEG complex. NisFEG is a tetrameric ABC transporter belonging to the lantibiotic transporter family LanFEG. It is constituted by the heterodimeric transmembrane region of NisE and NisG and the soluble homodimeric region formed by two NisF chains. The presence of this complex alone can confer protection against nisin, but the structural and mechanistic details of how it works remain elusive. In this work, by combining molecular modeling and molecular dynamics simulations, we aim to uncover the structural basis of nisin recognition and how it is exported in the nisin immunity complex NisFEG.Our results reveal the presence of distinctive clefts located between the interfaces of the transmembrane subunits NisE and NisG. Such clefts are also present in other ABC transporters involved in exporting hydrophobic peptides, but not in transporters mediating the transport of membrane components. To assess if the observed clefts could be involved in nisin transport, we performed co-solvent molecular dynamics simulations using multiple small-molecule probes. The probes accumulate specifically in a single cleft of the complex, which strongly suggests that it could act as a binding site for nisin. To further test the relevance of this site, we performed site-directed mutagenesis experiments targeting residues within it. Variants lacking aromatic residues in the putative binding cleft show impaired function compared to variants lacking aromatic residues in the opposite cleft. In conclusion, our results point towards an inter-protein surface cleft in NisFEG as a key region in nisin recognition.

7-POS Board 7

DEFINING THE ACTIVATING SIGNAL OF THE SOS RESPONSE – A MODULATOR OF BACTERIAL EVOLUTION.

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[2]University of Pennsylvania, Chemistry, Philadelphia, PA, USA
[3]University of Pennsylvania, Perelman School of Medicine, Infectious Diseases, Philadelphia, PA, USA

Bacteria's ability to rapidly acquire resistance-engendering mutations presents a formidable medical challenge. Understanding mechanisms that promote adaptation to antimicrobial stress could facilitate the development of strategies to preempt acquired resistance. One such pro-mutagenic bacterial pathway is the SOS response – a highly conserved genetic network that mediates DNA damage repair and tolerance. Activation of the SOS response is dependent on the interaction between the bacterial proteins RecA and LexA. RecA acts as a DNA damage sensor by forming active oligomeric filaments (RecA*) along single-stranded DNA in an ATP-dependent manner. RecA* can then interact with the repressor LexA, leading to its eventual degradation. Formation of the RecA*-LexA complex, termed the SOS signal complex, initiates downstream expression of SOS response genes. While understanding this complex is key to therapeutic intervention, the minimal determinants of SOS activation remain unknown, including the number of RecA monomers in RecA* that engage with LexA in the SOS complex and their requisite activation state. Here, we leveraged constrained RecA constructs as a tool to define the minimally sufficient RecA* filament for LexA engagement. By probing their capacity for filamentation, LexA binding, and induction of LexA degradation, we reveal unexpected insights into the SOS complex. In contrast with prevailing models, we found that two RecA units are sufficient for LexA cleavage, and that both DNA- and ATP-dependent activation of the filament is strictly required. Maximal activity is observed with four RecA units, suggesting auxiliary interactions may be optimal for LexA engagement. By introducing mutations into these RecA constructs, we further demonstrate that prior models based on mutations in monomeric RecA provided misleading results on the nature of the SOS complex. With this minimal system, we are now poised to investigate the precise nature of the SOS signal complex, ultimately supporting the goal of structure-guided design of potential SOS inhibitors.

10-POS Board 10

MOLECULAR MECHANISMS UNDERLYING PLAF PHOSPHOLIPASE ACTIVITY REGULATION BY FREE FATTY ACIDS IN P. AERUGINOSA

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The Gram-negative Pseudomonas aeruginosa is an opportunistic pathogen that causes nosocomial infections by producing numerous virulence factors. Among these factors, type A phospholipases (PLA) can contribute to host membrane damage and modulation of signaling networks in infected cells by modulating the membrane composition. In this context, we focus on PlaF, a phospholipase A1 (PLA1). This enzyme adopt a monomeric active and a dimeric inactive configuration. A crystal structure of the dimeric PlaF complexed with undecanoic and mirystic acid in the catalytic site of both monomers (PDB_ID 6i8w) is available. Computational studies evaluated the dynamics and energetics of the dimerization process. The results reveal that a single PlaF monomer can adopt a tilted configuration, which might facilitate phospholipid substrate access from the membrane. Furthermore, we elucidated the potential channeling mechanisms underlying substrate access and product egress in PlaF in accordance with the enzyme specificity and regioselectivity. Additionally, we revealed that medium-sized free fatty acids (FFAs) can inhibit PlaF activity according to a mixed inhibition kinetics. However, the detailed molecular mechanism that governs the inhibition of PlaF by FFAs has remained elusive. Here, we show by molecular simulations that the presence of FFAs in the membrane affects the dynamics and the energetics of both PlaF dimer dissociation and monomer tilting. Moreover, free energy computations reveal an energetic stabilization of the dimeric inactive configuration, which was correlated to an increased FFA concentration in the membrane. Using MMPBSA free energy calculations, we identified hot spot residues potentially involved in the binding of FFAs. Experimental studies also revealed that FFAs in the periplasmic space can inhibit PlaF activity. We propose a potential FFA-related mechanism of PlaF inhibition using free ligand diffusion simulations. Combined with experimental validations, the identification of FFA-binding site(s) involved in the inhibition of PlaF can help design novel drugs against P. aeruginosa.

13-POS Board 13

NOVEL ANTIBIOTICS TARGET BAMA LATERAL GATE OPENING AS MECHANISM OF ACTION

Katie M. Kuo[1]; Jinchan Liu[3]; Anna Pavlova[2]; James C Gumbart[2];
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BamA, the core component of the β-barrel assembly machinery (BAM) complex, is an outer membrane protein (OMP) in Gram-negative bacteria. Its function is to insert and fold substrate OMPs into the outer membrane (OM). Evidence suggests that BamA follows the asymmetric-hybrid- barrel model, where the first strand of BamA preferentially interacts with the substrate OMP compared to the last strand. More specifically, the threading model states that the separation of the first and last strands of BamA, known as lateral gate opening, allows nascent substrate OMP β-strands to sequentially fold through β-augmentation. Recently, multiple lead compounds that target and interfere with BamA function have been identified. In our models and simulations of BamA with the lead compounds bound, we found that the lateral gate was affected. In addition, we simulated mutants of BamA that are resistant to one or more of the identified lead compounds for 20 μs each in aggregate, for a total time of 100 μs. In these simulations, we observed a notably larger degree and/or frequency of opening of BamA's lateral gate, suggesting that the mutations grant resistance by making the gate more permissive. The lead compounds act by inhibiting BamA lateral gate opening and, thus, subsequent OMP folding and insertion. These simulations validate the lateral gate opening as a mechanism of action to target in antibiotic development.

16-POS Board 16

POLYMYXIN ANTIBIOTIC SOLIDIFIES THE BACTERIAL OUTER MEMBRANE BY ARRANGING LIPOPOLYSACCHARIDE INTO CRYSTALLINE STRUCTURES

Selen Manioglu[1]; Seyed Majed Modaresi[2]; Noah Ritzmann[1]; Johannes Thoma[3]; Sarah A. Overall[4]; Alexander Harms[2]; Gregory Upert[5]; Anatol Luther[6]; Alexander B. Barnes[4]; Daniel Obrecht[5]; Daniel J. Müller[1]; Sebastian Hiller[2];
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[2]University of Basel, Biozentrum, Basel, Switzerland
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[6]Bachem AG, Basel, Switzerland

The escalation of multi-drug resistance (MDR) in gram-negative bacteria has become an increasing concern for global health. Polymyxins are the last-resort antibiotics that stand out due to their potent activity against MDR pathogens. They result in the deformation of the bacterial outer membrane by interacting with lipopolysaccharide (LPS), but despite decades of studies, the mechanistic details of this interaction at the molecular level remain unclear. Here, we characterize the interaction of polymyxins with native, LPS-containing outer membrane patches of Escherichia coli by high-resolution atomic force microscopy imaging, along with structural and biochemical assays. We find that polymyxins arrange LPS into hexagonal assemblies to form crystalline structures while altering the biophysical properties of the membrane. The occurrence of the crystalline structures appears to be correlated with the antibiotic activity of polymyxins and absent in polymyxin-resistant strains. In addition, crystal lattice parameters alter with variations of the LPS, and modifications on the polymyxin backbone determine the occurrence of crystalline structures. Quantitative measurements show that the crystalline structures decrease membrane thickness and increase membrane area as well as stiffness. In conclusion, these findings suggest the formation of rigid LPS–polymyxin crystals and subsequent membrane disruption as the mechanism of polymyxin action and provide a benchmark for optimization and de novo design of LPS-targeting antimicrobials.

19-POS Board 19

DYNAMICS OF THE INTERACTION BETWEEN THE BACTERIAL NHEJ REPAIR PROTEINS KU AND LIGD AND DNA STUDIED USING SINGLE MOLECULE NANOFLUIDICS

Evgeniya Pavlova[1]; Anusha Budida[1]; Robin Öz[1]; Fredrik Westerlund[1];
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The non-homologous end-joining (NHEJ) pathway for repair of DNA double-stranded breaks was only recently shown to exist in some prokaryotes, where its machinery is minimal. For Bacillus Subtilis the homodimer Ku and the Ligase D (LigD) have been shown both in vitro and in vivo to be the only two essential factors. The mechanism of the LigD/Ku/DNA complex formation has not been studied in detail, in particular on the single DNA molecule level. Uncovering the molecular details of the process would open possibilities for future development of antibiotics that target bacterial NHEJ. In this work, we visualize and characterize interactions between the bacterial NHEJ proteins and DNA on the single DNA molecule level.Since NHEJ occurs on DNA ends, traditional single molecule techniques, such as magnetic and optical tweezers that require the DNA molecule to be attached to a surface of a bead, are challenging to use. We instead use a nanofluidic setup, in which the DNA is stretched only due to confinement in nanochannels. By mixing Ku with λ-phage DNA and confining the samples in nanochannels we show that Ku alone brings DNA ends together to form DNA circles and concatemers in a concentration and DNA/protein ratio dependent manner. By performing EMSA we demonstrate that DNA-binding of Ku is cooperative and that LigD stabilizes DNA end-joining by Ku. Moreover, by performing a ligation reaction and imaging the sample 4 hours post reaction we show that Ku remains bound to DNA after ligation has occurred, suggesting a system is available in vivo for removing Ku when ligation is completed. Together these results open opportunities for antibiotic research, e.g. novel drugs that target Ku-DNA or LigD-Ku interactions.

22-POS **Board 22**

MUTATIONS IN THE RND TRANSPORTER ADEJ OF ACINETOBACTER BAUMANNII REDUCING THE ACTIVITY OF ADEIJK EFFLUX PUMP

Aysegül Saral Sariyer[2]; Emrah Sariyer[3]; Inga V. Leus[1]; Helen I. Zgurskaya[1];
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Acinetobacter baumannii is a gram-negative and opportunistic pathogen that causes infections in the blood, urinary tract, and lungs. The World Health Organization identified A. baumannii as one of the global priority list of antibiotic resistant bacteria. Although A. baumannii utilizes several different antibiotic resistance mechanisms, one of the most effective is antibiotic efflux mediated by RND type efflux pumps such as AdeABC, AdeIJK and AdeFGH. In this study, the critical residues in the inner membrane transporter AdeJ were identified and the effect of substitutions in these residues on the activity of AdeIJK complex was analyzed experimentally and in silico. Substitutions G721I, R701A, N81A, E675A, F618A, F136A and A134I were introduced into AdeJ expressed from a plasmid carrying the AdeIJK operon under an arabinose-inducible promoter. Plasmids producing the mutated pump were electroporated into the efflux-deficient A. baumannii AbΔ3-pore strain and minimal inhibitory concentrations (MIC) of antibiotics novobiocin and chloramphenicol were determined. Cells producing five AdeJ mutants R701A, N81A, E675A, F618A, A134I became by 4-8 fold more susceptible to chloramphenicol, whereas cells carrying mutant with the F136A substitution were more susceptible to novobiocin. Mutations of R701A in the proximal binding site of AdeJ and N81A, F136A and E675A mutations in the distal binding site of AdeJ were analyzed using molecular dynamics simulations during 200 ns. The mobility and loop dynamics of AdeJ, hydrogen bonding of chloramphenicol with the residues and the path the antibiotic followed in the pocket were examined using trajectory files. E675A, F136A and N81A had a higher deviation than AdeJ-WT but R701A had lesser. In addition, calculations of the binding affinity of chloramphenicol to the wild type and mutant AdeJ variants using the MMGBSA method showed that the binding affinity increased in mutants. The mutations changed the hydrogen bonding profile of AdeJ.

25-POS Board 25

ENZYMATIC SYNTHESIS AND CHARACTERIZATION OF ARYL IODIDES OF SOME PHENOLIC ACIDS WITH ENHANCED ANTIBACTERIAL PROPERTIES

Ewa Olchowik-Grabarek[1]; Frédérique Mies[2]; Szymon Sekowski[1]; Alina T Dubis[3]; Pascal Laurent[4]; Maria V Zamaraeva[1]; Izabela Swiecicka[1]; **Vadim Shlyonsky**[2];
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[2]Université libre de Bruxelles, Laboratory of Physiology and Pharmacology, Faculty of Medicine, Bruxelles, Belgium
[3]University of Bialystok, Department of Organic Chemistry, Faculty of Chemistry, Bialystok, Poland
[4]Université libre de Bruxelles, Laboratory of Chemistry Instruction, Faculty of Medicine, Bruxelles, Belgium

Phenolic acids represent a class of drugs with mild antibacterial properties. We have synthesized iodinated gallic and ferulic acids and together with commercially available iodinated forms of salicylic acids studied their cytotoxicity, bacteriostatic and anti-virulence action. Out of these, iodogallic acid had lowest minimal inhibitory concentration against S. aureus (MIC=0.4 mM/118.8 μg/ml). At non-bacteriostatic and non-cytotoxic concentrations (<0.1 mM), it had strongest effect on erythrocyte membrane lipid ordering (at concentrations below 10 μM) and on α-hemolysin secretion by the bacteria (at a concentration of 40 μM). It formed strong complexes with α-hemolysin in solutions (logKb =4.68±0.60) and, accordingly, inhibited its nano-pore conduction in artificial lipid bilayers (IC50=37.9±5.3 μM). These effects of iodogallic acid converged on prevention of hemolysis induced by α-hemolysin (IC50= 41.5±4.2 μM) and point to enhanced and diverse antivirulence properties of iodinated aryl iodides. The analysis of molecular surface electrostatic charge distribution, molecular hydrophilicity, electronegativity and dipole moment of selected compounds suggests the importance of the number of hydroxyl groups and their proximity to iodine. A particular electrostatic surface charges modification in iodogallic acid led to higher electrostatic hydrophilicity, while it preserved at the same the overall molecular electronegativity and dipole moment as in non-iodinated gallic acid. This study may show new directions for the development of antibacterial/antivirulence therapeutics.

28-POS Board 28

SUBSTRATE TRANSLOCATION ACROSS BACTERIAL SUGAR TRANSPORTER: FROM SINGLE-MOLECULE DETECTION TO BIOLOGICAL RELEVANCE

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[1]Rajiv Gandhi Centre for Biotechnology, Transdisciplinary Research Program, Thiruvananthapuram, India
[2]Manipal Academy of Higher Education, Manipal, India

The passage of biomolecules across the bacterial cell is facilitated by porins that form hydrophilic channels in the outer membrane. Substrate-specific porins are evolved naturally to uptake specialized essential molecules and act as a regulated gateway. We focus on a sugar-specific porin called CymA present in the outer membrane of bacteria Klebsiella oxytoca. The crystallographic structure revealed a peculiar geometry comprising a monomeric beta-barrel with the presence of an N terminus segment inside the barrel lumen, constricting the pore diameter. We quantified the translocation kinetics of cyclic sugars of different charge, size, and symmetry across native and truncated CymA, a counterpart, devoid of the N terminus segment, using single-channel recordings. The chemically divergent cyclic hexasaccharides bind to the native and truncated CymA with different interaction affinities with translocation more rapidly through truncated pore. In contrast, larger cyclic octasaccharides completely bind to native and truncated CymA, interestingly, with distinct binding kinetics in agreement with liposome assays. This highlights the importance of symmetry match with pore axis as hexasaccharides fits in to the pore to translocate efficiently whereas heptasaccharides and octasaccharides enters the pore and completely occludes the lumen. This can shed light to understand the basic mechanism behind efficient molecular uptake across bacterial membrane transporters. For example, the occlusion of pore lumen by octasaccharides can be developed as a blocker against the multi-drug resistant pathogens. Finally, we introduced native CymA as a large nanopore sensor for simultaneously sensing a variety of biomolecules such as polypeptides, highlighting the structural and functional versatility of membrane porins. Ref: Vikraman, D.; Satheesan, R.; Kumar, K. S.; Mahendran, K. R. Nanopore Passport Control for Substrate-Specific Translocation. ACS Nano 2020, 14, 2285-2295.

Tuesday, August 16
POSTER SESSION II
14:30 – 16:00
Level 3, Exhibition Area

All posters are available for viewing during all poster sessions, however, below are the formal presentations for Tuesday. Presenting authors with odd-numbered poster boards should present from 14:30 – 15:15 and those with even-numbered poster boards should present from 15:15 – 16:00. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 14:30 – 15:15 | Even-Numbered Boards 15:15 – 16:00

Athar, Mohd	**2-POS**	**Board 2**
Chapagain, Prem	**5-POS**	**Board 5**
Darendeli Kiraz, Büsra Nur	**8-POS**	**Board 8**
Jeon, Tae-Joon	**11-POS**	**Board 11**
Li, Allen	**14-POS**	**Board 14**
Menon, Anjana Peethambaran	**17-POS**	**Board 17**
Phonrat, Kanasanun	**20-POS**	**Board 20**
Sariyer, Emrah	**23-POS**	**Board 23**
Shubeita, George	**26-POS**	**Board 26**

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2-POS Board 2

INVESTIGATION OF THE BINDING PROPENSITIES OF ANTIBIOTICS ON THE SURFACE OF MEX TRANSPORTERS

Mohd Athar[1]; Attilio Vittorio Vargiu[1]; Giuliano Malloci[1]; Paolo Ruggerone[1];
[1]University of Cagliari, Physics Department, Cagliari, Italy

The World Health Organization (WHO) has identified antimicrobial resistance as one of the main global public health concerns. Efflux pumps of the RND (Resistance Nodulation-cell Division) superfamily such as MexB, MexD, MexF, and MexY of Pseudomonas aeruginosa play an essential role in multidrug resistance (both intrinsic and acquired) in Gram-negative bacteria Structural studies performed on MexB and homologous transporters in other Gram-negative bacteria revealed the presence of two main substrate recognition sites, accessed by substrate molecules through up to four (to date) different entry channels. Efforts at understanding correlations between the physico-chemical properties of compounds and their behaviour as substrates, non-substrates, or inhibitors of RND transporters have mostly relied on data about the interaction of compounds with the two main recognition
sites, namely the access pocket and the deep binding pocket. However, considering also their interaction with protein channels and possibly other undetected sites could provide precious hints regarding both specificity and susceptibility. Under these premises, we performed a systematic blind ensemble-docking study addressing the binding propensities of several classes of antibiotics to the whole surface of the Mex transporters of P. aeruginosa. Our findings were employed to rationalize experimental data about the specificities of the compounds towards the different Mex transporters.

5-POS Board 5

COMPUTATIONAL INVESTIGATIONS OF THE DUAL MECHANISMS OF ACTION OF ANTIMICROBIAL LENTHIPEPTIDES

Prem P. Chapagain[1,2]; Rudramani Pokhrel[1]; Nisha Bhattarai[1]; Prabin Baral[1]; Bernard Gerstman[1,2]; Jae Park[3]; Martin Handfield[3];
[1]Florida International University, Physics, Miami, FL, USA
[2]Florida International University, Biomolecular Sciences Institute, Miami, FL, USA
[3]Oragenics Inc., Alachua, FL, USA

The alarming rise in antibacterial resistant infections in recent years due to the widespread use of antibiotics has underscored a dire need for the development of new antibiotics utilizing novel modes of action. Due to their diverse bioactivities, the post-translationally modified peptides known as lanthipeptides are promising candidates against drug-resistant bacteria. The modes of action of lanthipeptides include interference with cell wall synthesis by binding to lipid II and creating pores in bacterial membranes. We use atomic-scale molecular dynamics computational studies to compare the lipid II binding abilities as well as their transmembrane behaviors of five different lanthipeptides that are commonly used in antimicrobial research. These include, nisin, MU1140, gallidermin, NVB302, and NAI107. Among the five peptides investigated, nisin is found to be the most efficient at forming water channels through a membrane, whereas gallidermin and MU1140 are found to be better at binding the lipid II molecules. This study provides insights into the dual mechanisms of the action of lantibiotic peptides and can facilitate the design and development of novel lanthipeptides.

8-POS Board 8

DEVELOPMENT OF EVOLUTIONARY ESCAPE MODEL TO IDENTIFY DRUGS THAT DELAY RESISTANCE

Büsra Nur Darendeli Kiraz[1,2]; Enes Seyfullah Kotil[1];
[1]Bahcesehir University, Medical School, Istanbul, Turkey
[2]Yildiz Technical University, Bioengineering, Istanbul, Turkey

Antimicrobial resistance is an inevitable situation. Many approaches can be used to defeat resistance. One of these approaches is that considers the heterogeneity of the bacterial population. Resistance can be overcome if bacteria that show resistance in the bacterial population can be predicted before drug use. Here, we provided a technique with an applied experimental and computational approach to predict bacterial evolution against the drugs. For this purpose, we produced the dataset that includes known compounds enriched with twenty-one novel compounds that we identified for this work. We applied two methods to measure bacterial resistance against these drugs. In our first method, we have measured the concentration that kills the single-step mutant, known as a mutant prevention concentration (MPC). Our second method involved evolution experiments performed for five days with serial passages at pre-determined drug concentrations. The change in growth rate during the experiments was taken as the output. The data obtained from these two methods we developed our predictive model, the evolutoinary escape model (ESM). According to the model's prediction, drugs that develop low resistance were tested in vitro. Among the molecules tested in vitro, it was determined that KL-4 had superior properties in delaying the development of resistance. Estimating drug escape routes may be a promising method to delay resistance. Overall, our approach can foresee antibiotic resistance and contribute to drug design that delays resistance.

11-POS Board 11

ANTIMICROBIAL SUSCEPTIBILITY DIAGNOSTICS USING A CHROMATIC BIOSENSOR FOR POINT-OF-CARE TESTING

Seoyoon Song[1]; Huisoo Jang[1]; Woojin Jung[1]; Deborah Lee[2]; Sun Min Kim[1,3]; **Tae-Joon Jeon**[1,2];
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[2]Inha University, Biological Engineering, Incheon, South Korea
[3]Inha University, Mechanical Engineering, Incheon, South Korea

Sepsis is a life-threatening condition with systemic inflammatory responses caused by bacteria. In addition to its high mortality rates, the emergence of antibiotic-resistant bacteria, such as MRSA (methicillin-resistant Staphylococcus aureus), is accelerating, precluding the treatment of patients with proper antibiotics in a timely manner. To overcome the limitations of the conventional ASTs (antimicrobial susceptibility testings), we devised a chromatic biosensor of which color changes could be seen by the naked eyes for point-of-care testing where needed. The chromatic biosensors enable in situ ASTs because metabolites from bacteria result in the color changes of biosensors. The biosensor demonstrated its ability to yield comparable MIC (minimal inhibitory concentration) to the preestablished MIC determined via a standard broth dilution method. Moreover, when the AST was directly carried out on human serum spiked with MRSA, the time required for the whole process greatly reduced to as short as 15 hours as favorable compared to the conventional ASTs that usually take up to a few days. With the significantly shortened required time, our biosensor will contribute to helping the medical experts to prescribe appropriate antibiotics in a timely manner, thus reducing the mortality of sepsis patients.

14-POS Board 14

BIOCHEMICAL CHARACTERIZATION OF ENGINEERED RECA OLIGOMERS AND THEIR ROLE IN THE BACTERIAL SOS RESPONSE

Allen Li[1,3]; Michael B Cory[1]; Christina M Hurley[1]; Rahul M Kohli[1,2];
[1]University of Pennsylvania, Biochemistry and Biophysics, Philadelphia, PA, USA
[2]University of Pennsylvania, Medicine, Philadelphia, PA, USA
[3]University of Pennsylvania, Chemistry, Philadelphia, PA, USA

The rapid acquisition and development of antibiotic resistance in bacteria poses a critical obstacle. While a variety of mechanisms promote antibiotic resistance, one core mechanism is the bacterial SOS response to DNA damage. This system controls key processes in bacterial survival following DNA damage, including biofilm formation, but most importantly, expression of error-prone translesion DNA polymerases. The SOS response is controlled by the interaction between two key proteins, LexA and RecA. Without DNA damage, LexA represses the expression of SOS genes. Upon DNA damage, single-stranded DNA (ssDNA) segments are generated, followed by RecA oligomerization on the ssDNA and formation of extended nucleoprotein filaments. LexA can bind to these filaments, forming the 'SOS signal complex', and LexA autoproteolysis then relieves repression of SOS genes. Given the nature of the LexA-RecA interaction, studies to understand the molecular basis for complex formation have been hampered by the fact that any mutations in a single RecA monomer propagate throughout the filament. Previous models of the SOS signal complex have suggested that two to seven RecA monomers are required for the LexA-RecA interaction. Our work, by engineering covalently linked RecA oligomers of controlled lengths, has offered us the opportunity to identify the minimal quantity of RecA units needed for the SOS response. Furthermore, these engineered oligomers have allowed us to circumvent mutation propagation. We investigated specific residues in specific protomers of our engineered RecA oligomers and introduced mutations to probe their role in the SOS response. Our results demonstrated that point mutations in wild-type RecA can lead to a complete knockout of activity, while mutation in specific protomers of the engineered RecA can be tolerated. Ultimately, our engineered RecA oligomers lay the foundation for detailed characterization of the SOS signal complex and insights into the associated central bacterial survival mechanism.

17-POS Board 17

CONTROLLED PARTITIONING OF RIFABUTIN INTO THE MYCOBACTERIAL ENVELOPE LAYERS DURING THE LATENT INFECTION STAGE

Anjana Peethambaran Menon[1,2,3]; Tzong-Hsien Lee[2,3]; Marie-Isabel Aguilar[2,3]; Shobhna Kapoor[1,3,4];
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Antitubercular chemotherapy has been successful in bringing down the death rate due to tuberculosis, however there are cases of patients surviving with persistent mycobacterium at the end of their treatment cycles. The combination of drugs fails to surpass the mycobacterial membrane at the latent stage of infection, leading to increased cases of resistance. This demands for the need to consider new markers or agents that could overcome the drug resistant variants and its occurrence. Mycobacterium has a highly complex membrane envelope which has spatially resolved outer and inner membrane layers with distinct compositions and membrane properties at both early and latent stage of infection. In this work, Rifabutin's interaction with the cell envelopes of Mycobacterium smegmatis cultured to represent both the early and late infection stages were studied using a combination of biophysical methods to study the impact of membrane composition on drug interaction profile and hence resistance. UV-visible spectroscopic studies revealed a reduction in drug partitioning in the outer membrane, while the fluorescence quenching studies indicated a hindrance in the drug penetration across the inner membrane during the latent infection stage. However, surface plasmon resonance studies indicated a transient interaction of rifabutin, while it gets partitioned within different membranes. Altogether we demonstrated that the cell envelopes at different infection stages could control the drug entry into the cytoplasm, paving paths that lead to drug resistance. By leveraging the membrane-drug interaction profiles unique to mycobacteria at both the infection stages, specific chemotypes and anti-tubercular drugs can be designed to offer tractable insights into new combinational TB therapy.

20-POS Board 20

DETECTING BACTERIA NANOSCALE INTRACELLULAR MOVEMENT USING SUB-CELLULAR FLUCTUATION IMAGING (SCFI) TECHNIQUE AS A RAPID ANTIBIOTIC SUSCEPTIBILITY TEST (AST)

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The gold standard of an Antimicrobial Susceptibility Test (AST) takes 24-48 hours, leading to empirical prescription and mandating the misuse of antibiotics. A rapid AST is crucial to provide optimal treatment and prevent the spreading of antimicrobial resistance. The single-cell imaging technique, Sub-Cellular Fluctuation Imaging (SCFI), is capable of detecting intracellular fluctuations that can be linked to bacterial viability. The method is based on Total Internal Reflection Microscopy (TIRM) and does not require fluorescent labelling. In SCFI, a single bacterium is illuminated using an evanescent field and produces a changing scattering pattern representing the nanoscale motion within the cytoplasm. A 20-second video of a bacterium is recorded and analysed to quantify the SCFI-fluctuations. Here we demonstrate how SCFI-fluctuations can be used as a biomarker indicating bacteria susceptibility to an antibiotic. We found that Escherichia coli (E. coli) DH1 treated with 100 µg/mL kanamycin showed significant lower SCFI-fluctuations than a kanamycin resistance isolate derived by E. coli DH1 (p-vale < 0.05, one-way ANOVA). We also found that the bacteriostatic trimethoprim in 20 µg/mL concentration caused a detectable reduction in SCFI-fluctuations in a susceptible E. coli suspension (p-vale < 0.05, one-way ANOVA). Furthermore, methicillin-susceptible Staphylococcus aureus (MSSA) and methicillin-resistant Staphylococcus aureus (MRSA) exhibited a statistically significant difference in SCFI-fluctuations when exposed to a solution containing 100 µg/mL methicillin (p-vale < 0.05, one-way ANOVA). Additionally, we were able to monitor the SCFI-fluctuations in real-time and observed the effect of the antimicrobial polymyxin B (500 µg/mL) on a single E. coli bacterium. In this case, we observed a significant reduction (> 90%) in SCFI-fluctuations within 10 minutes. These results support the use of the SCFI technique to perform rapid AST on bacteria suspensions containing Gram-positive or Gram-negative bacteria. Further development of SCFI combining machine learning and automation will further reduce time and improve sensitivity.

23-POS Board 23

CHROMOSOMAL DELETION OF ADEL GENE IN ACINETOBACTER BAUMANNII ATCC 17978 LEADS TO OVEREXPRESSION OF ADEAB EFFLUX PUMP

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Multi-drug efflux pumps are one of the mechanisms that contribute to the MDR A. baumannii phenotype. Efflux pumps AdeIJK, AdeABC and AdeFGH contribute to the MDR phenotype in A. baumannii. A LysR-type transcriptional regulator (LTTR), AdeL, is encoded upstream from the adeFGH operon and is known as a negative regulator. The elucidation of efflux pump regulatory mechanisms may contribute to the discovery of new antibiotics. In this study, we deleted the 734 bp region of adeL gene in A. baumannii ATCC 17978 (AbWT) and its ΔAdeIJK mutant and characterized the effect of this chromosomal deletion on the antibiotic susceptibility profile and growth physiology. For deletion of the adeL gene, a gentamicin resistance gene cassette in pMo130-Gm and recombineering primers were used. The gentamicin resistance gene cassette was removed from ΔAdeIJK ΔAdeL:Gm and ΔAdeL:Gm by incorporating FLP recognition target (FRT) sites. Removal of the resistance gene cassette was confirmed by PCR. Broth microdilution method was used for determining minimal inhibitory concentrations (MIC) of antibiotics against ΔAdeIJK, ΔAdeIJK ΔAdeL, AbWT and ΔAdeL. Growth curves were analyzed for these strains grown in LB broth and in the presence of chloramphenicol (MIC/2). Compared to ΔAdeIJK, the ΔAdeL mutant had no changes in MIC values of antibiotics. The difference in MIC values was observed only between AbWT and its ***Δ***AdeL mutant for azithromycin, zeocin and gentamicin. According to growth curve assays, chromosomal deletion caused a growth defect in AbWT and its ΔAdeIJK mutant. This defect was more visible in the presence of chloramphenicol. Our results suggest that AdeL may have a different physiological role besides the control of AdeFGH expression. The changes in antibiotic susceptibility profile suggest that AdeL inactivation may cause the overexpression of AdeAB without changes in the expression of AdeFGH.

26-POS Board 26

A HIGH-THROUGHPUT APPROACH TO THE DISCOVERY OF NOVEL DNA-INTERCALATING ANTIMICROBIAL AND ANTINEOPLASTIC DRUGS

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Molecules that bind DNA by intercalating its bases remain among the most potent antimicrobials and cancer therapies due to their interference with DNA-processing proteins. To accelerate the discovery of novel intercalating drugs, we designed a Fluorescence Resonance Energy Transfer (FRET)-based probe that reports on DNA intercalation, allowing rapid and sensitive screening of chemical libraries in a high-throughput format. We demonstrate that the method correctly identifies known DNA intercalators in approved drug libraries and discover previously unreported intercalating compounds. When introduced in mammalian cells, the oligonucleotide-based probe rapidly distributes in the cytosol and nucleus, allowing direct imaging of the dynamics of drug entry and its interaction with DNA in its native environment. This enabled us to directly correlate the potency of intercalators in killing cultured cancer cells with the ability of the drug to penetrate the cell membrane. The combined capability of the single probe to identify intercalators in vitro and follow their function in vivo can play a valuable role in accelerating the discovery of novel DNA-intercalating drugs or repurposing approved ones.

Wednesday, August 17
POSTER SESSION III
14:30 – 16:00
Level 3, Exhibition Area

All posters are available for viewing during all poster sessions, however, below are the formal presentations for Wednesday. Presenting authors with odd-numbered poster boards should present from 14:30 – 15:15 and those with even-numbered poster boards should present from 15:15 – 16:00. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 14:30 – 15:15 | Even-Numbered Boards 15:15 – 16:00

Can, Dilan	**3-POS**	**Board 3**
Chaudhury, Anurag	**6-POS**	**Board 6**
Garcia Vazquez, Alba	**9-POS**	**Board 9**
K, Ratnasri	**12-POS**	**Board 12**
Mamou, Gideon	**15-POS**	**Board 15**
Ojkic, Nikola	**18-POS**	**Board 18**
Ryoo, David	**21-POS**	**Board 21**
Sauer, David	**24-POS**	**Board 24**
Sousa, Carla	**27-POS**	**Board 27**

Posters should be set up on the morning of Monday, August 15 and removed by noon on Thursday, August 18. All uncollected posters will be discarded.

3-POS Board 3

POST-EVOLUTIONARY 'RESCUER' GENES

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When bacteria are forced to grow under stressors or changed environmental conditions, those with the most suitable genotype for these conditions quickly replicate, forming most of the current population. Thus, bacterial evolution takes place. Therefore, there are many theoretically suitable solutions for many challenging conditions. The biggest problem among these solutions is the resistance developed by bacteria against antibiotics, and it is a worrying global problem. Knowing the evolutionary trajectories that mediate resistance will be critical to winning this battle. In a situation that is not emphasized enough, which genes are more critical in evolved bacteria for their current 'new' situation? Bacteria that have become resistant to a drug depend on the presence of specific genes to normalize their growth rate to stabilize their adaptation. Thus, the importance of that gene in the evolved bacteria changes.To test this hypothesis, We identified ten genes that affect fitness cost involved in cellular stress response, central carbon, amino acid, and lipid metabolism. The genes were selected based on flux balance analysis and other quantitative methods. We inhibited these ten genes with CRISPR interference (CRISPRi) in E.coli strains, and we applied adaptive laboratory experiments (ALE) against four antibiotics with different mechanisms of action. We then examined the effect of these genes on the growth rate after evolution. Decreased expression of targeted genes and decreased growth rates exhibited coordination. The results obtained reflected these targeted genes 'rescuer' role in the evolution. When bacteria evolve against an antibiotic, these genes become essential for the bacteria's new self. Highlighting these genes is promising in finding practical solutions against antibiotic resistance. In addition, these 'post-evo genes' will be essential in accelerating identifying candidate genes for new antibiotic discovery or cellular pathways to target.

6-POS Board 6

E.COLI MEMBRANE STRUCTURE AND NANOSCALE DYNAMICS AT SUB-INHIBITORY ANTIBIOTIC CONCENTRATIONS: CORRELATIONS TO EMERGENCE OF ANTIMICROBIAL RESISTANCE

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Exposure of bacteria to sub lethal concentration of antibiotics gives rise to antimicrobial resistance. Various drugs target the bacterial membrane directly or indirectly, and understanding the bacterial membrane response thus becomes very important. Our Fluorescence Correlation Spectroscopy (FCS) results demonstrate the E.coli membrane getting loosely packed showing a higher diffusion co-efficient after adding antibiotics[1,2]. But growing E.coli in progressively increasing concentrations of antibiotics allows it to grow even at the minimal inhibitory concentration (MIC). Using super-resolution stimulated emission depletion (STED) nanoscopy coupled with FCS reveals the emergence of length scale dependent E.coli membrane dynamics for increasingly higher antibiotic concentrations starting from a sub-MIC value. It implies that the E.coli tries to adapt its membrane in order to grow even at the MIC value before finally becoming susceptible to concentration >1 MIC. Our AFM results show different patterns of roughness of the outer membrane during the process of the bacteria being allowed to grow at different antibiotic concentrations. These interesting results can be an important step in understanding how this gram-negative bacterium tries to develop antimicrobial resistance at short time scales upon exposure to antibiotics.[1] Ilanila Ilangumaran Ponmalar, Jitendriya Swain and Jaydeep K. Basu. Biomaterials Science 20, 2609-2617, 2022 (https://doi.org/10.1039/D2BM00037G)[2] Ponmalar, Ilanila Ilangumaran, Jitendriya Swain, and Jaydeep K. Basu. Biochimica et Biophysica Acta (BBA)-Biomembranes 1864, no. 8 (2022): 183935. * The authors thank the financial support from the Indian Institute of Science, (IISc), Bengaluru, through the Ministry of Human Resources Development (MHRD)-funded Institute of Eminence (IOE) project.

9-POS Board 9

PLANET EARTH-LIKE PATTERNS IN THREE-DIMENSIONAL MULTI-SPECIES BACTERIAL COLONIES.

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Bacteria typically grow in communities and this provides them substantial advantages compared to solitary cells. These communities are often comprised of multiple bacterial species leading to the emergence of complex spatial patterns. The emergence of these complex spatial patterns can have a profound effect on bacterial function and survival within the communities. Most experimental studies investigating the mechanism behind this pattern formation have focused in two-dimensional systems. Here we propose a novel approach to study three-dimensional multi-species bacterial colonies. This three-dimensional setting replicates better some environmental bacterial habitats such as soil and intestines. Our results indicate that in three-dimensional multi-species colonies just the cells from the outer part of the colony are able to grow while the center of the colony remains static. We anticipate our protocol to be a starting point for further studies. For example, the protocol could be used to bring some light to many different biological and physical questions which are still unanswered such as the mechanism behind horizontal gene transfer and the social interactions arising within multi-species three-dimensional bacterial colonies. Furthermore, understanding how bacteria thrive in competitive habitats and their cooperative strategies for surviving extreme stress can be instructive, for instance, to inspire new investigations for developing a more rational approach for battling pathogenic bacteria resistant to antibiotics.

12-POS Board 12

ANTIMICROBIAL ACTION ON PERSISTER PHENOTYPES

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Bacterial persisters are a subset of phenotypic variants that exist in an isogenic bacterial population. They are characterised by their tolerance to harsh environments such as starvation and antibiotic exposure. Not only have persister bacteria been implicated in the recalcitrance of many infections, but recent evidence points towards persister cells serving as a precursor for onset of antibiotic resistance. Hence, understanding the formation and behaviour of persister bacteria is important for insights into emergence of antimicrobial resistance.Persistence is known to be a non-heritable and transient phenomenon occurring at very low frequencies, making the isolation and study of persisters harder. In addition, several different molecular pathways and physiological conditions have been shown to be connected to the persister phenotype, including cell dormancy or slow growth, reduced metabolic activity, activation of the stringent response and the involvement of toxin-antitoxin modules. In this study, we test the idea that a bacterial population when subjected to stress conditions, can enter persister-like state via multiple pathways. We enrich for persisters originating from different conditions from an exponentially growing bacterial culture. The enrichment is followed by a time-kill assay using the antibiotic Ofloxacin to measure the level of Ofloxacin tolerant persisters. While the enrichment based on reduced metabolic activity generates a 100 - fold increase in persisters, a prior antibiotic exposure yields only a 10-fold increase. We then examine the efficacy of various antibiotics and antimicrobial peptides (AMPs) in inhibiting the growth of the persister populations.

15-POS Board 15

COORDINATED CELL ENVELOPE BIOGENESIS IN GRAM-NEGATIVE BACTERIA

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The cell envelope of Gram-negative bacteria is a tripartite structure composed of the symmetric phospholipid inner membrane, an asymmetric outer membrane (OM) and an intervening peptidoglycan layer. Linkages between the OM and the peptidoglycan are crucial to the maintenance of cellular integrity and enable survival in challenging environments. The functionality of the outer membrane relies on a variety of outer membrane proteins (OMPs), which are inserted by the β-barrel assembly machine, BAM. Previous studies have shown that growing Escherichia coli cells segregate old OMPs towards the cell poles by a process known as binary partitioning, the basis of which is unknown. Here, we demonstrate that peptidoglycan underpins the spatiotemporal organization of OMPs. Mature, tetrapeptide-rich peptidoglycan binds to BAM components and suppresses OMP foldase activity. Nascent peptidoglycan, which is enriched in pentapeptides and concentrated at septa, associates with BAM poorly and has little impact on its activity, leading to preferential insertion of OMPs at division sites. Consequently, OMP biogenesis is synchronized with cell wall growth and results in binary partitioning of OMPs as cells divide. Our study reveals that Gram-negative bacteria coordinate the assembly of two major cell envelope layers by rendering OMP biogenesis responsive to peptidoglycan maturation, a potential Achilles heel that could be exploited in future antibiotic design.

18-POS Board 18

ANTIBIOTIC RESISTANCE VIA BACTERIAL CELL SHAPE SHIFTING

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Antibiotic resistance is one of the major threats to human society prompting urgent global response. Bacteria developed multiple strategies for antibiotic resistance by effectively reducing intracellular antibiotic concentrations or antibiotic binding affinities to their specific targets. Resistance commonly occurs via a reduction in porin expression, modulation of membrane lipid composition, horizontal gene transfer, increased expression level of efflux pumps and proteins that inactivate antibiotics, or via SOS response. Here we present a recently discovered pathway to antibiotic resistance that depends on the bacterial morphological transformation that promotes bacterial decrease of antibiotic influx to the cell. By analysing cell morphological data of different bacterial species under antibiotic stress, we find that bacterial cells robustly reduce surface-to-volume ratio in response to most types of antibiotics. Using quantitative modelling we show that by reducing the surface-to-volume ratio, bacteria can effectively reduce intracellular antibiotic concentration by decreasing antibiotic influx. The model predicts that bacteria can increase the surface-to-volume ratio to promote antibiotic dilution for membrane targeting antibiotics, in agreement with data on membrane-transport inhibitors. Using the particular example of ribosome-targeting antibiotics, we present a systems-level model for the regulation of cell shape under antibiotic stress and discuss feedback mechanisms that bacteria can harness to increase their fitness in the presence of antibiotics.

21-POS Board 21

TIGHTLY PACKED: INTERROGATING CELL ENVELOPE MECHANICS WITH A PRE-STRAINED CELL WALL

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The cell envelope of Gram-negative bacteria acts as a physical and chemical barrier against harmful small molecules via selective diffusion. It also must withstand the turgor pressure resulting from the high concentration of solutes inside the cell. It is composed of three components: the inner membrane, the outer membrane (OM), and the peptidoglycan cell wall between them. The cell wall is connected to the outer membrane by Braun's lipoprotein (Lpp). Previously, we determined how each component of the cell envelope responds to induced strain, concluding that most of the turgor pressure is borne by the OM and the cell wall. However, how these two components work together remains to be elucidated. Using molecular dynamics, we have simulated the combined system of the OM and the cell wall linked by multiple Lpp copies. We also constructed and simulated the system with pre-strained cell wall that was stretched 2x and 2.5x its original size to observe how the overall system adjusts, finding that the OM is resistant to the induced stress. We also calculated the area compressibility of the combined OM and cell wall system under different degrees of cell wall strain. Our results provide key insights into cell envelope stability and suggest promising directions for disrupting it.

24-POS Board 24

STRUCTURAL BASIS FOR INHIBITION OF THE DRUG EFFLUX PUMP NORA FROM STAPHYLOCOCCUS AUREUS

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Membrane protein efflux pumps confer antibiotic resistance by extruding structurally distinct compounds and lowering their intracellular concentration. Yet, there are no clinically approved drugs to inhibit efflux pumps, which would potentiate the effi- cacy of existing antibiotics rendered ineffective by drug efflux. Here, we identified synthetic antigen-binding (Fab) fragments that inhibit the quinolone transporter NorA from methicillin-resistant Staphylococcus aureus (MRSA). Structures of two NorA– Fab complexes determined using cryo-electron microscopy (cryo-EM) reveal a Fab loop deeply inserted in the substrate-binding pocket of NorA. An arginine residue on this loop interacts with two neighboring aspartate and glutamate residues essential for NorA-mediated antibiotic resistance in MRSA. Peptide mimics of the Fab loop inhibit NorA with submicromolar potency and ablate MRSA growth in combination with the antibiotic norfloxacin. These findings establish a class of peptide inhibitors that block antibiotic efflux in MRSA by targeting indispensable residues in NorA without the need for membrane permeability.

27-POS Board 27

MODELLING THE EFFECT OF HYDROPHOBICITY ON ANTIBIOTIC PASSIVE PERMEATION AND ITS IMPACT ON BACTERIAL BIOAVAILABILITY

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Antibiotic bioavailability in the bacterial cell is crucial for drug efficiency. Specifically, permeation by passive diffusion may be highly relevant, since facilitated routes of influx (i.e. protein channels or transporters) are frequently hindered in resistant bacteria. Here, we use molecular dynamics simulations to investigate the impact of changes in hydrophobicity on the permeability of a series of Pseudomonas quorum sensing inhibitors across membrane models. This set of drugs have a common 2-nitrophenyl scaffold, connected to an alcohol with an alkyl chain of increasing length, corresponding to increasing hydrophobicity.[1]We have determined a direct correlation between hydrophobicity and permeability, using the inhomogeneous solubility-diffusion model. This correlation does not fit, however, the experimental results, indicating an overestimation of the permeability of the most hydrophobic compounds. On the other hand, an analysis of the difference between ΔG_{max} and ΔG_{min} led to a permeability ranking that better agrees with experimental results, pointing to similar permeabilities for this set of molecules. Additionally, we showed that drug orientation across the bilayer has impact on the permeation process, as solutes that do not tend to flip at the membrane center permeate more favorably.Hence, using in silico methods, we were able to assess the impact of hydrophobicity on drug's passive diffusion and provide additional atomistic detailed information on the permeation process. Overall, our results indicate that hydrophobicity should not play a crucial role for the passive permeation of this set of drugs. This study evidences the potential of combining computational and experimental data to study drug permeability and contribute to the development of more effective drugs.1. Storz, M. P.; Allegretta, G.; Kirsch, B.; Empting, M.; Hartmann, R. W., From in vitro to in cellulo: structure–activity relationship of (2-nitrophenyl)methanol derivatives as inhibitors of PqsD in Pseudomonas aeruginosa. Organic & Biomolecular Chemistry 2014, 12 (32), 6094-6104.